

## Study on the Ageing Method and Antioxidant Activity of Black Garlic Residues

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### Abstract

Xiong F., Dai Ch.-H., Hou F.-R., Zhu P.-P., He R.-H., Ma H.-L. (2018): A study on the ageing method and antioxidant activity of black garlic residues. Czech J. Food Sci., 36: 88–97.

Garlic residue (GR), a co-product of garlic oil extraction, contains most of the nutrients of raw garlic (RG). Preparation of black garlic residue (BGR) is considered to be an effective method of processing GR. The main objective of this study was to optimise the ageing conditions of GR based on moisture, polyphenol and 5-hydroxymethyl-2-furaldehyde (HMF) levels. In addition, the antioxidant capacity of BGR was also evaluated *in vitro* and *in vivo* and compared with black garlic (BR) and RG. The results indicate that optimum ageing resulting in polyphenol and HMF contents of 25.80 mg/g and 3.84 mg/g, respectively, were achieved using a temperature of 90°C and humidity of 95% for four days. Both BGR and BR had stronger capacities to scavenge  $\alpha, \alpha$ -diphenyl- $\beta$ -picrylhydrazyl (DPPH) than RG with half maximal inhibitory concentration ( $IC_{50}$ ) values of 0.454, 0.514 and 4.236 mg/ml, respectively. Experiments on mice demonstrated that there was no obvious difference in antioxidant activity between BGR and BR *in vivo*. BGR and BR consumption significantly decreased malondialdehyde (MDA) levels in serum and liver, in addition to markedly increasing superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities.

**Keywords:** ageing conditions; antioxidant ability; garlic residues product

Garlic is widely grown in Asia due to its nutritional and medicinal value, especially in China. Among all garlic products, garlic oil is the most prevalent and is mainly applied to processed meat and chicken products in the form of natural antimicrobials, flavourings and antioxidants (HALLIWELL *et al.* 1990; SALLAM *et al.* 2004; LEONG *et al.* 2010). Garlic residue (GR), a co-product after oil extraction, has long been considered as waste. The utilization of GR in food and animal feed is limited owing to its characteristic odour, taste and tendency to cause an upset stomach.

Most nutrients and bio-active components are retained in GR after garlic oil extraction. In order to

utilise GR effectively, many investigations have been conducted with the aims of developing GR-containing products and investigating the biological effects of GR. For example, GR has been successfully used in the production of condiments. ZONG *et al.* (2014) optimised conditions for the ultra-high pressure-assisted extraction of polysaccharides from GR. The yield of polysaccharides reached 14.18% at a pressure of 400 MPa, extraction time of 4.5 min and a ratio of GR to distilled water of 1 : 16 (g/ml). ZHANG *et al.* (2014) discovered that the serum levels of total cholesterol and low-density lipoprotein in rat decreased significantly, whereas high density lipoprotein level

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Supported by the Primary Research & Development Plan of Jiangsu Province, Projects No. BE2017308 & 201635, Jiangsu Province Postdoctoral Science Foundation, Grant No. 1402001B, and Science and Technology Support Program (Agriculture) of Jiangsu Province, Project No. BE2016355.

<https://doi.org/10.17221/420/2016-CJFS>

increased, after the feeding of GR for seven days, revealing its bio-activity in lowering blood lipids.

As another form of garlic, black garlic (BG) is a very popular functional food in Asia. In general, it is produced by heating the whole raw garlic (RG) bulbs at a high temperature and a controlled humidity for about one month. Physical-chemical characteristics such as colour, flavour, texture and nutrients compositions are thus altered to greater or lesser extents, resulting in the production of aged BG (NENCINI *et al.* 2011). In marked contrast to RG, the appearance of garlic turns dark brown during the production of BG due to the non-enzymatic browning (Maillard reaction) which occurs during the heating process. In addition, BG tastes sweeter and softer than RG, as much of the pungent and unpleasant odour has been eliminated (KIM *et al.* 2012). Furthermore, the levels of bio-active compounds like S-Allyl cysteine, phenolic acid and flavanols increase during the ageing process (BAE *et al.* 2012; KIM *et al.* 2013a; LI *et al.* 2015). Several reports have demonstrated that BG harbours anti-hyperglycaemic, hypolipidaemic, anti-oxidative, immune-enhancing and anti-inflammatory effects, etc. (WANG *et al.* 2010; ANGELES *et al.* 2016; MOUSTAFA & HUSSEIN 2016).

To date, only a small number of studies have described BG processing although many publications have reported its beneficial effects on health. In order to utilise GR effectively, black garlic residue (BGR) was produced in this study and ageing conditions were optimised with reference to total polyphenol and 5-hydroxymethyl-2-furaldehyde (HMF) levels. In addition, the antioxidant activity of BGR was also evaluated *in vitro* and *in vivo*.

## MATERIAL AND METHODS

Garlic devoid of any diseases, insect injuries or mechanical damage was obtained from Luzhiye Co., Ltd. (China). The contents of moisture, crude protein and total ash of the whole garlic bulb were 67.5, 6.4, and 1.3%, respectively (AOAC 1990). Total sugar content, determined with the 3,5-dinitrosalicylic acid method, was 21.8% (HAN & ZHANG 2008). Garlic oil content (which contained some sulfur compounds) was 1.3% and was analysed according to the method of HUANG and ZHUO (2008).

The following reagents were used in this study: gallic acid, Folin-Ciocalteu reagent, coumaric acid and 5-hydroxymethyl-2-furaldehyde (HMF) were from

Sigma-Aldrich, Inc. (USA). The malondialdehyde (MDA) kit (A001-1-1), superoxide dismutase (SOD) kit (A005), and glutathione peroxidase (GSH-Px) kit (A003-1) were from Jiancheng Bioengineering Institute (China). The other reagents were of analytical grade and were purchased from Sinopharm (China).

**Ageing process of RG and GR.** RG was peeled and washed using distilled water and then immersed in a 0.2% NaCl solution for 30 min. The garlic was aged under different temperatures (70, 80, and 90°C) and levels of humidity (85, 90, 95, and 100%) at constant humidity or temperature for several days.

RG was peeled and crushed in a high-speed smashing machine, then incubated at 40°C for 1 h in order to fully transform alliin into allicin. Garlic oil was extracted using supercritical carbon dioxide extraction equipment with a pressure 35 MPa and temperature of 35°C for 200 minutes. After the separation of garlic oil, the GR was acquired. The subsequent GR ageing process was performed as above.

**Determination of moisture.** The moisture of the samples was determined with an oven method by drying to a constant weight at 105°C.

**Determination of polyphenol content.** Polyphenols were measured using the Folin-Ciocalteu method (LI *et al.* 2011), based on the reduction of polyphenols by oxidants. After mixing 10 g of samples with 10 ml of distilled water and homogenisation, the mixture was diluted to 100 ml with 50% acetone. Then, extraction of polyphenol was conducted at 30°C in an ultrasound water bath for 30 minutes. Filtration was performed before measuring polyphenol levels. Sample extracts (0.2 ml) and 0.5 ml of Folin-Ciocalteu reagent were mixed. After standing for 5 min, 1.5 ml of 20% sodium carbonate were added followed by dilution with distilled water to 10 ml. After incubation at 50°C for an hour, the absorbance was measured at 765 nm and the total polyphenol levels were calculated from a calibration curve, using gallic acid as the standard. The phenolic content was reported as gallic acid equivalents (mg) using the following linear equation based on the calibration curve:

$$Y = 0.063X - 0.008, R^2 = 0.9960 \quad (1)$$

where:  $Y$  – absorbance at 765 nm;  $X$  – concentration of gallic acid equivalents (mg/ml)

**Determination of HMF content.** HMF was determined spectrophotometrically according to the method described by LI *et al.* (2015). Sample extract (2 ml) (prepared with the same method as for polyphenol

nol extraction) was mixed with 5 ml of 4-methylaniline solution (5 mg/ml) and 1 ml of barbituric acid (5 mg/ml). After mixing fully and standing for an hour, absorbance at 550 nm was measured against a blank that had been prepared using deionised water instead of the sample. HMF content was calculated from a calibration curve using 5-hydroxymethyl-2-furaldehyde as a standard:

$$Y = 0.00067X - 0.001440, R^2 = 0.9979 \quad (2)$$

where:  $Y$  – absorbance at 550 nm;  $X$  – HMF content (mg)

The optimum temperature and humidity employed during the ageing procedure were determined based on the contents of moisture, polyphenol and HMF. BGR and BG prepared in this way were then further analysed for their phenolic compounds using HPLC and their antioxidant activities *in vitro* and *in vivo* were also determined.

**HPLC analysis.** The polyphenol extracts (prepared as above) were vacuum-concentrated and dissolved in ethyl acetate. After removing moisture with anhydrous sodium sulphate, ethyl acetate solution was concentrated again up to dryness, the residue was redissolved in methanol and the phenolic ingredients were analysed using HPLC LC-6A (Shimadzu Corporation, Japan).

After injection of 20  $\mu$ l of sample solution, separation was performed with a C18 column (4.6  $\times$  250 mm, 5  $\mu$ m) at 30°C with an elution of methanol/water/glacial acetic acid (5:95:1, v/v/v) at a flow rate of 0.8 ml/min and detection at 280 nm. Phenolic ingredients were quantified by determining the peak area of their maximum absorption wavelength.

**$\alpha, \alpha$ -Diphenyl- $\beta$ -picrylhydrazyl (DPPH) radical scavenging ability.** DPPH radical scavenging ability was measured according to the method of ZHANG *et al.* (2011) with some modifications. After mixing 200 g of ground samples and 3000 ml of distilled water, the extraction was conducted in a boiling water bath for 2 h and then centrifuged at 5000 g for 30 minutes. After that, the supernatant was vacuum-concentrated and lyophilised for the further analysis of antioxidant activities. Sample solutions (2 ml) with different concentrations were added to 2 ml of DPPH ethanol solution (0.1 mM). The mixture was shaken and left to stand for 30 min in the dark. The absorbance at 517 nm was measured using a spectrophotometer. Ascorbic acid was used as a positive control. DPPH radical scavenging activity was calculated according to the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [1 - (A_1 - A_2/A_0)] \times 100$$

where:  $A_1$  – absorbance of the mixture of sample and DPPH solution;  $A_2$  – absorbance of the mixture of sample and ethanol solution;  $A_0$  – absorbance of the mixture of distilled water and ethanol solution

The half inhibitory concentration ( $IC_{50}$ ) of samples for scavenging DPPH radicals represents the concentration that caused a 50% inhibition of radical formation.

**Design of the mice experiments.** BRG (200 g) was extracted with 3 l of distilled water in a boiling water bath for 2 h; after centrifugation at 5000 g for 30 min, the supernatant was then vacuum-concentrated and lyophilized for the mice experiments. BG and RG extracts were produced in the same way.

One hundred and twenty Kunming female mice weighing  $40 \pm 2$  g (six months old) were used (Jiangsu University Laboratory Animal Center). They were fed a standard laboratory diet purchased from Sebia Biological Technology Co., Ltd. (China), consisting of more than 18.0% crude protein, 4.0% crude fat, less than 8.0% moisture, 5.0% coarse fibre, and 6.5% total ash, as well as an appropriate mineral mixture, and tap water was freely available. Mice were randomly divided into 12 groups (10 mice per group): a tap water control group, a model group (fed with the same volume of physiological saline), a Vc group in which vitamin C was administered at 100 mg/kg of body weight (BW), three BGR groups, three BG groups and three RG groups at dosages of 130, 520, and 1300 mg/kg BW, respectively. After overnight fasting, all groups were fed their respective diets by gavage once daily for 30 consecutive days.

**Biochemical assays.** On the last day, gavage was performed as before. One hour later, the mice (except for the control group) were administered bromobenzene (dissolved in olive oil) at a concentration of  $6 \times 10^{-7}$  g/kg and a dosage of 0.01 ml/g BW. Eighteen hours after the administration, mice were euthanised. Blood samples were collected and centrifuged at 4000 g for 10 min at 4°C to produce the sera. The livers were quickly removed, washed and homogenised in ice-cold physiological saline to prepare a 10% (w/v) homogenate. The homogenate was centrifuged at 4000 g for 10 min at 4°C to remove cellular debris, and the supernatant was collected for analysis.

The levels of SOD, GSH-Px, and MDA were determined following the instructions of the respective kit. Briefly, SOD activity was estimated through the

<https://doi.org/10.17221/420/2016-CJFS>

inhibition of hydroxylamine oxidation by the superoxide radicals generated in the xanthine-xanthine oxidase system. GSH-Px activity was measured on the basis of the reaction of GSH and 5,5'-dithiobis-(2-nitrobenzoic acid). MDA content was determined with the 2-thiobarbituric acid method using 1,1,3,3-tetraethoxypropane as a standard.

**Statistical analysis.** The experiments were performed three times. The data were expressed as the mean  $\pm$  standard deviation and were analysed using one-way analysis of variance (ANOVA) and Student's *t*-test. All graphs were generated with Origin 9.0.

## RESULTS AND DISCUSSION

**Effect of ageing temperature on moisture, polyphenol and HMF content.** The effect of ageing temperature on moisture of BGR and BG at the ageing humidity of 95% is shown in Figure 1. BGR and BG moisture content increased slightly at 70°C with increasing processing time with a non-significant difference between BGR and BG. This might be due to water permeating garlic tissue to achieve a humidity equilibrium between the inside and outside of a cell under conditions of high humidity and low temperature. At 80 and 90°C, meanwhile, the moisture content of BGR and BG both decreased slightly to about 50%. It has been reported that BG tastes soft and elastic when moisture is kept at levels of 50–70% (ZHANG *et al.* 2016).

Polyphenol is an important indicator of the quality of BG products. Figure 2 show that polyphenol levels in both BGR and BG increased during the

initial phase of ageing, but then decreased with increasing ageing time. The incremental increase in polyphenol levels in BGR was faster than in BG, potentially due to the bigger surface of the material promoting a faster ageing process. The polyphenol content of BGR reached a maximum of 25.80 mg/g when processed at 90°C for four days, 19.71 mg/g when processed at 80°C for 12 days, and 15.62 mg/g when processed at 70°C for 20 days, respectively. The maximal polyphenol content of BGR increased about five-fold compared with raw material, consistent with the results of LI *et al.* (2015), who discovered that total polyphenol levels were about 7.6-fold greater when raw garlic was converted into BG. ROBARDS *et al.* (1999) considered that browning reactions could produce more complex polyphenols. Although the existence of other reductive ingredients could hamper the determination of polyphenols, the effect is not significant due to their low contents; ascorbic acid, as the main reductive ingredient, exhibits a concentration of 31.5 mg/100 g fresh garlic (POKLUDA & PETRIKOVA 2001).

HMF is not only a product of the Maillard reaction that occurs between an amino acid and a reducing sugar during heat-processing but is also a product of degradation reactions of carbohydrates (without the participation of proteins or other nitrogen compounds), which are one of the main antioxidant ingredients in black garlic products. The accumulation of HMF was linked with the rate of formation of black colour in the garlic sample (DURLING *et al.* 2009). Therefore, changes in HMF content can serve as an important monitoring index for predicting the rate of black formation in garlic samples.

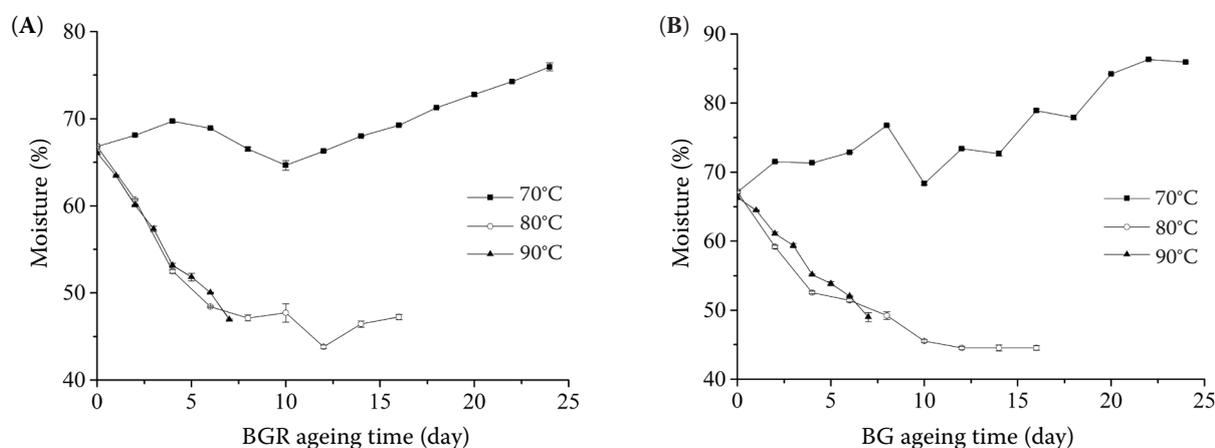


Figure 1. Effect of ageing temperature on the moisture of (A) black garlic residue (BGR) and (B) black garlic (BG) (values are mean  $\pm$  SD)

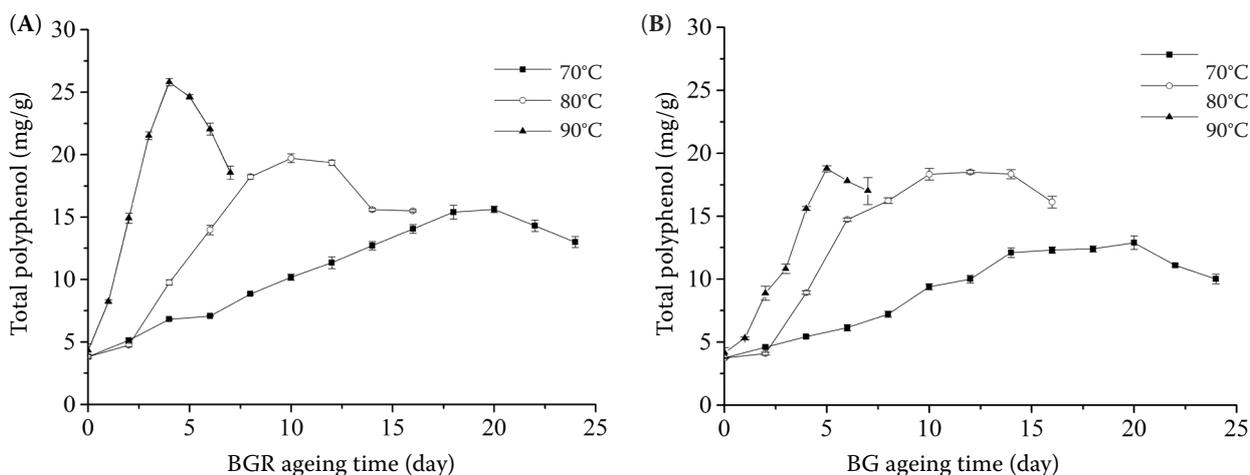


Figure 2. Effect of ageing temperature on total polyphenol content of (A) black garlic residue (BGR) and (B) black garlic (BG) (values are mean  $\pm$  SD)

As seen in Figure 3, HMF content increased significantly in BGR and BG during ageing at a temperature of 90°C and humidity of 95% demonstrating that the rate of the Maillard reaction was fast; in contrast, it was slow at 70 and 80°C. Maximum HMF levels reached 3.84 mg/g in BGR and 3.25 mg/g in BG; both values were measured on the 7<sup>th</sup> day at 90°C. These observations are consistent with those of ZHANG *et al.* (2016), who reported that garlic becomes black when the HMF content reaches about 4 g/kg.

Taken together, these results suggest that 90°C is the optimal ageing temperature for the preparation of BGR and BG.

**Effect of ageing humidity on moisture, polyphenol and HMF content.** The effect of humidity on the moisture content of BGR and BG at an ageing

temperature of 90°C is shown in Figure 4. The trends of the changes in moisture were similar for both BGR and BG. The moisture of BGR decreased with increased ageing time at humidity levels of 85, 90, and 95%; however, this parameter increased slightly at a humidity of 100%.

The effect of humidity on the polyphenol content of BGR and BG is shown in Figure 5. The results showed that the maximum levels of polyphenol reached in BGR were 28.05, 23.67, 25.80 and 26.00 mg/g, respectively, at levels of humidity of 85, 90, 95, and 100% and at an ageing temperature of 90°C. It has been reported that garlic is rich in phenolic compounds and that the content of polyphenol is increased by about four- to ten-fold in black garlic compared with fresh garlic (KIM *et al.* 2013b).

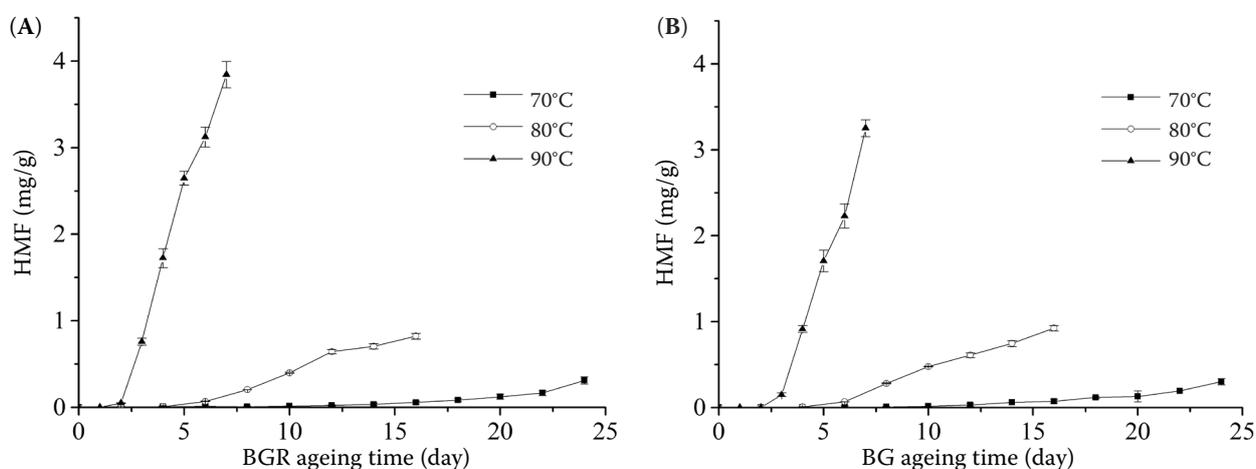


Figure 3. Effect of ageing temperature on 5-hydroxymethyl-2-furaldehyde (HMF) levels of (A) black garlic residue (BGR) and (B) black garlic (BG) (values are mean  $\pm$  SD)

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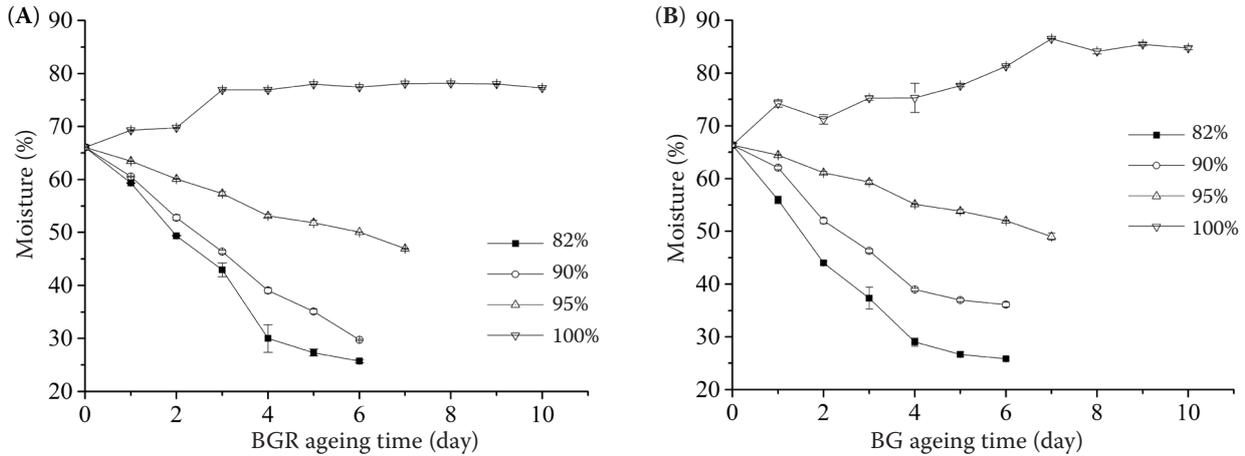


Figure 4. Effect of humidity level on the moisture of (A) black garlic residue (BGR) and (B) black garlic (BG) (values are mean  $\pm$  SD)

Figure 6A and B show that the changes of HMF in BGR and BG were similar. A lower level of humidity resulted in a faster accumulation of HMF. The maximum HMF content in BGR reached 3.84 mg/g on the 7<sup>th</sup> day at a humidity of 95%.

On the basis of the above results, 95% was determined as the optimal ageing humidity for garlic residue and raw garlic. Therefore, BGR and BG were prepared under the conditions of a temperature of 90°C, humidity of 95% and an ageing time of four days for further analysis.

**HPLC analysis.** Figure 7 indicates that BGR was rich in phenolic compounds. Gallic acid and coumaric acid were identified using HPLC by comparison with standard phenolics. The HPLC chromatogram indicated that coumaric acid was the main ingredient in BGR. Phenolic compounds are known to be powerful

chain-breaking antioxidants and have a strong scavenging ability due to their hydroxyl groups. These results indicated that the pronounced *in vitro* and *in vivo* antioxidant activities of BGR and BG were possibly due to their high phenolic content.

**DPPH radical scavenging ability.** DPPH radical scavenging activity is often used to evaluate the *in vitro* antioxidative property of a given substance (MACDONALD-WICKS *et al.* 2006). As seen from Figure 8, DPPH radical scavenging abilities increased with sample concentrations. The IC<sub>50</sub> values of BGR, BG and RG for scavenging DPPH were 0.454, 0.514, and 4.236 mg/ml according to their regression equations, while it was 0.005 mg/ml for ascorbic acid (data not shown). Both BGR and BG had stronger DPPH scavenging activities than RG, which is consistent with the studies of

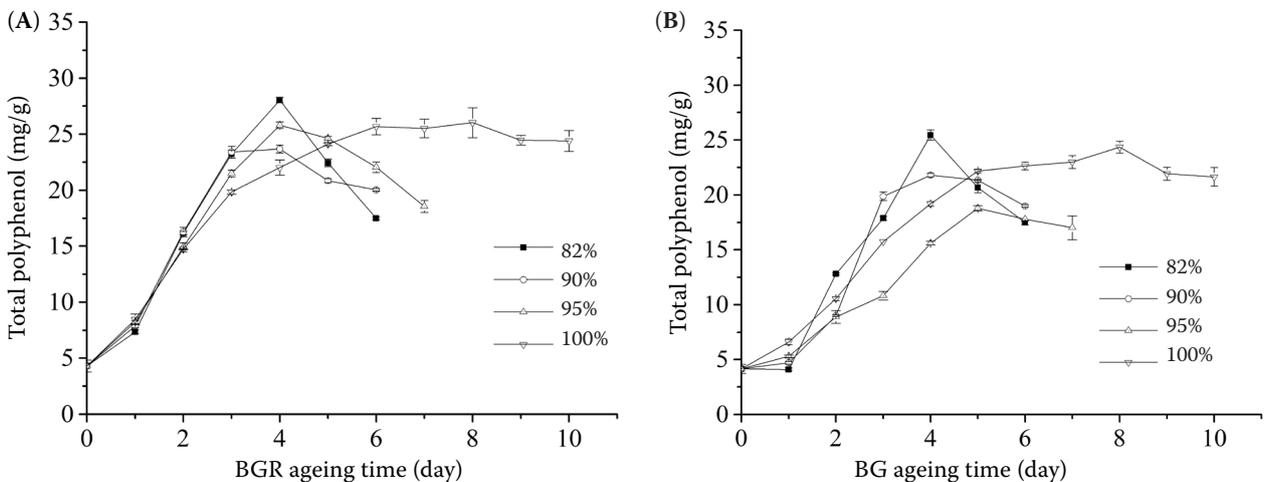


Figure 5. Effect of humidity level on total polyphenol content of (A) black garlic residue (BGR) and (B) black garlic (BG) (values are mean  $\pm$  SD)

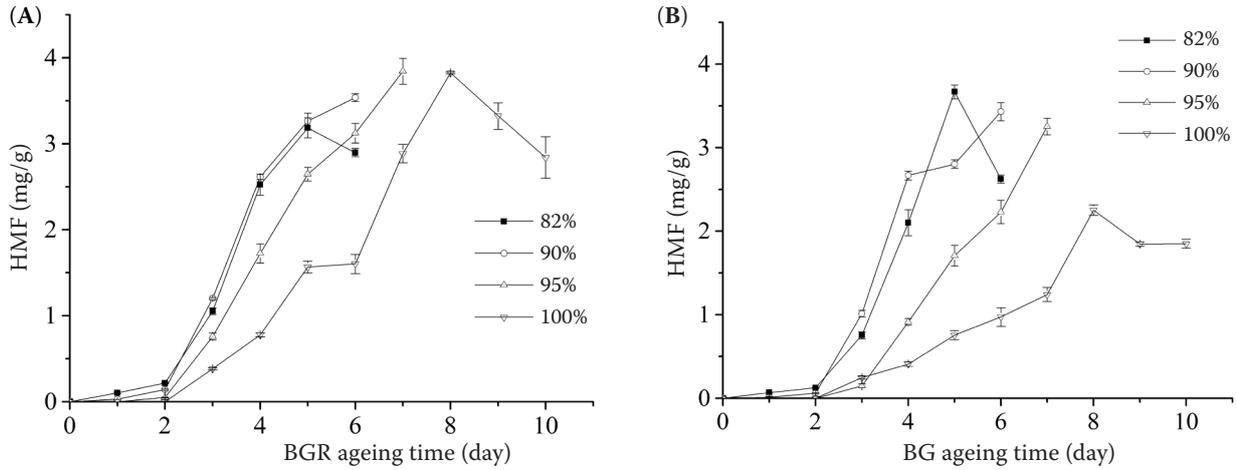


Figure 6. Effect of humidity level on 5-hydroxymethyl-2-furaldehyde (HMF) levels of (A) black garlic residue (BGR) and (B) black garlic (BG) (values are mean ± SD)

Table 1. Antioxidative effects of black garlic residue (BGR) in rat serum and liver

Group	Antioxidant activity			
	dosage (mg/kg)	MDA (nmol/ml)	SOD × 10 <sup>3</sup> (U/g)	GSH-Px (U/ml)
<b>Serum</b>				
Control	0	8.95 ± 0.88	32.10 ± 7.52	492.32 ± 53.61
Model	0	10.13 ± 0.88	26.71 ± 8.63	485.62 ± 56.31
Vc	100	9.25 ± 0.67	27.95 ± 13.32	503.51 ± 74.79
RG-L	130	9.63 ± 0.90	35.98 ± 11.24	468.78 ± 32.45
RG-M	520	9.41 ± 0.37 <sup>a,c</sup>	42.10 ± 9.46 <sup>b</sup>	480.82 ± 35.38
RG-H	1300	9.28 ± 0.83 <sup>b</sup>	32.62 ± 13.45 <sup>b</sup>	506.84 ± 80.72
BR-L	130	8.82 ± 0.35 <sup>a,c,e</sup>	27.75 ± 12.22	486.83 ± 37.16
BR-M	520	8.50 ± 1.00 <sup>a,d,f</sup>	30.88 ± 6.24	511.24 ± 70.23
BR-H	1300	7.25 ± 1.20 <sup>a,f</sup>	32.44 ± 8.34	536.71 ± 44.43 <sup>b</sup>
BGR-L	130	8.91 ± 0.44 <sup>a,c,e</sup>	28.16 ± 9.42	485.63 ± 33.42
BGR-M	520	8.44 ± 0.51 <sup>a,d,f</sup>	30.53 ± 12.59	512.39 ± 78.50
BGR-H	1300	7.96 ± 0.76 <sup>a,f</sup>	32.59 ± 9.82	537.15 ± 34.36 <sup>b</sup>
<b>Liver</b>				
Control	0	7.55 ± 2.09	196.48 ± 55.82	4050.92 ± 1197.02
Model	0	11.63 ± 3.91	174.75 ± 57.72	3361.73 ± 1318.95
Vc	100	10.68 ± 2.06	193.94 ± 48.62	4699.78 ± 1424.01
RG-L	130	10.40 ± 2.36	223.30 ± 50.66	3767.27 ± 1055.07
RG-M	520	10.06 ± 2.37	252.36 ± 66.25 <sup>b</sup>	3895.69 ± 1061.99
RG-H	1300	9.94 ± 3.23	274.69 ± 82.69 <sup>a</sup>	3917.08 ± 2071.86
BG-L	130	8.05 ± 1.61 <sup>a,c,f</sup>	210.84 ± 36.22	4975.28 ± 1410.47
BG-M	520	7.87 ± 1.22 <sup>a,d,f</sup>	286.25 ± 69.08 <sup>a,d,e</sup>	6510.59 ± 1908.13 <sup>a,d,f</sup>
BG-H	1300	7.03 ± 1.88 <sup>a,d</sup>	291.46 ± 52.95 <sup>a,d,e</sup>	6844.67 ± 1594.76 <sup>a,c,e</sup>
BGR-L	130	7.98 ± 1.07 <sup>a,c,f</sup>	212.16 ± 59.42	4977.30 ± 996.52
BGR-M	520	7.77 ± 1.46 <sup>a,d,f</sup>	301.43 ± 59.02 <sup>a,d,e</sup>	6127.27 ± 1562.07 <sup>a,d,f</sup>
BGR-H	1300	6.96 ± 1.19 <sup>a,d</sup>	306.58 ± 63.49 <sup>a,d,e</sup>	6825.51 ± 1383.63 <sup>a,c,e</sup>

MDA – malondialdehyde; SOD – superoxide dismutase; GSH-Px – glutathione peroxidase; values are mean ± SD; different letters indicate differences among samples: <sup>a</sup>*P* < 0.01, <sup>b</sup>*P* < 0.05, compared with the model group; <sup>c</sup>*P* < 0.01, <sup>d</sup>*P* < 0.05, compared with the raw group, <sup>e</sup>*P* < 0.01, <sup>f</sup>*P* < 0.05, compared with the Vc group

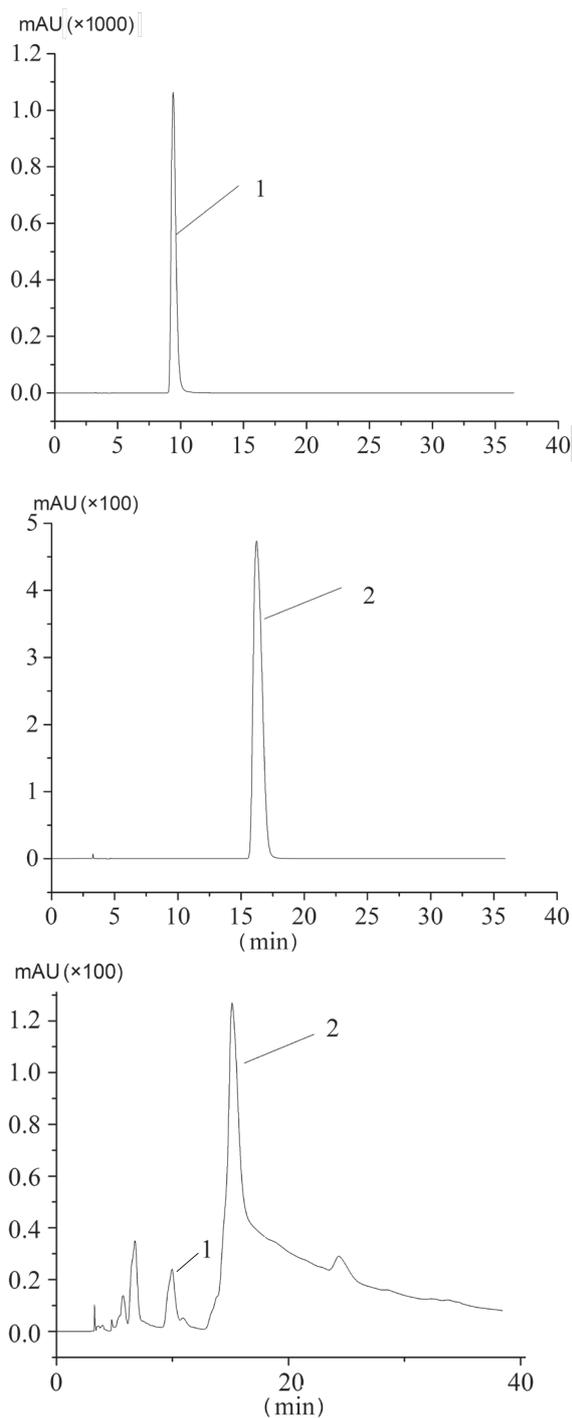


Figure 7. Phenolic compounds in black garlic residue (BGR) analysed using HPLC (280 nm): (1) gallic acid (9.967 min); (2) coumaric acid (16.217 min)

KIM *et al.* (2012) and ZHANG *et al.* (2015). These authors showed that BG had a stronger capacity for scavenging free radicals than fresh garlic.

**Antioxidant activity in vivo.** The free radical theory of ageing states that the excessive generation of reactive

oxygen species (ROS) or free radicals can lead to cell and tissue damage or death (RAMALINGAM & KIM 2014; SAYIN *et al.* 2014). It has been reported that SOD and GSH-Px are the key antioxidant enzymes that protect organisms against oxidative stress and tissue damage because SOD can catalyse superoxide radicals into hydrogen peroxide and oxygen, while GSH-Px participates in the detoxification of hydrogen peroxide (QUEGUINEUR *et al.* 2012; YANG *et al.* 2013). Hence, enhanced activity of SOD and GSH-Px may provide an effective defence against the damaging effects of free radicals. In addition, MDA is a principal product of lipid oxidation and has been found to be elevated in various diseases related to free radical damage; for this reason, it has been widely used as an index of lipid peroxidation (LIAO *et al.* 2014).

As shown in Table 1, SOD activities in both serum and liver were significantly increased by BGR, BR and RG at different dosages ( $P < 0.05$ ), by 22.01% in serum and by 75.86% in liver in the BGR-H group compared with the model group, respectively. BG and BGR administration have been described to lead to dosage-dependent increases in SOD levels. NADERI *et al.* (2015) reported that the treatment of diabetic rats with garlic increased SOD levels in blood and heart homogenates.

GSH-Px is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the host organism from oxidative damage.

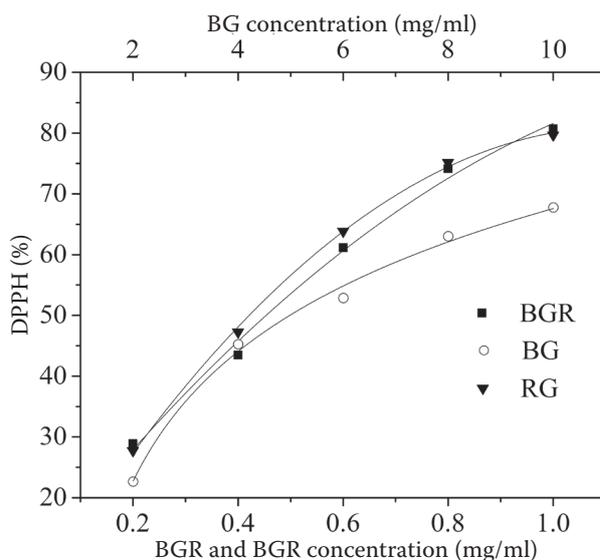


Figure 8.  $\alpha$ ,  $\alpha$ -Diphenyl- $\beta$ -picrylhydrazyl (DPPH) radical scavenging activities of black garlic residue (BGR), raw garlic (RG), and black garlic (BG)

As illustrated in Table 1, there were no significant differences between BG and BGR groups in GSH-Px levels in the serum and liver and GSH-Px activities were increased significantly in both groups. Compared with the model group, GSH-Px activities in liver increased about two-fold after administration of 1300 mg/kg BW BG and BGR, indicating that highly concentrated BGR can protect molecules from free radical-mediated attack.

Serum and liver levels of MDA decreased with increasing BGR, BG and RG concentrations (Table 1). Both BGR and BG effectively decreased MDA levels. Compared with the model group, serum and liver levels of MDA in the BGR-L groups were decreased by 12.04 and 31.38%, respectively ( $P < 0.05$ ), whereas MDA levels were decreased by 21.42 and 40.86%, respectively, in the BGR-H groups ( $P < 0.05$ ). NADERI *et al.* (2015) investigated the protective effects of garlic (*Allium sativum*) in the blood and heart of streptozotocin-induced diabetic rats, and demonstrated that homogenised garlic could decrease MDA levels significantly. The reduction of MDA levels in all BGR-treated groups suggested that BGR might inhibit the generation of cytotoxic chemicals, such as MDA, during lipid peroxidation.

Although how exactly BG or RG affect the levels of SOD, GPX-Px, and MDA is still unclear, some studies have considered that many biologically active compounds might be produced during the ageing process. RAJANI *et al.* (2008) considered that the probable protective mechanism of garlic against oxidative damage may be linked to its bioactive components which chelate metal ions (Cu, Zn, and Mn) thereby scavenging superoxide ions, subsequently inhibiting the oxidation of protein moieties and thus contributing to healthy cellular redox status.

## CONCLUSIONS

This study showed that the optimal conditions for the production of BGR were a temperature of 90°C and a humidity level of 95% for four days. BGR was found to have a strong DPPH scavenging activity with an  $IC_{50}$  of 0.454 mg/ml. Our data clearly demonstrated that mice administered 1300 mg/kg BW BGR extract exhibited significant decreases in MDA content and marked increases in SOD and GPH-Px activities in both serum and liver. These results suggest that BGR has a potent antioxidant activity and

thus has potential as a novel natural antioxidant in food and therapeutics.

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Received: 2016–11–10

Accepted after corrections: 2018–01–08

Published online: 2018–02–12