Antioxidant Activity of Various Soluble Melanoidins Isolated from Black Garlic after Different Thermal Processing Steps

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ABSTRACT: To gain insight into the antioxidant activity of various soluble melanoidins isolated from black garlic after different thermal processing steps, the antioxidant activity was evaluated. Black garlic was produced in a ripening chamber using a programmed stepwise heating schedule as follows: Step 1, 90°C and 100% (RH) for 34 h; Step 2, 60°C and 60% RH for 6 h; Step 3, 75°C and 70% RH for 48 h; Step 4, 70°C and 60% RH for 60 h; Step 5, 65°C and 50% RH for 192 h. The melanoidins isolated from black garlic after the different thermal processing steps were divided into different melanoidin fractions, i.e., melanoidins, pure melanoidin, bound melanoidin compounds (BMC). The antioxidant activity of the melanoidins bound to low molecular weight compounds (BMC fraction) was generally higher than those of the pure melanoidins. Notably, the antioxidant activity of various soluble melanoidins differed according to the thermal processing steps. The results may be useful in predicting the behavior of various soluble melanoidins during thermal processing of garlic.

Keywords: antioxidant activity, black garlic, melanoidins, thermal processing

INTRODUCTION

Garlic (Allium sativum L.) has remained one of the most important foods and spices for centuries. Extensive studies have shown that garlic can provide favorable biological and pharmacological effects in ‘in vitro’ and animal models in vivo, such as antimicrobial and anticancer activity (Kodera et al., 2002; González et al., 2011) as well as hypoglycemic and antioxidant effects (Banerjee et al., 2003).

Black garlic is simply a fresh garlic that has been fermented for a period at high temperature under high humidity. This results in a color change from white to dark brown due to enzymatic browning and the Maillard reaction, which is a result of condensation between a reducing sugar carbonyl and an amino group (Venir et al., 2009). The primary end products of this reaction are melanoidins that contribute to the flavor and color, and add nutritional value. These compounds are also responsible for certain biological effects, including antitumor, antioxidant, and antihypertensive functions, as well as blood sugar modulation (Rufián-Henares and Morales, 2007; Langner and Rzeski, 2014). Although black garlic is rich in melanoidins, there are few studies on the positive bioactive properties and applications of melanoidins in black garlic, and most researchers have focused on the polyphenol, flavonoid, and S-allyl cysteine content (Kim et al., 2013; Bae et al., 2014).

Melanoidins are present in widely consumed dietary components (e.g., coffee, cocoa, bread, malt, honey). Melanoidins possessing antioxidant (Delgado-Andrade and Morales, 2005; Morales, 2005), antinociceptive (Rufián-Henares and Morales, 2006; del Castillo et al., 2007), and prebiotic activity (Borrelli and Fogliano, 2005), are responsible for the development of color in heat-processed food products (Rizzi, 1997), may contribute to food texture, and are likely to play a role in the binding of nutritionally important metals (O’Brien and Morrissey, 1997) and potentially undesirable dietary (Yen and Hsieh, 1994) and flavor compounds (Hofmann and Schieberle, 2002). Therefore, the objective of the present study is to gain additional insight into the antioxidant activity of various soluble melanoidins isolated from black garlic after different thermal processing steps. The present findings may clarify on the impact of melanoidins on the shelf-life of melanoidin-containing foods and their possible functional properties.
MATERIALS AND METHODS

Chemicals and reagents
2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-S-triazine (TPTZ), and 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All chemicals used were of analytical grade and were obtained from Merck (Darmstadt, Germany), unless otherwise stated.

Sample preparation
Fresh garlic bulbs were purchased from the Namhae Bophysical Agricultural Association (Namhae, Korea) in 2019. Black garlic was produced in a ripening chamber (MBGAM-1500, Minyoung Chemical Co., Ltd., Gimpo, Korea), without removing the outer layers, using a programmed stepwise heating schedule as follows: Step 1: 90°C and 100% RH for 34 h; Step 2: 60°C and 60% RH for 6 h; Step 3: 75°C and 70% RH for 48 h; Step 4: 70°C and 60% RH for 60 h; Step 5: 65°C and 50% RH for 192 h. The samples tested in this study were as follows: raw garlic cloves, black garlic cloves at step 1, black garlic cloves at step 2, black garlic cloves at step 3, black garlic cloves at step 4, and black garlic cloves at step 5. These samples are designated as BG1, BG2, BG3, BG4, and BG5, respectively. To prepare the garlic powder, the fresh garlic and black garlic cloves were peeled, frozen in liquid nitrogen, and immediately freeze-dried. The resulting lyophilized garlic samples were ground into a powder with a mortar and pestle. The resulting powder was stored in sealed plastic bottles at −20°C until analysis.

Ultrafiltration
The water-soluble melanoidins were isolated from the black garlic after the different thermal processing steps described above by ultrafiltration (Morales and Jiménez-Pérez, 2004). An aliquot of each sample was subjected to ultrafiltration using an Amicon ultrafiltration cell, model 8400 (Amicon, Beverly, MA, USA), equipped with a 10,000 Da nominal molecular mass cut-off membrane. The retentate was filled up to 200 mL with water and were washed again. The washing step (dialfiltration) was repeated at least three times. The high-molecular-weight fraction corresponding to melanoidins (M) was freeze-dried and stored in a desiccator at 4°C until analysis. A diagram-chart of the extraction procedure for melanoidin and related fractions is depicted in Fig. 1.

DPPH radical scavenging activity
The antiradical activity of the different samples in methanolic medium was estimated according to the procedure reported by Delgado-Andrade et al. (2005). A 200 μL aliquot of sample (M, PM, or BMC) was added to 1 mL of DPPH’ (74 mg/L in methanol). The DPPH’ solution was prepared daily to give a final absorption of 1.8 AU at 520 nm. The temperature in the measurement chamber was set at 30°C. The antiradical activity of the sample is expressed as the percentage disappearance of the initial purple color. The higher the disappearance, the greater the antiradical activity. Aqueous solutions of Trolox at various con-
Metal-ion chelating activity

The ability of the sample (M, PM, or BMC) to chelate the prooxidative transitional metal ion (Fe²⁺) was investigated according to Dinis et al. (1994) with slight modifications. One-hundred microliters of sample (M, PM, or BMC) was added to 600 μL of distilled water and 100 μL of 0.2 mM FeCl₃·4H₂O. The mixture was allowed to rest at room temperature for 30 s. The reaction mixture containing 100 μL distilled water instead of sample served as a control. The reaction mixture was added with vigorous stirring to form an emulsion. After evaporation to dryness under vacuum at room temperature, distilled water (100 mL) and Tween-20 (400 μL) were added with vigorous stirring to form an emulsion. The antioxidant activity of the sample was evaluated using a spectrophotometer (Synergy HTX; Biotech Instruments) at 470 nm, immediately after sample preparation (t=0 min), and at 10 min intervals until completion (t=120 min) of the experiment. The rate of bleaching of β-carotene was calculated by linear regression fitting of the data over time. All samples were assayed in triplicate. Various concentrations of butylated hydroxyanisole (BHT), butylated hydroxytoluene (BHA), and α-tocopherol in 80% methanol were used as standards, and 80% methanol was used as the control.

The antioxidant activity was calculated in four different ways. First, the absorbance was plotted against time, as a kinetic curve, and the absolute value of the slope was expressed as the antioxidant value (AOX) described by Al-Saikhan et al. (1995) using the following equations:

\[
\text{AOX} = \ln \left( \frac{A_{t=0}}{A_{t=10}} \right) \times \frac{1}{t}
\]

where \(A_{t=0}\) is the initial absorbance (470 nm) of the emulsion at time 0, \(A_{t=10}\) is the absorbance (470 nm) at 10 min intervals until completion (t=20 min), and \(t\) is the time in min. The rates for different times were averaged to obtain one value for the sample.

The antioxidant activity (AA) was also calculated as the % inhibition relative to the control using the following relationship:

\[
\text{AA} = \frac{\text{degradation rate of control} - \text{degradation rate of sample}}{\text{degradation rate of control}} \times 100
\]

The third method of expression based on the oxidation rate ratio (ORR) was calculated by using the approach: Chelating activity (%) = \(\frac{A_0 - A_t}{A_0} \times 100\)

where \(A_0\) and \(A_t\) are the absorbance of the control and extract samples, respectively.

β-Carotene bleaching assay

The antioxidant activity of the sample was evaluated using a β-carotene-linoleic acid (linolate) model system (Miller, 1971) with slight modifications. β-Carotene (1 mL, 0.2 mg/mL dissolved in chloroform) was added to a round-bottom flask containing linoleic acid (20 μL) and Tween-20 (400 μL). After evaporation to dryness under vacuum at room temperature, distilled water (100 mL) was added with vigorous stirring to form an emulsion. The emulsion (5 mL) was added to individual tubes containing 0.2 mL of 80% EtOH (as control) or sample (M, PM, or BMC), or standard. The samples were then subjected to thermal autoxidation at 50°C for 2 h. The absorbance of the solution was monitored using a spectrophotometer (Synergy HTX; Biotech Instruments) at 470 nm, immediately after sample preparation (t=0 min), and at 10 min intervals until completion (t=120 min) of the experiment. The rate of bleaching of β-carotene was calculated by linear regression fitting of the data over time. All samples were assayed in triplicate. Various concentrations of butylated hydroxyanisole (BHT), butylated hydroxytoluene (BHA), and α-tocopherol in 80% methanol were used as standards, and 80% methanol was used as the control.

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\]

The third method of expression based on the oxidation rate ratio (ORR) was calculated by using the approach.
presented by Marinova et al. (1994) using the equation:

$$\text{ORR} = \frac{B_S}{B_0}$$

where $B_S$ is the rate of bleaching of $\beta$-carotene in the presence of the sample, and $B_0$ is the bleaching rate in the absence of the sample (blank).

In the fourth method, the antioxidant activity coefficient (AAC) was calculated as described by Mallet et al. (1994):

$$\text{AAC} = 1,000 \times \frac{A_c(120) - A_c(0)}{A_c(120) - A_c(0)}$$

where $A_c(120)$ and $A_c(0)$ are the absorbance of the antioxidant mix and control at $t = 120$ min, respectively, and $A_c(0)$ is the absorbance of the control at $t = 0$ min.

**Statistical analysis**

The experimental data were subjected to analysis of variance (ANOVA), and the significant differences between the mean values, determined from measurements carried out in five replicate tests (i.e., $P < 0.05$), were obtained by Duncan's multiple range test using statistical analysis software (SPSS 20.0, IBM, New York, NY, USA).

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**RESULTS AND DISCUSSION**

**DPPH radical-scavenging activity**

The quality of black garlic including its bioactivity and texture depends on the temperature during thermal processing. Briefly, black garlic is produced through processing fresh garlic at a controlled high temperature (60 to 90°C) and controlled high humidity (50 to 100%) for a period involving spontaneous fermentation and a number of chemical reactions. Owing to the variations in processing conditions, including processing temperature and humidity, pretreatment, and postproduction handlings, a range of black garlic products have been produced with varied compositions and physicochemical properties. The antioxidant activity of various soluble melanoidins isolated from black garlic after the different thermal processing steps were determined using the DPPH assay, and the results are summarized in Table 1. For the melanoidins, the results are expressed as mmol equivalents of Trolox (TE) per gram (TE/g) of melanoidin. However, for the PM or BMC, the results are expressed as mmol TE per unit PM or BMC released from one gram of melanoidin, in order to compare their antioxidant activity with that of the parent melanoidins at the concentration found in that melanoidin. For these fractions, the exact antioxidant activity (expressed in mmol TE/g of pure melanoidin or bound melanoidin compounds) could be calculated by considering the mean recovery of 82.2% and 17.8% for PM and BMC, respectively. In this sense, for example, the TEAC$_{\text{DPPH}}$ of the PM and BMC for the BG1 sample would be 0.17 and 0.90, respectively, instead of 0.14 and 0.16, respectively (Table 1). For the M, the antioxidant activity ranged between 0.22 and 2.05 mmol of TE/g of sample for BG1 and BG4, respectively. The antioxidant activity of the PM was consistently lower than that of the corresponding melanoidin, while the BMC exerted higher antioxidant activity than that of the corresponding melanoidin. In addition, the DPPH radical scavenging activity of the BMC increased with each successive thermal processing step, whereas that of the PM was similar at each step. In this regard, del Castillo et al. (2007) pointed out that bread-derived melanoidins have stronger peroxyl radical scavenging activity than low molecular weight compounds, which have been previously reported to be able to bind to the melanoidin skeleton (Cämmerer et al., 2002; Delgado-Andrade et al., 2005) and contribute to the final antioxidant activity. The fraction of BMC isolated from brewed coffee (Delgado-Andrade et al., 2005) and glucose-amino acid multidrug resistance proteins (MRPs) (Rufián-Henares and Morales, 2007) demonstrated higher antiradical activity than the pure melanoidins. However, Tagliazucchi et al. (2010) reported that the BMC fraction of high-molecular-weight (HMW) melanoidins found in balsamic vinegar consistently exhibited lower antioxidant activity than the pure melanoidins to which they are linked. Moreover, polyphenols, which are BMCs found in commercial foods such as coffee, are partially lost during heating (Delgado-Andrade et al., 2005). The results obtained herein indicate that the antiradical activity of melanoidins may be due to the variety

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**Table 1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of various soluble melanoidins isolated from black garlic after different thermal processing steps**

| Samples | TEAC$_{\text{DPPH}}$ (mg TE/g) |
|---------|--------------------------------|
| M       | PM                             | BMC                           |
| BG1     | 0.22±0.02                     | 0.14±0.01                     | 0.16±0.01                     |
| BG2     | 0.28±0.01                     | 0.12±0.02                     | 0.13±0.01                     |
| BG3     | 1.03±0.01                     | 0.16±0.01                     | 0.20±0.01                     |
| BG4     | 2.05±0.01                     | 0.15±0.01                     | 0.53±0.01                     |
| BG5     | 1.73±0.01                     | 0.15±0.01                     | 0.76±0.02                     |

Values are mean±SD. Different letters (a-e) within a column indicate significant differences ($P<0.05$).

M, melanoidin; PM, pure melanoidin; BMC, bound melanoidin compounds.

1 Data are expressed as mmol equivalents of Trolox per gram of sample.

2 Data are expressed as mmol equivalents of Trolox/pure melanoidin released from 1 g of melanoidin.

3 Data are expressed as mmol equivalents of Trolox/bound melanoidin compounds released from 1 g of melanoidin.
of structural properties. Thus, melanoidins are probably the predominant contributors to antioxidant activity in severely heat-treated samples.

FRAP assay
The FRAP assay measures the reduction of ferric irons (Fe\(^{3+}\)) to ferrous irons (Fe\(^{2+}\)) in the presence of antioxidants, which are reductants with half-reaction reduction potentials above that of the Fe\(^{3+}/Fe^{2+}\) couple. This assay is also commonly used for the routine analysis of single antioxidants and the total antioxidative activity. The ferric reducing ability of various soluble melanoidins isolated from black garlic after different thermal processing steps is summarized in Table 2. The results obtained in the ferric reducing ability test were in parallel with the data from the DPPH method, where the pure melanoidins show the lowest activity, while melanoidins and bound melanoidin compounds showed high activity. Furthermore, the FRAP of the M and BMC increased with each successive thermal processing step. Among the melanoidins, the BG4 sample exhibited the maximum ferric reducing ability, whereas among the BMC, the BG5 sample exhibited the maximum ferric reducing ability. However, the PM all showed similar activity. Oracz and Zyzelewicz (2019) reported that the accumulation of HMW MRPs-like melanoidins during roasting may increase the antioxidative capacity of the HMW fractions. In addition, the observed behavior can be explained by the presence of residues of certain active compounds, which spontaneously form covalent bonds with the functional groups of melanoidins during roasting (Brudzynski and Miotto, 2011; Sacchetti et al., 2016). Therefore, these results suggest that the antioxidative capacity of melanoidins may be affected by the synthesis effect between different bioactive compounds present in the structure of the melanoidins, which could determine their biological properties during thermal processing.

Determination of total antioxidant capacity using the TEAC assay
The ABTS\(^{+}\) assay is a widely used method for the assessment of the antioxidant activity of many vegetable and food matrices (Rameshwar Naidu et al., 2012; Giuffrè et al., 2017; Shannon et al., 2018). This method is based on the quenching of stable colored radicals and is an indicator of the free-radical quenching activity of antioxidants even when present in complex biological matrices such as plant or food preparations (extracts or fractions). The total antioxidant activity of the various soluble melanoidins isolated from black garlic after different thermal processing steps was determined using the TEAC assay, and the results are presented in Table 3. The total antioxidant activity of M ranged from 0.78 to 3.65 mmol TE/g of sample for BG1 and BG4, respectively. The M showed the highest activity in the BG4 sample, while the TEAC val-

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Table 2. Fe\(^{3+}\)-2,4,6-tripryidyl-S-triazine reducing ability of various soluble melanoidins isolated from black garlic after different thermal processing steps

| Samples | M\(^{1}\) | PM\(^{2}\) | BMC\(^{3}\) |
|---------|--------|--------|--------|
| BG1     | 0.65±0.01\(^{a}\) | 0.62±0.01\(^{c}\) | 0.61±0.01\(^{a}\) |
| BG2     | 0.83±0.01\(^{d}\) | 0.68±0.01\(^{a}\) | 0.65±0.01\(^{a}\) |
| BG3     | 2.42±0.01\(^{c}\) | 0.62±0.01\(^{c}\) | 0.68±0.01\(^{c}\) |
| BG4     | 5.20±0.02\(^{a}\) | 0.63±0.01\(^{c}\) | 1.32±0.01\(^{a}\) |
| BG5     | 3.46±0.01\(^{b}\) | 0.66±0.01\(^{b}\) | 1.71±0.01\(^{a}\) |

Values are mean±SD. Different letters (a-e) within a column indicate significant differences (P<0.05).
M, melanoidin; PM, pure melanoidin; BMC, bound melanoidin compounds.
\(^{1}\)Data are expressed as mmol equivalents of Trolox per gram of sample.
\(^{2}\)Data are expressed as mmol equivalents of Trolox/pure melanoidin released from 1 g of melanoidin.
\(^{3}\)Data are expressed as mmol equivalents of Trolox/bound melanoidin compounds released from 1 g of melanoidin.

Table 3. 2,2’-azino-bis(ethylbenzo-thiazolin-6-sulfonic acid) (ABTS) radical scavenging activity of various soluble melanoidins isolated from black garlic after different thermal processing steps

| Samples | M\(^{1}\) | PM\(^{2}\) | BMC\(^{3}\) |
|---------|--------|--------|--------|
| BG1     | 0.78±0.02\(^{a}\) | 0.63±0.02\(^{a}\) | 0.66±0.01\(^{a}\) |
| BG2     | 0.93±0.01\(^{a}\) | 0.64±0.01\(^{a}\) | 0.63±0.01\(^{a}\) |
| BG3     | 1.88±0.03\(^{a}\) | 0.69±0.02\(^{a}\) | 0.58±0.01\(^{a}\) |
| BG4     | 3.65±0.06\(^{a}\) | 1.11±0.02\(^{a}\) | 0.63±0.01\(^{a}\) |
| BG5     | 3.05±0.05\(^{a}\) | 1.48±0.02\(^{a}\) | 0.64±0.01\(^{a}\) |

Values are mean±SD. Different letters (a-e) within a column indicate significant differences (P<0.05).
M, melanoidin; PM, pure melanoidin; BMC, bound melanoidin compounds.
\(^{1}\)Data are expressed as mmol equivalents of Trolox per gram of sample.
\(^{2}\)Data are expressed as mmol equivalents of Trolox/pure melanoidin released from 1 g of melanoidin.
\(^{3}\)Data are expressed as mmol equivalents of Trolox/bound melanoidin compounds released from 1 g of melanoidin.
ues of the PM increased with each successive thermal processing step, and the BMC showed similar activity. The results differ from those obtained with the DPPH assay, likely due to the different reaction media (aqueous and methanolic for ABTS and DPPH, respectively). This behavior is like that observed by other researchers (Pastoriza and Rufián-Henares, 2014), who reported stronger scavenging activity against DPPH· than ABTS· radicals in the case of chocolate melanoidins. The differences between the ABTS and DPPH radical scavenging activity could also be attributed to the different reaction media (aqueous and methanolic for ABTS and DPPH, respectively). Moreover, the reaction mechanism with DPPH· radical involves the transfer of a hydrogen atom, while the reactions with ABTS· radicals involve an electron transfer process (Ichikawa et al., 2019). The observed differences indicate the complexity of the mechanism of action of melanoidins formed in real food.

**Metal-ion chelating activity**

The ferrous ion (Fe²⁺) in the Fenton reaction can catalyze the generation of potentially toxic reactive oxygen species (ROS), such as hydroxyl radicals, that initiate lipid peroxidation (Abbes et al., 2013). The metal ion chelating activity of the various soluble melanoidins isolated from black garlic after different thermal processing steps is presented in Table 4. All melanoidins isolated from black garlic after different thermal processing steps were able to chelate ferrous ions, which are the most powerful pro-oxidants among various species of metal ions. The ferrous ion chelating activity ranged from 78.34 to 89.51 for M, from 84.43 to 89.85 for PM, and 89.17 to 89.85 for BMC. The BMC were the strongest chelators, followed by PM and M. The difference in the chelating activity of the M and BMC was not significant (P>0.05), whereas the differences in the chelating activity of the PM were statistically significant (P<0.05) for the different thermal processing steps. Gu et al. (2010) found that HNW MRPs had higher metal-chelating potential than LMW MRPs. They

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Table 4. Metal ion chelating activity of various soluble melanoidins isolated from black garlic (BG) after different thermal processing steps. (Unit: %)

| Samples | Fe²⁺ chelating activity |
|---------|-------------------------|
|         | M          | PM          | BMC         |
| BG1     | 89.51±0.04a | 89.85±0.04a | 89.85±0.04a |
| BG2     | 89.34±0.04b | 89.68±0.04b | 89.85±0.04a |
| BG3     | 82.74±0.07c | 87.65±0.05d | 89.68±0.04b |
| BG4     | 78.34±0.08d | 86.29±0.05e | 89.17±0.04c |
| BG5     | 78.34±0.08d | 84.43±0.06e | 89.68±0.04b |

Values are mean±SD. Different letters (a-e) within a column indicate significant differences (P<0.05). M, melanoidin; PM, pure melanoidin; BMC, bound melanoidin compounds.
also suggested that the metal-chelating activity of MRPs may be affected by the presence of hydroxyl or pyrrole groups in their structures. The nitrogen atoms in melanoidins were proposed to be responsible for the chelation of copper ions (Ćosović et al., 2010). Wen et al. (2005) showed that the zinc-chelating activity of brewed coffee decreased as the intensity of the roasting increased, suggesting that the chromophoric groups of melanoidins may not be the main coordination sites for metal complexation, but the metal chelating activity of melanoidins may be due to other, as yet unknown, structures of these species.

**β-Carotene bleaching assay**

The antioxidant activity determined by the β-carotene bleaching method for the various soluble melanoidins isolated from black garlic after different thermal processing steps is shown in Table 5. The decrease in the absorbance of β-carotene in the presence of various soluble melanoidins (and well-known antioxidants used as standards) with the oxidation of β-carotene and linoleic acid are shown in Fig. 2, 3, and 4. For M, the AOX, AA, ORR, and AAC values were 0.004, 78.84%, 0.21, and 683.49, respectively, for BG2, which showed exceptionally high antioxidant activity, even higher than that of BHT and BHA at 0.05 mg/mL, and vitamin E at 0.1 mg/mL, while BG3 showed the lowest antioxidant activity. Among the PM, BG5 and BG2 exhibited the highest and lowest AOX, AA, ORR, and AAC antioxidant activities, respectively. Moreover, BG5 and BG1 had high antioxidant activity, even higher than those of BHT, BHA, and vitamin E. However, BG2 showed the lowest antioxidant activity, contrary to the case of melanoidins. In addition, among the BMC, BG2 and BG5 had high antioxidant activity, even higher than that of well-known antioxidants used as standards. However, BG3 showed the lowest antioxidant activity due to the M. Thus, BG2 showed the highest antioxidant activity among the M and BMC, and BG5 showed the highest antioxidant activity for PM. Notably, the antioxidant activity determined by the β-carotene bleaching method was highest for the BMC. It is important to note that the β-carotene bleaching assay only provides an indication of the level of lipophilic compounds because the β-carotene bleaching test is similar to an oil-in-water emulsion system (Ouchikh et al., 2011). However, the emulsifier may change the antioxidant distribution in the emulsified me-
dium, and in turn the antioxidant activity, making it more difficult to interpret the results. Moreover, emulsifiers form micelles, which may trap antioxidants in these self-assembled structures and carry them to the water phase (Shahidi and Zhong, 2015).

In conclusion, the present study reports different in vitro antioxidants in the melanoidins and melanoidin fractions, considering different assays. It is proposed that the BMC fraction exerts antioxidant activity that is generally higher than that of the pure melanoidins to which they are linked. More specifically, the results have shown that the processing temperature and time have an important influence on the antioxidant activity of black garlic. The results obtained here cannot be generalized, but they might be of considerable interest in predicting the behavior of various soluble melanoidins during thermal processing of garlic.

**AUTHOR DISCLOSURE STATEMENT**

The author declares no conflict of interest.

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