Prev. Nutr. Food Sci. 2016;21(4):398-405
https://doi.org/10.3746/pnf.2016.21.4.398
pISSN 2287-1098 · eISSN 2287-8602

Research Note

Evaluation of Melanoidins Formed from Black Garlic after Different Thermal Processing Steps

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ABSTRACT: The objective of this study was to evaluate the characteristics of melanoidins formed from black garlic (BG) after different thermal processing steps. The melanoidins formed from BG during thermal processing were produced in large amounts, and the initial (280 nm), intermediate (360 nm), and final stage product (420 nm) had similar tendencies. Compounds like degraded proteins, peptides, and phenolic acids were present in the melanoidins during thermal processing. All the melanoidin samples showed different absorptions in the UV-visible spectra, although these had similar shapes. Moreover, the carbon, hydrogen, and oxygen content of melanoidins formed from BG during thermal processing decreased initially, and then increased. However, the nitrogen content increased during thermal processing. As thermal processing progressed, the molecular weight of all the melanoidin samples showed increasing intensities, whereas the major peaks of each melanoidin sample had different retention times. Furthermore, the melanoidins formed from BG after different thermal processing steps contained -OH, -CH, amide I, and III groups. The crystallinity of the melanoidins was majorly formed at 31.58° and 43.62° (2θ).

Keywords: black garlic, thermal processing, melanoidins

INTRODUCTION

Garlic (*Allium sativum* L.) is a species of the onion genus that has long been used as both a culinary seasoning and medical herb (1). However, the consumption and application of fresh garlic in foods and medicines are limited due to its characteristic odor, spicy flavor, and tendency to cause an upset stomach. Black garlic (BG) is formed by aging whole garlic at high temperatures and humidity, causing the garlic to turn black because of the browning compounds produced. Thermal processing induces many chemical reactions in garlic, such as enzymatic browning and the Maillard reaction, causing its color to change from white and yellow to dark brown.

The Maillard reaction refers to the interaction initiated between the terminal α- or ε-amino groups of lysine residues in peptides or proteins and the carbonyl moieties of reducing sugars. The Maillard reaction may produce colored or colorless reaction products, depending on the stage of the reaction, as well as other factors such as pH, type and concentration of reactants, temperature, and water activity (2,3). In this reaction, melanoidins (brown nitrogenous polymers and co-polymers) are formed as the main end products of the reaction. These brown polymers have a significant effect on the quality of food, since color is an important food attribute and a key factor in consumer acceptance. Also, melanoidins are of interest not only due to their contribution to color formation, but also for their flavor-binding properties (4-8), antioxidative capacity (9-11), and metal chelating properties (11,12). Melanoidins have been studied in recent years because of their nutritional, biological, and health implications. Although more information about the structure of melanoidins has been revealed in recent years, the chemical structure of melanoidins has not been elucidated to date. At present, there are 3 main proposals for the structure of melanoidins (13,14): Heyns and Hauber (15) and Tressl et al. (16) suggested that melanoidins are polymers made up of repeating units of furans and/or pyrroles (linked via polycondensation reactions) formed during the advanced stages of the Maillard reaction. Hofmann (17) detected low-molecular-weight, colored substances, which could crosslink proteins via the ε-amino groups of lysine or arginine to produce high-molecular-weight colored melanoidins. Kato and Tsuchida (18), and more recently, the group of Cämmerer (13,14), suggested that the melanoidin skeleton is mainly built up of sugar degradation products formed in the early

Received 8 November 2016; Accepted 2 December 2016; Published online 31 December 2016

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stages of the Maillard reaction, and polymerized through aldol-type condensation reactions, and possibly linked by amino compounds. Although these proposals provide valuable information on what melanoidins might look like, it is important to note that these suggested melanoidin structures are mostly based on model studies. There is very limited information on the melanoidins formed from BG during the different thermal processing steps. Therefore, the objective of this study was to evaluate the characteristics of melanoidins formed from BG after different thermal processing step. These results might contribute to our understanding of the role of thermal processing on the Maillard reaction of BG.

MATERIALS AND METHODS

Chemicals and reagents

Dichloromethane, blue dextran, bovine serum albumin, albumin, β-lactoglobulin, myoglobin, ribonuclease A, and cytochrome C were purchased from Sigma Chemical Co. (St. Louis, MO, USA). High performance liquid chromatography (HPLC)-grade water was purchased from JT Baker (Phillipsburg, NJ, USA). Reagents were of the highest grade and used without further purification.

Sample preparation

Garlic was cultivated in Namhae, Korea. Fresh garlic bulbs were purchased from the Namhae Bomulsum Agricultural Association (Namhae, Korea) in 2015. BG was produced in a ripening chamber (MBGAM-1500, Minyoung Co., Ltd., Korea), without removing the outer layers, using a programmed, stepwise heating schedule as follows: Step 1, 90°C and 100% relative humidity (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; and Step 5, 65°C and 50% RH for 192 h. Samples of the BG cloves were tested after each of the 5 steps, and were designated as BG1, BG2, BG3, BG4, and BG5, respectively. To prepare the garlic powders, BG cloves were peeled, frozen in liquid nitrogen, and immediately freeze-dried. The lyophilized garlic samples were ground with a mortar and pestle, and the resulting powders were stored in sealed plastic bottles at −20°C until analysis.

Preparation of melanoidins extract and pure melanoidins extract from BG

BG power (100 g) was stirred in 300 mL of distilled water at 4°C for 2 h. The resulting mixture was then filtered (Whatman filter paper no. 40, ashless, Whatman, Maidstone, UK). The residue obtained was extracted again with 300 mL of distilled water at 4°C. After filtration, both filtrates were combined and defatted by extraction with dichloromethane (2×200 mL). The melanoidins solution was then dialyzed using a wet cellulose dialysis tube (33 mm of flat width, 21 mm of diameter, 12.4 kDa of molecular weight cutoff) for 2 days against running tap water and 1 day against demineralized water. The high molecular weight fraction corresponding to melanoidins was freeze-dried and stored in a desiccator until analysis.

Obtention of pure melanoidins was performed by preparing solutions containing 5 mg (to obtain a representative amount of product) of different melanoidins per milliliter in 2 M NaCl. NaCl was used to release low-molecular-weight compounds (such as chlorogenic acid) ionically attached to the melanoidin skeleton. After overnight incubation, the solutions were again subjected to ultrafiltration using an Amicon ultrafiltration cell model 8400 (Amicon, Beverly, MA, USA) equipped with membranes having a nominal molecular mass cut-off of 10 kDa. The retentates, which contained pure melanoidins, were resuspended in water and then freeze-dried and stored in a desiccator at 4°C until analysis.

Absorbance measurement

The degree of browning associated with the progression of the Maillard reaction was determined at 280, 360, and 420 nm. In the first stage of the Maillard reaction, reducing sugars react with amino acids, giving rise to non-color compounds, which do not absorb in the visible region (19). For this reason, the formation of the early compounds was monitored at 280 nm, and the pool of compounds formed at a more advanced stage at 360 nm. The progress of the reaction involves the production of final and high molecular weight compounds, termed melanoidins, with chromophore groups that have characteristic absorbance maximum at 420 nm (20). Briefly, browning measurements were performed as follows: Each sample of freeze-dried pure melanoidins (0.5 g) was suspended in 10 mL of distilled water, and the mixture was vortexed vigorously for 15 s and then sonicated in an ultrasonic disintegrator (Powersonic 620, Hwa Shin Technology Co., Seoul, Korea) for 10 min. The tube was then vortexed again for 5 s and centrifuged (14,000 *g*) for 10 min at 4°C. The supernatant obtained was suitably diluted and measured at 280, 360, and 420 nm in a spectrophotometer (Epoch; BioTech Instruments, Winooski, VT, USA). Analyses were performed in triplicate.

Specific extinction coefficient at 280, 325, and 405 nm

A 1.5 mg/mL sample solution was prepared by dissolving 45 mg of freeze-dried, pure melanoidins in 30 g of water. Subsequently, various dilutions were prepared from this melanoidins solution. To minimize errors that may occur during dilution based on volumes, the melanoidins solutions were prepared by weighing. The quartz cuvette used for absorption measurements was rinsed

thoroughly with the melanoidins dilution prior to measuring absorption at 280, 325, and 405 nm (Epoch; BioTech Instruments). The specific extinction coefficient (K_{mix}) was calculated using the Lambert-Beer law:

$$E\ (-) = K_{mix}\ (L/g/cm) \times \text{concentration}\ (g/L) \times \text{length of light path}\ (cm).$$

The use of the specific extinction coefficient (K) was preferred over the molar extinction coefficient (ε), since the molecular weights of melanoidins are unknown and are probably variable. By using K, the concentration parameter used in the Lambert-Beer law is expressed in L/g/cm, which makes it applicable to melanoidins.

Wavelength spectra

Wavelength spectra of melanoidins were recorded using a UV-visible spectrophotometer (Epoch; BioTech Instruments), with the wavelength ranging from 200 nm to 700 nm.

Elemental analysis

An elemental analyzer (Model CE440, PerkinElmer, Inc., Waltham, MA, USA) was used to measure the carbon, hydrogen, and nitrogen contents of the samples, and the oxygen content was calculated by difference. The results were reported in percentages by weight.

Molecular weight measurement

The molecular weight distribution profiles of the melanoidins were measured using high performance gel-permeation chromatography on an Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a differential refractive index detector (RID), and an automatic sample injector was used. The mobile phase was 0.02% NaN_3/water solution, and the flow-rate was 1.0 mL/min. All separations were performed with a PL Aquagel-OH Mixed column (300×7.5 mm I.D., 8 μm particle size, Agilent Technologies), and the retention times were plotted against the logarithms of their corresponding average molecular weights. All spectra were acquired using a ChemStation software system (Agilent Technologies). Seven standards were used as molecular weight calibration standards ($\log MW = -0.1654R_t + 6.5726$, with R_t=retention time, expressed in min): blue dextran (2,000 kDa), bovine serum albumin (66 kDa), albumin (44.287 kDa), β-lactoglobulin (36 kDa), myoglobin (17 kDa), ribonuclease A (13.7 kDa), and cytochrome C (12.380 kDa). The data analysis was performed using the ChemStation software (Hewlett Packard, Palo Alto, CA, USA).

Fourier-transform infrared spectroscopy (FT-IR) analysis

FT-IR analysis was performed using an FT-IR spectrometer (FT/IR-6300, JASCO, Tokyo, Japan) using the KBr pellet method. Spectra were recorded at a resolution of 4 cm^{-1} between 400 and 4,000 cm^{-1}, with a total of 100 scans.

X-ray powder diffraction (XRD) analysis

The XRD pattern was obtained from a PANalytical X'Pert PRO MPD X-ray diffractometer (PANalytical, Almelo, The Netherlands) that employed a conventional copper target X-ray tube set to 40 kV and 30 mA. The X-ray source was Cu Kα radiation. Data were collected in the range of $2\theta = 3.00$ to 50.00° (θ being the angle of diffraction), with the following parameters at room temperature: step width, 0.02°; step time, 0.4 s; scanning speed, 5°/min; divergence slit width, 0.2 mm; scatter slit width, 0.6 mm; receiving slit width, 0.2 mm. Samples were freeze-dried, and then 50 mg samples were added into the slide for packing prior to X-ray scanning.

Statistical analysis

Each experiment was performed in triplicate. Data were reported as the means±standard deviations and were analyzed by SPSS (version 20.0, IBM Inc., Armonk, NY, USA). Analysis of variance (ANOVA) and Duncan's multiple range test were used to determine significant differences among the means, and differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Absorbance measurement

The changes in the absorbances (at 280, 360, and 420 nm) of melanoidins formed from BG after different thermal processing steps are presented in Fig. 1. Comparatively, the values obtained at 280 nm were higher than those obtained at 360 or 420 nm for each pair of melanoidins formed from BG during thermal processing. In addition, the absorbances of BG4 at 280, 360, and 420 nm were significantly higher than those of the other samples ($P<0.05$). Although this fact possibly indicates a great abundance of early Maillard reaction products (MRPS) in all samples, it has been shown that a single molecule can absorb at both 420 and 280 nm, and higher absorbances are usually observed at 280 nm (21). Moreover, according to the properties of the reactants involved, the MRPS are usually associated with the absorbance increases at 280 nm, 320~360 nm, and 420~450 nm, corresponding to the initial, intermediate, and final stages of MRPS formation, respectively (22). The initial stage of the Maillard reaction produces colorless (about 280 nm) intermediates arising from sugar-amine condensation and Amadori rearrangement. The intermediate stage (320~360 nm) produces colorless or yel-

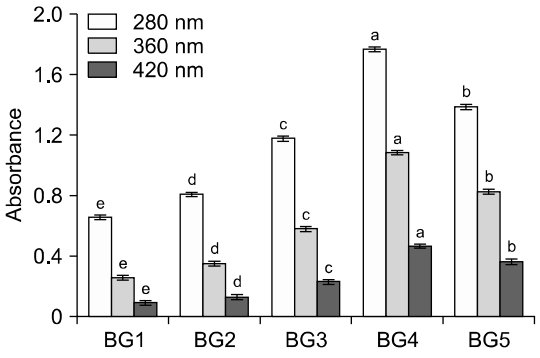

Fig. 1. Browning measurements at 280, 360, and 420 nm of melanoidins formed from black garlic after different thermal processing steps. BG1, black garlic cloves at step 1; BG2, black garlic cloves at step 2; BG3, black garlic cloves at step 3; BG4, black garlic cloves at step 4; BG5, black garlic cloves at step 5. Different letters (a-e) within same absorbance are significantly different at $P<0.05$, as analyzed by Duncan's multiple range test.

low products via several reactions, such as sugar dehydration, sugar fragmentation, and amino acid degradation (Strecker degradation). The final stage (420~450 nm) yields highly colored products via aldol condensation, aldehyde-amine condensation, and formation of heterocyclic nitro compounds. The results suggested that intermediate products were produced to a large extent, and the initial (280 nm), intermediate (360 nm), and final stage product (420 nm) had similar tendencies in BG during thermal processing.

K_{mix}

The absorption maximum at 280 nm can be explained by the presence of proteins and phenolic acid. The absorption maximum at 325 nm can be attributed to the presence of phenolic acid. It is generally accepted that melanoidins contain conjugated systems, which result in light absorption throughout the whole spectrum (23). The wavelength selected for measuring melanoidins is most often 405 nm (24). On the basis of these absorption spectra, it can be stated that absorption measurements at 280, 325, and 405 nm provide useful information on the relative amounts of melanoidins and other compounds formed. To be able to compare absorption values of different BG samples at a specific wavelength, the absorption of melanoidins at a specific wavelength was expressed as K values. Since the melanoidin samples used in this study were most likely a mixture of various compounds, the specific extinction coefficient was defined as K_{mix}. The value of K_{mix} provides information on the relative amounts of melanoidins (K_{mix} 405 nm) and other compounds like proteins (K_{mix} 280 nm) and phenolic acid (K_{mix} 280 and 325 nm) present in melanoidins formed from BG. In Table 1, the changes in the K_{mix} values at 280, 325, and 405 nm for the melanoidins formed from BG after different thermal processing steps

Table 1. K_{mix} values of melanoidins of garlic at different thermal processing steps (unit: L/g/cm)

Samples	$K_{mix\ 280\ nm}$	$K_{mix\ 325\ nm}$	$K_{mix\ 405\ nm}$
BG1	1.43±0.26^{e}	0.51±0.01^{e}	0.12±0.01^{e}
BG2	1.58±0.01^{d}	0.67±0.01^{d}	0.16±0.01^{d}
BG3	2.31±0.01^{c}	1.04±0.01^{c}	0.29±0.01^{c}
BG4	3.63±0.01^{a}	1.84±0.01^{a}	0.59±0.01^{a}
BG5	2.78±0.01^{b}	1.39±0.01^{b}	0.45±0.01^{b}

Values are means of three replicates±standard deviation. Different letters (a-e) within a column indicate significant differences at $P<0.05$.
BG1, black garlic cloves at step 1; BG2, black garlic cloves at step 2; BG3, black garlic cloves at step 3; BG4, black garlic cloves at step 4; BG5, black garlic cloves at step 5.

are given. The K_{mix} values of BG4 at 280, 325, and 405 nm were significantly higher than those of the other samples ($P<0.05$). The K_{mix} values increased with increasing thermal processing steps, the BG4 sample exhibited values of 3.63, 1.84, and 0.59 for K_{mix} 208 nm, K_{mix} 325 nm, and K_{mix} 405 nm, respectively. This can be explained by the fact that compounds like degraded proteins, peptides, and phenolic acids are present in melanoidins during the thermal processing steps. In addition, K_{mix} 405 nm was the highest for BG4, suggesting that BG4 is relatively rich in melanoidins. However, the question remains whether these compounds are incorporated into the melanoidins complex or not.

Wavelength spectra

The changes in the wavelength spectra of melanoidins formed from BG after different thermal processing steps are shown in Fig. 2. The absorbance of melanoidins increased markedly as the thermal processing steps increased. The BG4 displayed the highest absorbance of all the samples. In particular, the band intensities of melanoidins formed from the BG4 step were higher than those of melanoidins formed from the other samples during the thermal processing steps. All the melanoidin samples showed different absorptions in the UV-visible spectra, although these had similar shapes. Higher intensities of the melanoidin samples were indicative of increased absorbance in the ultraviolet region, while the absorbance intensities gradually decreased in the visible region. Every peak had a maximum absorbance that appeared in the range of 260~320 nm, which is characteristic of melanoidins. This trend for melanoidin-type colorants was also described by other authors (25,26). Moreover, this wavelength shift was supposedly due to a possible increase in the extent of chromophore conjugation in melanoidins, resulting in a copigmentation effect. The UV-visible spectra, exhibiting both featureless end absorption and increased intensity with a decrease in the wavelength, were typical of melanoidins (27).

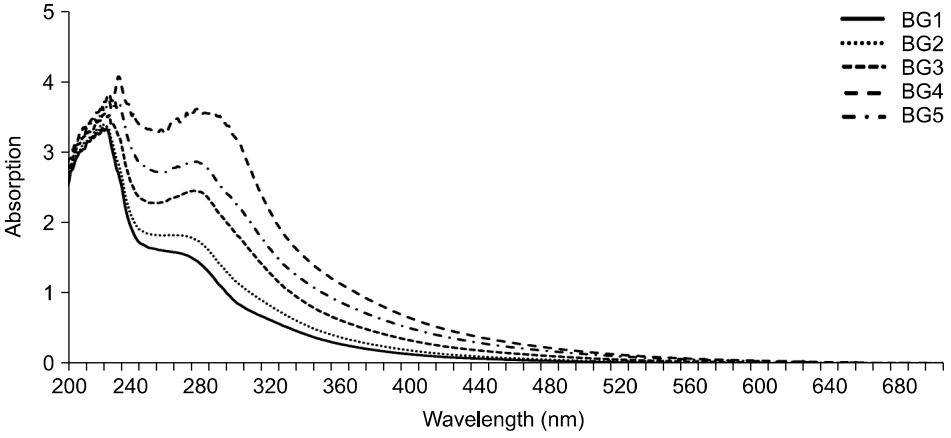

Fig. 2. Wavelength spectra of melanoidins formed from black garlic after different thermal processing steps. BG1, black garlic cloves at step 1; BG2, black garlic cloves at step 2; BG3, black garlic cloves at step 3; BG4, black garlic cloves at step 4; BG5, black garlic cloves at step 5.

Elemental analysis

Changes in the carbon, hydrogen, oxygen, and nitrogen contents were used to estimate which major chemical transformations take place during thermal processing. As shown in Table 2, the carbon, hydrogen, and oxygen contents of melanoidins formed from BG during thermal processing decreased in the fourth step of thermal processing (BG4), and then increased in BG5. However, the nitrogen content increased during thermal processing. The nitrogen content of BG ranged from 0.3 to 2.7%, while the carbon content ranged from 31.8 to 38.9%, and so it had a C/N ratio of >12.2. The nitrogen content of BG4 was high, resulting in a low C/N ratio. The increase in the nitrogen content relative to carbon suggests that the incorporation of carbon-rich substances such as carbohydrates and polyphenols could occur to result in the final polymeric melanoidin structure. Furthermore, it suggests a sudden loss or degradation of nitrogen-rich substances, such as amino acids, peptides, and proteins via reactions such as deamidation. The principal reaction in the formation of a high-molecular-weight melanoidin structure, therefore, should be the coupling of carbon-rich compounds, such as carbohydrates, polyphenols, and their degradation products, to a nitrogen-rich protein skeleton. Similar results were found by Bekedam et al. (28), who observed a positive correlation between roasting and the content of galactomannans in the melanoidin fraction of a coffee brew. Also, Cämmerer and Kroh (13) suggested that the melanoidin skeleton was mainly built up from sugar degradation products formed in the early stages of the Maillard reaction. The hypothetical structure proposed was based on the reactions of dicarbonyl compounds (dehydrated, sugar-derived intermediates) that can react among themselves (aldol reaction or nucleophilic addition) as well as undergo substitution reactions with amino compounds.

Table 2. Elemental composition of melanoidins from black garlic after different thermal processing steps

Samples	Elements (wt%)				
	C	H	O	N	C/N
BG1	37.9	6.4	44.3	0.3	126.3
BG2	38.9	6.3	45.6	0.6	64.8
BG3	34.8	5.5	38.2	1.3	26.8
BG4	31.8	4.9	31.5	2.6	12.2
BG5	34.5	5.2	35.0	2.7	12.8

BG1, black garlic cloves at step 1; BG2, black garlic cloves at step 2; BG3, black garlic cloves at step 3; BG4, black garlic cloves at step 4; BG5, black garlic cloves at step 5.

Molecular weight measurement

The changes in the gel permeation chromatograms of the melanoidins formed from BG after different thermal processing steps are shown in Fig. 3. Gel permeation chromatography allowed separation of melanoidins on a molecular weight basis. Using this technique, low-molecular-weight compounds were retained in the pores of the gel beads and eluted later than the molecules of high-molecular-weight compounds. As thermal processing progressed, the molecular weights of all the melanoidin samples increased; the major peaks of each melanoidin sample had different retention times. With regard to the molecular size distribution of the melanoidins formed, 3 peaks of different molecular masses were observed, corresponding to the peak apices. The major peaks, appearing at 8.95, 9.41, and 9.80 min, corresponded to molecular weights of 4.29, 4.07, and 3.98 kDa, respectively. In particular, the molecular weights of compounds with molecular weights of 4.07 and 3.98 kDa increased markedly as thermal processing progressed, while those of compounds with molecular weights of 4.29 kDa decreased markedly. Hofmann (29) reported that the reaction between casein and glucose led to a drastic increase in molecular weights by carbohydrate-induced oligomerization of the protein backbone. Furthermore, these results confirm the earlier findings of Clark and Tannenbaum (30) that the reaction between proteins and carbohydrates leads to polymerization of the reaction mixture. The results showed that the melanoidins formed from

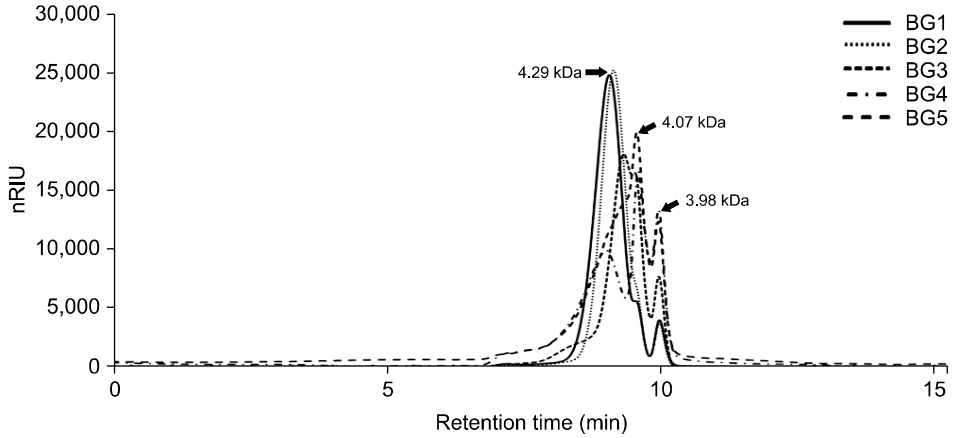

Fig. 3. Molecular weights of melanoidins formed from black garlic after different thermal processing steps. BG1, black garlic cloves at step 1; BG2, black garlic cloves at step 2; BG3, black garlic cloves at step 3; BG4, black garlic cloves at step 4; BG5, black garlic cloves at step 5.

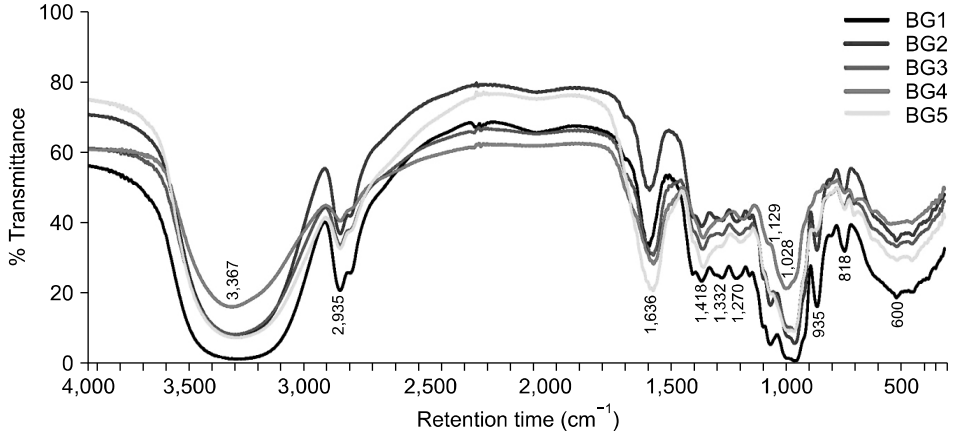

Fig. 4. FT-IR spectra of melanoidins formed from black garlic after different thermal processing steps. BG1, black garlic cloves at step 1; BG2, black garlic cloves at step 2; BG3, black garlic cloves at step 3; BG4, black garlic cloves at step 4; BG5, black garlic cloves at step 5.

BG after different thermal processing steps had different size exclusion chromatography elution patterns.

FT-IR analysis

The identification of compounds using infrared spectroscopy is based on the existence of characteristic group frequencies that have roughly the same values regardless of the compound in which the functional group appears. The changes in overlay of the infrared spectra of the melanoidins formed from BG after different thermal processing steps are shown in Fig. 4. The same functional groups were present in the melanoidins formed during the different steps, and their infrared spectral differences were mainly due to variations in absorption intensities. These results are consistent with previously reported spectra for other synthetic melanoidins (31). As the thermal processing steps increased, the spectra of all the melanoidin samples showed a broad and intense band for the stretching vibration of the -OH group at 3,367 cm^{-1}. An extremely broad band attributable to hydrogen-bonded hydroxyl groups appeared at 3,200~3,500 cm^{-1} (32). The main bands (such as those representing -OH groups) at 3,367 cm^{-1} were due to oxygen-containing functionalities. The low contribution of the CH groups was reflected by the weak peak at 2,935 cm^{-1}. The characteristic absorption bands between 4,000 and 2,500 cm^{-1} were 3,480~3,440, 3,260~3,270, and 2,960~2,878 cm^{-1} for the -OH, -NH, and -CH stretching regions, respectively (33). The absorption bands around 1,636, 1,418, 1,332, 1,270, 1,129, and 1,028 cm^{-1} were attributed to the C=C, CH, CN, CO, C-O-C, and C-C groups, respectively. The absorption bands around 1,650, 1,560, and 1,310 cm^{-1} were attributed to the amide I, II, and III groups (34), respectively. In addition, there were several discernible absorbances (at 1,159, 1,082, and 1,014 cm^{-1}), which were attributed to C-O bond stretching (35). Additional characteristic absorption bands appeared at 934, 818, and 600 cm^{-1} due to the stretching vibrations of the entire anhydroglucose ring. This suggested that the melanoidins formed from BG after different thermal processing steps contained -OH, -CH, amide I, and amide III groups.

XRD analysis

XRD measurements were performed to examine if chemical modifications altered the crystallinity of the melanoidins. The XRD spectra of the melanoidins formed from BG after the different thermal processing steps are shown in Fig. 5. As thermal processing progressed, the melanoidin samples showed 6 major peaks at 27.26°, 31.58°, 42.08°, 43.62°, 45.26°, and 49.1° (2θ). As thermal processing progressed, it is interesting to note that the intensity of the peaks at 27.26° and 31.58° (2θ) increased, while the intensity of the peaks at 42.08°, 43.62°, and

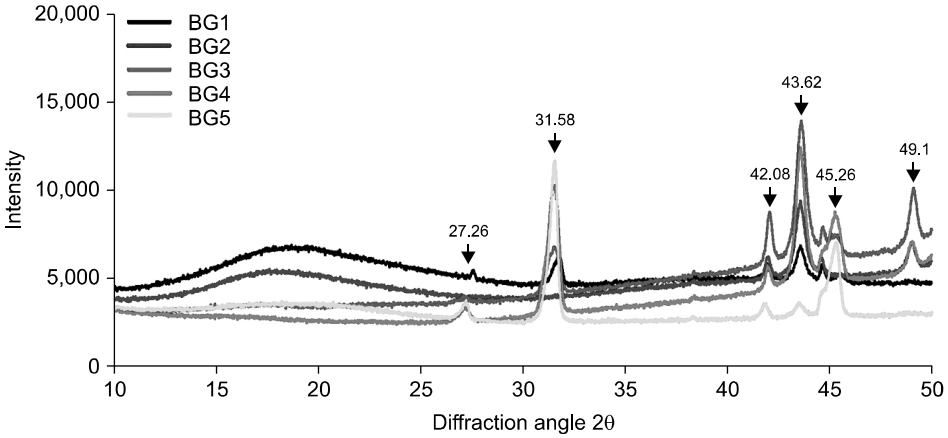

Fig. 5. X-ray diffraction patterns of melanoidins formed from black garlic after different thermal processing steps. BG1, black garlic cloves at Step 1; BG2, black garlic cloves at Step 2; BG3, black garlic cloves at Step 3; BG4, black garlic cloves at Step 4; BG5, black garlic cloves at step 5.

45.26° (2θ) increased and then decreased. Moreover, the peak at 49.1° (2θ) disappeared during the process. This observation suggests that crystallinity of the melanoidins was mainly developed at 31.58° and 43.62° (2θ). As thermal processing progressed, the degree of crystallinity also increased, but had a different magnitude.

In conclusion, the melanoidins formed from BG during thermal processing were produced to a large extent, and the initial (280 nm), intermediate (360 nm), and final stage product (420 nm) had similar tendencies. Compounds including degraded proteins, peptides, and phenolic acids were present in the melanoidins formed during thermal processing. All the melanoidin samples showed different absorptions in the UV-visible spectra, although these had similar shapes. Moreover, the carbon, hydrogen, and oxygen contents of the melanoidins formed decreased and then increased. However, the nitrogen content increased during thermal processing. As the thermal processing steps increased, the molecular weights of all the melanoidins samples increased, whereas the major peaks of each melanoidin sample had different retention times. Furthermore, the melanoidins formed from BG after the different thermal processing steps contained OH, CH, amide I, and amide III groups. The crystallinity of the melanoidins was mainly developed at 31.58° and 43.62° (2θ). These results contribute significantly to our understanding of the effects of thermal processing on the Maillard reaction in BG.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Butt MS, Sultan MT, Butt MS, Iqbal J. 2009. Garlic: nature's protection against physiological threats. *Crit Rev Food Sci Nutr* 49: 538-551.
2. Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol* 28: 25-30.
3. Chang HL, Chen YC, Tan FJ. 2011. Antioxidative properties of a chitosan-glucose Maillard reaction product and its effect on pork qualities during refrigerated storage. *Food Chem* 124: 589-595.
4. Andriot I, Le Quéré JL, Guichard E. 2004. Interactions between coffee melanoidins and flavour compounds: impact of freeze-drying (method and time) and roasting degree of coffee on melanoidins retention capacity. *Food Chem* 85: 289-294.
5. Charles-Bernard M, Kraehenbuehl K, Roberts DD. 2003. Interactions between volatile and non-volatile coffee components. In *Melanoidins in Food and Health*. Vegarud G, Morales FJ, eds. European Commission, Directorate-General for Research, Brussels, Belgium. Vol 4, p 51-58.
6. Hofmann T, Czerny M, Calligaris S, Schieberle P. 2001. Model studies on the influence of coffee melanoidins on flavor volatiles of coffee beverages. *J Agric Food Chem* 49: 2382-2386.
7. Hofmann T, Schieberle P. 2002. Chemical interactions between odor-active thiols and melanoidins involved in the aroma staling of coffee beverages. *J Agric Food Chem* 50: 319-326.
8. Obretenov C, Demyttenaere J, Tehrani KA, Adams A, Keršiene M, De Kimpe N. 2002. Flavor release in the presence of melanoidins prepared from L-(+)-ascorbic acid and amino acids. *J Agric Food Chem* 50: 4244-4250.
9. Delgado-Andrade C, Morales FJ. 2005. Unraveling the contribution of melanoidins to the antioxidant activity of coffee brews. *J Agric Food Chem* 53: 1403-1407.
10. Steinhart H, Luger A, Piost J. 2001. Antioxidative effect of coffee melanoidins. In *19éme Colloque Scientifique International surle Café*. Association Scientifique Internationale du Café. Trieste, Italy.
11. Wen X, Takenaka M, Murata M, Homma S. 2004. Antioxidative activity of a zinc-chelating substance in coffee. *Biosci Biotechnol Biochem* 68: 2313-2318.
12. Morales FJ, Fernández-Fraguas C, Jiménez-Pérez S. 2005. Iron-binding ability of melanoidins from food and model systems. *Food Chem* 90: 821-827.
13. Cämmerer B, Kroh LW. 1995. Investigation of the influence of reaction conditions on the elementary composition of melanoidins. *Food Chem* 53: 55-59.
14. Cämmerer B, Jalyschko W, Kroh LW. 2002. Intact carbohydrate structures as part of the melanoidin skeleton. *J Agric Food Chem* 50: 2083-2087.
15. Heyns K, Hauber R. 1970. Strukturermittlung spezifisch ^{14}C-markierter sorbosebräunungspolymerisate durch thermische fragmentierung. *Justus Liebigs Ann Chem* 733: 159-169.
16. Tressl R, Wondrak GT, Garbe LA, Krüger RP, Rewicki D. 1998. Pentoses and hexoses as sources of new melanoidin-

like Maillard polymers. *J Agric Food Chem* 46: 1765-1776.
17. Hofmann T. 1998. Studies on melanoidin-type colorants generated from the Maillard reaction of protein-bound lysine and furan-2-carboxaldehyde—chemical characterisation of a red coloured domaine. *Eur Food Res Technol* 206: 251-258.
18. Kato H, Tsuchida H. 1981. Estimation of melanoidin structure by pyrolysis and oxidation. *Prog Food Nutr Sci* 5: 147-156.ill.
19. Renn PT, Sathe SK. 1997. Effects of pH, temperature, and reactant molar ratio on L-leucine and D-glucose Maillard browning reaction in an aqueous system. *J Agric Food Chem* 45: 3782-3787.
20. Morales FJ, Jiménez-Pérez S. 2004. Peroxyl radical scavenging activity of melanoidins in aqueous systems. *Eur Food Res Technol* 218: 515-520.
21. Fogliano V, Monti SM, Musella T, Randazzo G, Ritieni A. 1999. Formation of coloured Maillard reaction products in a gluten-glucose model system. *Food Chem* 66: 293-299.
22. Nursten HE. *The Maillard reaction: hemistry, biochemistry and implications*. The Royal Society of Chemistry, Cambridge, UK. p 2-4.
23. Bekedam EK, Schols HA, van Boekel MA, Smit G. 2006. High molecular weight melanoidins from coffee brew. *J Agric Food Chem* 54: 7658-7666.
24. Borrelli RC, Visconti A, Mennella C, Anese M, Fogliano V. 2002. Chemical characterization and antioxidant properties of coffee melanoidins. *J Agric Food Chem* 50: 6527-6533.
25. Kim JS, Lee YS. 2008. Effect of reaction pH on enolization and racemization reactions of glucose and fructose on heating with amino acid enantiomers and formation of melanoidins as result of the Maillard reaction. *Food Chem* 108: 582-592.
26. Rafik M, Mas A, Elharfi A, Schue F. 1997. Decoloration de solutions sucrees par ultrafiltration sur une membrane a base de poly(organocyclophosphazene). *Eur Polym J* 33: 679-690.
27. Clark AV, Tannenbaum SR. 1970. Isolation and characterization of pigments from protein-carbonyl browning systems. Isolation, purification, and properties. *J Agric Food Chem* 18: 891-894.
28. Bekedam EK, Loots MJ, Schols HA, Van Boekel MAJS, Smit G. 2008. Roasting effects on formation mechanisms of coffee brew melanoidins. *J Agric Food Chem* 56: 7138-7145.
29. Hofmann T. 1998. Studies on the relationship between molecular weight and the color potency of fractions obtained by thermal treatment of glucose/amino acid and glucose/protein solutions by using ultracentrifugation and color dilution techniques. *J Agric Food Chem* 46: 3891-3895.
30. Clark AV, Tannenbaum SR. 1974. Isolation and characterization of pigments from protein-carbonyl browning systems. Models for two insulin-glucose pigments. *J Agric Food Chem* 22: 1089-1093.
31. Rubinsztain Y, Ioselis P, Ikan R, Aizenshtat Z. 1984. Investigations on the structural units of melanoidins. *Org Geochem* 6: 791-804.
32. Fang JM, Fowler PA, Sayers C, Williams PA. 2004. The chemical modification of a range of starches under aqueous reaction conditions. *Carbohydr Polym* 55: 283-289.
33. Mucha M, Miśkiewicz D. 2000. Chitosan blends as fillers for paper. *J Appl Polym Sci* 77: 3210-3215.
34. Sannan T, Kurita K, Ogura K, Iwakura Y. 1978. Studies on chitin: 7. IR spectroscopic determination of degree of deacetylation. *Polymer* 19: 458-459.
35. Goheen SM, Wool RP. 1991. Degradation of polyethylene-starch blends in soil. *J Appl Polym Sci* 42: 2691-2701.