

Research Article

The Physicochemical Changes of Black Garlic during Thermal Processing

^{1,2}Mengmeng Lei, ²Zesheng Zhang, ²Rui Liu, ²Min Zhang and ²Mengying Xu

¹College of Food Science and Technology, Henan Agricultural University, Zhengzhou 450002,

²Key Laboratory of Food Nutrition and Safety, Tianjin University of Science and Technology, Ministry of Education, Tianjin 300457, China

Abstract: To explore the physicochemical changes of black garlic during the thermal processing steps and further reveal the role of Maillard reaction in the formation mechanism of black garlic. The physicochemical changes including UV-Vis absorbance, fluorescence and color difference were determined. The UV absorbance at 294 nm and browning intensity at 420 nm gradually increased with increasing heating time, while the fluorescence intensity showed a maximum value at the heating time of 3 days. The color value of black garlic increased after heating at 70-80°C for 10 days. These results indicated Maillard reaction was primarily responsible for the formation of black garlic.

Keywords: Black garlic, physicochemical, thermal processing

INTRODUCTION

Garlic (*Allium sativum* L.) is widely used as foods and spices in many countries for thousands of years. It has also been used as herbal remedies for the treatment of various epidemic diseases, such as fever, headache, cholera and dysentery (Kodera *et al.*, 2002; Corzo *et al.*, 2007). Recently, garlic has attracted much attention for disease prevention because of its beneficial effects, including antibacterial, antioxidant and anticancer activities. However, the consumption and application of fresh garlic in foods and medicines is limited due to its penetrating odor and spicy taste.

Black garlic, a novel fermented garlic product originating from Japan, has been recently developed for eliminating the unpleasant odor and improving the flavor of fresh garlic. Many investigations have demonstrated that black garlic, compared with fresh garlic, also has a wide range of beneficial health effects, such as anti-tumor (Wang *et al.*, 2012), antioxidant (Sato *et al.*, 2006), anti-allergic (Kim *et al.*, 2012), anti-inflammatory (Purev *et al.*, 2012), hypocholesterolaemic and hepatoprotective activities (Kim *et al.*, 2011a). Furthermore, black garlic was found to improve insulin sensitivity and dyslipidemia in db/db mice significantly (Lee *et al.*, 2009), thereby possibly managing diabetes and enhancing the immune system (Wang *et al.*, 2010).

Black garlic, as a novel food product, is generally produced by placing fresh garlic in a humidity-controlled room at high temperature for several weeks without adding any additives (Kim *et al.*, 2011b). During the thermal processing steps, significant

changes in the physicochemical properties of garlic have taken place, eventually leading to the formation of black garlic with characteristics of non-irritating smell and fruit-like sweetness. It was suggested that processing methods had a significant impact on garlic's antioxidant activities (Querioz *et al.*, 2009). However, the exact formation mechanism of black garlic is still unknown. Maillard reaction, the most common type of non-enzymatic browning, has been recognized as a major factor in producing black garlic. Maillard reaction typically occur between reducing sugars and proteins, peptides or amino acids upon thermal processing (Beniakul *et al.*, 2005; Laroque *et al.*, 2008) and can produce a variety of intermediates and brown pigments with a wide range of potentially promising antioxidant activities (Moreno *et al.*, 2006; Xiang *et al.*, 2008). Until now, there have been few studies on the chemical changes in the formation period of black garlic, which has a close relationship to the formation of black garlic and its bioactivities.

The purpose of this study was to explore the physicochemical changes of black garlic during the thermal processing steps and further reveal the role of Maillard reaction in the formation mechanism of black garlic. The changes in UV absorbance, fluorescence intensity and color were determined by a combination of physical techniques.

METHODOLOGY

Materials: Garlic was purchased from a local market in Tianjin, China. All other chemicals and solvents were of analytical grade and were purchased locally.

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

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Sample preparation: Black garlic was produced according to Chinese patent ZL 201110154928.1. Briefly, fresh garlic was maintained in a humidity-controlled room at 70-80°C for 10 days without any additional treatments and additives. The fresh garlic without any treatments was used as the reference blank (day 0). Samples were removed after 1-10 days of heat treatment and stored at -20°C prior to extraction. The garlic and black garlic samples were peeled, mixed with 1.5 volumes (g/mL) of triple-distilled water and mashed in a mortar and pestle. It was then kept at room temperature for 30 min and filtered to obtain the garlic extracts for further analysis.

UV-Vis and fluorescence analysis: UV-Vis analysis of garlic samples was performed using an UVmini-1240 spectrophotometer (Shimadzu, Kyoto, Japan). The UV absorbance and browning intensity of garlic samples was measured at 294 and 420 nm, respectively, according to the method of Ajandouz *et al.* (2001). The triple-distilled water was used as the blank solution.

Fluorescence analysis was performed on a Fluoroskan-FL Ascent fluorometer (Thermo labsystems, Helsinki, Finland). The fluorescence intensity of garlic samples was measured using the method reported with slight modification (Morales and Perez, 2001). Briefly, each sample was excited at 355 nm and emission at 460 nm was recorded at 20°C with triple-distilled water as blank solution.

Determination of color: The apparent color of garlic samples was measured using a DC-P3 colorimeter (Xingguang Color-Measurement Instrument, Beijing, China). Color was expressed in CIE system. The total color difference (ΔE) was given by the Hunter-Scottfield equation (Claude and Ubbink, 2006):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^* , Δa^* , Δb^* represents the difference between the black garlic and garlic sample (day 0) on L^* (lightness), a^* (redness and greenness) and b^* (yellowness and blueness) values, respectively.

Statistical analysis: The results are presented as the means \pm Standard error (S.D.). The data were analyzed by one-way ANOVA and Student's t-test using SPSS statistical software (version 16.0 for windows, SPSS Inc., Chicago, IL, U.S.A.). Differences were considered statistically significant at $p < 0.05$ for all tests.

RESULTS AND DISCUSSION

UV-Vis absorbance analysis: UV-Vis and fluorescence analysis were employed to evaluate the role of Maillard reaction in the formation of black garlic and browning development. Changes in UV-Vis

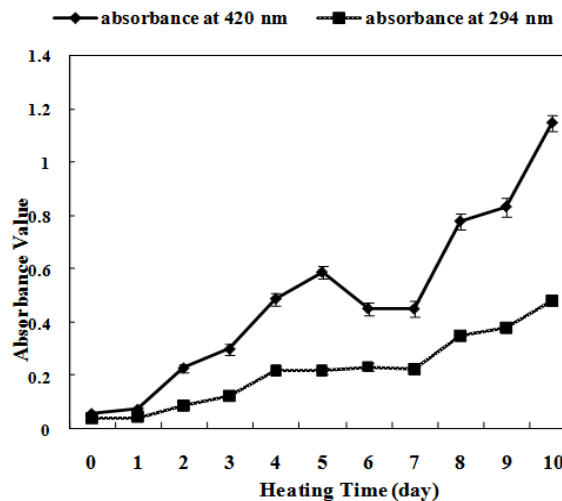


Fig. 1: Changes in UV-Vis absorbance of black garlic extracts during heat treatment

absorbance of black garlic extracts during heat treatment were shown in Fig. 1. The UV-Vis absorbance development is an important and obvious feature of the Maillard reaction after initial stage. UV absorbance at 294 nm was used to determine the intermediate stages of the non-enzymatic browning reaction as described by Zhuang and Sun (2011). Figure 1 showed that the absorbance at 294 nm increased from 0.039 to 0.217 during the first 4 days, stabilized at ~0.22 in the following 3 days and then sharply increased to 0.489 in the last 3 days. The results suggested that a non-colored and non-fluorescent compound, which could be the precursor of the maillard reaction, was formed during the heat treatments (Ajandouz *et al.*, 2001). Yilmaz and Toledo (2005) considered that thermally processed foods contained various levels of maillard reaction products. It has been reported that maillard reaction can even take place in the dehydrated garlic during storage (Moreno *et al.*, 2006) and can also occur in the concentrated aged garlic extract when stored at room temperature for longer than 10 months (Ide *et al.*, 1999).

UV absorbance at 420 nm is often used as an indicator of the extent which the Maillard reaction takes place in foods and it symbolizes the final stages of the browning reaction. Its intensity is the most convenient measurable index of the Maillard reaction as it may be estimated visually (Yilmaz and Toledo, 2005; Fogliano *et al.*, 1999). Furthermore, the appearance of pre-melanoidins and the formation of melanoidins could be monitored by UV absorbance at 420 nm, which has been used as the end-point measurement for quantifying the yield of high molecular weight melanoidins. As can be seen in Fig. 1, the browning intensity gradually increased from 0.058 to 0.589 during the first 4 days, slightly decreased to 0.451 on from 5 to 7 days and then sharply increased to 1.149 in the last 3 days. The

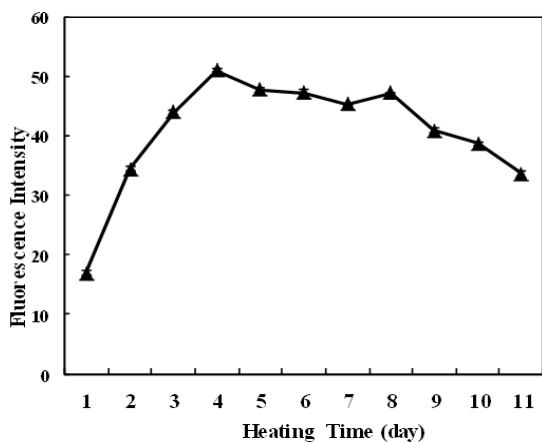


Fig. 2: Changes in fluorescence intensity of black garlic extracts during heat treatment

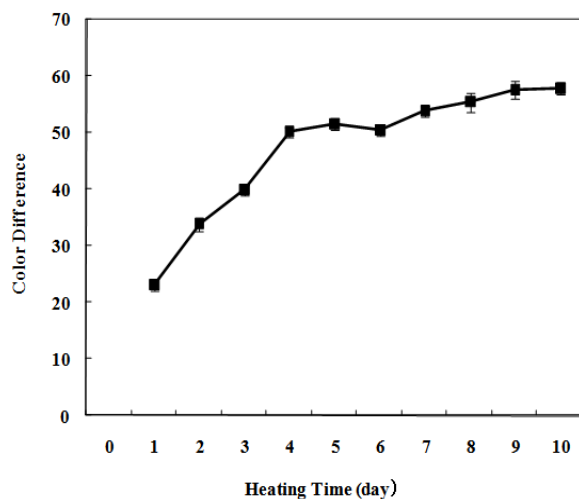


Fig. 3: Changes in color difference of garlic samples during heat treatment

change in absorbance at 420 nm was similar to that at 294 nm, suggesting that a large proportion of the intermediates are precursors of brown products of the Maillard reaction and which were in agreement with the reported results (Xiang *et al.*, 2008; Hwang *et al.*, 2011). The highly UV-absorbing and colorless compounds are formed at intermediate stages, whereas the brown polymers are formed at final stages of the Maillard reaction. It may be supposed that a great quantity of intermediate UV-absorbing compounds were converted into brown products during the final several days.

Fluorescence analysis: Changes in fluorescence intensity of black garlic extracts during heat treatment were shown in Fig. 2. Fluorescence compounds developed prior to the formation of brown pigments in the Maillard reaction. Figure 2 showed that the fluorescence intensity of black garlic reached a

maximum at a heating time of 3 days and then decreased gradually as heating time increased. This result was in line with previous studies on Maillard reaction (Bosch *et al.*, 2007; Lertittikul *et al.*, 2007), which suggested that fluorescence intermediates other than non-color intermediates were formed. In contrast to non-fluorescent compounds, the fluorescent compounds were presumably more reactive in formation of brown products, as shown by the decrease of fluorescence intensity over time. Fluorescence results strengthen the role played by Maillard reaction in browning development.

The significant role of Maillard reaction in browning development and formation of black garlic was indicated by UV-Vis and fluorescence analysis, which showed typical Maillard reaction features as described in the previous work (Moreno *et al.*, 2006; Bosch *et al.*, 2007). Changes in UV absorbance and fluorescence intensity suggested the formation of intermediates and brown products of the Maillard reaction.

Color analysis: Changes in color of garlic samples during thermal processing were shown in Fig. 3. The total color difference (ΔE) increased over time, indicating that the color of garlic changed from white to brown and eventually became black with the increasing of thermal processing time. This result was accordance with the changes of UV-Vis absorbance and the color characteristics of the Maillard reaction. A variety of intermediate products (pre-melanoidins) and their brown pigments (melanoidins) were produced via the Maillard reaction (Beniakul *et al.*, 2005; Lertittikul *et al.*, 2007). Therefore, the results indicated that Maillard reaction rendered the color of black garlic.

The antioxidant activities of black garlic samples gradually increased during the thermal processing and it exhibited a trend similar to that of the UV-Vis assay. This result indicated that the increase in antioxidant activity might be related to the presence of maillard reaction products (Beniakul *et al.*, 2005; Morales and Perez, 2001; Hwang *et al.*, 2011).

CONCLUSION

Changes in UV absorbance, fluorescence intensity and color were consistent with the characteristic of these indicators in Maillard reaction. The UV absorbance at 294 nm and browning intensity at 420 nm gradually increased over 10 days of thermal processing, while the fluorescence intensity showed a maximum value at the heating time of 3 days. The color value of black garlic increased after heating at 70-80°C for 10 days. These results indicated that Maillard reaction was primarily responsible for the formation of black garlic.

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