Food Chemistry 239 (2018) 56–61

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

An analysis of the changes on intermediate products during the thermal processing of black garlic

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ARTICLE INFO

Article history:
Received 7 February 2017
Received in revised form 5 May 2017
Accepted 13 June 2017
Available online 15 June 2017

Chemical compounds studied in this article:
Fructose (PubChem CID: 5984)
Sucrose (PubChem CID: 5988)
Glucose (PubChem CID: 107526)
Fructosylproline (PubChem CID: 119856)
Fructosylvaline (PubChem CID: 71777427)
Fructosylvaline (PubChem CID: 71316981)

Keywords:
Black garlic
Thermal processing
Amadori compounds
Heyns compounds
Sugar

ABSTRACT

The thermal processing of black garlic was simulated. Fresh garlic was incubated at 55 °C with 80% humidity and sampled every 5 or 10 days. The changes in relevant products were as follows: the fructan content was decreased by 84.79%, and the fructose content was increased by 508.11%. The contents of Maillard reaction intermediate products were first increased and then decreased. The colour of garlic gradually became dark and the pH decreased from 6.13 to 4.00. By analyzing these changes, the mechanism of black garlic formation and the changes on the Maillard reaction were revealed. The sweetness of black garlic resulted mainly from the fructose that was produced, and the black colour was largely due to the Maillard reaction between fructose/glucose and amino acids. An understanding of this process is useful to explain the formation mechanism of black garlic and could lead to better control of the quality of black garlic.

1. Introduction

Garlic is a pungent condiment that is widely used, and it also has certain biological activities, such as antioxidant and antibacterial activities (Horita et al., 2016; Noda, Asada, Sasaki, Hashimoto, & Nakamura, 2013). However, the use of garlic can be limited because of its pungent flavour. Black garlic is made by the thermal processing of garlic. The unpleasant flavour of garlic can be removed after 60–90 days of thermal treatment. In the processing of black garlic, many physical and chemical properties of the garlic are altered, such as colour, texture, and taste, among others (Zhang et al., 2015). In addition, the flavour and nutritional properties of the garlic have been changed, during the thermal processing of black garlic, due to the Maillard reaction (Kim, Kang, & Gweon, 2013a, 2013b). Many reports have shown that black garlic has wide biological activities, such as *anti*-cancer and antioxidant activities, and the antioxidant activity, in particular, is higher in black garlic than in raw fresh garlic (Dong et al., 2014; Kim, Nam, Rico, & Kang, 2012).

There are a large number of polysaccharides in garlic, accounting for more than 75% of its dried material, and the primary component of the polysaccharides in garlic is fructan (Baumgartner, Dax, Praznik, & Falk, 2000). The fructan in garlic is a member of the 1-kestose family, and it is composed of fructose and glucose at a ratio of 14:1. The primary chain of garlic fructan is composed of (2→1)-β-D-fructopyranose linked to a terminal (2→1)-α-D-glucopyranose at the non-reducing end and a (2→6)-β-D-fructopyranose branched chain (Chen, Cheong, Song, Shi, & Huang, 2013). Though there have been some reports regarding fructan structure and content determination, there have been few studies of the fructan in black garlic. Most reports have only demonstrated that polysaccharides or fructan are degraded into simple sugars, such as glucose, fructose or disaccharide, during the processing of garlic (Li, Lu, Pei, & Qiao, 2015; Zhang et al., 2015), and the change in fructan content during the processing of garlic has not been reported in detail. Zhang et al. measured the changes in the molecular weights of polysaccharides in black garlic extracts at different thermal processing periods and noted

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http://dx.doi.org/10.1016/j.foodchem.2017.06.079
0308-8146/

that a sweeter black garlic taste was caused by increases in glucose, fructose and sucrose contents (Zhang et al., 2015). The Maillard reaction is a non-enzymatic reaction between carbonyl groups and amino groups (Hwang, Kim, Woo, Lee, & Jeong, 2011). Heyns and Amadori compounds are very important intermediate products of the Maillard reaction, being produced by the Maillard reaction between fructose or glucose, respectively, with amino acids. Amadori compounds are *N*-substituted 1-amino-1-deoxyketoses, and Heyns compounds are *N*-substituted 2-amino-2-deoxyaldoses (Mossine, Barnes, Glinsky, & Feather, 1996; Yaylayan & Sporns, 1987). Amadori and Heyns compounds can generate different products during thermal processing (Zhang, Yin, & He, 2006). Yuan et al. determined the content of Amadori and Heyns compounds in black garlic using high-performance liquid chromatography–tandem mass spectrometry (Yuan, Sun, Chen, & Wang, 2016). Which kind of sugar is primarily responsible for the sweetening of black garlic? After the degradation of fructan in garlic, the fructose content is above that of glucose in the system; however, as it is a kind of ketose, the reaction speed of fructose is slower than that of glucose (a kind of aldose) (Hwang et al., 2011). Therefore, we were interested in which pathway the Maillard reaction in black garlic occurs primarily via the Amadori or Heyns. To further examine the mechanism of the Maillard reaction in black garlic, we simulated the thermal processing of garlic and described the mechanism of the formation of black garlic by determining the changes that occur in the levels of fructan, fructose, and Maillard reaction intermediates as well as the pH and colour changes in black garlic, which provides a scientific reference for the formation mechanism of black garlic. The analysis results are useful for the evaluation and control of the processing of black garlic.

2. Materials and methods

2.1. Materials

Materials: 20 kg of fresh garlics, which were produced from Shandong province, were obtained from a local market in Beijing, China.

Reagents: Fru-Pro (fructose-proline), Fru-Val (fructose-valine) and Fru-Leu (fructose-leucine) were bought from Toronto Research Chemicals (Toronto, Ontario, Canada). Formic acid (98%) and acetonitrile (≥99.9%) were bought from Acros Organics (Geel, Belgium). The Dowex 50WX4 ion-exchange resin (H^+, 200–400 mesh) was purchased from Acros Organics (Geel, Belgium). The fructan assay kit was bought from Megazyme Inc. (Chicago, Illinois, U.S.A.). Sodium borohydride and maleic acid were bought from Sigma-Aldrich (St. Louis, Mo., U.S.A.). All other analytical grade reagents were bought from Beijing Chemical Works in China.

Instruments: The ESI-MS/MS spectra were acquired by a QQQ mass spectrometer (Agilent 6460, California, USA). The Agilent-1260 system incorporated a binary pump, ELSD-detector, autosampler, thermostatted column compartment and controller. The sample solution was obtained by using ultrapure deionized water from a Milli-Q system (Millipore, Bedford, USA). The analytical balance (Adam PWC 214, d = 0.0001 g) was made in England. The ultrasonic extraction apparatus (KQ-500DE) was produced by Kunshan Ultrasonic Instruments Company in China. The centrifuge (TDI-S-A) was bought from Shanghai Anting Scientific Instrument Plant in China. The freeze-drying machine (FD-1A-50) was produced by Beijing Boyikang Experimental Instrument Co., Ltd., in China. The solid phase extraction (ASE-12) was made by Tianjin Aotesaisi Instrument Co., Ltd., in China. The high speed grinder (FW-100) was produced by Beijing Yongguangming Experimental Instrument Co., Ltd., in China.

2.2. The preparation and simulated thermal processing of black garlic

The fresh raw garlic was stored at −20 °C for 24 h before thermal processing. For the simulated thermal processing, the garlic was incubated in a humidity-controlled room at 80% humidity and 55 °C for 90 days. During the first 60 days, the black garlic was sampled every 5 days, and during the last 30 days, the black garlic was sampled every 10 days. The garlic samples were peeled and freeze-dried for 24 h. The freeze-dried garlic was kept in liquid nitrogen for 2 min, and the garlic was then immediately ground into powder with a high speed grinder, strained through an 80 mesh sieve, and stored.

2.3. The measurement of the fructan content in black garlic

The fructan content was measured with a Megazyme Fructan Kit (the method is referred to that of AOAC 999.03 and AACC 32.32) (Yuan et al., 2016). A 0.1000 g powdered sample was accurately weighed and put into a 100 ml round-bottom flask, and then 80 ml of hot deionized water was added. This solution was stirred and heated at 80 °C for 15 min until the solid had completely dispersed. The solution was cooled to room temperature, transferred to a volumetric flask (100 ml), and diluted with deionized water. The solution was filtered for further analyses. First, the starch and sucrose in the solution were enzymatically hydrolyzed into reducing sugars with sucrase/amylase, and then the reducing sugars were reduced to sugar alcohols with alkaline sodium borohydride. Next, the solution was treated with fructanase, and the fructan in the solution was enzymatically hydrolyzed into glucose and fructose. Finally, the solution was reacted with p-Hydroxybenzoic acid hydrazide reagent in a boiling water bath for exactly 6 min, and then all tubes were immediately placed in cold water for 5 min. The absorbance of all solutions was measured at 410 nm against a reagent blank as soon as possible after cooling.

2.4. The measurement of the soluble sugar in the samples

2.4.1. Pretreatment of the samples

The pretreatment of the samples was according to the previous study experience (Yuan et al., 2016). The freeze-dried black garlic powder was put in a blender and 100 ml of deionized water was added and then mixed. After 2 min, the samples were extracted in deionized water by ultrasonication for 20 min. Then, the samples were centrifuged for 10 min at 1500*g*. The solid residue was reextracted with 50 ml of deionized water and centrifuged again. The supernatants were combined, filtered, transferred to a 200 ml volumetric flask and then brought to the appropriate volume with deionized water. 1 ml of the sample solution was added to the prepared column, which combined ion-exchange resin (Dowex 50WX4) and C18 resin. Deionized water was used to elute the column and remove water-soluble sugars, and the eluate was collected in a volumetric flask to determine the sugar contents.

2.4.2. Chromatography conditions

The sugar compounds in the samples were analyzed by an Agilent-1260 system equipped with ELSD-detector. An amino column (Inertsil 250 × 4.6 mm, 5 μm) was used to analyze the sugar contents, and the column temperature was set at 30 °C. The injection volume was set at 10 μl. The mobile phase was a 25/75 (v/v) isocratic mixture of water and acetonitrile, and the flow rate was 1 ml/min. Each cycle lasted 20 min, and the data were analyzed using the Chemstation for LC system B.04.03 software.