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Comparison of phenolic acids and flavonoids in black garlic at different thermal processing steps

Ji-Sang Kim[a,*], *Ok-Ju Kang*[a], *Oh-Cheon Gweon*[b]

[a]*Department of Food and Nutritional Science, Kyung Nam University, Changwon 631-701, Republic of Korea*
[b]*Department of Curinary Arts & Bakery, Namhae College, Namhae 668-801, Republic of Korea*

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ABSTRACT

The phenolic acid and the flavonoid constituents of garlic subjected to different thermal processing steps were examined. Black garlic was produced in a ripening chamber by 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 results of the present investigations showed that thermal processing affected quantities of each phenolic acid and flavonoid component. The total phenolic content (TPC) and total flavonoid content (TFC) of the garlic subjected to different thermal processing steps were higher than those of fresh garlic. In particular, the black garlic cloves ripened using Step 1 (BG1), black garlic cloves ripened using Step 2 (BG2), black garlic cloves ripened using Step 3 (BG3), and black garlic cloves ripened using Step 5 (BG5) samples exhibited levels of TPC that were higher than the TFC, while the TFC in the fresh garlic (FG) and black garlic cloves ripened using Step 4 (BG4) samples were higher than the TPCs. Hydroxycinnamic acid derivatives were found to be the major phenolic acids of garlic at different processing steps. Among the four major flavonoid subgroups in garlic, flavanols were found at the highest concentrations followed by flavanones and flavones, except in the FG sample.

1. Introduction

Polyphenols are the most abundant antioxidants in our diet and are common constituents of foods of plant origin and are widespread constituents of fruits, vegetables, cereals, olive, dry legumes, chocolate and beverages, such as tea, coffee, cocoa and wine (Ferri & Grassi, 2010; Grassi et al., 2008, 2009). Polyphenols comprise a wide variety of molecules that have a polyphenol structure (i.e., several hydroxyl groups on one or more aromatic rings), but also molecules with one phenol ring, such as phenolic acids and phenolic alcohols.

Polyphenols are divided into several classes, according to the number of phenol rings that they contain and to the structural elements that bind these rings to one another. Phenolic acids present in plants are hydroxylated derivatives of benzoic and cinnamic acids (Herrmann, 1989; Shahidi & Naczk, 1995, Chapter 5). Flavonoids are divided into many categories, including flavonols, flavones, catechins, proanthocyanidins, anthocyanidins and isoflavonoids (Havsteen, 1983; Shahidi & Naczk, 1995, Chapter 5). Many of the flavonoids and related compounds are known to possess strong antioxidative characteristics (Dziedzic & Hudson, 1983) and widely investigated as

* *Corresponding author*. Address: Department of Food and Nutritional Science, Kyung Nam University, 85(Woryeong-dong) Munhwanam 11-gil, Masanhappo-gu, Changwon-si, Gyeongsangnam-do 631-701, Republic of Korea. Tel.: +82 55 249 2185; fax: +82 55 245 5001.
E-mail address: jisangkim@kyungnam.ac.kr (J.-S. Kim).
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a new source of bioactive ingredient that can be incorporated into foods in the development of functional foods.

Garlic (*Allium sativum* L., Alliaceae) has been playing one of the most important dietary and medicinal roles in human beings for centuries. It has been cultivated since ancient times, used as a spice and flavouring and, due to its potential benefits in preventive and curative medicine, has been used in many cultures (Rivlin, 2001). Health properties of garlic depend on its bioactive compounds, especially the organosulphur compounds, which are also responsible for its pungent flavour. In addition to these compounds, garlic is also characterized by phenolic compounds (Lanzotti, 2006), which have interesting pharmacological properties and are present in relatively high amounts. Moreover, some garlic products such as aged garlic extract or black garlic have been found to contain an increased level of polyphenols compared to raw garlic (Nencini, Menchiari, Franchi, & Micheli, 2011; Park, Park, & Park, 2009).

Black garlic is a processed garlic product that is prepared by heat treatment of the raw garlic at high temperature under controlled humidity for more than 1 month. Black garlic products have emerged as one of the fastest-growing health-oriented food product in Korean market with the growing awareness of the health benefits of garlic (Bae, Cho, Won, Lee, & Park, 2012). Moreover, thermal processes are commonly used in food manufacturing. One of the important objectives of thermal processes is to raise the sensory quality of foods, their palatability and to extend the range of colours, tastes, aromas and textures in food (Capuano & Fogliano, 2011). In addition, heating processes lead to the formation of biological compounds that are not originally present in food. However, influences of thermal processes on the concentration of individual flavonoids and phenolic acids in garlic are unknown. Therefore, the objective of the present study was to measure the content of phenolic compounds (total amount as well as individual flavonoids and phenolic acids) in garlic and to analyze the influence of thermal processes on garlic.

2. Materials and methods

2.1. Chemicals and reagents

Gallic acid, *p*-hydroxybenzoic acid, chlorogenic acid, catechin, caffeic acid, epicatechin, epigallocatechin gallate, *p*-coumaric acid, ferulic acid, *m*-coumaric acid, *o*-coumaric acid, quercitrin, myricetin, resveratrol, morin, quercetin, naringenin, apigenin, vanillic acid, kaempferol and formic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and HPLC-grade water were purchased from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals and solvents were of analytical reagent grade.

2.2. Sample preparation

Garlic was cultivated in Namhae-gun, Korea. Fresh garlic bulbs were purchased from the Namhae Bomulsum agricultural association (Namhae, Korea) in 2011. Black garlic was produced in a ripening chamber (MBGAM-1500, Minyoung, Co. Ltd., Korea), without removing the outer layers, by 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 were designated as FG, BG1, BG2, BG3, BG4, and BG5, respectively. To prepare the garlic powder, fresh garlic and black garlic cloves were peeled off. They were 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.

2.3. Analysis of total polyphenols

2.3.1. Extraction of polyphenols

The method described by the International Organization for Standardization (ISO) 14502-1 was used (ISO, 2005). Briefly, 0.200 ± 0.001 g of each sample was weighed in an extraction tube, and 5 mL of 70% methanol at 70 °C was added. The extract was mixed and heated at 70 °C for 10 min. After cooling at room temperature, the extract was centrifuged at 7840*g* for 10 min. The supernatant was decanted in a graduated conical tube. The extraction step was repeated third times. Both extracts were pooled and the volume adjusted to 10 mL with cold 70% methanol. One millilitre of the extract was diluted with water to 5 mL.

2.3.2. Determination of total polyphenol

The total polyphenol content (TPC) was determined spectrophotometrically, using gallic acid as a standard, according to the method described by the International Organization for Standardization (ISO) 14502-1 (ISO, 2005). Briefly, 1.0 mL of the diluted sample extract was transferred in duplicate to separate tubes containing 5.0 mL of a 1/10 dilution of Folin–Ciocalteu's reagent in water. Then, 4.0 mL of a sodium carbonate solution (7.5%, w/v) was added. The tubes were then allowed to stand at room temperature for 60 min before absorbance at 765 nm was measured against water. All values were expressed as mg gallic acid equivalents (GAE) per kg dry matter of the garlic sample.

2.4. Determination of total flavonoid content

Total flavonoid content was determined using a colourimetric method described previously (Woisky & Salatino, 1998). Garlic powder (0.2 g) was added to 20 mL of 80% methanol, extracted for 2 h at room temperature and centrifuged at 18,000*g* for 15 min. The volume of the extract was made up to 100 mL with 80% methanol. A portion of 0.5 mL was taken and 0.5 mL of 2% ethanolic solution of $AlCl_3$ was added to it. After 1 h at room temperature, the absorbance was read at 420 nm. All values were expressed as mg quercetin equivalents (QE) per kg dry matter of garlic sample.