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Short communication

Extraction method for increasing antioxidant activity of raw garlic using steam explosion

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ABSTRACT

Novel extraction method for increasing the antioxidant activity of raw garlic was proposed using steam explosion. Raw garlic was hydrolyzed by high temperature (183–258 °C) and pressure steam (10–45 atm), and then crushed by the rapid decompression. The antioxidant activity of raw garlic treated by steam explosion was higher than that of black garlic, i.e. aging garlic. The lowest EC_{50} value, i.e. the highest antioxidant activity, of extract from raw garlic was obtained at a steam pressure of 45 atm for a steaming time of 5 min, but the highest amount of phenolic compounds, i.e. 93.7 mg-catechin equiv./g-dry raw garlic, was obtained at a steam pressure of 30 atm for a steaming time of 5 min.

1. Introduction

Metabolic syndrome becomes a big social problem in Japan, and one of two people of man and one of five people of woman in old and middle ages are metabolic syndrome or spare groups [1]. It seems that the oxidation stress caused by harm of reactive oxygen species participates in mechanism of the metabolic syndrome deeply. When internal organs fat increases, the amount of adipocytokine secreted from cells is disturbed and not only hyperlipidemia, hyperglycosemia, and high blood pressure are caused but also the reactive oxygen species increases in this process and let their symptoms progress. There are two types of adipocytokines; good adipocytokines have antioxidant activities and bad adipocytokines has the inflammatory nature and increase the reactive oxygen species [2]. When the reactive oxygen species increases in the body, the synergetic effect of their oxidation stress and bad adipocytokines causes hyperlipidemia and high blood pressure with insulin resistance. Furthermore, the reactive oxygen species recommends the oxidation of blood LDL cholesterol and increases participation LDL as results in lets arteriosclerosis progress. For reducing internal reactive oxygen species, an intake of the food including the nourishment ingredient with the antioxidant activity is desired.

A lot of plant food have an antioxidant activity and show a free radical scavenging ability, and they have been used as healthy food. Garlic, i.e. *Allium sativum*, is one of the highest antioxidant and hypoglycemic food and used for not only culinary but also medicinal purposes because it contains polyphenol-based antioxidant materials [3]. In recent years black garlic, i.e. aged old garlic, has been attracted as a higher antioxidant food compared to raw garlic [4,5]. However, in order to manufacture black garlic, it takes a long time for aging, i.e. 30–60 d, at high temperature, i.e. 55–80 °C, and high humidity, i.e. 70–95% [6,7]. Therefore, it is desired to not only shorten the manufacturing time but also simplified the manufacturing process.

In this study, the conversion method of raw garlic into garlic pieces with a higher antioxidant activity than black garlic was developed using a steam explosion. Steam explosion consisted of steam hydrolysis at high temperature and pressure followed by sudden reduction of the pressure for a mechanical treatment of the hydrolyzed product has been known as an effective treatment method for degrading and depolymerizing the components of biomass [8–10]. Therefore, this study examined the effect of steam explosion for increasing the antioxidant activity of product and compared the antioxidant activity of steam-exploded raw garlic with that of black garlic.

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2. Methods

2.1. Sample

Raw garlic was purchased from a local market in Aomori Prefecture, Japan. Raw garlic was peeled and used as a sample in this experiment.

2.2. Steam explosion

For increasing an antioxidant activity of raw garlic, steam explosion was carried out in a batch apparatus equipped with a 2 l reactor (Steam explosion apparatus NK-2L; Japan Chemical Engineering and Machinery Co. Ltd, Osaka, Japan) [11]. One hundred grams of raw garlic was introduced into the reactor and exposed to saturated steam at a pressure of 10 (183 °C), 15 (200 °C), 20 (214 °C), 25 (225 °C), 30 (235 °C), and 45 atm (258 °C) for a steaming time of 1–10 min. The prescribed temperature was reached in a few seconds. After exposure to the saturated steam, a ball valve at the bottom of the reactor was suddenly opened to rapidly bring the reactor to atmospheric pressure. The liquid–solid reaction product, i.e. a steam-exploded raw garlic, was collected in the receiver.

The severity factor of steam explosion treatment is expressed by a correlation between steam temperature and steaming time [12,13]. Since the volume of reactor (2 l) is small, the steam pressure may reach the target value in a very short time. In this case, the severity factor can be calculated by the following equation:

$$S = \mathrm{Log}\left[t \cdot \exp\left\{\frac{T-100}{14.75}\right\}\right] \quad (1)$$

where S is the severity factor, T is the steam temperature (°C), and t is the steaming time (min). 14.75 is the activation energy value under conditions where process is first order kinetics and obeys the Arrhenius law.

2.3. Extraction and separation method

After freeze-drying steam-exploded raw garlic, one gram of dry sample was extracted in a 300 ml Erlenmeyer flask with 100 ml distilled water and the extract was separated by a filtration used for determining radical scavenging activity and amount of total phenolic compounds.

2.4. Determination of radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl radical) is a stable nitrogen-centered free radical whose color changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation [14]. Radical scavenging activity was calculated based on the change of absorbance due to the decrease in DPPH in relation to the control value [15,16]. The extract (2 ml), ethanol (2 ml), and 0.5 mM DPPH in ethanol solution (1 ml) were mixed in the test tube, and the decrease in absorbance at 517 nm was measured after 30 min of reaction. Considering the color of extract, the ethanol solution (1 ml) instead of 0.5 mM DPPH in ethanol (1 ml) was used as a blank. As a control water (1 ml) was added instead of the extract. The radical scavenging activity can be calculated by the following equation:

$$\text{Radical scavenging activity}\ (\%) = \left[\frac{X_0 - (X - X_a)}{X_0}\right] \times 100 \quad (2)$$

where X is the absorbance of the extract and DPPH at 517 nm after 30 min of reaction, X_0 is the absorbance of DPPH at 517 nm as a control, and X_a is the absorbance of the extract at 517 nm as a blank.

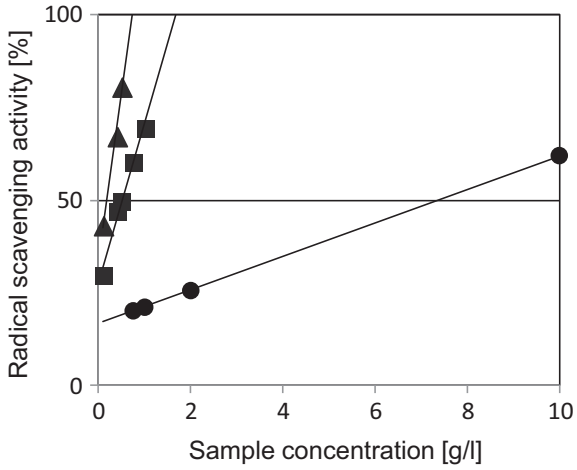

Fig. 1. Relationship of radical scavenging activity and sample concentration of extract obtained from steam-exploded raw garlic under various steaming times at a steam pressure of 15 atm. Symbols: ●, 1 min; ■, 3 min; ▲, 5 min.

2.5. Determination of total phenolic compounds

Amount of phenolic compounds in the extract was measured according to the Folin–Ciocalteu method [17]. The extract (200 μl) was added to the test tube containing 4 ml of distilled water, followed by addition of 1 ml phenol reagent. The mixture was thoroughly stirred. In addition, 1 ml of 10%(w/v) sodium carbonate was added to this solution. The absorbance of reaction was measured at 760 nm after 1 h of reaction. Estimations were carried out in triplicate and calculated from a calibration curve obtained with catechin. The amount of phenolic compounds was expressed as catechin equivalent (mg-catechin equiv./g-dry raw garlic).

3. Results and discussion

Fig. 1 shows the relationship of radical scavenging activity and sample concentration of extract obtained from steam-exploded raw garlic at a steam pressure of 15 atm for a steaming time of 1, 3, and 5 min. The proportional correlation between radical scavenging activity and sample concentration was observed and EC_{50}, a concentration at a radical scavenging activity of 50%, of each sample was obtained from the straight lines of Fig. 1. The EC_{50} values were 7.444, 0.538, and 0.202 g/l at steaming time of 1, 3, and 5 min, respectively. Since the EC_{50} value is a widely used parameter to measure the free radical scavenging activity and a lower value indicates a higher antioxidant activity [18,19], it seems that a longer steaming time increases the antioxidant activity of extract from raw garlic.

Fig. 2 shows the EC_{50} value of extract from the raw garlic treated under various steam pressures and steaming times. The EC_{50} values changed significantly with steam pressures and steaming times. At a steam pressure of 15 and 20 atm the EC_{50} value decreased rapidly with the increase of steaming time reaching about 0.5 g/l at 3 min and then decreased gradually, but at a steam pressure of 30 and 45 atm the EC_{50} value decreased very slowly from about 0.3 to 0.14 g/l at a steaming time of 1–10 min and no significant decrease was observed. It suggests that the part of polyphenols contained in raw garlic was low-molecularized and dissolved as low molecular phenolic compounds in water by steam explosion with a high temperature and pressure steam followed by the rapid decompression to the atmospheric pressure. Furthermore, it seems that the polysaccharides were also degraded and pyrolysis products like furfural and 5-HMF were produced at a steam pressure of 30 and 45 atm while protein, lipids, and other extractives suffered