The changes of antioxidant properties from fresh into black garlic using rice cooker

U Fitrotin[1], Mardiana[1] and B N Hidayah[1]

[1] Institute for Assessment of Agricultural Technology (IAAT), West Nusa Tenggara Province, Indonesia. Jalan Raya Peninjauan Narmada, West Lombok, West Nusa Tenggara Province, Republic of Indonesia 83371

Email: ulyaelariefy@gmail.com

Abstract. The processing methods and tools influenced the quality of black garlic. The objectives of this study were to measure the changes of phenolic content and antioxidant activity from fresh into black garlic extract using rice cooker and the changes of its antioxidant properties during storage. The phenolic content was determined with Folin-Ciocalteu and antioxidant activity was examined using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Ascorbic acid was used as antioxidant reference. Sangga sembalun (white) and Chinese varieties were used as black garlic raw material using rice cooker. The radical scavenging activity (RSA) of Sangga sembalun (white) and Chinese varieties reached highest valuees at 12 and 10 days aging respectively using rice cooker and decreased slowly after those days. The antioxidant activities of black garlic which were stored in refigerator were better than in room temperature. This results provide useful information for understanding the optimum aging for maximazing the antioxidant properties of black garlic using rice cooker and the best stored condition after harvesting.

1. Introduction

Garlic (*Alliun sativum* L.) have been known as a famous foods that can provide pharmachological effects [1] and favorable biological [2]. Some researchs have shown that the antioxidant activity of garlic can be enhanced through black garlic. The antioxidant activity of black garlic (10 µg/mL) is better than fresh garlic in the same dose [3]. The main reasons for enhanced bioactivity of black garlic compared with fresh garlic are the changes of physicochemical properties. During the thermal process of black garlic, some chemical compounds from fresh garlic are converted into amadori/heyns compounds, which are key intermediate compounds of maillard reaction that have been known as antioxidant agent. Some researchers also reported that many valuable components within black garlic against diseases increased during the aging process, especially polyphenol, flavonoids, and some intermediates of maillard reaction.

The contents of chemical compounds of black garlic depend on the conditions during thermal processing. Processing methods influenced the antioxidant activity of black garlic [4]. Black garlic can be produced in conditions under controlled temperature (60-90˚C) and humidity (80-90%) [5]. Based on sensory evaluation, processing at 70-80˚C produced better quality of black garlic and homogeneous black color. Even though black garlic was produced faster at 90˚C, but it produces nonideal tastes, such as bitter and sour taste. Processing at 60˚C the color of garlic was not completely black, therefore 60˚C is also not an ideal condition for the aging process [6]. Black garlic which were incubated in a thermohygrostatic chamber (THPE 025, Jeio Tech, Seoul Korea) at 70˚C in 90% relative humidity for

21 days reached higher antioxidant activity than 28 and 35 days [7]. Fresh garlics were wrapped alluminiumfoil and incubated in oven at 65-80˚C for 15 days reached radical scavenging activity 65,19% in IC_{50} 16,44 ppm [8].

Rice cooker is one of the tools for cooking rice in Indonesia. It is favourite tools for housewife because of simple and quick therefore it always in their kitchen. The inlet temperature of rice cooker is between 70-80˚C at warm condition and more 100˚C at cooking condition. Generally, many housewifes in Indonesia making black garlic using rice cooker and harvested it at their preference time. However, little has been reported about the changes of antioxidant properties from fresh into black garlic using rice cooker and the ideal storage to decrease damage of antioxidant activity. These information are needed in harvesting of black garlic from rice cooker at an ideal aging where its antioxidant activities are at optimum level. Also, avoid from harvesting it at a lower antioxidant activities.

The objectives of this study were to measure the changes of phenolic content and antioxidant activity of fresh garlic extract into black garlic using rice cooker and the changes of its antioxidant properties during storage.

2. Materials and Methods

2.1. Materials and Reagents

Sangga Sembalun variety was used as local fresh garlic which was harvested from Sembalun Highland of Lombok Island, West Nusa Tenggara Province of Indonesia in April 2018 and import fresh garlic (Chinese variety) was purchased from a local market in Narmada, West Lombok. Folin ciocalteu's reagent, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma –Aldrich Chemical Co. (St. Louis. MO.USA).

2.2. Preparation and extraction of black garlic extract

Fresh garlics were incubated in rice cooker (Miyako MCM 508) at ±70˚C in 75% relative humidity for 2, 4, 6, 8, 10, 12, and 14 days. Extraction of fresh and black garlic according to [7] with slight modification. The fresh and black garlic cloves were peeled from the bulb and ground by a high-speed blender. The ground black garlic was mixed with deionized water for 1 h at room temperature in a shaker (CR300, Fine PCR, Seoul, Korea). The extracts were centrifuged (400 rpm, 10 min) and supernatant were collected. Finally the supernatant was analysed as soon as possible.

2.3. DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to [9]. DPPH solution in methanol (1.5 mM) was prepared, and then 3 ml DPPH solution was mixed with 1 ml crude extract of fresh and black garlic. The mixtures were incubated for 1 h in the dark room. Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) was used to measure the absorbance at λ 524 nm. The increasing of DPPH radical scavenging activity was shown by decreasing of the DPPH solution absorbance. The equation to calculate antioxidant activity was given as percent DPPH radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = \left[\frac{\textbf{control absorbance} - \textbf{sample absorbance}}{\textbf{control absorbance}}\right] \mathbf{X\ 100\%}$$

The control contained 3 ml of 1.5 mM DPPH solution and 1 ml of methanol. Ascorbic acid was used as positive controls. Data were reported as means ± SD from three replications.

2.4. Determination of total phenolic content

The crude extract (2 ml) was placed in a tube, and 1 ml Folin-Ciocalteu reagents was added, mixed, and allow to stand for 1 min. Then 4 ml of 15% of sodium carbonate (Na_2CO_3) solution were added, mixed and placed in a dark room for 2 h at room temperature [10]. Absorbance of the resulting blue complex was then measured at 760 nm using a shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). Methanol was used as the blank and gallic acid used as standard. The used of gallic acid based on its stability and purity. The results were expressed as mg gallic acid equivalents (GAE)/g of dry sesame seed. Data were reported as means ± SD from three replications.

2.5 Statistical analysis

A completely randomised design was used for the experiment. The data were analysed with SPSS version 12.0 using one way ANOVA followed by least significant different (LSD) test at the 95% confident level ($p<0.05$).

3. Results and discussion

3.1. The changes of antioxidant activity of black garlic extract during aging period

3.1.1. The changes of DPPH radical scavenging activity of black garlic extract during aging period

The antioxidant activity of black garlic extract was evaluated by DPPH radical scavenging activity. Fig. 1 showed the percentage of DPPH radical scavenging activity of extract black garlic. The DPPH radical scavenging activity of black garlic extract from local fresh garlic was significantly higher than from the imported one. This possibly due to the phenolic content of local garlic is higher than the imported one (Fig 2).

During the aging periods, the antioxidant activity of black garlic extract from local and imported fresh garlic were gradually increased up to 12 and 10 days respectively and decreased after those days. Means that black garlic from local and imported fresh garlic reach its optimal antioxidant properties at the 12th and 10th days of aging respectively. On the other hand, it was said that the antioxidant activity of black garlic measured by DPPH radical scavenging activity highest on the 21st day of aging under controlled conditions of 70˚C and 90% relative humidity [7]. This is possible due to differences of relative humidity conditions of rice cooker which is ±70˚C and 75% relative humidity. The enhanced biological activity of black garlic when compared with fresh garlic lies in conversion of phytochemical compounds during the aging process. These differences possibly due to during aging process the chemical compounds of local fresh garlic which were changed to polyphenol, flavonoids and some intermediate of maillard reaction were more than the imported one. It was argued that many valuable components within black garlic increased during the aging process, especially polyphenol, flavonoids and some intermediate maillard reaction have been known as antioxidant agent [11].

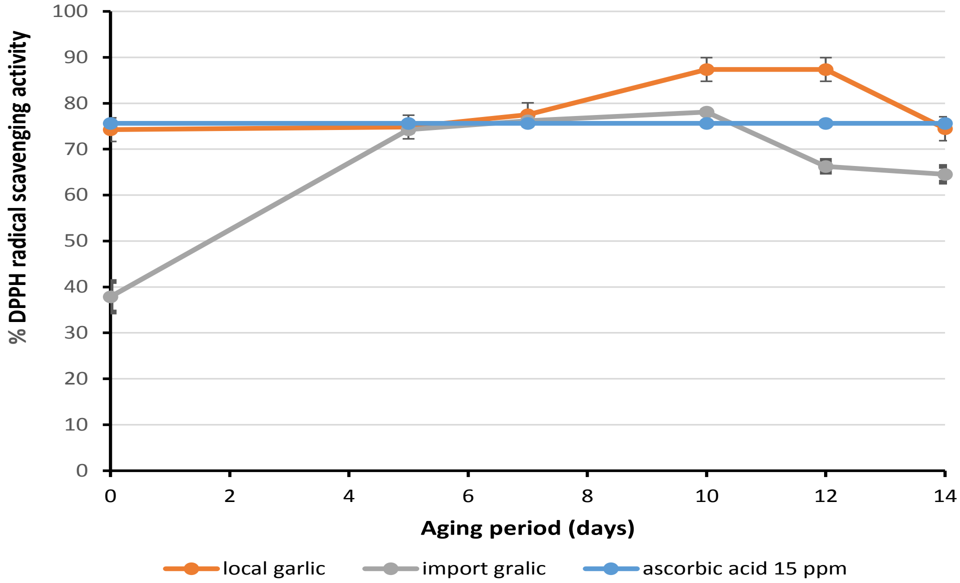

Fig. 1. The changes of radical scavenging activity of black garlic extract during aging period

3.1.2. The changes of phenolic content of black garlic extract during aging period

The phenolic content of black garlic increased during aging period. This is in line with research by [7] which found that the polyphenol content of black garlic increased until 21 days of aging and decreased after that. The phenolic content of black garlic from local variety was increased higher than the imported one. This possibly due to the conversion of phytochemical compounds during the aging process of local variety is more optimal than the imported one. It was reported by other researcher [5] that during the thermal process, some chemical compounds from fresh garlic are converted into amadori/heyn compounds, which are key intermediate compounds of maillard reaction. One of the main antioxidant compounds in black garlic is 5-hydroxymethylfurfural (5-HMF), and it is also an important intermediate product in maillard reaction. The amount of 5-HMF is increased during the aging process [6].

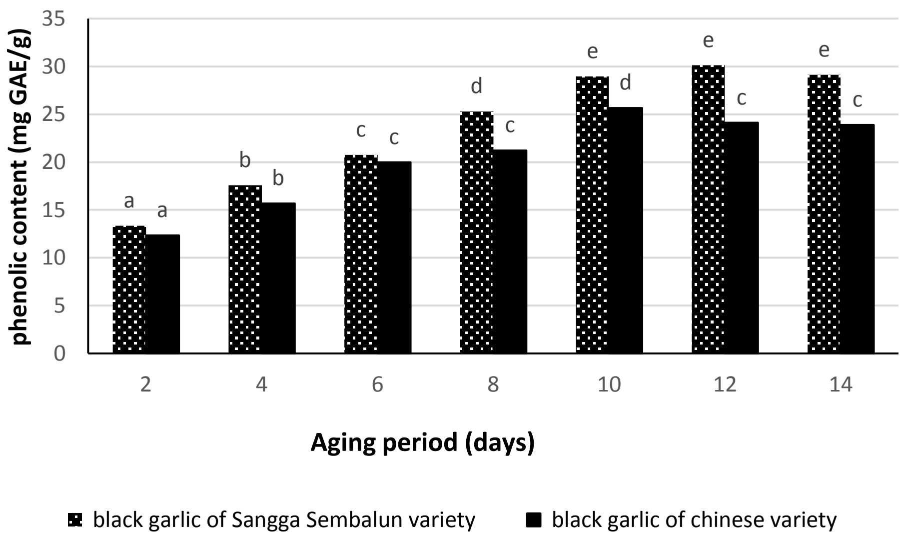

Fig 2. The changes of phenolic content in black garlic during the aging process

The chemical compounds of aged black garlic are complicated, and the quality of its products depends on the manufacturing process. The contents of chemical compounds of black garlic depend on the conditions during thermal processing.

Based on the findings on antioxidant compounds and antioxidant activities, we were conclude that the optimum aging for maximazing the antioxidant properties of local and import varieties using rice cooker are 12 and 10 days respectively.

3.2. The changes of antioxidant properties of black garlic extract during storage

The antioxidant activity of black garlic extract decreased during storage. The decrease of radical scavenging activity of black garlic which stored at room temperature was faster than in refrigerator. This possible due to high temperature was one of the main factors that can decreased antioxidant activity. Other research reported that the storage of *Amenopsis californica* extract at 25˚C for 90 days can decreased 45% of phenolic content and 26% of radical scavenging activity [12]. While the sorage at 4˚C for 90 days can decreased it 35,98% and 17% of phenolic content and radical scavenging activity respectively (Table 1).

Table 1. The differences of black garlic % RSA in different storage condition

	Storage period (days)									
	0	10	20	30	40	50	60	70	80	90
% RSA (room temperature)	87,35	64,75	61,38	42,71	40,31	38,31	30,66	28.57	21,28	16,48
% RSA (in refrigerator)	87,35	84,36	80,75	77,34	70,35	68,38	66,96	65,20	63,09	57,12

4. Conclussion

The antioxidant activities of black garlic from local and import fresh garlic were increased up to 12th and 10th aging days respectively and decreased after those days. The antioxidant activities of black garlic during storage in a room temperature decreased up to 81.13% while in refrigerator decreased only 34,61% both after three months.

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