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Evaluation of Antioxidant Activity, Phenolic and Vitamin C Content of Effect Drying Temperature in Black Garlic Kating Type

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ABSTRACT

Fermented garlic or also referred to as black garlic goes through heating process so that it has antioxidants activity both in vivo and in vitro. Antioxidant activity of black garlic may be affected by the processing method. Black garlic has a dark brown color and fresh sweetness, different from aroma, taste and color, as well as antioxidant potential in garlic. This study was aimed to evaluate antioxidant activity, phenolic and vitamin C content in black garlic after it undergoes drying process at temperature of 60-90°C to improve sensory quality. The method used to determine phenolic content used Folin reagents, antioxidant content with radical scavenging ability DPPH method, and vitamin C level with Iodometric titration. The phenolic content of black garlic and vitamin C at temperature of 80°C showed noticeable difference ($P<0.05$) with value of 21.0982 ± 0.089 mg GAE/g and 0.5632 ± 0.009 mg/g respectively. Antioxidant activity showed the highest IC50 value at 80°C warming with value of 146.2954 ± 121.849 ppm ($P>0.05$). It can be concluded that heating affected IC50, phenolic, and vitamin C content of black garlic.

Keywords: *Antioxidant, Black garlic, Heating, Total phenolic, Vitamin C.*

1. INTRODUCTION

Garlic (*Allium sativum* L.) is a species of onion genus that have been widely used, an herb that has been used as traditional medicine in Indonesia. [1] In addition to its potential as antioxidant, garlic have potential as antibacterial, antivirus, antifungal, anticancer [2], antidiabetic and anti-inflammatory [3,4]. The use of garlic as medicine is less in demand by the public, this is due to the pungent smell and bitter taste in garlic dur to allicin compounds [5]. Several technological developments have been made to improve the sensory quality of garlic, one of which is by fermentation to obtain black garlic [6,7].

Black garlic product has a dark brown color and fresh sweetness because of Maillard reaction. Maillard reaction will give changes to aroma, taste and color as well as antioxidant potential in foodstuffs [8]. The texture of black garlic will also be more sticky, chewy and have a sweeter taste compared to garlic before fermentation, with antioxidant content of 65.195% , while garlic at about 46.386% [9]. Bioactive compounds contained in black garlic include SAC (S-allyl cysteine), polyphenol and flavonoids. The three compounds are formed through heating process. The length of heating used is responsible for the increased content of antioxidant compounds in black garlic [10]. During the fermentation process, allicin compounds turn into antioxidant components such as S-allyl cystein (SAC), tetrahydro-β-carbolines, alkaloids, and flavonoids so that black garlic does not have strong flavor like raw garlic. Black garlic tastes sweeter than garlic. The amount of SAC in black garlic is five to six times higher than fresh garlic [11]. SAC is formed from the process of catabolism γ-Glutamyl-S-allylcysteine. SAC is white powder-shaped with a characteristic odor and is stable for up to 2 years. SAC content in black garlic was able to ameliorate oxidative damage and various diseases such as cardiovascular changes, cancer, stroke, Alzheimer's disease, and other degenerative diseases related to age [12]. The reaction which changes γ-glutamyl-s-allylcysteine into s-allylcysteine is presented in Figure 1.

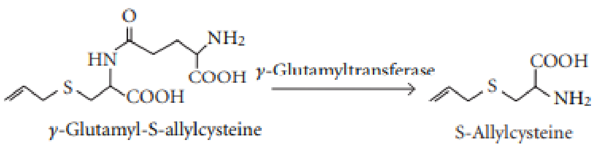

Figure 1 Reaction changes γ-glutamyl-s-allylcysteine into s-allylcysteine [12].

Not everyone can consume black garlic directly because of its taste or appearance, so innovation is needed to make black garlic easy to use like cooking spice. Sensory quality improvement is expected to increase the functional value of black garlics. Some garlic-related research has been done to do drying or making into powder[13, 14]. Powdering technology is one of the methods to expand the utilization of materials in the form of powder, while being able to increase shelf life. Vacuum drying technology is one of the drying methods using low pressure so that it can remove water from the material faster than conventional methods. Vacuum drying technology has also been used to dry agricultural ingredients such as radish, carrots, shallots, garlic, cabbage, and others. Processing by drying food ingredients can affect the increase in vitamin C content and antioxidant [15].

2. METHODS

2.1. Material

Some of the ingredients used among others were kating type black garlic obtained from distributors in Sidoarjo, distilled water, methanol p.a (Fulltime), ethanol p.a (Fulltime), Folin-Ciocalteu reagent (Merck), Na2CO3 (Merck), error acid (Merck), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich).

2.2. Requirement

The tools used were glassware (Iwaki Pyrex), oven (Daihan Labtech), thermometer, infrared moisture determination balance (Kett FD-610), blender (Miyako BL-102-PL), 60 mesh filter, knives, baking sheets, analytical scales (Ohaus), vortex (Labnet VX-200), incubators (Memmert), and UV-Vis spectrophotometer (Shimadzu UV-800).

2.3. Onion Preparation

Garlic and *kating* type black garlic were purchased commercially and powdered using heating temperatures of 60, 70, 80, and 90°C. The heated black garlic was then ground into powder and filtered using 100-sized mesh.

2.4. Determination of Total Phenolic Content

Total phenolic content was determined using modified method [16]. About 0.1 mL sample was placed in a test tube, 50 percent Folin Ciocalteu reagents were added, and then sample was mixed for 1 minute. The solution contained 2 mL of 2% sodium carbonate solution (Na2CO3). For 30 minutes, the mixture was kept in a dark room. The absorbance of the extract solution was measured using UV-vis spectrophotometer at a wavelength of 750 nm.

2.5. Determination of DPPH Free Radical Capture Activity

Determination of DPPH free radical capture activity was done in using modified method [16]. About 0.5 mL garlic sample was poured into the test tube and added 2 mL of DPPH solution 0.2 mM. Blank solution was made by means of 2 mL 0.2 mM DPPH solution put into a test tube and added 0.5 mL ethanol. After solution was incubated in a lightless chamber for 30 minutes, DPPH absorption was measured using UV-Vis spectrophotometer at 517 nm. Antioxidant ability was measured through decrease of DPPH absorption due to sample addition. The absorption value of DPPH solution before and after the addition of the extract is calculated as percentage of antioxidant activity using following formula:

$$\text{DPPH scavenging ability (\% Inhibition)} = \frac{(Control\ Absorption - Sample\ Absorption)}{Control\ Absorption}\ x\ 100\%\ [16]$$

2.6. Analysis of Vitamin C Levels

Vitamin C levels in the sample were determined by redox titration method (iodimetry) with the addition of 1 mL of amum indicator 1% (1 g/100 mL of aqueous) on 10 mL of garlic samples that had been dissolved in aqueous. The titrant used was iodine 0.01 N. Titration was stopped when the solution changed color into blue-blackish lasting for 1 minute, indicating that the titration end point had reached. The result was calculated by the formula [17]:

$$\text{Vitamin C} = \frac{Vol.Titran\ x\ N12\ x\ Mr\ Vit\ C\ x\ 100}{Valensi\ Vitamin\ C}$$

3. RESULTS AND DISCUSSION

Several types of garlic are known in the Indonesian community, one of which is kating garlic. The origin of this garlic was from China, and is very popular with the public, especially Indonesian because of its strong aroma and taste. The characteristic of kating garlic is its small size but large clove. The flesh has dense and wet texture, as well as clean white color resembling paper outer skin (Figure 2a). Kating garlic can be processed by heating at high temperatures resulting in black garlics. The heating process causes various chemical reactions in garlic, for example enzymatic browning and Maillard reactions which cause garlic color to change from white and yellow to dark brown or almost black [18] as shown in Figure 2b.

3.1. Total Phenolic Onion

Garlic and black garlic have phenolic content that can act as an antibacterial substance. The results of the analysis of variety, drying temperature had effect on total phenolic content of black garlic (P<0.05) as presented in Table 1. The data showed that the total phenolic of garlic without drying differed significantly compared to black garlic. After fermentation process, the content of polyphenol compounds in black garlic was higher [6]. Black garlic had higher total phenolic content compared to raw garlic. Dried black garlic showed increasing total phenolic with highest value from 80°C drying temperature with total phenolic content of 21.0982 ± 0.089 mg GAE/g. At 90°C, total phenolic decreased considerably at 14.1072 ± 0.078 mg GAE/g.

(a) (b)

Figure 2 (a) Original garlic kating, and (b) black garlic kating.

Table 1. Total phenolic analysis of black garlic.

No	Sample	Total Phenolic (mg GAE/g)^{a}
1.	Control original garlic	11.8929 ± 0.018^{a}
2.	Control black garlic	12.3393 ± 0.032^{b}
3.	Black garlic temperature 60°C	14.6548 ± 0.089^{d}
4.	Black garlic temperature 70°C	15.6458 ± 0.044^{e}
5.	Black garlic temperature 80°C	21.0982 ± 0.089^{f}
6.	Black garlic temperature 90°C	14.1072 ± 0.078^{c}

a The number in the table is the standard mean ± deviation value.
The numbers followed by the letter differences showed significant differences at the same level in one column of Duncan's advanced test results (sig<0.05).

The presence of heating in phenolic compounds increases the content of free phenols due to reduced esters, glycosides, and ester [19] The decrease in total phenolic compounds that occur at a temperature treatment of 90°C can be caused because the length of time during heating can affect bioactive content, such as polyphenols and flavonoids [18][20] Heat treatment has a great influence on the availability of flavonoids depending on the length of time of treatment, sensitivity to heat, and the physicochemical environment of foodstuffs [21]. Rising temperatures would increase permeability of cell walls, resulting in increased solubility and diffusion of phenolic compounds. Increase of temperature also leads to increase of total phenolic content up to a certain temperature, but as temperature increases it will cause to decrease of phenolic contentc. This is due to decomposition of phenolic compounds after boiling point is reached, thus new compounds are quite more volatile. The number of studied bioactive compounds in the garlic is relatively high. The level of polyphenols and tannins were found to be significantly higher in fresh red onion compared to raw garlic and raw white onion [20, 22].

3.2. Antioxidant activity of black garlic

The results of the analysis of antioxidant activity of black onion kating which is expressed as IC50 are shown in Table 2 using black garlic control without drying. The smaller the IC value of50, the higher the antioxidant activity in the sample. On the determination of antioxidant activity is carried out based on the radical scavenging ability method DPPH (2,2-diphenyl-1-picrylhydrazyl). In this method used free radical compound commonly used as a model in measuring the capture power of free radicals in DPPH. DPPH is a stable free-radical compound used as a reagent in free radical capture tests. The DPPH free radical suppression method is based on the reduction of the DPPH free radical methanol solution colored by the inhibition of free radicals. When the DPPH solution reacts with the electron donor material, the DPPH compound will be reduced. This can be indicated by the change in the purple color of the DPPH to yellow. The yellow color comes from the picryl group that is formed as in Figure 3 [23].

The decrease in absorbance occurs due to additional electrons coming from antioxidant compounds in unpaired electrons in the nitrogen group in the structure of DPPH compounds. The DPPH solution is purple. The intensity of purple will decrease when the DPPH radical is bonded with hydrogen. The stronger the antioxidant activity of the sample, the greater the decrease in the intensity of its purple color [24].

Table 2. IC_{50} analysis of black garlic

No	Sample onions	IC value$_{50}$ (ppm)[a]
1.	Control Black garlic	328.5375 ± 245.203[a]
2.	Black garlic temperature 60°C	265.1772 ± 198.921[a]
3.	Black garlic temperature 70°C	209.9721 ± 180.055[a]
4.	Black garlic temperature 80°C	146.2954 ± 121.849[a]
5.	Black garlic temperature 90°C	336.9376 ± 203. 788[a]

[a] Number is mean ± standard deviation. Numbers followed by different letter showed significant differences based on post-hoc Duncan's test (*sig*<0.05).

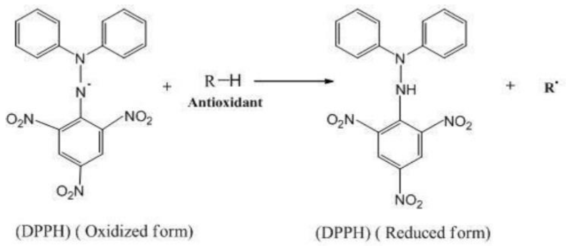

Figure 3 Reaction of DPPH with antioxidant compounds (Source: pubs.rsc.org).

Table 2 shows drying of black garlic resulted in unnoticeable difference of antioxidant activity (P>0.05) and antioxidant activity in black garlic was categorized as weak. The higher the drying temperature in black garlic, antioxidant activity was higher. Highest IC_{50} value was shown from drying temperatures of 80°C at 146.2954 ± 121,849. Antioxidant activity decreased after drying at 90°C which amounted to 336.9376 ± 203. 788. Heating or drying can have an influence on antioxidant activity. The number of DPPH radical antidotes significantly increased as temperature increased from 40 to 85°C [14]. Other researchers mentioned that onion extract could affect the ability of electron donors to bind radical DPPH in a certain temperature variation where the ability of electron donors (%) from onion extract gradually increased along with the increase in temperature from 100 to 120 °C, so it was stated that the ability of binding electron donors (%) increased at higher temperatures [25]. Other research had also shown that long warming provides significantly increased antioxidant activity of black garlic compared to fresh garlic. Black garlic extract had a very strong antioxidant activity with IC_{50} values for each 15 days, namely 2.41 g/mL; 25 days ie 2.93 g/mL; 35 days which is 2.27 g/mL. IC_{50} value <10 g/mL indicates that black garlic extract with heating time of 15, 25, and 35 days has very strong antioxidant potential [5].

3.3 Vitamin C levels of kating garlic

Garlic is one of the plants that contain a wide variety of vitamins. One of the vitamins contained in white and black garlic is vitamin C. Vitamin C acts as an antioxidant and effectively overcomes free radicals that damage cells or culture [17]. Garlic extract has been shown to have antioxidant activity. Strong activity in amino acid fractions was found and the main active compound was identified as N-alpha (1-deoxy-D-fructose-1-il)-L-arginine (Fru-Arg). Fru-Arg's anti-oxidant activity was comparable to ascorbic [26]. Analysis of vitamin C levels in *kating* garlic with variations of drying is shown in Table 3. Results of analysis had a noticeable difference on the level of vitamin C of black garlic (P<0.05).

Vitamin C levels increased with increasing drying temperatures. The highest level was at 80°C by 0.5632 ± 0.009 and decreased to 0.3104 ± 0144 after drying at 90°C. Vitamin C levels could increase up to five-fold. Previous research showed that rising temperature can lead to the increase of water-soluble vitamins, such as vitamin C, but lower the content of fat-soluble vitamins [27]. Vitamin content such as aspantothenic acid (VB5) and niacin (VB3) can increase during garlic processing. The increase in VB3 content is associated with the release of VB3 in garlic cells caused by disruption of cell membranes during thermal processing. VB5 is relatively stable and slightly sensitive to heat, oxygen, and light during processing. The increase in the content in garlic after processing can also be caused by a reduction in moisture content during the production of black garlic [28].

Table 3. Results of vitamin C analysis in black garlics

No	Sample onions	Vitamin C Levels (mg/g)
1.	Control Black Garlic	0.1848 ± 0.009[a]
2.	Black garlic temperatur 60°C	0.1907 ± 0.013[a]
3.	Black garlic temperature 70°C	0.2904 ± 0.009[b]
4.	Black garlic temperature 80°C	0.5632 ± 0.009[d]
5.	Black garlic temperature 90°C	0.3104 ± 0. 144[c]

[a] Number is mean ± standard deviation. Numbers followed by different letter showed significant differences based on post-hoc Duncan's test (*sig*<0.05).

4. CONCLUSION

Black garlics that have been fermented and turned into powder form by heating had different total phenolic and vitamin C content, and antioxidant activity. Phenolic content, IC50, and protein all followed the same pattern, with the highest value at heating temperature of 80°C and decreased at 90°C temperature.

AUTHORS' CONTRIBUTIONS

Nuniek Herdyastuti: conceptualizasion, method and drafting manuscript. Prima Retno Wikandari and Dina Kartika Maharani: collecting data and editing manuscript. Mirwa Adi Prahara: review and editing of manuscript. Amalia Putri Purnamasari: data curation and editing.

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