Differences of Bio (Chemical) Characterization of Garlic and Black garlic on Antibacterial and Antioxidant Activities

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Abstract. Black garlic is a fermented garlic product that produces black color from the fermentation process at 80°C for 15 days. Interestingly, black garlic has pharmacological effects, but the properties possessed by black garlic are inseparable from the processes that occur during its manufacture which involve the heating process. This study aimed to examine the differences in biochemical characterization of garlic and black garlics that affect the antibacterial and antioxidant activity. Analysis of bio-(chemical) ingredients was conducted by mass spectroscopy method, whereas antibacterial behaviour was carried out against *Streptococcus pneumoniae* and *Klebsiella pneumoniae* using the Kirby-Bauer diffusion method. The antioxidant testing was done using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The results showed that black garlics had a different mass spectrum display on the mass/charge (m/z) ratio compared to garlic. The result showed that there was an elevation in the m/z ratio of 39.48, 104.74, 112.69, 175.79, 381.95 and 544.03. These was predicted to cause a change in color, texture and different taste in black garlics. The antibacterial activity of black garlic was not significantly different from garlic both against *Streptococcus pneumoniae* and *Klebsiella pneumoniae*. Likewise with the DPPH antioxidant activity of black garlic is not significantly different from garlic. Overall, the results of this study indicate that the garlic fermentation process with a heating method at 80°C for 15 days can increase the m/z ratio of black garlics, change color, texture, and taste but do not significantly change the antibacterial activity or antioxidant activity.

Keywords: Black Garlic; Fermentation; Mass/charge ratio; Antibacterial activity; Antioxidant Activity.

1. Introduction
Garlic has been widely known as a plant that is used both for food and as a medicinal plant. Garlic has been known as an alternative medicine for high blood pressure, loss of appetite, lung problems, snakebite, whooping cough, and baldness. Even during the second world war, Russian and German soldiers consumed garlic as a treatment on the battlefield. 1,2 Black garlic is a fermented garlic product that produces black on the final product of the fermentation process. The health benefits of black garlic have long been developed in Japan and Korea. Black garlic or also known as Aged Black Garlic is made by storing fresh garlic at a certain temperature and humidity within a period of several days to several months without treatment and the addition of certain additives until the color of the garlic turns black. The result
of the process of making black garlics is intended to increase the activity of the active substances contained in garlic, eliminate the distinctive taste and aroma of garlic, and produce a sour-sweet taste in black garlic.

Black garlics have pharmacological activities, but the activities of black garlics are inseparable from the processes that occur during their manufacture. Black garlic extract is known to have antibacterial, antioxidant, hypo-allergenic, anti-diabetic, anti-inflammatory, hypocholesterolemic, hypolipidemic, and anticarcinogenic activities. However, these activities show different effects depending on the fermentation conditions, starting from the specific temperature control and the different fermentation time. 3,4 Black garlic is processed by involving the heating process. The content of thiosulfinate in garlic after different thermal processing has been shown to decrease. Thiosulfinate is the main flavoring agent in raw garlic. Therefore, the typical odor that is in fresh garlic will be reduced in black garlics. During the heating process at a temperature of 60-90°C changes in levels of allicin in black garlics. Increasing and reducing the levels of active substances garlics also occur in phenol compounds. This occurs related to the activity of γ-glutamyl-transpeptidase (γ-GTP) which is thermodabile.5,6 The formation of S-allyl cysteine (SAC) is also influenced by the water content that helps the γ-Glutamyl-S-allylcysteine (GSAC) and γ-GTP reaction through the hydrolysis process in the formation of SAC. Therefore, the higher the fermentation temperature, the higher the evaporation of water. So the process of forming SAC will be reduced. 5,6 In general, this study aimed to examine the differences in biochemical characterization of black garlic and garlic that affect the antibacterial and antioxidant activities.6

2. Method

This research was a experimental laboratory study with research design, using a completely randomized design research design (CRD) with one treatment factor, namely fermentation. In this study uses the Mass Spectrometry (MS) method for biochemical testing, DPPH for antioxidant test and Kirby-Bauer test for antibacterial.

The test material used in this study was fresh garlic obtained in traditional markets. Furthermore, fresh garlic fermented at 80°C for 15 days in the oven. During the fermentation process, the oven's humidity is always monitored at 70%-80% to prevent dryness, then garlic and black garlics are extracted using 96% alcohol respectively.

Analysis of organic material components from garlic and black garlic analyzed by mass spectroscopy is an analytical technique with the basic principle of making a neutral molecule charged so that it can be detected. The main purpose of mass spectroscopy is to find out the molecular weight. Information obtained from the MS spectrum is the weight of the ion, the molecular mass of the isolate added or reduced by the ion source.

Testing for antibacterial effects was carried out using the Kirby Bauer Diffusion Modification method with Muller Hinton Agar (MHA) media for the bacteria Klebsiella pneumoniae, and the Blood Agar Plate (LAD) media for the Streptococcus pneumoniae media. The positive control was Amoxicillin antibiotic and the negative control was DMSO. Furthermore, the agar medium was incubated for 24-48 hours at 37°C. Yields can be measured using a caliper or a ruler to measure the diameter of the clear colored inhibition zone formed around the well. 9

Antioxidant Activity Test was tested by 1,1-diphenyl-2-picrylhydrazyl (DPPH.) Method. Reduction in DPPH absorbance was measured using a spectrophotometer at a wavelength of 515 nm. The control solution was made from a mixture of 0.1 mL methanol and 2.9 mL of DP25 0.025 mg / mL solution. Vitamin C solution with the same concentration as the concentration of the test extract solution was used as standard. This work was carried out 3 times.10,11
3. Results and Discussion

Antibacterial measurements of ethanol extract of garlic and ethanol extract of black on the growth of \textit{S. pneumoniae} and \textit{K. pneumonia} were measured by using the Kirby-Bauer diffusion method. The measurement results can be seen in Table 1.

\textbf{Table 1. Kirby-Bauer Test Results on garlic and Black garlic against \textit{S. pneumoniae} and \textit{K. pneumonia}}

| Sample   | Inhibition (mm) | Control (+) (mm) | Control (-) (mm) |
|----------|-----------------|-----------------|-----------------|
|          | 100% 75% 75%   |                 |                 |
| Garlic   |                 |                 |                 |
| \textit{S. pneumoniae} | 15.5 13.5 13 | 12.2 0          |                 |
| BlackG   | 11 15 9        | 11 10 0        |                 |
| Garlic   |                 |                 |                 |
| \textit{K. pneumoniae} | 30 29 25 24 | 17.5 0        |                 |
| BlackG   | 22 24 12 13 17.5 | 17.5 0 |                 |

These results indicated that the ethanol extract of black onion had a higher inhibition zone average value on the growth of \textit{S. pneumoniae} and \textit{K. pneumonia} compared to ethanol extract of single garlic.

Antibacterial test results demonstrated that inhibitory zones in the growth of \textit{S. pneumoniae} and \textit{K. pneumonia} which were marked by the presence of clear areas on all agar given by a single ethanol extract garlic paper disk and a single black garlic ethanol extract with 75% and 100% concentrations respectively. Inhibition zones were also formed in the positive control i.e. amoxicillin antibiotic at 30 μg dose, but inhibition zones were not formed in the negative control of DMSO.

Eventhough, the results of data analysis using the independent T-test method showed that garlic and black garlic did not have a significant difference in antibacterial activity (p> 0.05).

\textbf{Table 2. Garlic DPPH Antioxidant Activity Test Results}

| Concentration (ppm) | Absorbance | Inhibition (%) | IC50 (ppm) | IC50 (ppm) |
|---------------------|------------|----------------|------------|------------|
|                     | 1          | 2              | 1          | 2          | 1          | 2          |
| 0                   | 0.828      | 0.844          | 0          | 0          |           |            |
| 1100                | 0.673      | 0.674          | 18.74      | 20.14      |           |            |
| 3299                | 0.529      | 0.526          | 36.13      | 37.68      | 2985.7     | 2887.9     | 2936.8     |
| 4399                | 0.375      | 0.347          | 54.72      | 58.89      |           |            |
|                     | 0.212      | 0.212          | 74.40      | 74.88      |           |            |

Antioxidant test measurements of garlic ethanol extract and ethanol extract of black garlic were measured using the DPPH method. The following are the average results of the measurement of the antioxidant activity of a single garlic ethanol extract which can be seen in Table 2.

The following inhibition curves of garlic extract against DPPH are illustrated in Figure 1 as follows.
Table 3. Black Garlic DPPH Antioxidant Activity Test Results

| Concentration (ppm) | Absorbance | Inhibition (%) | IC50 (ppm) |
|---------------------|------------|---------------|------------|
|                     | 1          | 2             | 1          | 2          | 1          | 2          |
| 0                   | 0.799      | 0.794         | 0          | 0          | -         | -          |
| 1122                | 0.658      | 0.647         | 17.65      | 18.51      | -         | -          |
| 2244                | 0.488      | 0.477         | 38.92      | 39.92      | 3131.3     | 3083.6     | 3107.5     |
| 3365                | 0.352      | 0.349         | 55.94      | 56.05      | -         | -          |
| 4487                | 0.252      | 0.246         | 68.46      | 69.02      | -         | -          |

The following inhibition curves of black garlic extract against DPPH are depicted in Figure 2 as follows.

Based on the calculation results of IC50 value of ethanol extract of garlic obtained the average value of IC50 in a single garlic ethanol extract was 2936.8 ppm which means that a solution of ethanol extract of single garlic as much as 2936.8 ppm was needed to capture 50% DPPH free radicals. Whereas based on the calculation result of IC50 value of ethanol extract of black garlic the average value of IC50 on ethanol
extract of single black garlic is 3107.5 ppm which means it needs a solution of ethanol extract of single black garlic as much as 3107.5 ppm to capture 50% DPPH free radicals.

The results of testing the antioxidant activity of ethanol extract of garlic and ethanol extract of black garlic can be concluded that the IC50 value of ethanol extract of garlic is smaller than ethanol extract of black onion. The smaller the IC50 value, the greater the ability to capture free radicals which can be seen in table 4.

|   | Garlic | Black Garlic | Difference |
|---|--------|--------------|------------|
| Average IC50 | 2936.8 ppm | 3107.5 ppm | 170.7 ppm |

The parametric statistical test is performed using the T-independent test. However, the results of the test showed that the antioxidant activity of garlic and black garlic was not significantly different (p > 0.05).

The results of the Bio (chemical) Test with the Mass Spectroscopy (MS) Method can be seen in figure 3 and 4.

![Garlic mass spectrum diagram](image)
Mass spectroscopy is an analytical technique with the basic principle of making a neutral molecule charged so that it can be detected. The main purpose of mass spectroscopy is to find out the molecular weight. The spectrum of the mass spectrometer is a "bar chart", where this diagram shows the relative magnitude of the current generated by ions from several variations in the mass/charge ratio. The vertical scale is related to the current received by the recorder, associated with many ions reaching the detector. Diagram 1 shows the mass spectrum of unfermented garlic. As can be seen that the most ions are at a mass/charge ratio of 175.83 while other ions have a mass/charge ratio, 39.48, 60.58, 70.57, 104.70, 116.69, and so on. While in Diagram 2 shows the mass spectrum of black garlics that look different in the mass/charge ratio compared to garlic. Diagram 2 shows that there is an increase in ions in the mass/charge ratio of 39.48, 104.74, 112.69, 175.79, 381.95, and 544.03 and so on.

The difference in mass/charge ratio in Diagrams 1 and 2 shows changes in the chemical composition of the components of garlic due to the fermentation process at 80°C for 15 days. The heating process causes the thiosulfinate content in garlic to decrease. Thiosulfinate is the main flavoring agent in raw garlic. Therefore, the typical odor that is in fresh garlic will be reduced in black garlics. The heating process reduces thiosulfinate levels due to the Maillard reaction involving fructose, fructans, and other carbohydrates in garlic samples. Decreasing thiosulfate in black garlic samples is conversion to SAC, S-allyl-cysteine, arginine, and other undefined compounds when subjected to thermal processing. Likewise, alliinase released from vacuole cells from garlic during thermal processing can reduce thiosulfinic to cytotoxic and alkyl alkane-thiosulfinic, such as allicin. 12

During the heating process at a temperature of 60-90°C changes in levels of allicin in black garlics. The amount of allicin increases at 80°C. However, after reaching a temperature of 90°C the content of allicin will decrease. Increases and decreases in the levels of active black garlics also occur in phenol

![Black Garlic mass spectrum diagram](image-url)
compounds. In a similar study, it was also found that the higher the fermentation temperature of eating the lower the SAC content of black garlcs. In fermentation at 40°C, the highest SAC content was obtained, while the lowest SAC content was contained in fermented garlcs at 85°C at the same heating time. This happens related to the -GTP activity which is ther molabile.6

The formation of SAC is also influenced by the water content which helps the reaction of GSAC and TP-GTP through the hydrolysis process in the formation of SAC. Therefore, the higher the fermentation temperature, the higher the evaporation of water. So that the process of forming SAC will be reduced.6

The changes caused by the fermentation process in this study have an impact on the antioxidant content, which is an increase even though the increase is not significant. But unlike the antibacterial activity, no significant changes occur due to the fermentation process.6

Taken together, the results of this study indicate that the garlic fermentation process with a heating method at 80°C for 15 days can increase the mass/load ratio of black garlcs, change color, texture, and taste but do not significantly change the antibacterial or antioxidant activity.

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