

# Lipid Profile and Blood Glucose Levels of Wistar Rats Fed a Non-High Fat Nutrient Supplemented with Black Garlic Extract

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## Abstract

Many studies related to the therapeutic effect of black garlic (BG) have been carried out on cancer, diabetes, neurodegenerative, cardiovascular, dyslipidemia and other diseases. This investigation was conducted to examine the effect of BG supplements on the lipid profile and blood glucose of rats fed a customary diet (non-high fat diet). A fermented black garlic product was extracted by a maceration method and its phytochemical components were analyzed using LCMS. Black garlic extract was given to healthy rats with a normal feed for 14 days. Twenty-four rats were divided into 4 groups of 6 rats. As a control, group A was given aquadest (placebo), and groups B, C, and D were given BG extract at a dose of 15 mg/kg BW, 30 mg/kg BW, and 45 mg/kg BW, respectively. On day 15, blood was taken from the retro-orbital plexus of the rats to measure the total levels of cholesterol, High-Density Lipoprotein-Cholesterol (HDL-C), Triglyceride (TG), Low-Density Lipoprotein-Cholesterol (LDL-C) and glucose. Black garlic made by fermentation at 80°C for 8 days contained more monosaccharides, disaccharides and oligosaccharides than fresh garlic. Black garlic contained 32 types of organosulfur compounds, and the 5 most abundant compounds were allicin (5.813%), allin (4.993%), isoalliin (3.77%), cycloalliin (3.163%) and (-) S-allyl-L-cysteine (2.022%). Black garlic extract administration was able to maintain blood glucose homeostasis in rats fed a normal diet (non-high fat diet). Levels of total cholesterol, triglyceride, and LDL-C were significantly decreased in groups administered black garlic compared to the control group, whilst the level of HDL-C increased significantly in groups administered black garlic compared to the control group.

**Keywords:** Cholesterol; Fermentation; Phytochemical; Supplement; Triglyceride

## 1 Introduction

Health is very important to humans as it is central to life. Various efforts are made, including plant-based nutrition approaches, to enhance well-being and prevent or defer progression of chronic degenerative diseases. Vegetable food

ingredients and their various derivative products contain numerous micronutrients (minerals and vitamins), fiber and phytochemicals. A plant-based diet has been linked with a decreased risk for chronic diseases, such as cancer (Clem & Barthel, 2021), cardiovascular diseases (Kim et al., 2019) and neurodegenerative

diseases (Katonova et al., 2022), and the modulation of gut microbiota which can have a positive influence on body health (Dore & Blottiere, 2015; Mozaffarian, 2016).

Many types of spices, fruits, vegetables and other plants are used as nutritional supplements, and are also acknowledged for their health benefits, such as preventing disease. For example, garlic (*Allium sativum*), considered to be a basic ingredient in Indonesian cuisine, is one of these plants. There are 4 types of garlic found in the Indonesian market, namely local garlic, kating (compound) garlic, lanang (single) garlic and Shin Chung garlic. Fresh garlic contains 63% water, 2.3% organosulfur compounds, 28% carbohydrates (fructans), 2% protein (alliinase), 1.2% free amino acids (arginine) and 1.5% fiber (Lu et al., 2017, 2018). The amount and type of bioactive compounds in garlic can be increased through fermentation for a certain period-of-time, and also promoted by high temperature (60-90°C) and high humidity (80-90%) (Kimura et al., 2017). The fermentation process produces a product called black garlic (BG). BG has been consumed in Japan, South Korea and Thailand for centuries (Bae et al., 2014), and was brought into Taiwan and other countries about a decade ago. BG has also been widely used as a condiment for chicken, fish, soups and risotto (Bradley, 2009).

Garlic fermentation at high temperatures and high humidity causes different flavors, textures and colors (Kimura et al., 2017). The aging process changes the nutritional and sensory properties of BG and increases its bioactivity (Ahmed & Wang, 2021). The health advantages of BG consumption are mostly due to bioactive substances, such as phenols and organosulfur compounds (Najman et al., 2020). Bioactive compounds of BG have various physiological functions in the body, and thus have potential as supplements and for therapeutic use. Many studies related to the therapeutic effect of BG have been carried out on cancer (Al-Shehri, 2021), diabetes (Ha & Kim, 2017), neurodegenerative diseases (Nillert et al., 2017), cardiovascular diseases (Valls et al., 2022), dyslipidemia (Tran et al., 2018) and other diseases. The therapeutic potential is related to the function of BG extract as an antioxidant, anti-inflammatory agent

(Jeong et al., 2016), antiallergic agent (Yoo et al., 2014), antidiabetic agent (Kalhotra et al., 2020) and anticarcinogenic agent (Park et al., 2014). However, the effect of BG as a supplement for health and disease prevention has not been widely investigated. This research is directed to determine the effect of supplementation of a standard nutriment (non-high fat diet) with black garlic (BG) extract on the lipid profile and blood glucose of rats. This research sought to reveal the potential of BG as a natural growth promoter that can be consumed daily as a feed additive (or supplement) to maintain health. Lipid profile and blood glucose are indicators or parameters for early detection of diabetes and atherosclerosis. In developing countries, these two diseases are increasing in number every year and are closely related to lifestyle and diet.

## 2 Materials and Methods

### 2.1 Materials

Kating (compound) garlic (*Allium sativum*) was acquired from local farmers in Central Java, Indonesia. Animal feed BR-1 (PT Japfa Comfeed Indonesia), glucose kits, total cholesterol kits, HDL-C kits and Triglyceride kits (DiaSys Diagnostic Systems GmbH, Holzheim, Germany) were also acquired.

### 2.2 Garlic thermal fermentation

Kating (compound) garlic (*Allium sativum*) was fermented. A total of 10 kg of fresh unpeeled garlic were washed and physically/thermally fermented at a humidity level of 90% and temperature of 80°C for 0 days, 4 days and 8 days to produce black garlic (BG).

### 2.3 Black garlic extraction

The fermented black garlic products were chopped and then mashed using a blender. Extractions were carried out by a maceration method using water as a solvent for 4 hours. All extractions were carried out with three replicates. After extractions were stopped, samples

Table 1: Setup for LCMS analysis

Setup for LCMS analysis	
LCMS apparatus model	: Shimadzu LCMS – 8040 LC/MS
Column	: Shimadzu Shim Pack FC-ODS (2 mm x 150 mm, 3 $\mu$ m)
Volume of injection	: 1 $\mu$ l
Voltage of capillary	: 3.0 kV
Column temperature	: 35 °C
Mode of Mobile-phased	: Isocratic
Rate of flow	: 0.5 ml/min
Sampling cone	: 23.0 V
Solvent	: Ethanol 90\%
MS focused ion mode	: Io type [M]+
Collison energy	: 5.0 V
Desolvation gas flow	: 60 ml/hr
Desolvation temperature	: 350 °C
Fragmentation method	: Low energy CID
Ionization	: ESI
Scanning	: 0.6 sec/scan (mz: 10-1000)
Source temperature	: 100 °C
Run time	: 120 minutes

were filtered through a linen filter cloth. The filtrates from the three extractions were combined and all filtrates were concentrated in a rotary evaporator (RE 100-Pro, DLAB Scientific Inc. USA, China) and freeze-dried. Freeze-dried samples were placed in dark glass bottles and sealed and then stored at -20°C until the samples were used.

## 2.4 Quantification of black garlic compounds using liquid chromatography-mass spectrometry (LCMS)

Samples of garlic incubation for 0 days, 4 days and 8 days were analyzed for their compounds using an LCMS system equipped with a pump (LC-10Advp = 2 units), Autosampler (SIL-10Advp), PDA Detector (SPD-M10Avp), Mass spectrometer (LCMS-2010A) and Column oven (CTO-10ASvp). Table 1 outlines the setup for LCMS analysis while the analytical procedure was carried out according to Habibah et al. (2021).

## 2.5 Black garlic treatment of rats

This experimental study has a randomized control group posttest design. Black garlic extract (BG) was given to healthy rats to determine its effect on lipid profiles and blood sugar levels. This research phase was carried out at the Biology Laboratory, under the ethical clearance number 141/KEPK/EC/2022 from the Health Research Ethics Committee, Universitas Negeri Semarang. Twenty-four white rats (*Rattus norvegicus*), meeting the following selection criteria: Wistar strain, male, 2-month-old with body-weight 210-240g, were acclimatized in a cage with humidity 68% and 12 hours/day of light exposure. Four groups of six rats were formed. Control group (A) was prescribed with aquadest (placebo). Groups B, C, and D were given BG extract at a dose of 15 mg/kg BW, 30 mg/kg BW and 45 mg/kg BW, respectively. Treatments were given every day for 14 days. During treatments, rats were given food and drink ad libitum. The rats were fed animal feed BR-1 which contained water 12%, crude protein 21%, crude fiber 4.5%, phosphorus 0.7-0.9%, crude fat 4% and calcium 0.9-1.1% (PT Japfa Comfeed Indonesia).

The blood of rats from the retro-orbital plexus (eye corner) was collected using a microhematocrit, according to the procedure of Sharma et al. (2014), on day 15. Before the blood was collected, the rats were anesthetized by inhalation with chloroform. Blood plasma samples were obtained in tubes which contained anticoagulant (3ml EDTA). The tube was put into a cooler bag and taken to the laboratory to be centrifuged at 3000 rpm for 15 minutes. The blood plasma fluid in the supernatant is separated and put into a new tube. Then, measurements for High-Density Lipoprotein-Cholesterol (HDL-C), total cholesterol, Low-Density Lipoprotein-Cholesterol (LDL-C), Triglyceride (TG), and glucose in the blood plasma of rats were taken.

## 2.6 Quantification of total cholesterol, HDL-C, triglyceride, LDL-C and glucose levels

Total cholesterol, triglyceride, HDL-C and glucose levels were quantified enzymatically and colorimetrically, using a UV-Visible Spectrophotometer (Lambda 25, PerkinElmer, Inc., Waltham, Massachusetts, United States). Assessment of HDL-C and total cholesterol levels were carried out using the CHOD-PAP (Cholesterol oxidase-4-Amino-antipyrine) method according to the manufacturer's manual kit (DiaSys Diagnostic Systems GmbH, Holzheim, Germany). Measurement of triglyceride levels used the GPO-PAP (Glycerol-3-phosphate-oxidase-4-Amino-antipyrine) method according to the manufacturer's manual kit (DiaSys Diagnostic Systems GmbH, Holzheim, Germany). LDL-C levels were calculated using the theory of Friedewald et al. (1972), which is:  $LDL-C = \text{total cholesterol} - (\text{HDL-C} + \text{triglyceride}/5)$ . Calculation of glucose levels was carried out using the GOD-PAP (Glucose oxidase-4-Amino-antipyrine) method according to the manufacturer's manual kit (DiaSys Diagnostic Systems GmbH, Holzheim, Germany).

## 2.7 Data analysis

Phytochemistry constituent data were analyzed descriptively. Data on HDL-C, cholesterol, triglyceride, LDL-C and glucose levels were scrutinized for their normality using the Shapiro-Wilk test and for their homogeneity of variance using the Levene test. The data was then subject to one way ANOVA (Analysis of Variance) testing, with a 95% confidence level. Differences between treatment groups were analyzed by LSD (Least Significant Difference), with a 95% confidence level. Statistical Product and Service Solution (SPSS) 24 installed on a Microsoft Windows machine was used to conduct analysis.

## 3 Results and Discussion

Heating caused an enzymatic browning reaction and a Maillard reaction, resulting in a change of garlic color to dark brown (Figure 1). The Maillard reaction is a non-enzymatic browning reaction that causes an increase in red color, a decrease in brightness and a decrease in yellow color. The results of LCMS analysis showed that the thermal process produced monosaccharides (arabinose, glucose, galactose) and disaccharides (sucrose) (Table 2) which gave a sweet taste and soft texture to black garlic. Black garlic was also noted to be able to increase oligosaccharides (Table 2) that act as prebiotics (marked by †). Black garlic is a source of organosulfur compounds. The LCMS analysis result showed that there were 32 types of organosulfur compounds detected and the 5 most abundant compounds were allicin (5.813%), allin (4.993%), isoallin (3.77%), cycloalliin (3.163%) and (- S-allyl-L-cysteine (2.022%) (Table 3).

Concentrations of triglycerides, total cholesterol, HDL-C, LDL-C and blood glucose for rats in this study are shown in Figure 2. Black garlic administration tended to decrease concentrations of total cholesterol, triglycerides and LDL-C. Concentrations of HDL-C tended to increase, with increasing dose of BG given. However, BG administration did not tend to show an increase or decrease in blood glucose concentrations compared to the control group.

Results of normality and homogeneity of vari-



Figure 1: Changes in color of garlic. (A) fresh; (B) 4 days fermentation at 80°C; (C) 8 days fermentation at 80°C

Table 2: The carbohydrate composition of fresh and black garlic

No	Peak number*	RT*	Carbohydrate compound	Concentration (%)			
				Fresh garlic	Black garlic 4 day	Black garlic 8 day	Balance**
1.		1.602	Arabinose	nd	0.453	0.493	<b>0.493</b>
2.		4.709	Glucose	nd	0.209	0.227	<b>0.227</b>
3.		4.713	Galactose	nd	0.286	0.311	<b>0.311</b>
4.	7	1.209	Furfural	nd	Nd	0.053	<b>0.053</b>
5.	48	4.729	Mannose	1.059	1.153	1.277	0.218
6.	73	12.137	Inulobiose†	1.122	1.222	1.353	0.231
7.		12.289	Sucrose	nd	0.238	0.259	<b>0.259</b>
8.	102	26.303	1-Kestose†	2.058	2.241	2.482	0.423
9.	103	26.305	Raffinose†	0.729	0.794	0.880	0.150
10.	104	26.309	Inulotriose†	0.996	1.084	1.200	0.205
11.	108	43.21	Inulotetraose†	0.349	0.380	0.421	0.072
12.	109	43.213	Nystose†	1.304	1.420	1.573	0.268
13.	110	46.176	Stachyose	1.624	1.768	1.958	0.334
14.	111	46.18	1(F)- $\alpha$ -D-galactosyl raffinose†	0.410	0.447	0.495	0.084
15.	113	49.954	1F-Fructofuranosyl nystose†	0.730	0.795	0.881	0.150

Note: asterisk mark (\*) is based on LCMS data from fresh garlic; two-asterisk mark (\*\*) shows the difference between 8 days black garlic and fresh garlic; bold number represents compound found only in black garlic; nd: not detected; dagger mark (†) indicates prebiotic.

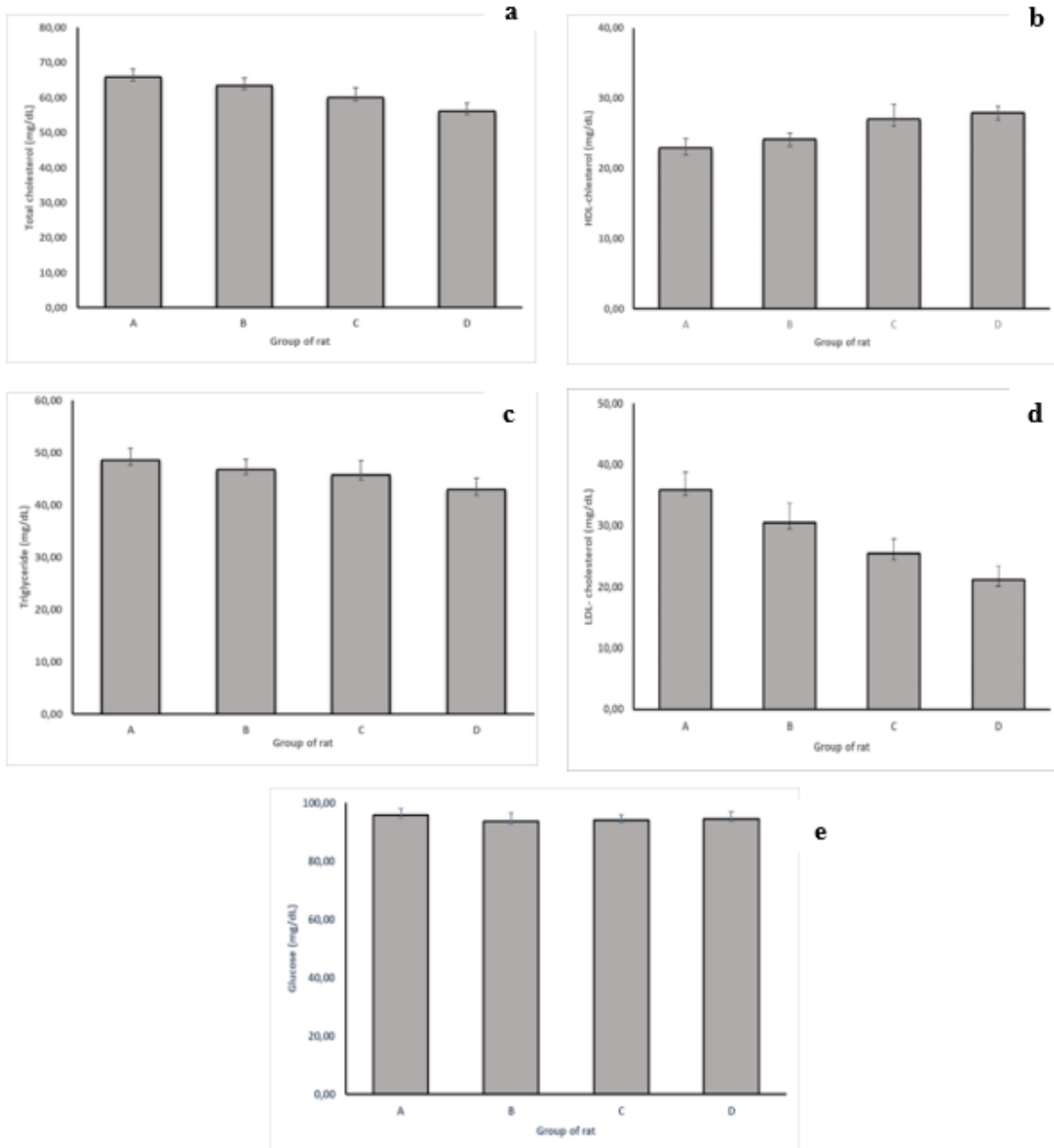


Figure 2: Lipid profile and blood glucose levels of rats given various doses of black garlic (BG). a. total cholesterol; b. HDL-cholesterol; c. Triglyceride; d. LDL-cholesterol; e. Glucose. Group A as control group. Group B was given BG extract at a dose of 15 mg/kg BW. Group C was given BG extract at a dose of 30 mg/kg BW. Group D was given BG extract at a dose 45 mg/kg BW.

Table 3: Organosulphur composition of black garlic on day 8

No	Peak number	RT	Organosulphur compound	Concentration (%)
1.	1	1.046	allyl mercaptan	0.635
2.	4	1.166	allyl methyl sulfide	0.189
3.	5	1.203	dimethyl disulfide	0.235
4.	11	1.254	diallyl sulfide	0.560
5.	12	1.285	methyl allyl disulfide	0.466
6.	13	1.286	Cystein	0.090
7.	14	1.287	methyl propyl disulfide	0.347
8.	17	1.484	dimethyl trisulfide	0.593
9.	19	1.536	diallyl disulfide	0.585
10.	20	1.545	S-methyl 2-propene-1- thiosulfinate	0.187
11.	21	1.645	dipropyl disulfide	0.400
12.	22	1.673	Allicin	<b>5.813</b>
13.	23	1.68	Methiin	0.728
14.	24	1.688	(-) S-allyl-L-cysteine	<b>2.022</b>
15.	25	1.69	trans-S-(1-propenyl)-L- cysteine	0.871
16.	33	2.538	methionine	0.260
17.	40	3.208	Alliin	<b>4.993</b>
18.	41	3.211	cycloalliin	<b>3.163</b>
19.	42	3.216	Isoalliin	<b>3.770</b>
20.	43	3.225	di-(2-propenyl) trisulfide	0.858
21.	45	3.502	diallyl trisulfide	0.315
22.	46	3.505	allyl trisulfide	0.643
23.	49	4.733	3,5-diethyl-1,2,4-trithiolane	0.543
24.	55	5.823	diallyl tetrasulfide	0.461
25.	57	7.935	Ajoene	<b>1.765</b>
26.	58	8.027	allyl pentasulfide	0.638
27.	59	9.045	Thiamine	0.036
28.	62	9.803	diallyl hexasulfide	0.460
29.	65	10.505	$\gamma$ -glutamyl-S-trans-1- propenyl-cysteine	1.169
30.	68	11.508	diallyl heptasulfide	0.858
31.	77	12.487	allithiamine	0.287
32.	79	13.205	S-(2-carboxypropyl) glutathione	0.461

Note: numbers in bold indicate the 5 most concentrated types of organosulfur.

Table 4: Test results for lipid profile and blood glucose level of rats

Groups	Total Cholesterol (mg/dL)	LDL-cholesterol (mg/dL)	HDL-cholesterol (mg/dL)	Triglyceride (mg/dL)	Glucose (mg/dL)
A (n=6)	65.87 <sup>a</sup>	35.86 <sup>a</sup>	22.91 <sup>a</sup>	48.5 <sup>a</sup>	95.79
B (n=6)	63.40 <sup>a</sup>	30.48 <sup>b</sup>	24.11 <sup>a</sup>	46.74 <sup>a,b</sup>	93.57
C (n=6)	60.05 <sup>b</sup>	25.42 <sup>c</sup>	26.96 <sup>b</sup>	45.74 <sup>b</sup>	94.08
D (n=6)	56.16 <sup>c</sup>	21.11 <sup>d</sup>	27.91 <sup>b</sup>	42.89 <sup>c</sup>	94.55

Different superscript letters in same column showed significant differences based on 95% confidence level or  $p < 0.05$  from LSD examination. Group A as control group. Group B was given BG extract at a dose of 15 mg/kg BW. Group C was given BG extract at a dose of 30 mg/kg BW. Group D was given BG extract at a dose 45 mg/kg BW.

ance testing showed that the concentration data of triglycerides, total cholesterol, HDL-C, LDL-C and blood glucose of rats had normal distribution ( $p > 0.05$ ) and were homogeneous ( $p > 0.05$ ). Results of the one-way ANOVA test showed that total cholesterol, triglycerides, HDL-C and LDL-C concentrations were significantly affected by BG administration. Meanwhile, BG administration had no significant effect on the blood glucose concentration of rats fed a normal diet (non-high fatty diet). In this study, it is possible that the BG doses administered did not contain high enough concentrations of S-allyl cysteine (SAC) (Table 3) to exert a hypoglycemic effect.

Results of the LSD follow-up test showed that total cholesterol and LDL-C concentrations of groups C (BG dose of 30 mg/kg BW) and D (BG dose of 45 mg/kg BW) were significantly lower than the control group. The triglyceride concentrations of groups B (BG dose of 15 mg/kg BW) and D (BG dose of 45 mg/kg BW) were significantly lower than the control group. Meanwhile, HDL-C concentrations of groups C (BG dose of 30 mg/kg BW) and D (BG dose of 45 mg/kg BW) were significantly higher than the control group (Table 4). Black garlic (BG) in this study was made by fermentation at 80°C for 8 days, without additives, in accordance with research by Kang (2016). Garlic fermentation at high humidity and high temperature causes garlic to become dark brown, tending towards black, with a soft and rubbery finish and also to have a slightly

sour-sweet taste (Qiu et al., 2018). The change in color of garlic to dark brown occurs due to the Maillard reaction. As revealed by Choi et al. (2014), the Maillard reaction is a non-enzymatic browning reaction at 70°C, producing a product that causes an increase in red color, a decrease in brightness and a decrease in yellow color. In addition to color changes, the heating reaction also causes unstable compounds to become more stable (Kang, 2016). The usual Maillard reaction occurs between 3 neutral amino acids (leucine, valine, and proline) and reducing sugars (glucose, fructose), producing intermediate products, namely Amadori compounds derived from glucose-amino acids and Heyns compounds derived from fructose-amino acids. Fru-Pro, Fru-Val, Fru-Leu, Glu-Pro, Glu-Val and Glu-Leu, the three pairs of Amadori and Heyns compounds, have a major impact on black garlic properties, including taste, shade and antioxidant activity (Yuan et al., 2016).

During thermal processes, fructans decompose successively into monosaccharides (mostly fructose and glucose) and disaccharides, as well as oligosaccharides due to thermal induction and an enzymatic reaction by fructan exohydrolases (Cheong et al., 2012; Liang et al., 2015). However, according to the research results of Lu et al. (2018), the breakdown of polysaccharides is caused by heat treatment and not due to enzymatic hydrolysis. It was also reported by Ryu and Kang (2017) that the amount of sucrose,



fructose and glucose in BG increased in contrast to raw garlic. Research results by Lei et al. (2014) also showed that monosaccharides were more dominant than polysaccharides and disaccharides in black garlic.

Alliin (S-Allyl-L-cysteine sulfoxide) is a natural sulfoxide derived from the fresh garlic amino acid cysteine. Raw garlic that is heated, chopped or mashed, causes alliin to be converted by the enzyme alliinase into allicin (S-Allyl prop-2-ene-1-sulfinothioate) which gives fresh garlic its scent. Allicin is a particularly responsive compound, with restricted thermal stability, that will decompose to S-allyl cysteine (SAC), diallyl sulfide (DAS), diallyl trisulfide, ajoene and diallyl disulfide, and then be converted into S-allylmercaptocysteine (SAMC). SAMC is inodorous and bland and is a stable molecule (Zhang et al., 2015). However, SAMC production is related to  $\gamma$ -GTP enzyme activity, which requires a 40°C prime temperature (Bae et al., 2014), and thus explains why this compound to not be found in this study (Table 3). SAC is the main organosulfur molecule, produced by  $\gamma$ -glutamyl-S-allyl cysteine (catalyzed by  $\gamma$ -GTP) through enzyme hydrolysis (Xu et al., 2015). In this study, the amount of SAC recorded was low (2.022%), probably because the fermentation process was carried out at 80°C for 8 days. As mentioned by Bae et al. (2014), S-Allylcysteine (SAC) is produced in large quantities throughout the aging process (fermentation) of garlic by enzymatic hydrolysis. The higher the fermentation temperature (aging), the lower the amount of SAC produced in black garlic. Increased levels of SAC and SAMC compounds may have a key role as antilipidemic agents and antioxidants (Ha et al., 2015). SAC content in BG can repair oxidative destruction and numerous diseases such as cardiovascular changes, Alzheimer's disease, cancer, stroke and other age-related deterioration diseases (Colin-Gonzalez et al., 2012).

BG extract given to rats was normal (not hyperglycemic, nor hypercholesterolemic nor hyperlipidemic), therefore BG was able to maintain blood glucose homeostasis. Research by Seo et al. (2009) also showed that BG treatment in hyperglycemic rats did not show any difference in glucose and plasma insulin levels compared to control rats. The hypoglycemic effect of black

garlic is due to S-allyl cysteine which can stimulate pancreatic beta cells to produce and secrete insulin, causing a decrease in blood glucose levels (Ha & Kim, 2017).

BG supplementation in rats fed a normal diet (non-high fat nutriment) significantly minimized levels of triglyceride, cholesterol and LDL-C. The number of triglycerides, cholesterol and LDL-C in blood plasma has a very major role in atherosclerosis pathogenesis. This research clearly indicated that BG had a hypolipidemic effect. This result was consistent with many previous studies. Sprague Dawley male rats on a high-fat diet (HFD) given 1.5% (w/v) of BG experienced a decrease in body weight and epididymal fat, a decrease in triacyl glycerides in serum and liver, along with an increase in HDL-C levels in serum (Ha et al., 2015). A previous study has also shown that BG supplementation of HFD-treated rats (Jung et al., 2011) improved the serum lipid profile, including triglycerides, LDL-C, total cholesterol and HDL-C. Jung et al. (2014) also demonstrated that BG can improve the blood lipid profile in cases with mild hypercholesterolemia.

Unrestricted ingestion of garlic is known to cause several adverse issues such as bad breath, allergic reactions and poisoning. The fermentation process of garlic into BG causes the loss of the characteristic garlic odor. The main bioactive compounds in BG are SAMC and SAC which are produced through the fermentation process (Kim et al., 2012; Shin et al., 2014). These water-soluble compounds (SAC and SAMC) are considered to account for the anti-hyperlipidemic effect of BG (Shin et al., 2014). The BG has no toxicity and can be consumed for a long time without causing side effects. Results of a previous study also found that triglycerides and total cholesterol in physiologically normal rats can reduced by S-allyl cysteine in BG (Tran et al., 2018). However, further research on the possible toxic impact of black garlic in long-term administration is needed.

The mechanism of decreasing cholesterol, triglyceride and LDL-L levels probably occurs because BG extracts decrease mRNA expression of the sterol regulatory element-binding protein-1c (SREBP-1C), thereby accelerating cholesterol metabolism and downregulation of lipid.

mRNA expressions of acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and glucose-6-phosphate dehydrogenase (G6PDH) are also decreased (Ha et al., 2015). Protein SREBPs are transcription elements that manage lipid biosynthesis and adipogenesis by commanding the expression of various enzymes which are needed for the synthesis of cholesterol, triacylglycerol, fatty acid and phospholipid (Bertolio et al., 2019). SREBP plays a key role in combining inflammation lipid metabolism, cell expansion, nourishment, energy stress, and other pathological and physiological processes (Shimano & Sato, 2017). BG extract is not only related to the fat synthesis mechanism but is also associated with other systems. BG extract supplementation significantly increases the faecal fat excretion, both TG and cholesterol. A significant decrease in blood lipid profile concentration of rats (treated with BG extract) was shown by fecal excretion escalation and a reduction of dietary fat absorption (Ha et al., 2015).

#### 4 Conclusions

Black garlic made by fermentation at 80°C for 8 days contained more monosaccharides, disaccharides, and oligosaccharides than fresh garlic. Black garlic contained 32 types of organosulfur compounds, and the 5 most abundant were allicin (5.813%), alliin (4.993%), isoalliin (3.77%), cycloalliin (3.163%) and (-) S-allyl-L-cysteine (2.022%). Black garlic extract administration was able to maintain blood sugar homeostasis in normal nutriment (non-high fat diet) rats. Levels of total cholesterol, triglyceride and LDL-C were significantly decreased in the groups administered black garlic compared to the control group. However, the HDL-C levels increased significantly in the groups administered black garlic compared to the control group.

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