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Functional properties of *Saccharomyces kluyveri* Y97-fermented solo black garlic

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Abstract. *Setiyoningrum F, Pribadi G, Afiati F, Herlina N, Solikhin A, Lisani N. 2018. Functional properties of* Saccharomyces kluyveri *Y97-fermented solo black garlic. Asian J Agric 2: 48-51. Saccharomyces kluyveri* Y97-fermented solo black garlic was made by fermentation of fresh solo garlic in medium containing *S. kluyveri* aging in 70°C and relative humidity close to 60%. The fermentation period of fresh solo garlic in the medium was 0, 2, 4 and 6 days. The black aging period was 0 (fresh garlic), 7, 14 and 21 days. Antioxidant capacity, flavonoid content, and total polyphenol were observed. *S. kluyveri* Y97-fermented solo black garlic had higher antioxidant capacity, flavonoid content and total polyphenol compared to solo black garlic without fermentation. Fermentation of fresh solo garlic by *S. kluyveri* Y97 before the aging process could increase solo black garlic's functional properties.

Keywords: solo black garlic, *S. kluyveri* Y97, antioxidant, flavonoid, polyphenol

INTRODUCTION

Black garlic is a product of garlic breeding development, especially for medicinal purposes. It has many bioactivities including inhibition of colon and gastric cancer cell growth, antioxidant, alteration of lipid profile in diabetes, anti-obesity, anti-inflammatory, and antiallergic activities (Seo et al. 2009; Ha et al. 2015; Kimura et al. 2017). Processing method and garlic cultivars affect the quality and bioactive value of black garlic (Kimura et al. 2017; Chen et al. 2013; Bae et al. 2014). Solo or single garlic has a higher benefit than normal garlic (Naji et al. 2017). Controlled heating at high temperature and humidity was widely used for black garlic processing (Ngan et al. 2017; Kang et al. 2008; Bae et al. 2014; Choi et al. 2014; Kimura et al. 2017). In addition, the use of enzyme treatment and curing can be applied to make black garlic (Wang and Su 2017). Those treatments are applied to eliminate unpleasant odor and increase its palatability (Pure et al. 2017; Bae et al. 2014; Lu et al. 2017; Zhang et al. 2015).

Phenol oxidation is the key to the success of black garlic production. Increasing antioxidant, phenol and flavonoid are due to phenol oxidation process. Such process is in line with the increasing antitumor effect (Wang et al. 2012) and immunostimulatory activity (Purev et al. 2012). S-allyl-cysteine is a special product from phenol oxidation and plays an essential role in black garlic's pharmacological effects (Ngan et al. 2017). Its amount is five to six times higher than fresh garlic (Bae et al. 2012; Wang et al. 2012). Brown color and sweeter taste on black garlic caused by non-enzymatic browning like Maillard reaction and caramelization (Bae et al. 2012; Zhang et al. 2016). Some antioxidant compounds are made using such process (Osada and Shibamoto 2006; Yilmaz and Toledo 2005).

Some experiments added the immersion/soaking process on microorganism medium before or after aging of black garlic i.e., kombucha and vinegar (Pure et al. 2017) *Lactobacillus plantarum* (*L. plantarum*) PN05 (*Ngan* et al. 2017) and *S. cerevisiae* KCTC 7910, *M. pilosus* KCTC 26768, and *Lactobacillus plantarum* KCTC 3104 (Kim et al. 2016), *Streptococcus thermophiles*, *Bifidobacterium*, *Candida utilis*, and *Saccharomyces cerevisiae* (CN104336550A). Although the purpose of the process was important to increase the value of black garlic benefit, the data about the physiochemical properties including functional properties such as antioxidant value, total flavonoid content and total polyphenol is still limited. The aim of this study was to examine the functional (bioactive) properties of solo black garlic fermented by *S. kluyveri* Y97 before aging process.

MATERIALS AND METHODS

Materials

Fresh solo garlic was obtained from local market in Bogor. The reagent used in this research were DPPH (α,α-diphenyl-β-picrylhydrazyl), garlic acid, quercetin (Sigma Aldrich), Follin Ciocalteu, sodium carbonate, ethanol, methanol aluminum chloride, potassium acetate (Merck). Isolate of *S. kluyveri* Y97 was obtained from Indonesian Culture Collection (InaCC).

DPPH (α,α-diphenyl-β-picrylhydrazyl) radical scavenging ability

Here, method from Muanda et al (2011) was used to determine DPPH radical scavenging ability. Garlic extract 0.2 mL were added to 0.8 mL DPPH methanol solution (0.2 mM). The mixture was shaken and left to stand for 30 minutes in dark conditions. After that, the absorbance was measured at 517 nm using a spectrophotometer. The inhibition percentage of DPPH radical scavenging ability was calculated by the following equation:

$$\% \text{ inhibition} = (A_0-A_1) *100 / A_0$$

Where:

A_0= absorbance of the mixture of DPPH and methanol solution,

A_1= absorbance of the mixture of DPPH and garlic extract.

***S. kluyveri* Y97-fermented solo black garlic processing**

Fresh solo garlic was fermented in medium containing *S. kluyveri* Y976 of 10 x10º7 cfu/ml for 0, 2, 4, 6 days. Solo garlic was cultivated and aged at the temperature of 70ºC with air moisture of about 60% for 0, 7, 14 and 21 days. Solo black garlic was stored in the freezer-20ºC until It was analyzed.

Determination of total polyphenol content

The polyphenol content was reported as garlic acid equivalents using following linear equation based on calibration curve: y=0.0015x+0.0007, R^2 = 0,9939, where y is the absorbance at 750 nm and x is the concentration of garlic acid equivalents (ppm). A garlic extract (50 µL) was diluted in 800 µL distilled water. Diluted garlic extract was mixed with 50 µL Follin-Ceucalteu (10 %) and 100 µL sodium carbonate (7%). After incubation for 30 minutes at room temperature, the absorbance was measured at 750 nm. The incubation process was done in dark conditions (Chang et al. 2002).

Determination of total flavonoid content

Total flavonoid content was determined according to Chang et al. (2002) with quercetin as standard. Quercetin equivalent was standardized using following linear equation based on calibration curve: y=0.0041x-0.0063, R^2=0.9968, where y is the absorbance at 415 nm and x is the concentration of quercetin (ppm). 50 µL garlic extract was mixed with ethanol absolute (30uL), 10% aluminum chloride (50µL), and 1M potassium acetate (50µL). The mixture was diluted in 600 µL distilled water and incubated at room temperature for 30 minutes. The absorbance was recorded at 415 nm.

Data analysis

All experiments were carried out in duplo. The data were expressed as mean values and analyzed using SPSS 16.0 software.

RESULTS AND DISCUSSION

Results

Fresh solo garlic had 3.76% of antioxidant capacity. Fermentation of fresh solo garlic in medium containing *S. kluyveri* Y97 10^7 cfu/ml for 2, 4 dan 6 days increased the antioxidant capacity about 9.24, 10.14 and 18.94% respectively (Figure 1). The 21 days-aging process increased the antioxidant capacity 9.08, 8.33, and 4.51 times of solo garlic fermented by *S. kluyveri* Y97 for 2, 4 and 6 days respectively, compared to *S. kluyveri* Y97 fermented solo garlic without aging. The antioxidant capacity of 21-days aging of solo black garlic for all fermentation treatments was around 83.90-85.6%. The highest antioxidant capacity was observed at solo black garlic fermented by *S. kluyveri* Y97 for 6 days. At 21 days aging time, the fermentation by *S. kluyveri* Y97 increased antioxidant capacity reaching 2 times compared to without *S. kluyveri* Y97 fermentation.

Fermentation by *S. kluyveri* Y97 for 2, 4 and 6 days increased the total flavonoid content of solo garlic 17.6, 22.2, and 25.2-fold respectively, compared to the fresh solo garlic. Fresh solo garlic had 0.53 mg QE/g wet basis of flavonoid content. In line with antioxidant capacity results, the highest total flavonoid content was obtained by 21-aged solo garlic with fermentation. Compared to 21 days aged solo black garlic without fermentation, the total flavonoid content of 21 days aged solo garlic increased 2.6, 2.9, and 2.9-fold for 2, 4 dan 6 days fermentation respectively. The highest was obtained by solo black garlic which was fermented for 6 days and continued by aging process for 21 days and the 85.58 mg QE/g wet basis. The total flavonoid of solo black garlic without fermentation and aged 21 days was 29.64 mg QE/g wet basis.

The highest of total flavonoid was obtained by 6 days fermented of *S. kluyveri*-21-days aged solo black garlic, 116.72 mg GAE/g wet basis. Its total polyphenol content increased 1.26-fold compared to 21-days aged solo black garlic without fermentation. In solo black garlic without fermentation, 21-days aging process induced the increase of total polyphenol content reached 3.6-fold. Fresh solo garlic had 26.13 mg GAE/g wet basis of total polyphenol content. Compared to 21 days aged solo black garlic, *S. kluyveri* Y97 fermented on 21-days aging process induced the increase of total polyphenol content 1.18; 1.26; 1.23-fold for 2, 4, 6 days of fermentation respectively.

Discussion

Fermentation of *S. kluyveri* Y97 before aging process on solo black garlic increased the antioxidant capacity. The analysis of variance showed that there was an interaction between fermentation time and aging time. Fermentation for 4 and 6 days gave the same results (not significant) on the quality of solo black garlic. An optimal antioxidant capacity was shown by solo black garlic solo fermented for 4 days and continuing aged for 21 days. There is no literature that reveals a clear mechanism for increasing bioactive properties due to fermentation yet.

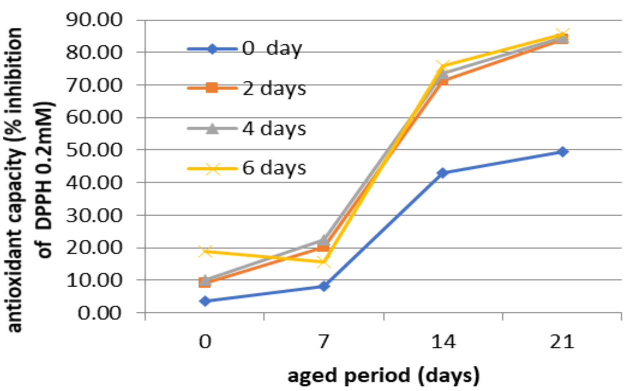

Figure 1. Antioxidant capacity of *S. kluyveri* Y97 fermented on solo black garlic

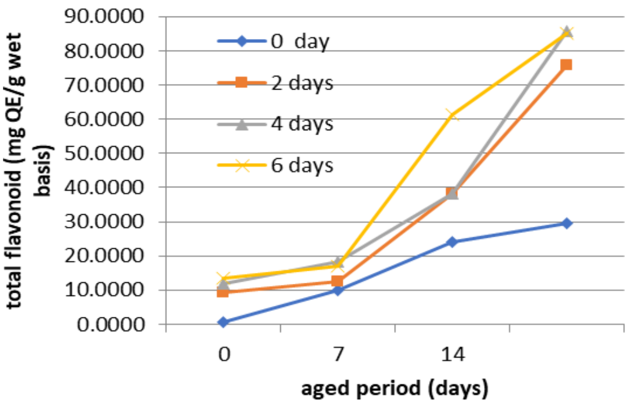

Figure 2. Total flavonoid content of *S. kluyveri* Y97 fermented on solo black garlic

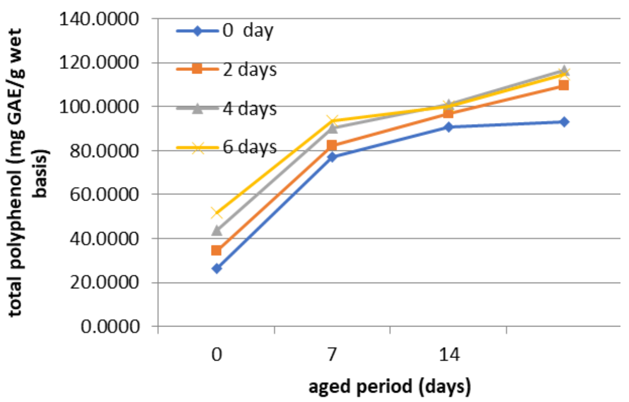

Figure 3. Total polyphenol content of *S. kluyveri* Y97 fermented on solo black garlic

Research by Jung et al. (2011) revealed that black garlic fermented by *S. cerevisiae* increased antioxidant activity in vitro by EDA (electron-donating activity) antioxidant activity, which the EDA of fermented black garlic was 13.65% and meanwhile the unfermented black garlic was 10.32%. Antioxidant capacity enhancement was alleged to those microbes convert several components in food or substrate and changes sugar to alcohol and lactic acid which can increase bioactivity of the components (Bae et al. 2004; Trint et al. 2007). Jung et al. (2017) mentioned the antioxidant capacity of red ginseng extract by the probiotic *Lactobacillus plantarum* KCCM 11613P. That phenomenon was like this research. In recent years, Hien-Trung et al. (2007) discovered that the bioactivity of ginseng could be enhanced by yeast fermentation. Therefore, they hypothesized that the bioactivity of black garlic may also be enhanced by yeast fermentation (Kimura et al. 2017). The presence of flavonoid and polyphenol compounds could increase the antioxidant capacity.

Some flavonoid compounds in black garlic are catechin, epicatechin, epigallocatechin gallate, quercetin, apigenin, myricetin, resveratrol, morin, quercitin, kaempferol and narigenin (Kim et al. 2013). Abundant concentration of myricetin, quercetin and apigenin was found in black garlic (Miean and Mohamed 2001). Heat thermal during the aging process induced the increase of flavonoid content in black garlic (Kim et al. 2013). The result of this research noted that there was interaction between fermentation and aging time ($p<0.05$). In this research, total flavonoid content increased about 1.23-fold on solo black garlic fermented by *S. kluyveri* Y97 for 6 days and aged for 21 days. The optimum process to produce an optimal quality of solo black garlic was 4 days fermented by *S. kluyveri* Y97 then continuing aged for 21 days.

Consistent with antioxidant results, total polyphenol content of *S. kluyveri* Y97-fermented on solo black garlic was increased along with fermentation and aging period. The increase of total polyphenols could be caused by an increase in the level of complex polyphenols from the final phase of browning reactions as revealed by Robards et al (1999). Moreover, Guihua et al (2007) found that the heating process increased the phenolic content due to cleavage of the bound form (esterification and glycosylation), which results in an increase in free form. In addition, another possible reason for the increase of phenolic content in heated samples was the reduction/ inhibition of enzymatic oxidation involving antioxidant compounds in raw plant material (Dewanto et al. 2002; Nicoli et al.1999). Kim et al. (2013) revealed that high concentration of hydroxycinnamic acid derivates was found in aged black garlic, such as chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, m-coumaric acid, o-coumaric acid.

The analysis of variance showed that there was no interaction between fermentation and aging time ($p>0.05$), but the fermentation and aging time affected the result significantly ($p<0.05$).

The optimum process to produce an optimal quality of solo black garlic based on total polyphenol content, was 4 days fermented by *S. kluyveri* Y97 then continuing to age for 21 days, producing about 116.72 mg GAE/g wet basis. At the time of writing, there was no literature that explained a clear mechanism of relation between TPC and fermentation by yeast. Other literature mentioned that fermentation is conducted to improve the storage period, nutrition, and sensory characteristics related to foods (Nout and Motarjemi 1997). Research in other raw plant materials showed that the total phenolic content in black ginseng fermented by *S. cerevisiae* was higher than that of raw ginseng and black ginseng (Jung et al. 2017). Jhan et al. (2015) reported that the total phenolic content increased after fermentation, when red beans were fermented by *B. subtilis* and *L. delbrueckii* sub sp. *bulgaricus.* Based on Jung et al. (2011), *S.cerevisiae*-fermented on black garlic exhibited much better bioactivity against syndromes such as hyperlipidemia, obesity, nephropathy, and hepatopathy than ordinary (aged) black garlic. Furthermore, fermented BG 400 mg/kg and 200 mg/kg revealed significantly higher effects than aged black garlic 400 mg/kg. In other words, fermented black garlic has more effective bioactivity against high feed diet-induced obesity, hyperlipidemia, nephropathy, and hepatopathy than ABG. Therefore, the bioactivity of BG could be enhanced by yeast fermentation, and fermented BG may be more qualified to improve diabetes and its related complications (Jung et al. 2011; Kimura et al. 2017). In addition, the functional properties, and the bioactivity of some natural products in increased by fermentation.

In conclusion, fermentation by *S. kluyveri* on solo black garlic processing could increase its functional properties due to enhanced bioactivity such as antioxidant capacity, total flavonoid content, and total polyphenol contents. The optimum quality of solo black garlic in this research was produced by 4 days of fermentation on *S. kluyveri* Y97 medium then continuing aged for 21 days.

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REFERENCES

Bae EA, Hyun YJ, Choo MK, Oh JK, Ryu JH, Kim DH. 2004. Protective effect of fermented red ginseng on transient focal ischemic rats. Arch Pharm Res 27: 1136-1140.

Bae SE, Cho SY, Won YD, Lee SH, Park HJ. 2012. A comparative study of the different analytical methods for analysis of S-allyl cysteine in black garlic by HPLC. LWT-Food Sci Technol 46 (2): 532-535.

Bae SE, Cho SY, Won YD, Lee SH, Park HJ. 2014. Changes in S-allyl cysteine contents and physicochemical properties of black garlic during heat treatment," LWT-Food Sci Technol 55 (1): 397-402.

Chang CC, Yang MH, Wen HM, Chern JC. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric method. J Food Drug Anal 10: 178-182.

Chen S, Shen X, Cheng et al. 2013. Evaluation of garlic cultivars for polyphenolic content and antioxidant properties. PLoS ONE, Vol. 8 (11): e79730. DOI: 10.1371/journal.pone.0079730.

Choi S, Cha HS, Lee YS. 2014. Physicochemical and antioxidant properties of black garlic. Molecules 19: 16811-16823.

Dewanto V, Wu X, Adam KK., Liu RH. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem 50: 3010-3014.

Ha AW, T. Ying T, Kim WK, 2015. The effects of black garlic (*Allium sativium*) extracts on lipid metabolism in rats fed a high fat diet. Nutr Res Pract 9 (1): 30-36.

Hien-Trung T, Han SJ, Kim SW, Lee YC, Kim DH. 2007. Bifidus fermentation increases hypolipidemic and hypoglycemic effects of red ginseng. J Microb Biotechnol 17: 1127-1133.

Jhan JK, Chang WF, Wang PM, Chou ST, Chung YC. 2015. Production of fermented red beans with multiple bioactivities using co-cultures of *Bacillus subtilis* and *Lactobacillus delbrueckii* subsp. b*ulgaricus.* LWT-Food Sci Technol 63: 1281-1287.

Jung K, An JM, Eom DW, Kang KS, Kim SN. 2017. Preventive effect of fermented black ginseng against cisplatin-induced nephrotoxicity in rats. J Ginseng Res 41: 188-194.

Jung YM, Lee SH, Lee DS, You MJ, Chung IK, Cheon WH, Kwon YS, Lee YJ, Ku SK. 2011. Fermented garlic protects diabetic, obese mice when fed a high-fat diet by antioxidant effects. Nutr Res 31: 387-396.

Kang MJ, Lee SJ, Shin JH, Kang SK, Kim JG, Sung NJ. 2008 Effect of garlic with different processing on lipid metabolism in 1% cholesterol fed rats. J Korean Soc Food Sci Nutr 37 (2): 162-169.

Kim JS, Kang OJ, Gweon OC. 2013. Comparation of phenolic acids and flavonoids in black garlic at different thermal processing steps. J Funct Food 5: 80-66.

Kim S, Park SL, Lee S, Lee SY, Ko S, Yoo M. 2016. UPLC/ESI-MS/MS analysis of compositional changes for organosulfur compounds in garlic (*Allium sativum* L.) during fermentation. Food Chem 211: 555-559.

Kimura S, Tung YC, Pan MH, Su NW, Lai YJ, Cheng KC. 2017. "Black garlic: A critical review of its production, bioactivity, and application, J Food Drug Anal 25 (1): 62-70.

Lu X, Li N, Qiao X, Qiu Z, Liu P. 2017. Composition analysis and antioxidant properties of black garlic extract. J Food Drug Anal 25: 340-349.

Muanda F, Kone D, Dicko A, Soulimani R, Younos C. Phytochemical composition and antioxidant capacity of three Malian medicinal plant parts. Evid Based Compl Altern Med 2011: 674320, 8 pages. DOI: 10.1093/ecam/nep109

Naji KM, Al-Shaibani ES, Alhadi FA, Al-Soudi SA, and D'souza MR. 2017. Hepatoprotective and antioxidant effects of single clove garlic against CCl4-induced hepatic damage in rabbits. BMC Compl Altern Med 17 (1): 411

Ngan N, Giang M, Tu N. 2017. Biological activities of black garlic fermented with Lactobacillus plantarum PN05 and some kinds of black garlic presenting inside Vietnam. Indian J Pharm Edu Res 51 (4): 672-678

Nicoli MC, Anes M, Parpinel MT, Franceschi S. 1999.Influence of processing on the antioxidant properties of fruits and vegetables. Intl J Food Sci Technol 10: 94-100.

Nout MJR, Motarjemi Y. 1997. Assessment of fermentation as a household technology for improving food safety: a joint FAO/WHO workshop. Food Control 8: 221-226.

Osada Y and Shibamoto T. 2006. Antioxidative activity of volatile extracts from Maillard model system. Food Chem 98: 522-528.

Pure AE, Mofidi SMG, Keyghobadi, ME Pure. 2017.Chemical composition of garlic fermented in red grape vinegar and kombucha. J Funct Foods 34: 347-355.

Purev U, Chung M, Oh D. 2012. Individual differences on immunostimulatory activity of raw and black garlic extract in human primary immune cells. Immunopharmacol Immunotoxicol 34: 651-660.

Seo YJ, Gweon OC, Im J, Lee YM, Kang MJ, Kim JI. 2009. Effect of garlic and aged black garlic on hyperglycemia and dyslipidemia in animal model of type 2 diabetes mellitus. J Food Sci Nutr 14 (1): 1-7.

Trinh HT, Han SJ, Kim SW, Lee YC, Kim DH. 2017. *Bifidus* fermentation increases hypolipidemic and hypoglycemic effects of red ginseng. J Microbiol Biotechnol 17: 1127-1133.

Wang W. and Sun Y. 2017. In vitro and in vivo antioxidant activities of polyphenol extracted from black garlic. Food Sci Technol 37 (4): 681-685.

Wang X, Jiao F, Wang Q, Wang J, Yang K, Hu R, Liu H, Wang H, Wang Y.2012. Aged black garlic extract induces inhibition of gastric cancer cell growth in vitro and in vivo. Mol Med Rep 5: 66-72.

Yilmaz Y and Toledo R. 2005. Antioxidant activity of water-soluble Maillard reaction products. Food Chem 93: 273-278.

Zhang X, Li N, Lu X, Liu P, Qiao X. 2016. Effects of temperature on the quality of black garlic. J Sci Food Agric 96: 2366-2372.

Zhang Z, Lei M, Liu R, Gao Y, Xu M, Zhang M. 2015. Evaluation of alliin, saccharide contents and antioxidant activities of black garlic during thermal processing, J Food Biochem 39 (1): 39-47.