APPLICATION OF GREEN TEA-KOMBUCHA FERMENTATION ON SOLO BLACK GARLIC PROPERTIES

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Abstract

In this study, fermentation by green tea-kombucha was applied on fresh solo garlic prior to aging process. Period fermentation was 0, 7, 14 and 21 days then continued aging process for 0, 7, 14 and 21 days. Some functional properties: antioxidant capacity, total flavonoid and total polyphenol were observed. There was an interaction between fermentation period and aging period on the antioxidant capacity and total polyphenol of solo black garlic significantly (p<0.05). Solo black garlic was produced by 7 days-kombucha fermented then continuing 21 days-aged had the highest functional properties, where as its functional properties were 64.72% of antioxidant capacity, 44.16 mg QE.g of total flavonoid and 193.39 mg GAE/g of total polyphenol.

Keywords: fermentation, green teakombucha, solo black garlic, functional properties,

1. Introduction

Black garlic is a famous herbal medicine that proven has a lot of activity such as antioxidant, anti cancer, anti hepatopathic, immunodulator, antimicrobial, anti-inflammatory, and neurotrophic activities (Seo et al., 2009; Kodera et al., 2012; Purevet et al., 2012; Ha et al., 2015). Black garlic is processed by heating of fresh garlic at high temperature and high or controlled humidity (Kimura et al., 2017; Choi et al., 2014; Bae et al., 2014; Kang et al., 2008). Aging process of
garlic induced increasing properties in black garlic compared with fresh garlic such as S-allylcysteine (SAC) increased 5 to 6 times (Bae et al., 2012; Wang et al., 2012), extended self life (Chu et al., 2007), higher phenolic content 5-8 times (Kim et al., 2012), lower off flavor (Kimura et al., 2017) and lower fructan content (Yuan et al., 2016).

In this research, solo garlic was used as raw material for black garlic producing. Solo garlic has an all medicinal components more than multi clove garlic, so it could produce more bioactive compounds than multi clove garlic in black garlic form. Najiet al. (2017) revealed that solo black garlic has higher benefit than multi clove black garlic. S-allyl-cystein (SAC) content of solo garlic is higher than multi clove garlic. Research by Bae et al. (2012) revealed SAC of multi clove garlic was 97.75 µg/g, whereas solo garlic has 228.48 µg/g (Gia-Buu et al., 2018).

Some research of fermentation garlic or black garlic has been reported, such as soaking in kombucha and vinegar (Pure et al., 2017); Lactobacilu sp lantarum PN05 (Ngan et al., 2017); Saccharomyces cerevisiae (Jung et al., 2011); mixture of S. cerevisiae KCTC 7910, M. pilosus KCTC 2676, and Lactobacillus plantarum KCTC 3104 (Kim et al. 2016); Streptococcus thermophiles, Bifidobacterium, Candida utilis, and Saccharomyces cerevisiae (CN104336550A); Saccharomyces kluyveri (Setiyoningrum et al., 2018).

Based on Jung et al. (2011), S.cerevisiae-fermented on black garlic exhibited much better bio activity against syndromes such as hyperlipidemia, obesity, nephropathy, and hepatopathy than ordinary (aged) black garlic. Furthermore, fermented BG 400mg/kg and 200mg/kg revealed significantly high effects than aged black garlic 400 mg/kg. In otherwords, fermented black garlic has more effective bioactivity against high feeddiet-induced obesity, hyperlipidemia, nephropathy, and hepatopathy than ABG. Therefore, the bioactivity of BG could beenhanced by yeast fermentation, and fermented BG maybe more qualified to improved diabetes and its related complications (Jung et al. 2011; Kimura et al. 2017).

Kombucha was used as a fermentation medium of solo garlic in this research. Pure et al. (2017) reported that there were changes of compounds in multi clove garlic during fermentation in kombucha medium. Kombucha was a typically sweetened tea
infusion, fermented with a symbiosis mixture of acetic acid bacteria and yeast. The final product is an acidified beverage (pH about 2) were acetic acid is a dominant acid, rich in phenolic compound and show high antioxidant activity (Pure et al., 2017). This aims of this research was to investigate the effect fermentation of solo garlic in green tea-kombucha on solo black garlic properties (antioxidant capacity, total flavonoid, total polyphenol) and determine its optimum condition.

2. Material and Methods

Fresh solo garlic was purchased from local market in Bogor. The reagent used in this research were: garcilic acid, quercetin (Sigma Aldrich), DPPH (α,α–diphenyl-β-picrylhydrazyl), follineucalteu, sodium carbonate, ethanol, methanol, aluminium chloride, potassium acetate (Merck). Green tea was obtained from Gambung, Indonesia and starter of kombucha was a lab collection.

2.1 Fermentation of solo garlic in green tea-kombucha medium

Green tea-kombucha was prepared by extracting green tea was extracted in hot water, then added amount of sugar. Starter of kombucha was added in green tea-extract and fermented for 7 days. Starter of kombucha was separated with the solution and ready to used as fermentation medium of fresh solo garlic. Fresh solo garlic was fermented in kombucha medium for 0, 7, 14, 21 and 28 days. Solo garlic was cultivated and aged in the temperature of 70°C with air moisture about 60% for 0, 7, 14 and 21 days. Solo black garlic was stored in the freezer -20°C until it was analyzed.

2.2 DPPH (α,α–diphenyl-β-picrylhydrazyl) radical scavenging ability (Muanda et al. 2011)

Method of Muanda et al. (2011) was adopted 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 stand for 30 minute in the dark condition. After that, the absorbance was measured at 517 nm using a spectrophotometer. Methanol was used as a blanco. The inhibition percentage of DPPH radical scavenging ability was calculated by the following equation:

\[
\% \text{ inhibition} = \frac{(A_0 - A_1) \times 100}{A_0}
\]
Where:

\[ A_0 = \text{absorbance of the mixture of DPPH and methanol solution,} \]
\[ A_1 = \text{absorbance of the mixture of DPPH and garlic extract.} \]

2.3 Determination of total polyphenol content (Chang et al. 2002)

The polyphenol content was measured 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 solo black garlic extract (50 µL) was eluted 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 and dark condition, the absorbance was measured at 750 nm.

2.4 Determination of total flavonoid content (Chang et al. 2002)

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). A solo black garlic extract of 50 µL were mixed with ethanol absolute (30 µL), 10% aluminium chloride (50 µL), and 1M potassium acetate (50 µL). The mixture was diluted in 600 µL distilled water and incubated at room temperature for 30 minutes. The absorbance was recorded at 415 nm.

2.5 Data analysis

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

3. Results And Discussion

In this study, solo garlic was fermented in green tea-kombucha prior to aging process. Fresh solo garlic had 3.76% of antioxidant capacity (Setiyoningrum et al. 2018). Fermentation of solo garlic in green tea-kombucha increased its antioxidant capacity reached of 2-3 fold. Aging process increased its antioxidant capacity reached of 6-7 fold. There was interaction between fermentation and aging period statistically \((p < 0.5)\). Aging period influenced antioxidant capacity of solo black garlic, 21 days of aging was the optimum aging period. Highest antioxidant capacity was obtained by solo garlic which fermented for 7 days then continued aging for 21 days. The result was in line with Setiyoningrum et al. (2018)
that reported increasing of antioxidant capacity due to fermentation of solo garlic. In that research, the highest antioxidant capacity was observed at solo black garlic fermented by *S. kluyveri* Y97 for 6 days.

Kombucha contains of yeast, lactic acid bacteria and acetic acid bacteria (Pure *et al.*, 2017). Antioxidant capacity enhancement was alleged to those microbes convert several components in food or substrat and changes sugar to alcohol and lactic acid or acetic acid which can increase bioactivity of the components (Bae *et al.* 2004; Trintet *et al.* 2007). Jung *et al.* (2017) mentioned that antioxidant capacity of red ginseng extract by the probiotic *Lactobacillus plantarum* KCCM 11613P.

![Antioxidant capacity of solo black garlic fermented by green tea kombucha](image)

**Figure 1.** Antioxidant capacity of solo black garlic fermented by green tea kombucha

Fresh solo garlichad 0.53 mg QE/g wet basis of flavonoid content (Setiyoningrum*et al.* 2018). In line with antioxidant capacity results, the highest of total flavonoid content was obtained by 21-aged solo garlic with fermentation. Analysis of variance shown that there was interaction between fermentation and aging period (p<0.05). Same with antioxidant capacity result, the highest was obtained by solo black garlic which was fermented for 7 days and continued aging process for 21 days, 44.16 mg QE/g wet basis, increased about 2 times. The total flavonoid of solo black garlic without fermentation and aged 21 days was 29.64 mg QE/g wet basis.
In this research was alleged that fermentation could increase the flavonoid content. The same phenomenon was reported by Setiyoningrum et al. (2018) that fermentation has an effect on total flavonoid of solo black garlic. Heat thermal on aging process also induced the increasing of flavonoid content in black garlic (Kim et al., 2013). Some flavonoid compounds in black garlic are quercetin, catechin, epicatechin, epigallocatechin gallate, apigenin, myricetin, resveratrol, morin, quercitin, kaempferol and narigenin (Kim et al., 2013), which is abundant concentration of myricetin, quercetin and apigenin was found in black garlic.

Based on analysis of variance, there was an interaction between fermentation and aging period significantly (p<0.05). Fresh solo garlic had 26.13 mg GAE/g wet basis of total polyphenol content (Setiyoningrum et al., 2018). Consistent with antioxidant and flavonoid result, the highest of total polyphenol was obtained by 7 days fermented on kombucha medium-21 days aged solo black garlic, 193.40 mg GAE/g wet basis. Its total polyphenol content increased 2-fold compared with 21 days aged solo black garlic without fermentation. Total polyphenol of solo garlic was higher than multi clove black garlic. Choi et al. (2014) has report total polyphenol of multi clove black garlic aged 21 days was 58 mg/g.
Phenol oxidation is the key to the success of black garlic production. Increasing antioxidant, phenol and flavonoid are due to phenol oxidation process. Research in other raw plant material 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). Research by 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*.

There were several reason, why the total flavonoid and polyphenol increased after aging process. First, the aging process break the bound form like glycosylated and esterified, thus leading to the increase of free forms. Secondly, it cuased by the decreaseon enzymatic oxydation involving the antioxidant compounds. Thirdly, it caused by an increase in the levels of complex polyphenol from the later phase of the browning reaction (Kim *et al*., 2013). Moreover, Kim *et al.* (2013) stated 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.

4. Conclusions

Fermentation solo garlic in green tea-kombucha prior to aging process could increase its functional properties due to enhanced bioactivity such as antioxidant tcapacity, total flavonoid content and total polyphenol contents. The optimum condition was fermentated for 7 days and continued aging for 21 days.
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