Characteristics of Solo Black Garlic Fermented in Kombucha Black Tea

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Abstract. A research has done on the characteristics of solo black garlic fermented by kombucha black tea. Fermentation is carried out for 0, 7, 14 and 21 days and continued to aging process for 0, 7, 14 and 21 days. Observation was done to see the characteristics of functional traits such as antioxidant capacity, total flavonoids and total polyphenols amount. There was a significant interaction (p<0,05) between the fermentation period and the aging period in the functional properties of a solo black garlic. The study resulted in 68,34 % of functional values characteristic of antioxidants capacity, 49,94 mg QE flavonoids total and 185.97 mg GAE/g of polyphenols total. Increased bioactivity resulting from fermented solo black garlic using kombucha tea for 7 days and continued by aging process for 21 days.

1. Introduction
Garlic is a clustered plant which has been utilized as an alternative medication for several illnesses such as hypertension, hypercholesterolemia, diabetes and pharmacologically potential as antibacterial, anti-hypertension and antithrombotic agent. Garlic contains at least 33 compounds, several enzymes, 17 amino acids and some minerals such as selenium [1]. It has higher sulfuric components than another Allium species as a result it has particular aroma and medication uses [2]. One treatment developed to boost garlic usefulness is a heating process with black garlic as the final result. Black garlic is garlic stored or heated at a certain temperature and under controlled humidity for a period of several days.

Tea is one of the most consumed beverages in the whole world. People look for its natural compounds, antioxidants of the flavonoids that can keep the body from the attacks of radical free, which makes this beverage considerably notorious around the globe [3]. Tea leaves can be processed into green and black tea. Black tea is a product of fully fermented tea leave and traditionally has been used to brew kombucha. Black tea is highly nutritious for scoby (Symbiotic culture of bacteria and yeast ) and the most consistent stabilizer of pH level [4]. Almost all kinds of tea been have found out essential for freshness, fitness as well as body healthiness. Tea contains beneficial compounds such as polyphenols, theophylline, flavonoids/ metixantin, tannins, vitamins C and E, catechins, and some minerals such as Zn, Se, Mo, Ge, Mg which also known as anti-mutagenic, anti-cancer, anti-bacterial and antioxidants [5].
Fermented tea beverages have been a tradition since hundreds of years ago and known to increase probiotics and creates refreshing taste. Kombucha is a refreshing beverage which traditionally resulted from the fermentation of sweet tea, symbiotic bacteria and strong yeast acetate. This beverage has been consumed intensively due to prophylactic and therapeutic nature [6].

Based on the potential mentioned above, this study aims to look at the combination of these ingredients towards the characteristics of solo black garlic that fermented by tea black kombucha.

2. Methods
This research uses the following materials in the study: solo white garlic bought from a traditional market, black tea (Gambung, Indonesia) and kombucha starter (a collection of Lab), quercetin (Sigma-Aldrich), DPPH (α, α-diphenyl-β-pikrillhidrazil), ceucalteu follin, natrium carbonate, ethanol, methanol, aluminum chloride, potassium acetate (Merck).

2.1 Fermented solo white garlic in a medium of Black Tea Kombucha
Black tea is extracted using hot water which then added sugar. Black garlic kombucha is used to soak garlic for 7 days. Scoby is then ready to be used as a ferment for 0, 7, 14, 21 and 28 days. White solo garlic is cultured at a temperature of 70°C, humidity of 60% during 0, 7, 14 and 21 days. The resulted products of black garlies are stored for 0, 7, 14 and 21 days. Black garlies are stored at a temperature of -20°C until ready for analysis. Heated at a temperature of 70-75°C with a humidity of 85-90%, brood white garlic for 5-25 days result in brown color characteristics at the intensity at OD 2.685, antioxidant capacity of 89-92%, flavonoids total of 16000-28000 ppm and phenolic total of 130000-160000 ppm [7].

2.2 Testing the antioxidant DPPH method (α, α-diphenyl-β-picrylhydrazyl) [8]
Black garlic extract as much as 0.2 mL was added to a 0.8 mL of DPPH methanol solution. Both solution then mixed and let to chill for 30 minutes in dark room then measured the absorbance level using a spectrophotometer. The antioxidant capacity is expressed as a percentage of inhibition against DPPH radicals. Antioxidants capacity (%) is calculated as follow:

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\text{The capacity of the antioxidant (\%) = \frac{\text{Blank Abs-Sample Abs}}{(\text{Blank Abs})} \times 100%}
\]

2.3 Testing of Total Polyphenol Content [9]
Linear equations through calibration curves \( y = 0.0015x + 0.0007, R^2 = 0.9939 \), where \( y \) is the absorbance at 750 nm and \( x \) is the concentration of garlic acid equivalents (ppm) is performed to measure the total polyphenol amount. Diluted garlic extract were mixed with 50 mL of Follin Ceucalteu (10%) and 100 mL of sodium carbonate (7%). After incubation for 30 minutes at room temperature and dark conditions, the absorbance was measured at 750 nm.

2.4 Testing Total Flavonoid Content [9]
Linear equations through calibration curves \( y = 0.0041x -0.0063, R^2 = 0.9968 \) where \( y \) is the absorbance at 415 nm and \( x \) is the concentration of quercetin (ppm) is performed to measure the total flavonoids. Solo black garlic extract of 50 μL were mixed with ethanol absolute (30uL), 10% aluminum chloride (50μL), and 1M of potassium acetate (50μL). The mixture was diluted in 600 μL of 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 analized using SPSS 16.0 software.
3.1 Antioxidant Capacity

Black tea that has been fermented using scoby, is used to ferment solo black garlic (SBG). The antioxidant capacity of SBG in this study showed improvement after run through aging process. The variance investigation found that the aging and fermentation period significantly effected the antioxidants levels (p<0.05), but the interaction of the two does not affect it significantly (p>0.05). Setiyoningrum [10] study resulted in 3.76% of antioxidants capacity in fresh white garlic. Once through the immersion and maturation process, the antioxidant capacity increased from 5.15% at D7 to 68.34% at D21 which is the maximum period of aging. This value is in line with [10] study which states that an increase in antioxidant capacity in SBG was due to fermentation of S. kluyveri Y97 for 6 days.

![Graph](attachment:graph.png)

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

Black garlic has strong antioxidant properties after controlled heating and humidity state. Garlic extract has been proven to have strong deterrence to free-radical (1,1-diphenyl-2-pikrillhidrazil (DPPH)) [11], all functional properties of black garlic function in synergic manner against all of the components [12]. The results of a study showed that HPLC of black garlic extract fractions F3 and F4 had the highest total phenolic content and antioxidant activity compared to F1 and F2. F3 and F4 fractions have a DPPH radical antidote level of around 30% at a concentration of 30mg / mL, close to 5mg / mL of ascorbic acid or gallic acid. At a concentration of 100 mg / mL, F3 and F4 produce 70% of the DPPH radical antidote level. The lowest DPPH radical scaling rate was shown by F1 at a concentration of 100 mg / mL, which is around 20.2% [11].

3.2 Total Flavonoids

An increase of total SBG flavonoids fermented in black kombucha tea for 7 days was evident, from 0.805 mgQE/g in SBG without aging to 49.94 mgQE/g after run through a ripening process for 21 days. The increase also occurred in SBG soaked in kombucha black tea for 28 days without aging (0.56 mQE / g) to 60.84 mQE / g after 21 days of aging.
The results of variance investigation has shown that the aging and fermentation time significantly affect the flavonoids levels (p<0.05), but the interaction of the two does not affect it significantly (p>0.05). Black garlic tastes sweet, sticky, and little bit similar to Javanese tamarind [13]. Diet of 5% of black garlics can reduce cholesterol levels in blood plasma and triglyceride levels and increase insulin resistance [14]. S-allyl cysteine component in addition to polyphenols and other flavonoids components allegedly became bioactive components as an antioxidant, anti-diabetic and anti-inflammatory [15]. Compared to fresh garlic, the antioxidants in black garlics i.e. polyphenols and flavonoids are higher [16]. The phenolic acid components found in black garlics are the following: gallic acid, vanillic acid, chlorogenic acid, caffeic acid, ferulic acid, acid m-coumaric, and o-coumaric acid. As for the flavonoids components detected on black garlic are the following: catechin, epicatechin, quercitrin, myricetin, quercetin, and morin [17].

3.3 Total Polyphenol

Tannins which are phenolic compounds found in various types of green plants on varying amount [18]. Tannins belong to polyphenol compound category. One of the benefits of tannin for human is as
an antibacterial. Tannin as an antibacterial can inhibit protein synthesis [19]. Tannin is a complex compound in the form of a polyphenol mixture which is difficult to separate as a result hard to crystallize. Phenol compound from tannins act as astrigensia, antiseptic and color contributor [20]. It is known that phenolic compounds belong to of plant bioactive products components and is beneficial for health [21]. It has been observed that the total amount of phenolic fractions of F3 and F4 is much higher than those of F1, F2, and F5 (p<0.05) [11]. The total amount of phenolic in black garlic rose four to 10 times compared to usual fresh garlic, and the derivative of hidroxykinamat acid found as principal phenolic acid of garlic under different processing-steps [17].

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

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