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Antioxidant Properties of Single Garlic (*Allium sativum*) Pickle

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Abstract

Single garlic is a type of garlic that has a single clove, also known as single clove garlic, monobulb garlic or single bulb garlic. Traditionally, single garlic was used as medicinal based on its properties as antimicrobial, antiprotozoal, antimutagenic, antiplatelet, antihyperlipidemic and antioxidant. The Alliin substance was presumably as an antioxidant, but it was changed easily to unstable allicin caused by Alliinase enzyme activity which is increased when garlic was chopped, crushed or processed. The change of Alliin to allicin will reduce the antioxidant activity of single garlic. A treatment that reduces the Alliinase activity will retain the Alliin substance and the antioxidant capacity could be maintained. The fermentation process will give a low pH as the result of carbohydrate metabolism in a fermentation medium, and reduce the alliinase activity. The aims of this research were to evaluate the growth of *Lactobacillus plantarum* B1765 as a starter culture in single garlic, pH and the effect of fermentation time process (3, 6 and 9 days)to antioxidant activity on single garlic pickle. The growth of *L.plantarum* B1765 was measured as Total Lactic Acid Bacteria (LAB) by Total Plate Count (TPC) using the MRS medium, and antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging methods, expressed as inhibition activity (%). Fermentation process along 9 days showed that *L.plantarum* B1765 growth on single garlic pickles for 1 log cycles from 10^6 CFU/mL to 10^7 CFU/mL, pH reduces from 5.6 to 4.4, closely related to increasing of TAT from 0.16 % to 0.28%. The fermentation process also showed an increase in the inhibition activity along the fermentation process from 10,67% to 11.17% and showed a higher inhibition than control (3.88%-7.39%). Single pickled garlic can be potentially developed as natural antioxidant health food.

Keywords

pickle, single garlic, alliin, antioxidant

1 Introduction

Garlic (*Allium sativum*) had been used as a traditional medicine in many cultures. In Indonesia, the use of single clove garlic is more familiar to be used for medicinal purposes. The health benefit of garlic is based on its properties as antimicrobial, antiprotozoal, antimutagenic, antiplatelet, antihyperlipidemic as well as antioxidant [1]. Antioxidants are found as a health-protecting factor. The organosulfur called alliin (S-allyl-cysteine sulfoxide) had demonstrated as a responsible antioxidant compound in garlic [2,3]. Fresh garlic cloves contain about 6 to 14 mg/g of alliin (0.6%-1.4% fresh weight) and account for approximately 80% of cysteine sulfoxides in garlic [4]. Unfortunately, alliin is not a stable compound, it changes easily to allicin that is caused by Alliinase activity when garlic cloves are chopped, crushed, or processed.

A technology process is needed to reduce the Alliinase activity so that the availability of alliin as an antioxidant compound could be retained. The activity of Alliinase enzyme could be inhibited by reducing pH, so as a protein, Alliinase will be denaturated at low pH and will lose its biological function. Lactic fermentation is well-known to produce a low pH as a result of glucose that is metabolized into lactic acid by lactic acid bacteria (LAB). The source of glucose in garlic is inulin [5] that can be metabolized by lactic acid bacteria to lactic acid to produce low pH and to inhibit Alliinase activity so that retained the alliin as an antioxidant. Fermentation of garlic in kombucha has IC50 factor for antioxidant activity was 10.25 %

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higher than fresh garlic [6] Some study showed that many strains of LAB growth well on inulin medium [7] Stimulation of fructan and inulin gave a high growth of exogenous *Lactobacillus plantarum No. 14 LP14* [8].

The objective of this research is to evaluate the growth of *L. plantarum* B1765, one strain of LAB, in the single garlic medium, resulting pH, and antioxidant activity of single garlic pickle compared to fresh garlic. The expected contribution of this research was to give information about single garlic pickles as a fermented product that potential to retain the antioxidant activity than of unfermented single garlic. The outcome of this study was to provide available data regarding the potency of pickle single garlic as a natural antioxidant.

2 Methods

2.1 Preparation of the culture starter

Isolate of bacteria *L. plantarum* B1765 from frozen stock glycerol and de Man, Rogosa, Sharpe (Oxoid) (1:1) at temperature 2°C was reconditioned to room temperature at 28°C. One percent of the isolate was inoculated to MRS broth (Oxoid) and incubated at 37^{0}C for 20 h in an anaerobic state. The suspension then was centrifuged (Eppendorf) for 15 minutes at 3500 rpm, the supernatant was discarded and the pellet was suspended in 50 mL of NaCl 0.85% sterile used as a culturing starter of LAB bacteria for single garlic pickle.

2.2 Preparation of the single garlic pickle

Single garlic was bought from a local market in Mojosari City, East Java, Indonesia. About 150 g of single cloves were weighted and moved into 500 ml sterile glass jars. Sterile aquadest then added until it covered the cloves in the jar and about 5% of NaCl (w/v) then was also added. *L.plantarum* B1765 of 5% (v/v) then was inoculated to the jar and lid was closed. The containers were covered with papers; jars were stored at room temperature for 3, 6, and 9 days fermentation.

2.3 Enumeration of total LAB

Enumeration of LAB was done by modification method of using enumerate the viable cells in the homogenate samples by serially diluted and plated aseptically using the poured method on solid MRS agar (Oxoid) that contains 1% CaCO3, and incubated anaerobically for 48 h at 37 °C. LAB growth was expressed in colony-forming units per milliliter (CFU/mL).

2.4 pH

pH was measured using an electronic pH meter (ATC Pen type).

2.5 Antioxidant activity

DPPH (Merck-USA) was prepared at a concentration of 2% (w/v) in ethanol. Different concentrations of extracts were prepared, and 2 ml taken in separate test tubes. About 2 ml of DPPH solution was added in each test tube and these solutions were kept in dark for 30 min. All the samples were tested in triplicate. Later optical density was recorded at 517 nm using spectrophotometer UV-VIS (Shimadzu 1800) Distilled water with DPPH (Merck–USA) was used as blank. Inhibition of DPPH activity% = (A-B/A) ×100 Where A= optical density of blank; B=optical density of sample.

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3 Results and Discussion

3.1 The growth of *L. plantarum* B1765, pH and TAT

Single garlic was known as a source of inulin. The use of *L. plantarum* B1765 as the starter culture to ferment single garlic was intended to ferment inulin in single garlic to produce low pH so could inhibit the Alliinase enzyme and the alliin as an antioxidant could be retained. The result showed that *L. plantarum* B1765 could growth in single garlic. One-way ANOVA represented that the fermentation time gave a significant effect to the number of LAB ($p<0.05$).

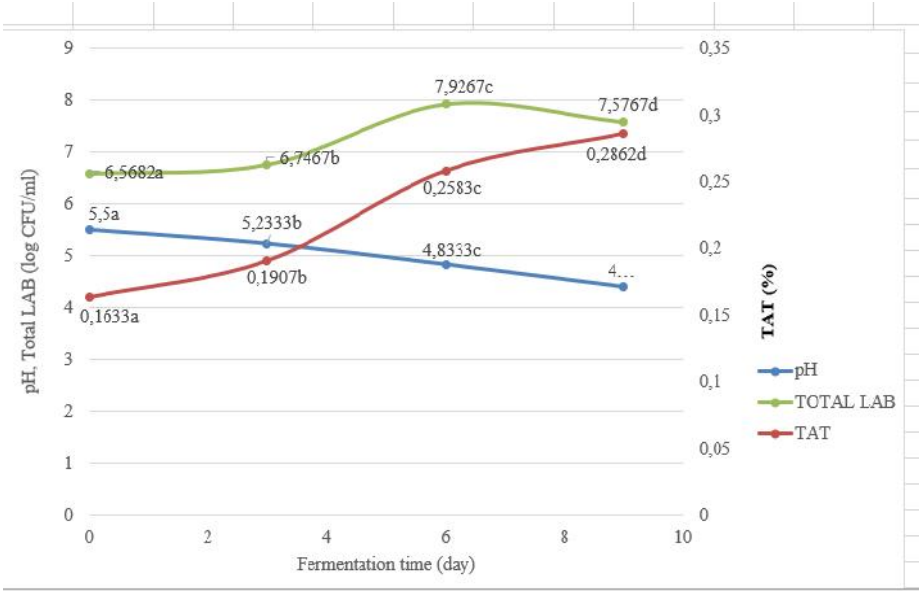

Fig 1. Value of pH, total LAB, and TAT at different fermentation time of single garlic pickle

Note: The upper-case letter on the figure indicates significantly different ($P<0.05$).

The growth of *L. plantarum* B1765 was fermentation time-dependent as listed in **Fig 1**. There was no increase of the number of *L. plantarum* B1765 from 0 days (6,5682 log CFU/ml) to 3 days fermentation, then an increase about 1 log cycle from 3 days (6.75 log CFU/ml) to 6 days (7. 93 log CFU/ml), but it was not significant for 6 days to 9 days of fermentation. The increasing number of total LAB was followed by decreasing in pH and increasing of TAT that all differ significantly for all the time of fermentation. From these data, it was known that single garlic pickle is a good medium for the growth of *L. plantarum* B1765. Inulin in single garlic was assumed as the source for the growth of *L. plantarum* B1765. Inulin is well known as the best source as prebiotics [9]. As dietary fiber inulin is not digested by digested enzyme and delivered to the colon, therefore, it can be used selectively by gut microflora as the growth medium. Inulin is degraded to FOS, glucose and fructose that stimulated the growth of bacteria including probiotics bacteria and usefully for the health of the host [10].

3.2 Antioxidant activity

Antioxidant of single garlic pickle had been evaluated by free radical scavenging activity methods using the stable radical 2, 2-diphenyl-1-picrylhydrazyl (DDPH) and expressed as % inhibition to DDPH. The determination of % inhibition was done using a spectrophotometric assay at wavelength 517 nm. **Fig 2** presents the percent of DPPH inhibited by single garlic pickle samples from various fermentation times (3 days, 6 days and 9 days).

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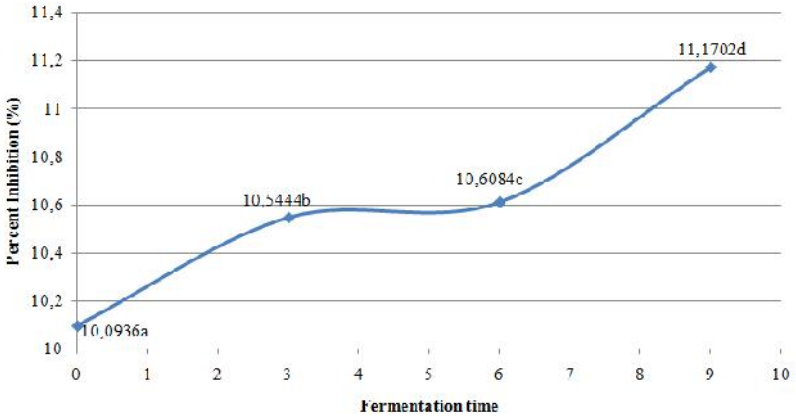

Fig 2. Percent inhibition of single garlic pickle at different fermentation times

Note: The upper case letter on the figure indicates significantly different (P <0.05).

One-way ANOVA shows that percent inhibition of DDPH was fermentation time-dependent ($p<0.05$). The percent inhibition increased by increasing fermentation time. The percent inhibition was 10.54%, 10.61% and 11.17% for 3, 6 and 9 days of fermentation respectively. This value was higher than the percent inhibition of control (3.88%- 7.39%). It is concluded that the fermentation process has a better inhibition activity than control. The fermentation process results in a lower pH that inhibits the Alliinase activity. Therefore, alliin compounds as an antioxidant could be retained and the percent inhibition to DDPH increased. Alliin as antioxidants had been studied in widely research [11]. The IC50 value of kombucha after fermentation with garlic was 0.14 ml which is 46.15% lower than fresh kombucha (0.26 ml); also vinegar after fermentation with garlic had an IC50 value of 0.15 ml which is 76.56% lower than of fresh vinegar (0.64 ml) [6].

4 Conclusion

Single garlic pickle has potential as a natural antioxidant. The percent inhibition of DDPH showed a higher value than the control of unfermented single garlic.

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