The Effect of Garlic (Allium sativum) on Inhibition of Escherichia coli Bacteria in White Tofu

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Abstract

White tofu is made from soybean seeds which has high protein content of 7.06 gram per 100-gram tofu and water of 8488%. The nature of tofu which contains much water and protein is a good medium for bacterial growth. Bacteria like Escherichia coli can cause spoilage in white tofu. High protein in white tofu has a short storage time so that it requires preservatives to inhibit the decay process. One of the natural ingredients which has antibacterial ability is garlic (Allium sativum). Allicin is the most potent compound contained in garlic (Allium sativum) which has antibacterial and fungal abilities. Thus, garlic (Allium sativum) can act as a preservative for white tofu. This study aimed to determine the effect of various concentrations of garlic (Allium sativum) on the inhibition of Escherichia coli bacteria in white tofu at room temperature storage. This study was a laboratory experiment with a completely randomized design. Data were obtained by counting the number of bacterial colonies using the SPC method. The data were analyzed by descriptive method. Meanwhile, in the samples inoculated on EMB agar, colonies which matched with Escherichia coli characteristics were still found especially starting at the 48th hour of storage. It can be concluded that there was an effect of differences in the concentration of garlic (Allium sativum) soaking water at 0 to 48 hours on the growth of Escherichia coli colonies marked by the absence of Escherichia coli colonies on EMB agar. There was an effect of garlic (Allium sativum) on inhibition of Escherichia coli bacteria in white tofu.

Keywords: Garlic, Allium sativum, Inhibition, Escherichia coli, White Tofu

1. Introduction

White tofu is a popular food in Indonesia. Many people demand it because it is cheap but nutritious [1]. According to the USDA (United States Department of Agriculture), white tofu is made from soybean seeds which has a high protein of 7.06 gram in 100 gram of tofu and a moisture content of 84-88%. The nature of tofu which has high water and protein contents is a good medium for bacterial growth [2]. Bacteria such as Escherichia coli can cause spoilage in white tofu [1,2]. One of the quality requirements for tofu can be seen from the contamination of Escherichia coli [3]. Escherichia coli can cause urinary tract infections and diarrhea [4].

High protein content in white tofu results in short storage time so it requires preservatives to extend the storage time by inhibiting the spoilage process [5]. One of the natural ingredients which have antibacterial ability is garlic (Allium sativum). Allicin compound is the most potent compound contained in garlic (Allium sativum) which has antibacterial and fungal abilities [6]. Thus, garlic (Allium sativum) can act as a preservative in white tofu [7]

2. Methods

This was a laboratory experimental research with a completely randomized design. The independent variables were garlic soaking water with concentrations of 0%, 30%, 40%, 50%, and 60%. The dependent variable was the amount of growth of Escherichia coli bacteria in white tofu.

2.1 Preparing White Tofu

The processes of white tofu preparation which includes (1) Sorting and washing soybeans, (2) Soaking soybeans for 12 hours in water with ratio of 1 (water): 2 (soybean), (3) Peeling soybeans and washing them again, (4) Milling soybeans with the addition of hot water (T±80oC) with a ratio of water and soybeans 4:1, (5) Filtering soybean slurry using a filter cloth so that the pulp and filtrate are separated, then the filtrate is boiled, (6) Boiling is carried out for 5 minutes, (7) Clumping with the addition of vinegar with a concentration of 25% as much as 20 ml, (8) Filtering the tofu curd with a filter cloth placed on the tofu printer, (9) Printing and pressing the tofu curd [2].

2.2 Preparing Garlic Soaking Water

Garlic was peeled and weighed with a mass of 0g, 45g, 60g, 75g, 90g, respectively. Then, it was added by 150ml, 105ml, 90ml, 75ml, 60ml of water, respectively. After that, it was mashed using a blender and then poured into the tofu storage container according to the treatment label. This resulted in a concentration of 0%, 30%, 40%, 50%, and 60% garlic soaking water.

2.3 Preparing Eosin Methylene Blue Agar

EMB Agar was weighed at 37.5 grams on a watch glass with an analytical balance, then it was put into an Erlenmeyer and added with 1 L of distilled water. Then, it was stirred using a spatula. The Erlenmeyer is covered with cotton and heated on a hot plate until it boiled. After that, the Erlenmeyer was covered with aluminum foil and sterilized by autoclaving at 121oC at 1 atm pressure for 15 minutes.

2.4 Sample Dilution

1g of tofu sample was taken from each treatment container and placed in a test tube containing 9 ml of sterile distilled water (10-1 dilution). Then, it was homogenized with a vortex for a few seconds until it looked homogeneous. The sample in the test tube was taken as much as 0.5 ml using a micropipette and then transferred to another test tube containing 4.5 ml of sterile distilled water (10-2 dilution) [8].

2.5 Inoculation of the sample on the medium

Samples in the 10-1 and 10-2 dilution test tubes were taken as much as 1 ml and each was inoculated in a sterile petri dish. The pour plate technique was used in inoculation process. The purpose of using this technique was to determine the approximate number of live bacteria expressed in the number of colonies. This technique was carried out by pouring the sample first and then followed by pouring the media into a petri dish. The media was poured after being sterilized by autoclaving and allowed to cool to a temperature of 4045oC [9]. After the sample and media are poured, it was homogenized by shaking the petri dish in a circular motion. Then, it was incubated at 37oC for 24 hours.

2.6 Colony Counting with the Standard Plate Count

Colonies were counted in the following way: 1) it was carried out on dishes with the number of colonies between 30-300; 2) several large colonies with doubtful colony counts were counted as one colony; and 3) a row (chain) of colonies seen as a line was counted as one colony. Data reporting according to SPC standards followed these rules: 1) the results consisted of two numbers namely the first number in front of the comma and the second number after the comma, if the third number is equal to or greater than five, it was rounded up one digit higher; 2) if the culture results of all dilutions yielded less than 30 colonies on a petri dish, count only on

the number of colonies at the lowest dilution; 3) if the culture results of all dilutions yielded more than 300 colonies on a petri dish, count only on the number of colonies at the highest dilution; 4) if the cup from two dilution levels produced colonies with an amount between 30-300, then compare the results of the calculation of the number of colonies per ml from the two dilutions. If the comparison result was 2, then it was reported using the smallest number of colonies per ml, but if the comparison was 2, it was reported using the average number of colonies per ml from both dilutions. 5) if using two petri dishes (duplo) per dilution, the data were collected from both plates [10].

3. Result

3.1 Escherichia coli Colony Morphology

At 24 hours in 0% sample (soaking water without garlic), colonies with characteristic round, slightly convex surface, and dark purple color with metallic green luster were seen. According to [11], this colony is in accordance with the characteristics of the Escherichia coli colony so that Escherichia coli colonies began to appear since the 24th hour of storage in 0% samples (water immersion without garlic).

3.2 Microscopic morphology of Escherichia coli

The result of gram staining on 0% sample of Escherichia coli colonies obtained gram-negative rods. This is in accordance with [12] who mentioned that Escherichia coli is a gram-negative rod bacterium. Gramnegative bacteria have a pink color due to their thin peptidoglycan layer and thick lipid layer so that the crystal violet dye which is mostly attached to the lipid layer will fade after rinsing with alcohol. This is due to the alcohol-soluble lipid layer resulting in the disappearance of the crystal violet dye attached to the lipid layer. After being washed off by alcohol, the sample is given a safranin dye so that it will appear pink [13].

3.3 Escherichia coli colony calculation results

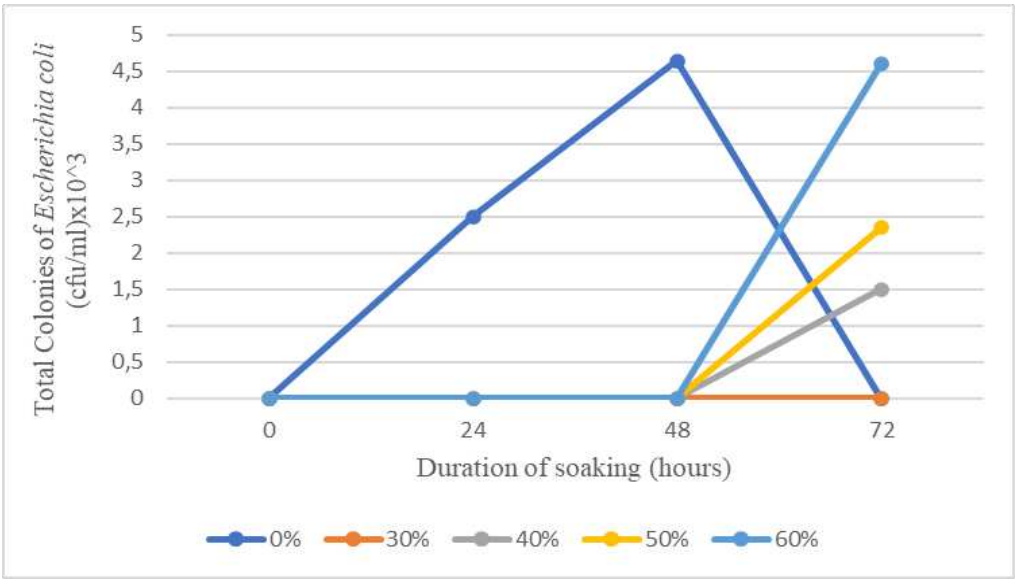

Fig. 1. Graph of total colonies of Escherichia coli on white tofu

The graph shows the growth activity of Escherichia coli colonies from 0 to 72 hours. In the 0% sample, the number of Escherichia coli colonies was 2.5 x 103 CFU/ml at the 24th hour and increased to 4.65 x 103 CFU/ml at the 48th hour, while in the other samples there was no colony growth activity at the 48th hour. At 72 hours, it was obtained 4.6 x 103 CFU/ml in the 60% sample; 2.35 x 103 CFU/ml in 50% sample; and 1.5 x 103 CFU/ml in the 40% sample. However, Escherichia coli colonies were not found in 0% and 30% samples. Thus, it can be concluded that no Escherichia coli colonies were found from 0 to 72 hours in 30% samples.

4. Discussion

An increase in the number of Escherichia coli colonies occurred in the 0% sample as much as 2.5 x 103 CFU/ml at the 24th hour and 4.65 x 103 CFU/ml at the 48th hour. This can be caused by water immersion in white tofu which can support the growth of Escherichia coli. The growth of microorganisms is affected by humidity and water content. Bacteria and fungi require humidity of more than 85% for their growth [14]. Therefore, Escherichia coli can immediately experience an exponential growth phase. While the samples of 60%, 50%, and 40% did not get colony growth activity. This is because there is an inhibition of the growth of Escherichia coli colonies by the antibacterial agent allicin and essential oil in Allium sativum.

At 72 hours, 60%, 50%, and 40% samples were found to have Escherichia coli colonies. This can be caused by the antibacterial ability of Allium sativum which decreases over time [15]. In addition, it can also be caused by bacteria which entered the exponential phase. In contrast to the 0% sample, Escherichia coli colonies in the 0% sample decreased until no colonies were perceived on EMB agar. This could be due to the bacteria in the 0% sample having lost viability or at the end of their life phase which is called the death phase [16]. Prior to the death phase, bacteria undergo a stationary phase which is characterized by the depletion of nutrients and the accumulation of toxic products. Thus, bacterial growth stops completely [4].

In the 30% sample, no Escherichia coli colonies were found for 0 hours. This could be due to the lack of storage of white tofu, so the bacteria are still in the adaptation phase. The adaptation phase can last from a few hours to a few days. In addition, it can also be caused by the content of allicin and essential oils which are antibacterial substances contained in Allium sativum so that it can inhibit the growth of Escherichia coli bacteria [17].

5. Conclusion

Based on this study, it can be concluded that there is an effect of various concentrations of garlic (Allium sativum) on the inhibition of Escherichia coli in white tofu characterized by the absence of Escherichia coli colonies on EMB agar from 0 to 48 hours in all samples of garlic soaking water with a certain concentration.

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