Inhibitory power of young coconut fiber ethanol extract (Cocos nucifera Linn) on the growth of Bacteria staphylococcus aureus and Escherichia coli in tofu

N K Sumarni 1*, Rahmawati 1 and Ruslan 1

1Department of Chemistry, Faculty of Mathematics and Natural Science, Tadulako University, Palu, Indonesia

*E-mail: syahparawan@gmail.com

Abstract. Analysis of the growth of bacteria Staphylococcus aureus and Escherichia coli has been carried out on tofu that is soaked in ethanol extract of coconut coir (Cocos nucifera Linn). This study aims to determine the lowest total growth of the bacteria Staphylococcus aureus and Escherichia coli in tofu that is immersed in ethanol extract of young coconut fibre-based on variation in concentration and storage time. Young coconut coir is extracted using 96% ethanol and applied in various concentrations from 1000 to 7000 ppm with a soaking time of 24, 48, 72, and 96-hour, respectively. The total bacteria in the tofu from each treatment was calculated by the total plate count method. Based on the results obtained, it can be seen that the lowest total growth of Staphylococcus aureus is 3751 CFU/g, which is immersed at a concentration of 7000 ppm for 24-hour. As for the Escherichia coli bacteria as much as 4737 CFU/g, which was soaked at the same concentration for 96-hour.

1. Introduction

Coconut is a tropical plant that has long been known by the people of Indonesia, its spread in almost all parts of the archipelago. Coconut plants become a strategic commodity that has a social, cultural, and economic role in the lives of Indonesian people. The benefits of coconut plants not only lie in the flesh of the fruit, which can be processed into coconut milk, copra, and coconut oil, but all parts of the coconut plant have great benefits. The main reason that makes coconut a commercial commodity is because all parts of the coconut can be used for various purposes so that the coconut tree is dubbed the Tree of life [1].

Young coconut fruit is one of the unique coconut tree products because in addition to the flesh of the fruit, it can be consumed coconut water directly can also be drunk directly without undergoing processing. The uniqueness can be supported by the physical properties and chemical composition of water and young coconut meat, so that it is very popular with consumers, including children and adults. On the other hand, the part of the young coconut that has not received serious attention in its processing is the coir part, which so far has only been used as firewood and allowed to accumulate lead to environmental hazard pollution.

Young coconut coir is the largest component of coconuts, the outer portion of the coconut which encloses the coconut shell. Coconut coir thickness ranges from 5-6 cm consisting of the outer layer (exocarpium) and the inner layer (endocarpium). Young coconut coir consists of fiber and cork that connects another fiber. Each coconut has 0.4 kg of coir, which consists of 30% fiber.[2] Young coconut coir contains tannin or tannic acid compounds around 4.28%, while the young coconut cork contains...
tannin compounds about 5.62%. Tannins have antibacterial activity against Escherichia coli and Staphylococcus aureus [3-4], the mechanism of inhibition of tannin compounds as antibacterial can occur by shrinking the bacterial cell membrane walls, disrupting bacterial cell permeability and damaging bacterial membranes so that growth inhibited bacteria [5]. It can be the basis in optimizing the utilization of young coconut coir extracts such as preservatives of tofu.

Tofu is a processed soybean product having a protein content of about 45.88 - 48.83% [6]. In addition to having advantages, tofu also has weaknesses, namely high water content (80% -88%), so that is easily overgrown with microbes [7]. Protein content that is high enough in tofu causes tofu, including products that are easy or fast to rot. Damage to tofu is caused by contamination by proteolytic bacteria (breaking down proteins). Changes in the number of germs in tofu during immersion can increase, but the effectiveness of decreasing the number of germs can reach 20%. According to [8], the threshold of microbial contamination in tofu was 4.3 x 105 CFU/g. [9], also reported that in the ethyl acetate extract of coconut coir, there were catechin and flavonoid compounds had antibacterial activity.

Natural antibacterial compounds can be obtained through the extraction process of plant parts. Some examples of natural antibacterials that can be used as food preservatives include wood smoke containing phenol and formaldehyde essential oils spices, and nisin produced by Lactococcus lactis [10]. Ethanol and water extracts from Eugenia jambos have antibacterial properties against bacteria such as: Staphylococcus aureus Yersenia enterocolitica, Staphylococcus hominis, Staphylococcus cohnii, Staphylococcus warneri[11]. The ethanol extract of Salvia pratensis leaves can inhibit Escherichia coli, Bacillus cereus and Saccharomyces cerevisiae [12].

That it has examined 28 types of plant parts from 24 species [13]. The results showed, 5 plant species that have the potential to be developed as an antibacterial source for food, namely Caesalpinia sappan, Thea sinensis, Rhus javanica, Pinus densiflara, and Prunus mume. Ethanol extracts from these plants have been tested for their ability to inhibit the development of the pathogenic bacterium Bacillus subtilis. Plants can synthesize various types of bioactive compounds that can act as antibacterial, including phenol compounds and their derivatives, terpenes, and terpenoids, alkaloids, polypeptides, and steroids [14]. It can be the basis for optimizing the utilization of young coconut coir extracts such as tofu preservatives.

Tofu is a food product in the form of soft solids made through soybean processing by the deposition of protein, having a protein content of about 45.88 - 48.83% [15]. In addition to having advantages, tofu also has weaknesses, namely highwater content (80% -88%), so that is easily overgrown with microbes [7]. Protein content that is high enough in tofu causes tofu, including perishable products that can last for 1-2 days, after which the tofu turns to acid and changes in aroma, color, and texture, so it is not suitable for consumption [16]. Damage to tofu is caused by contamination by proteolytic bacteria (protein breakers). According to [8], the threshold of microbial contamination in tofu is as much as 4.3 x 105 CFU/g. Efforts to extend the shelf life of tofu and suppress the growth of bacteria in tofu can be preserved but so far some people use formalin in the preservation of tofu even though the use of formalin in food can cause poisoning in the human body even in the long term can trigger the growth of cancer cells and disruption of the kidney.

Based on the background above, tofu preservation can be done naturally in coconut coir ethanol extract. This study aims to determine the inhibition of ethanol extract of young coconut fiber in inhibiting the growth of Staphylococcus aureus and Escherichia coli on tofu based on variations in the extract concentration.

2. Materials and methods

The basic ingredients used in this study were young coconut coir (green coconut type), 96% ethanol, aquades, tofu, Nutrient Agar (NA), aluminum foil, tissue, plastic wrap, and cotton.

Equipment used in the form of a blender, analytical balance, oven, rotary vacuum evaporator, UV-Vis spectrophotometer, spray bottles, dropper pipette, agitation shaking machine, Erlenmeyer 250 and 500 mL, magnetic stirrer, funnel, scissors, basin, 60 mesh sieve, beaker 1 L, beaker 100, 250, and 500
mL beaker, micropipette, petri dish, test tube, test tube rack, glass funnel, autoclave, incubator, laminar, bunsen.

2.1 Preparation of samples
Coconut coir used is young coconut coir taken from one coconut tree, and the sample is dried in the sun to remove the water content (water content between 5-10%) to dry (2-7 days), then mashed into powder and sieved with a 60 mesh sieve. The sample in powder form is then extracted with 96% ethanol.

2.2 Sample extraction [11]
Weighted 250 grams of dried coconut fiber samples and immersed in 5 L of ethanol solvent for 72-hour. Then extract using Whatman filter paper number 1. The filtrate obtained was evaporated using a rotary vacuum evaporator until a concentrated extract was obtained, then the yield was determined and carried out for further analysis.

2.3 Ethanol extract application in tofu
Concentrated extract was dissolved in distilled water so that the solution was obtained according to variations in concentration from 1000 to 7000 ppm with an increase of 1000 ppm. Tofu with a size of 20 grams is soaked in each variation of the concentration of the solution for 24, 48, 72, and 96 hours. Next, an analysis of total growth of Staphylococcus aureus and Escherichia coli bacteria in tofu has been immersed during this time.

2.4 Preparation of total microbial test material [19]
A total of 56 grams of nutrient agar (NA) was dissolved in 2000 mL of distilled water, then sterilized in an autoclave at 121°C at a pressure of 1 atm for 15 minutes. Then cooled and stored in the refrigerator for later use in the total microbial test.

2.5 Test the total microbes by the Total Plate Count method [19]
Test tubes are provided to make a dilution of the sample to be examined, ranging from $10^{-1}$ to $10^{-3}$ mL. A total of 1 g of tofu sample was dissolved in distilled water to 10 mL. Next, in a pipette of 1 mL and put into the first test tube and added 9 mL of distilled water and then shaken, so that the concentration of the first test tube is $10^{-1}$ mL. Next, 1 mL of solution is taken from the first test tube into the second test tube and shake until homogeneous, and then the second tube concentration becomes $10^{-2}$ mL. And so on so that the suspension is obtained to a dilution level of $10^{-3}$ mL. Nine petri dishes were provided containing agar nutrient medium and were marked according to the order of dilution of the test tube, and then each tofu sample was taken from the three tubes and put into a petri dish which already contained the medium. Furthermore, it was incubated at 37°C for 24 hours. Analysis of total bacteria was carried out by the total plate count (TPC) method. The number of bacterial colonies growing at each sample dilution was calculated using the following equation:

$$\text{Number of colony} = \sum \text{colony}_{cup} \times \frac{1}{\text{df}} \times \sum \text{Inoculum}$$  \hspace{1cm} (1)

Where, df = dilution factor

2.6 Organoleptic test
Organoleptic test conducted on tofu that was soaked in coconut fiber ethanol extract was a hedonic quality test and descriptive test. The parameters observed included aroma, color, and texture. Panelists were given containers containing tofu that had been soaked with coconut fiber ethanol extract. Hedonic quality test values use a range of 1-7 for each attribute. For example aroma test, for value = 1 very
dislike, 2 = dislike, 3 = rather dislike, 4 = neutral, 5 = somewhat like, 6 = like and 7 = very like. The test was conducted using 30 panellists of students who were randomly selected.

3. Results and discussion
3.1. Yield presentation of ethanol extract
Young coconut coir ethanol extract was obtained by maceration extraction at room temperature for 3 x 24 hours. The results were obtained by the extraction of young coconut coir ethanol extract by 12.43% of 250 g of coconut fiber. The yield presentation obtained is higher when compared to the results obtained by [20], who carried out a maceration extraction process for 24 hours at 40°C obtained an extract yield of 5% from 300 g of coconut fiber. The immersion time of the sample in the extraction process took longer, causing the active compounds in the sample to dissolve more in the solvent so that the yield produced was also higher [18].

3.2. Testing of microbial inhibition
The results of the total microbial analysis in tofu soaked in coconut coir of ethanol extract solution as shown in Figure 1 and Figure 2, show that the higher the concentration of the solution, the less the total bacteria Staphylococcus aureus and Escherichia coli are in tofu. The higher the concentration, the less the number of bacteria that can survive [21].

![Figure 1. The number of Staphylococcus aureus colonies from various concentrations of extracts](image1)

![Figure 2. The number of Escherichia coli colonies from various concentrations of extracts](image2)

From the above figures, it can also be seen that the duration of soaking in each variation of the extract concentration can affect the contamination of the two bacteria found in tofu. The extract concentration that most inhibited the growth of Staphylococcus aureus was obtained at a concentration of 7000 ppm with a 24-hour immersion time of 3751 CFU/g. As for the growth of Escherichia coli bacteria, the most
inhibiting extract concentration was obtained at a concentration of 7000 ppm, with 96 hours of immersion time of 4737 CFU/g. The threshold of microbial contamination in tofu that is still safe to consume is $5 \times 10^4$ CFU/g (according to SNI 2009 number 7388), and $4.3 \times 10^5$ CFU/g [8]. Thus tofu that is soaked with 7000 ppm young coconut coir of ethanol extract solution for 96-hour is still suitable for consumption because it does not exceed the specified microbial contamination threshold value.

ANOVA analysis results showed that ($\alpha = 0.05$) ethanol extract of coconut coir based on the time of tofu immersion on the growth of Staphylococcus aureus bacteria was not significantly different because the significant value was $0.660 < \alpha (0.05)$, so it could not be continued with the Duncan test.

For Escherichia coli in tofu, it shows that the growth value of bacteria that is not significantly different is the significant value of $0.988 < \alpha (0.05)$. However, the highest total growth of *Escherichia coli* in tofu was found at 96-hour immersion with a total bacterial growth of 27883.42 CFU/g. In contrast, the ethanol extract of coconut coir from variations in concentration on the growth of Escherichia coli bacteria showed no significant difference in the number of bacterial growth, i.e. sig value $0.774 < \alpha (0.05)$. The total growth of Escherichia coli bacteria which is mostly obtained in tofu that is immersed in ethanol extract solution with a concentration of 1000 ppm, is 33826.94 CFU/g, but it is still safe for consumption.

The difference in inhibition of antibacterial compounds against Gram-positive and Gram-negative bacteria is due to the cell wall structure of the two types of bacteria are different [22]. The cell wall of Gram-positive bacteria (*Staphylococcus aureus*) consists of several layers of peptidoglycan, which forms a thick and rigid structure and contains cell wall substance called treatic acid. In contrast, the cell wall of gram-negative bacteria (*Escherichia coli*) consists of one or more thin layers of peptidoglycan so that the cell wall of Gram-negative bacteria is more susceptible to physical shocks, such as the addition of antibiotics or other antibacterial agents.

### 3.3. Organoleptic quality

Descriptive sensory analysis is a sensory analysis method where the sensory attributes of a product food ingredient are identified. This analysis can be done for all sensory parameters and several aspects in determining the shape of the taste or texture profile. The parameters can be a variety of terminology both about attributes, characteristics, and product quality. Descriptive sensory analysis of tofu samples by immersing coconut coir ethanol extract can be described by product appearance, aroma, color, and texture of the tofu sample. Panellist response to sensory analysis in organoleptic testing involving 30 panellists.

#### 3.3.1. Aroma

Aroma is one of the analysis parameters used to classify the level of liking of tofu. During 1 hour of storage, there must be a change in an aroma that can change the level of liking for tofu, so it is necessary to test the aroma of tofu to find out the sample of tofu whose aroma is preferred as described in Figure. 3.

The aroma can be defined as something that can be observed with the sense of smell. In the food industry, testing of odors is considered important because it can quickly provide an assessment of the product regarding whether or not the product is received. In addition, the odor can also be used as an indicator of damage to the product [23]. Taste and aroma arise due to natural and synthetic chemical compounds and the reaction of these compounds with the nerve endings of the tongue and nose [24]. The panelist’s response to the aroma of tofu that was most chosen was at concentrations of 3000 ppm and 5000 ppm at 4.66, and the least was chosen at concentrations of 1000 ppm and 2000 ppm, which was 3.63.
3.3.2. Color. Determination of the quality of a food ingredient in general, is very dependent on the aroma, color, and texture, but the main factor is the color that, determines consumer acceptance and provides a clue about chemical changes in food.

The panelist's response to the color of tofu can be chosen in Fig. 4. It appears that the most chosen is the control sample code of 4.73, and the least one chooses it is at a concentration of 5000 ppm of 3.86. Color is a material property that originates from the spread of the spectrum of light, as well as the gloss of material affected by reflected light [25]. Color is not a substance or substance but a person's sensation because of the stimulation of a beam of radiation energy that falls into the sense of sight. If a food or product has an attractive color, it can cause a person's taste to try the product because the color is one of the visual profiles that becomes, the first impression of consumers in assessing a product.

3.3.3. Texture. Food texture is a collection of a number of different characters and it is felt by various members of the human body [2]. Texture is a matter that must be considered in determining the quality of tofu. The quality of tofu texture can be seen in Fig 5.

The panelist's response to the tofu texture was the most chosen at a concentration of 2000 ppm of 4.86 and the least at a concentration of 6000 ppm of 3.9.
4. Conclusion
Based on the results of research that has been done, it can be concluded that the ethanol extract of young coconut fiber can inhibit the growth of bacteria in tofu. The lowest total growth of *Staphylococcus aureus* bacteria was 3751 CFU/g, which was soaked with a 7000 ppm ethanol extract solution for 24-hour. As for the *Escherichia coli* bacteria as much as 4737 CFU/g which was soaked with 7000 ppm ethanol solution for 96-hour.

Acknowledgement
Thank you the Head of Chemistry Department at Tadulako University and the Chemical Students Association for their supports and assistances.

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