Comparison Effects Of Seaweed Concentrations On Total Bacteria And Yeast Kombucha *Gracilaria Verrucosa* During The Production Process

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**Abstract.** Yeasts and bacteria used in the fermentation of kombucha *Gracilaria verrucosa* require nutrients to be able to grow and function optimally. The carbohydrate content of seaweed *Gracilaria verrucosa* is one of the factors that affect the growth of bacteria and yeasts that play a role in producing kombucha beverage products. The purpose of this study was to determine the effect of comparison of seaweed concentrations on total bacteria and yeast kombucha during the production process. The method used in this study is an experimental method with a completely randomized design (CRD) with six treatments and 4 replications. The result of this research is that the concentration of seaweed affects the total bacteria and yeast during the kombucha production process. Tests on the total bacteria showed that the total number of bacteria treated by P0 increased up to day 3 with the highest total bacteria at 1.7x10⁷ CFU/ml. On the 3rd day total yeast also increased, the highest yield of total yeast in treatment P1 was 1.9x10⁷ CFU/ml.

1. Introduction

Indonesia has many types of seaweed, about 555 species of which are known as export commodities, namely *Euchema* sp., *Gracilaria* sp., *Gelidium* sp., and *Sargassum* sp. Many foods and drinks are made from seaweed, one of which is seaweed that is used as a drink such as kombucha. Kombucha is a traditional drink fermented from a solution of tea and sugar using kombucha microbial starter (*Acetobacter xylinum* and several types of yeast, such as *Saccharomyces cerevisiae*) which is fermented for 7-12 days.

Other basic ingredients of kombucha that are high in phenol include bay leaves, soursop leaves, betel leaves, guava leaves and coffee leaves which have been studied by Zubaidah and Wistiana (2015). According to Aryawati and Pratiwi, 2012 that the manufacture of kombucha is made from seaweed. The results obtained that the seaweed *Sargassum* sp. can be used as raw material for making kombucha. However, research on the total bacteria and yeasts that play a role in kombucha using *Gracilaria verrucosa* seaweed as raw material has not been carried out.
Kombucha is a drink-shaped concoction that is the result of a symbiosis of bacteria and yeast. One of the marine biological materials that has high nutritional value and is beneficial for health is seaweed. Seaweed contains carbohydrates (sugar or vegetable-gum), protein, a little fat, and ash, most of which are sodium and potassium salt compounds, vitamins A, B1, B2, B6, B12, and C; Beta carotene; chlorophyll pigment; as well as minerals, such as potassium, calcium, phosphorus, sodium, iron, iodine and antioxidants (Pratiwi, et al., 2011).

Kombucha culture, commonly known as SCOBY (Symbiotic culture of bacteria and yeast) contains various types of microorganisms. The microorganisms in it consist of the bacteria Acetobacter pateurianus, Lactobacillus sp., Pediococcus sp., Gluconacetobacter, Acetobacteriyxinum, Acetobacter aceti, and Gluconabacter. Then there are also several yeasts consisting of Zygossaccharomyces rouxii, Torulopsis, Candida fomata (Crum and Alex, 2016).

The production of kombucha shows that the longer the fermentation, the higher the total acid. This is because during the fermentation process, yeasts and bacteria metabolize sucrose and produce a number of organic acids such as acetic acid, gluconic acid, and glucuronic acid, thereby increasing levels of organic acids (Jasman and Widianto. 2012).

Microorganism activity requires a variety of sources, both sources of C (carbon), sources of N, sources of P, minerals and water that must be present in the media. The carbon source is obtained from the carbohydrate content in seaweed (Ardheniati et al., 2009). There are several types of microbes that require compounds such as oxygen for the growth of microorganisms in the media, vitamins, precursors of fermentation products, and growth factors. Nitrogen sources and carbon sources are important factors that can affect the growth and product of microorganisms (Zuhri et al., 2013). The content of seaweed is needed by microorganisms to be processed for fermentation.

2. Material and methods

2.1. Material

The materials used were seaweed Gracilaria verrucosa from Jabon Sidoarjo, granulated sugar, kombucha starter culture (SCOBY) obtained from UKM Senandung Sejuk Sidoarjo, mineral water, 1% chloramphenicol, 0.9% physiological NaCl diluent, Plate Count Agar (PCA).), Eosin Methylene Blue Agar (EMBA), Lactose Broth (LB), Potato Dextrose Agar (PDA), distilled water, boiling stone, NaOH, Phenolphthalein indicator (PP), pH meter, burette, static, Erlenmeyer tube, measuring flask, and measuring pipette.

2.2. Methods

The method used in this study is an experimental method with a completely randomized design (CRD). The use of RAL is because in this research process all conditions are the same except for the concentration of seaweed used. The treatment in this study consisted of 5 treatments with 4 replications.

2.3. Making Kombucha Drink

The dried Gracilaria verrucosa seaweed was then washed and cut into small pieces. Cooking can be done by mixing 600 ml of mineral water with a concentration of seaweed according to the treatment (3%, 4%, 5%, 6% and 7%) and 5 grams of tea. This seaweed boiling process uses a temperature of 89°C, the time required for boiling is 5 minutes. The decoction is then filtered as much as 500 ml and then put into a glass jar that has been sterilized with 70% alcohol. 100 grams of granulated sugar is then put into the solution and stirred until dissolved. Wait for the solution to a temperature of 30°C, then add 10 grams of nata kombucha (SCOBY) and 10% starter of the steeping volume. The jar was then immediately covered with a white calico cloth and tied with a rubber band.

2.4 Total Bacteria (Total Plate Number)

The total bacterial analysis method used is the plate count method by scattering using PCA (Plate Count Agar) media. The sample of kombucha drink was pipetted as much as 1 ml and put into 9 ml of 0.9% physiological NaCl diluent then homogenized. The homogenized solution was then diluted serially until a
dilution of 10-5 was obtained. The last three serial dilutions were aseptically pipetted 0.1 ml into a petri dish which already contained PCA (Plate Count Agar) media in duplicate, and leveled using a drigalski. The petri dishes were then incubated in an inverted position at an incubator at 30°C for 48 hours. The number of growing colonies was observed and counted. Sampling was carried out on days 0, 3, 6, 9 and 12 of fermentation.

2.5 Total Yeast
The total yeast analysis method used was the cup count method by spreading it using PDA (Potato Dextrose Agar) media. The sample of kombucha drink was pipetted as much as 1 ml and then poured into a test tube which already contained 9 ml of 0.9% physiological NaCl diluent and then homogenized. The homogenized solution was then diluted serially until a dilution of 10-5 was obtained. Then from the last three serial dilutions, 1 ml of dilution was pipetted in duplicate into a petri dish that already contained PDA media to which 1 ml of 1% chloramphenicol solution had been added. The petri dish was then inverted and incubated at 30°C for 48 hours. Sampling was carried out on days 0, 3, 6, 9 and 12 of fermentation.

3. Result and discussion
Microorganisms that play a role in the fermentation process are acetic acid bacteria and several types of yeast. These microorganisms convert the sugar content available in the media into cellulose. The presence of cellulose indicates that seaweed can be used as raw material for making kombucha. This is because the parameters of kombucha products can be fermented or not, namely by the formation of a strong network of cellulose called nata (Suhardini and Zubaidah, 2016).

Total bacteria tended to increase on days 0 and 3 of fermentation. In all treatments the increase in the number of bacteria until the 6th day of fermentation then decreased until the 12th day. The result of total bacteria according to the given seaweed concentration shows that the higher concentration of seaweed has a smaller total number of bacteria.

The duration of fermentation greatly affects microbial activity, especially Acetobacter xylenium bacteria in producing nata/cellulose. Growth can be stimulated from sufficient carbon sources for kombucha microbial life. In seaweed the main nutritional composition is carbohydrates, so apart from sugar, the carbon source also comes from Sargassum sp seaweed which can stimulate microbial growth in seaweed media (Pratiwi et al., 2011).

Table 1. Total Bacterial Value on Fermentation Time 0 days, 3 days, 6 days, 9 days and 12 days.

| Treatment | Total bacteria (CFU/ml) ± SD |
|-----------|-----------------------------|
|           | 0 Day                      | 3 Day                      | 6 Day                      | 9 Day                      | 12 Day                     |
| P0        | 4,5x10^6 ± 0,48a           | 1,7x10^7 ± 0,21a           | 5,1x10^6 ± 0,52a           | 5,9x10^5 ± 0,53b           | 9,8x10^4 ± 0,31bc          |
| P1        | 1,4x10^6 ± 0,44ab          | 4,1x10^6 ± 0,57ab          | 6,0x10^5 ± 0,08c           | 5,0x10^5 ± 0,20ab          | 5,8x10^4 ± 0,14c           |
| P2        | 2,2x10^6 ± 0,52ab          | 2,6x10^6 ± 0,54b           | 6,3x10^5 ± 0,15c           | 2,8x10^5 ± 0,16b           | 9,6x10^4 ± 0,17bc          |
| P3        | 1,8x10^6 ± 0,51ab          | 2,5x10^6 ± 0,44b           | 6,3x10^5 ± 0,15c           | 3,4x10^5 ± 0,12b           | 2,1x10^5 ± 0,18c           |
| P4        | 6,5x10^5 ± 0,07bc          | 2,1x10^6 ± 0,39b           | 2,5x10^5 ± 0,59ab          | 4,7x10^5 ± 0,10ab          | 2,1x10^5 ± 0,13a           |
| P5        | 3,4x10^5 ± 0,03c           | 4,0x10^5 ± 0,26c           | 1,3x10^6 ± 0,27bc          | 1,0 x10^6 ± 0,09b          | 1,7x10^5 ± 0,10ab          |

Notes: Different notation indicates that there is a significant difference between treatments (P<0.05). P1 (3% seaweed), P2 (4% seaweed), P3 (5% seaweed), P4 (6% seaweed), P5 (7% seaweed).
Day 3 has entered the logarithmic phase which is characterized by a significant growth of bacterial cells. The logarithmic phase of microbes will divide rapidly and constantly, the speed of growth is strongly influenced by the medium in which it grows such as nutrient content, pH, as well as environmental conditions including temperature and humidity (Safitri et al., 2016).

Total yeast showed an increase until the 3rd day, then decreased until the end of fermentation. At the beginning of the fermentation, the second treatment had a total yeast in the range of 1.7x10^6 CFU/ml. On the 3rd day of P1 treatment, the total yeast was the most which reached 1.9x10^7 log CFU/ml.

During the increase and decrease in fermentation, changes in the number of cells during fermentation can be influenced by the condition of the medium due to the production of metabolites and the activity of microorganisms, thus affecting the growth and interactions between microorganisms (Loncar et al., 2014).

The increase that occurred on the 3rd day was due to the condition of the medium that was in accordance with the growth needs of the bacteria so as to produce yeast cells that could degrade sucrose with the help of enzymes into glucose and fructose. In the presence of glucose and fructose, they become a source of nutrition for the growth of the bacteria Acetobacteri sp. (Efivani, 2018).

The decrease in the number of cells is due to the decreased pH of the medium and tends to be acidic, this can inhibit the activity of yeast cells and the number of cells will decrease, so that cell density will also decrease (Eric and Jessica, 2013).

Table 2. Total Yeast Value on Fermentation Time 0 days, 3 days, 6 days, 9 days and 12 days.

| Treatment | Total Yeast (CFU/ml) ± SD |
|-----------|----------------------------|
|           | 0 Day | 3 Day | 6 Day | 9 Day | 12 Day |
| P0        | 4.8x10^6 ± 0.33^a | 1.6x10^7 ± 0.09^a | 3.2x10^6 ± 0.57^a | 7.1x10^5 ± 0.17^a | 6.5x10^4 ± 0.25^b |
| P1        | 3.1x10^6 ± 0.61^ab | 1.9x10^7 ± 0.26^a | 2.1x10^6 ± 0.48^ab | 4.4x10^5 ± 0.17^ab | 1.2x10^5 ± 0.15^b |
| P2        | 1.7x10^6 ± 0.48^ab | 2.2x10^6 ± 0.51^b | 1.2x10^6 ± 0.23^ab | 7.8x10^5 ± 0.10^b | 3.4x10^5 ± 0.11^a |
| P3        | 3.3x10^5 ± 0.58^bc | 6.2x10^5 ± 0.06^c | 5.8x10^5 ± 0.08^c | 3.1x10^5 ± 0.21^bc | 1.3x10^5 ± 0.14^b |
| P4        | 7.2x10^5 ± 0.04^bc | 4.5x10^5 ± 0.31^c | 4.5x10^5 ± 0.10^c | 1.8x10^5 ± 0.14^c | 1.1x10^5 ± 0.33^b |
| P5        | 6.3x10^5 ± 0.07^cd | 3.2x10^5 ± 0.21^c | 6.9x10^5 ± 0.06^bc | 3.8x10^5 ± 0.17^b | 9.8x104 ± 0.35^b |

4. Conclusion

The result of this research is that the concentration of seaweed has an effect on the total bacteria and yeast during the kombucha production process. Tests on the total bacteria showed that the total bacteria treated P0 increased up to day 3 with the highest total bacteria at 1.7x10^7 CFU/ml. On the 3rd day total yeast also increased, the highest yield of total yeast in treatment P1 was 1.9x10^7 CFU/ml.
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