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Comparison Effects of Seaweed Concentrations on Total Bacteria and Yeast Kombucha (*Kappaphycus* Alvarezii) During the Production Process

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Abstract

The comparison of the concentration of *Kappaphycus alvarezii* seaweed is estimated to affect the total bacteria and yeast during the kombucha production process. The carbohydrate content in seaweed can be one of the factors for the formation of nata as a parameter for the success of making kombucha, as well as its effect on nutrition for bacterial and yeast growth. This study aims to determine the effect of comparing the concentration of seaweed on the total bacteria and yeast kombucha *Kappaphycus alvarezii*. The method used in this study is an experimental method with a completely randomized design (CRD) with six treatments and 4 replications. The results of this study indicate that the comparison of the concentration of *Kappaphycus alvarezii* seaweed affects the total bacteria and yeast during the kombucha making process. The total bacteria test showed that the total bacteria increased until the 3rd day and the highest total yield was found in the control treatment, which was $1,6x10^7$ CFU/ml on the 3rd day. Then the total yeast also increased until the 3rd day and the highest yield was found in the control treatment of $2,3x10^7$ CFU/ml.
Keywords: Bacteria, yeast, fisheries, kombucha, *Kappaphycus alvarezii*

Introduction

In the world of science, seaweed is known as algae, which is a chlorophyll plant, and all parts of the plant are called thallus. Seaweed is an aquatic commodity that has quite a high economic value (Nasmia et al., 2020). The use of seaweed is currently increasingly broad and diverse, due to the increasing number of research studies and knowledge of the benefits of this commodity. Seaweed also has various secondary metabolite compounds that have potential in the treatment of various diseases caused by pathogenic microbes. The use of seaweed bioactive compounds has begun to be developed to replace the use of synthetic chemical raw materials, which are harmful to humans and the environment (Nasmia, 2014). Currently, seaweed processing is carried out in the industry. Some products are made from seaweed, namely lumphead, jam, sweets, and crackers (Murdinah, 2013). Meanwhile, some drinks are made from seaweed, such as jelly drink and seaweed juice (Sanger, 2010). Apart from that, there is also kombucha, which is a fermented drink made from *Sargassum sp.* (Pratiwi et al., 2012). Kombucha is a drink made from

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fermented tea solutions using *Acetobacter* bacteria and several types of yeast (Marwati, 2013). In general, kombucha can be made with the basic ingredients of a black tea solution plus sugar. In the current innovation of developing kombucha products, much can be done by replacing tea ingredients with fruit or vegetables (Syakbandini, 2018), and kombucha is also made from banana peels (Pure, 2016).

Kombucha is a fermented drink with a high phenolic leaf solution (Suhardini and Zubaidah, 2016) or fruit media such as salah (Zubaidah et al., 2018), pomegranate (Yavari et al., 2018), apples (Akbarirad et al., 2017), and grapes (Ayed et al., 2017). With added sugar and fermented with kombucha starter, namely the symbiosis of *Acetobacter xylinum* bacteria and several types of yeast among *Saccharomyces cerevisiae*, the fermentation process is carried out for 1-2 weeks (Battikh et al., 2012; Sun et al., 2015). During the fermentation process, there are changes in physical and chemical properties, including sugar content, alcohol content, pH, and antioxidant levels (Nguyen et al., 2015).

Saccharomyces breaks down glucose to form ethanol, while the bacteria A. Xylinum is able to oxidise ethanol to acetic acid (Ayuratri and Kusnadi, 2017). The fermentation process makes it possible to increase the total free polyphenols due to microbial enzymatic activity, which can liberate chelated polyphenolic compounds so that more are detected during analysis (Zubaidah et al., 2012). Seaweed is processed into kombucha products because seaweed is an important source of macronutrients such as carbohydrates, protein, fat, fiber, vitamins, and minerals (micronutrients) (Muraguri et al., 2016). The content contained in the seaweed is used as a medium for making kombucha drinks, besides tea leaves. These various types of seaweed can produce kombucha, which is characterized by the formation of nata with the activity of Acetobacter bacteria, as well as the formation of organic acids and alcohol. So using this type of seaweed, *Sargassum sp*., can be used as a medium for producing kombucha (Pratiwi et al., 2012).

In general, kombucha tea for the substrate comes from the tea solution, and the carbon source is sugar (Fifendy et al., 2013). One of the nutrients that have an important role in the growth and product of microorganisms. The carbon source for the growth of bacteria and yeast can be obtained from the addition of sugar. The carbohydrate content of seaweed is expected to support the number of microbes that play a role in fermentation.

Low substrate concentrations can accelerate growth to low levels, but at certain concentrations growth can be constant, and concentrations that are too high can inhibit growth. So it is necessary to conduct research on the effect of the concentration of seaweed as a substrate in order to determine the total amount of bacteria and yeast during the kombucha fermentation process.

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Material and Method

Site study

This research was conducted in December 2018 - February 2019. Research and observations were carried out at the Chemistry and Analysis Laboratory and at the Microbiology Laboratory, Faculty of Fisheries and Marine, Airlangga University, Surabaya.

Material

The material used is *Kappaphycus alvarezii* seaweed obtained from UD. Karang Baru, Bluto, Sumenep Regency, Madura. Other materials needed include sugar, kombucha culture starter (Scoby) obtained from UKM Senandung Sejuk Sidoarjo, mineral water, 1% chloramphenicol, 0.9% physiological NaCl diluent, plate count agar (PCA), Eosin Methylene. Sampling was carried out on days 0, 3, 6, 9 and 12 of fermentation.

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 study all the conditions were the same except for the concentration of seaweed used. The treatment in this study consisted of 5 treatments with 4 replications.

Picture 1. (a): Media preparation, (b): Kombucha Starters, (c): Seaweed Drying, and (d):Coliform Testing

Beverage Making Kombucha

The first thing to make seaweed kombucha drink was the dried *Kappaphycus alvarezii* seaweed, washed and cut into small pieces. Cooking is done by mixing 600 ml of mineral water with a concentration of seaweed according to treatment (3%, 4%, 5%, 6% and 7%) and 5 grams of black tea. The process of boiling seaweed using a temperature of 89°C with a boiling time of 5 minutes. The decoction is then filtered as much as 500 ml and put into glass jars that have 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% of the starter liquid. The jar was then immediately covered with a white calico cloth and tied with a rubber band. Storage was carried out at room temperature for 12 days.

Total Bacteria

The total bacterial analysis method used is the plate count method, which involves spreading it on PCA 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

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10 - 5 was obtained. The last three serial dilutions were aseptically pipetted (as much as 0.1 ml) to be put into a petri dish that already contained duplicate Plate Count Agar (PCA) media and leveled using a drigalski (SNI 01-2332.3-2006, 2006). The petri dishes were then incubated upside down in an incubator at 30^{o}C for 48 hours (Figure 1). The number of growing colonies was observed and counted. Sampling was carried out on days 0, 3, 6, 9, and 12 of fermentation.

Total Yeast

The total yeast analysis method used is the cup count method, which involves spreading it on Potato Dextrose Agar (PDA) media. The sample of kombucha drink was pipetted into a test tube that 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, 0.025 ml of dilution was pipetted in duplicate into a petri dish that already contained PDA media to which 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.

Data Analysis

Data on total bacteria, coliforms, and yeasts obtained in this study were statistically analyzed using analysis of variance (ANOVA). If there were significant differences between treatments, then it was continued with Duncan's multiple range comparison test.

Results and Discussion

The raw material used in this research is dried *Kappaphycus alvarezii* seaweed. The main parameters were total bacteria and total yeast during the *Kappaphycus alvarezii* seaweed kombucha production process. *Kappaphycus alvarezii* seaweed showed changes in the total growth curve of bacteria and yeast. The results of the calculation of the Analysis of Variance (ANAVA) of total bacteria showed that there was a significant difference between the concentrations of seaweed ($P<0.05$). The use of *Kappaphycus alvarezii* seaweed as a substrate can be used as an alternative fermentation medium that can produce kombucha drinks other than tea. Through a series of processes, the glucose and galactose content of seaweed will be a source of carbon and nutrients for the growth of microorganisms in kombucha culture. The cellulose formed in *Kappaphycus alvarezii* kombucha indicates that *Kappaphycus alvarezii* seaweed can be fermented and is also used as raw material for making kombucha (Nurhidayah, 2018).

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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	$2,5x10^{6} \pm 0,54^{a}$	$1,6x10^{7} \pm 0,09^{a}$	$1,4x10^{7} \pm 0,51^{a}$	$4,9x10^{5} \pm 0,21^{a}$	$6,0x10^{4} \pm 0,13^{ab}$
P1	$1,5x10^{5} \pm 0,66^{c}$	$2,5x10^{5} \pm 0,36^{d}$	$1,9x10^{5} \pm 0,15^{d}$	$3,6x10^{4} \pm 0,23^{c}$	$2,1x10^{4} \pm 0,14^{c}$
P2	$4,4x10^{5} \pm 0,64^{bc}$	$7,6x10^{5} \pm 0,53^{cd}$	$4,3x10^{5} \pm 0,10^{cd}$	$4,9x10^{4} \pm 0,39^{c}$	$4,4x10^{4} \pm 0,59^{ab}$
P3	$7,6x10^{5} \pm 0,48^{ab}$	$2,0x10^{6} \pm 0,05^{bc}$	$2,0x10^{6} \pm 0,52^{bc}$	$2,9x10^{5} \pm 0,09^{ab}$	$6,8x10^{4} \pm 0,65^{a}$
P4	$1,6x10^{6} \pm 0,10^{ab}$	$3,2x10^{6} \pm 0,11^{bc}$	$3,2x10^{6} \pm 0,25^{ab}$	$1,5x10^{5} \pm 0,13^{b}$	$3,2x10^{4} \pm 0,44^{ab}$
P5	$1,9x10^{6} \pm 0,32^{ab}$	$8,9x10^{6} \pm 0,30^{ab}$	$1,6x10^{6} \pm 0,16^{bc}$	$1,7x10^{5} \pm 0,09^{b}$	$7,9x10^{4} \pm 0,24^{a}$

The total growth pattern of bacteria and yeast during fermentation was almost the same, increasing at the beginning of fermentation and decreasing in number at the end of fermentation. Changes in the number of microorganisms during fermentation can be influenced by the condition of the medium due to metabolites produced from microorganism activity, so that it can affect the growth and interactions between microorganisms. The highest increase in total bacteria was found in kombucha with 7% seaweed concentration in the fifth treatment on the third day of fermentation, which was 8.9×10^{6} CFU/ml.

However, when compared with the control treatment using black tea, it will produce a higher total of bacteria, which is 1.6×10^{7}. A large increase in the number of bacteria indicates a phase of bacterial growth in the log growth phase (exponential phase), which is a phase where microorganisms grow and divide at maximum speed so that the number also increases. Meanwhile, on the 6th to the 12th day of treatment, P1, P2, P4, and P5 decreased total bacteria. The decrease in total bacteria was caused by reduced nutrients and sugar content as a carbon source for bacterial growth, as well as other content that could inhibit bacterial growth, such as an increase in alcohol content (Nurhidayah, 2018). The average total yeast data with fermentation times of 0 days, 3 days, 6 days, 9 days, and 12 days obtained the analysis of variance (ANOVA), which showed that there was a significant difference between seaweed concentrations ($P < 0.05$).

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	$1,1x10^{7} \pm 0,58^{a}$	$2,3x10^{7} \pm 0,21^{a}$	$7,1x10^{6} \pm 0,41^{a}$	$5,6x10^{5} \pm 0,13^{ab}$	$1,3x10^{5} \pm 0,18^{cd}$
P1	$2,8x10^{5} \pm 0,68^{c}$	$5,0x10^{5} \pm 0,56^{c}$	$3,2x10^{5} \pm 0,18^{c}$	$2,7x10^{5} \pm 0,15^{b}$	$8,9x10^{4} \pm 0,25^{cd}$
P2	$3,2x10^{5} \pm 0,26^{c}$	$1,0x10^{6} \pm 0,05^{bc}$	$5,6x10^{5} \pm 0,08^{ab}$	$1,0x10^{5} \pm 0,51^{c}$	$6,6x10^{4} \pm 0,17^{d}$
P3	$5,8x10^{5} \pm 0,05^{bc}$	$1,3x10^{6} \pm 0,08^{bc}$	$1,0x10^{6} \pm 0,06^{b}$	$4,2x10^{5} \pm 0,20^{ab}$	$1,7x10^{5} \pm 0,33^{bc}$
P4	$1,3x10^{6} \pm 0,45^{b}$	$4,5x10^{6} \pm 0,49^{b}$	$7,2x10^{5} \pm 0,16^{b}$	$5,0x10^{5} \pm 0,04^{ab}$	$3,6x10^{5} \pm 0,27^{ab}$
P5	$1,4x10^{6} \pm 0,50^{b}$	$3,3x10^{6} \pm 0,62^{b}$	$7,8x10^{5} \pm 0,12^{b}$	$8,3x10^{5} \pm 0,06^{a}$	$5,1x10^{5} \pm 0,15^{a}$

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Tests on total yeast showed that there was an increase in total yeast at the beginning of the fermentation. The administration of 6% *Kappaphycus alvarezii* (P4) seaweed produced the highest amount of yeast in the seaweed addition treatment of 4.5 x 10^6 CFU/mL on the 3rd day of fermentation, but this amount was still smaller than the control treatment. This indicates that the yeast has utilized the nutrients in the medium to grow and reproduce, thereby increasing the yeast's total value.

The decrease in the amount of yeast occurred from the 3rd day of fermentation to the 12th day of fermentation. Giving 4% of *Kappaphycus alvarezii* (P2) seaweed resulted in the lowest amount of yeast, 6.6 x 10^4 CFU/mL, on the 12th day of fermentation. The decrease in the number of cells was due to the decreased pH of the medium, which tended to be acidic, which could inhibit the activity of the yeast cells and cause the number of cells to increase. Decreases, so that the cell density will also decrease (Aditiwati and Kusnadi, 2003).

CONCLUSION

The concentration of seaweed affects the total bacteria and yeast kombucha seaweed (*Kappaphycus alvarezii)* during the production process because the total growth pattern of bacteria and yeast during fermentation is almost the same, which increases at the beginning of fermentation and decreases in number at the end of fermentation. Changes in the number of microorganisms during fermentation can be influenced by the condition of the medium due to metabolites produced from microorganism activity, so that it can affect the growth and interactions between microorganisms.

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