

ANALYSIS OF CHEMICAL CHARACTERISTICS OF KOMBUCHA TEA SEAWEED *Eucheuma cottonii*

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ABSTRACT

Seaweed is a marine plant that is found in many Indonesian waters. Seaweed has various types, one of which is *Eucheuma cottonii* seaweed. So far, *Eucheuma cottonii* is only used as functional food in the food sector, but there are still few who use it as a functional drink. The purpose of this study was to determine the chemical characteristics of kombucha tea seaweed *Eucheuma cottonii*. The research method used is experimental. The results of the chemical characteristics obtained in the best treatment are treatment D (12%) with a total sugar content value of 7.67%, total ash content of 0.773%, polyphenol content of 0.177% and tannin content of 0.202%.

Keywords: : Seaweed; Kombucha tea; *Eucheuma cottonii*; Chemical characteristics

INTRODUCTION

A country that has abundant natural resources is the State of Indonesia. Many island countries are surrounded by waters that cause the country to have natural resources from abundant waters, namely marine life. One of the marine biota in the waters of the State of Indonesia is marine plants which are often called seaweed (Rachmawati and Siti, 2022). This seaweed is one of the abundant catches, this can be seen from the data in 2020 seaweed produced as much as 200,000 kg and in 2021 it was 350,000 kg. This data continues to increase because many small fishermen are also cultivating seaweed to meet market needs for seaweed (Rachmawati *et al.*, 2021).

Eucheuma cottonii seaweed is one type of red algae that has a cylindrical thalus, bright green, reddish brown and light green and has a slippery surface. The nutritional composition of *Eucheuma cottonii* seaweed includes carbohydrates, proteins, fats, ash, salt compounds (sodium and calcium), and bioactive compounds (alkaloids, flavonoids, terpenoids, antioxidants, etc.) (Sanger and Yan, 2018). In addition, *Eucheuma cottonii* also contains several vitamins, namely vitamins A, B1, B2, B6, B12 and vitamin C as well as several minerals such as K, Ca, Na, Fe and iodine. With various nutritional content of *Eucheuma cottonii* seaweed, many people use this seaweed for food processing (Budiyanti and Supasman, 2021).

One of the food processing made from *Eucheuma cottonii* seaweed is kombucha tea. Kombucha tea is one of the beverage products obtained from the fermentation of beverages added by SCOBY to the processing process. SCOBY itself is a fermenter obtained from bacteria and yeast. Kombucha drinks have good benefits for health including as antioxidants, antibacterials, improving intestinal microflora, lowering blood pressure, neutralizing or detoxing from toxic substances, and increasing immunity (Rosida *et al.*, 2021). With the various benefits that exist in kombucha tea compared to ordinary tea which has many organic acids, vitamins and amino acids (Purnami *et al.*, 2018). This study aims to determine the

chemical characteristics of kombucha tea seaweed *Eucheuma cottonii* with different sugar concentrations.

RESEARCH METHODS

Materials

The equipment in this study used is: cabinet dryer, digital scales, measuring cups, beaker glass, stoves, glass jars, spatulas, trays, choppers, basins, pH meters. While the materials in this study used are: *Eucheuma cottonii* seaweed, latex gloves, sugar, baking paper, blanch cloth, aquades.

Research Methods

This research was conducted at the Faperta Laboratory of PGRI University Banyuwangi and the Food Laboratory of the Department of Agricultural Technology, Jember Polytechnic University. Making kombucha seaweed refers to Gustishio *et al.*, (2023) with treatment modification. Before making kombucha tea, the equipment must be ensured to be clean and sterile, especially the kombucha tea jar container (sterile). Put 25 grams of dried *Eucheuma cottonii* seaweed into a sterile jar which is then added 200 ml of hot water and different sugar concentrations according to each treatment, and stirred until well dissolved. Then after all is dissolved, 800 ml of water is added. After that, SCOBY is added as a kombucha stater and fermented for 12 days, to get the results of good quality kombucha tea and a new SCOBY can be fully formed. The treatment used was a difference in different sugar concentrations, namely in treatment A (9%), treatment B (10%), treatment C (11%) and treatment D (12%) in making kombucha drinks and carried out 3 repetitions. The research to be carried out is in the form of experimental research using Complete Randomized Design. With the purpose of the study was to determine the chemical characteristics of kombucha drinks with differences in sugar concentration. The chemical characteristics carried out include analysis of polyphenol levels, levels total ash, tannins, sugar content. The data obtained were analyzed statistically using variety analysis (ANOVA) then carried out a BNT (Smallest Real Difference) test of 5%.

Polyphenol Analysis

Polyphenol testing according to Evitasari and Erna (2021), the procedures include:

1. Manufacture of gallic acid master solution

Weighed 10 mg gallic acid, dissolved with ethanol p.a to 10 mL so that obtained the concentration of the mother solution is 1000 µg/mL. A solution of 1000 µg/mL was diluted to concentrations of 50, 100, 150, 200 and 250 µg/mL.

2. Preparation of 7.5% Na₂CO₃ solution

Weigh 7.5g Na₂CO₃ plus 80 mL aquades, then boil until all dissolved Na₂CO₃ powder. Next, Na₂CO₃ that has been boiled, then allowed to stand for 24 hours, filtered, and diluted with aquades to a volume of 100 mL.

3. Preparation of sample solution

Weighing 100 mg of extract, then dissolved with ethanol p.a 25 mL until the concentration of the mother solution was 4000 µg/mL. Then the 4000 µg/mL solution was diluted to concentrations of 600 µg/mL, 800 µg/mL, 500 µg/mL, 400 µg/mL, and 200 µg/mL.

4. Determination of maximum wavelength of Error Acid

A total of 0.1 mL of standard solution was included in the cuvette, supplemented with 1 mL of Follin-Ciocalteu reagent. which is liquid and diluted (1 : 10 V / V water), allowed to stand 5 minutes. The mixture is added 0.8 mL Na₂CO₃ (75 g / l water), and allowed to stand for 30 minutes at room temperature. Then the absorbance is measured at a wavelength of 500 to 850.

5. Calibration curve creation

A total of 0.1 mL of standard solution was inserted in the cuvette, plus 1 mL of Folin-Ciocalteu reagent (1:10 v/v water), allowed to stand 5 minutes. The mixture plus 0.8 mL Na₂CO₃ (75 g/L water), allowed to stand for 30 minutes at room temperature. Then the absorbance is measured on the selected wave.

6. Total Polyphenol Testing on Green Tea Extract

A total of 0.1 mL of sample solution was inserted in the cuvette, plus 1 mL of Follin-Ciocalteu reagent (1:10 v/v water), allowed to stand for 5 minutes. The mixture plus 0.8 mL Na₂CO₃ (75 g / L water), allowed to stand for 30 minutes at room temperature. Then the absorbance is measured at wavelengths of 600-850 nm. The absorbance of the extract solution is measured using 3-repeat UV-Vis spectrophotometer. Total polyphenol content expressed in mg GAE (gallic acid equivalence) in 1 gram of extract. The largest nm and absorbance indicate the peak point of the spectrum. Wavelength at this point that gives the highest absorbance is used as the selected wavelength.

This test according to Marsell *et al.*, 2021 with the following procedure: the porcelain cup is dried in a 105°C oven for 3 hours. After that cup weighed with Sartorius' balance sheet (a). Then into the cup is added as much as 1-2 g of the sample of the preparation (b). The cups and samples are dried in a 600°C electric furnace for 18-24 hour. The ash-finished sample is then placed in a desiccator for 1 hour. The weight of the cup and ash is weighed (c).

$$\% \text{ Dry weight (BK)} = (c - a) / b \times 100\%$$

a = weight of porcelain cup; b = weight of porcelain cup + sample; c = weight of porcelain dish + sample after ovened

Tannin Analysis

Determination of tannin levels in kombucha tea. *Eucheuma cottonii* seaweed kombucha tea each pipette as much as 1 mL into the test tube, added 3 mL FeCl₃ 0.1 M. Then homogenized, then added another 3 mL K₃Fe(CN)₆ 0.008 M. Let stand for 10 minutes and filtered using paper Strain. Measured using UV-Vis spectrophotometry at length 720 nm wave (Mutmainnah, 2018).

Total Sugar Analysis

Total sugar analysis procedures according to Mandjoro *et al.*, (2019) include: samples of research results are weighed a total of 4 g, then added 80% alcohol in a ratio of 1:1 to in beakers and filtered Using whatman filter paper. The filtrate is measured pH, if it is acidic added NaOH 0.1N until sufficient alkaline (pH about 9). Heated solution on a water bath temperature of 100°C for 30 minutes, the solution is filtered again, next the mixture is heated at ± 85°C up to alcohol-free solutions. If present precipitate, re-filtration. The filtrate is transferred into a measuring flask 25 mL, then added 3 mL of lead acetate (Pb(CH₃COO)₂) carefully until the solution is clear, the volume is corrected with aqueous, shaken until homogeneous and filtered with filter paper Whatman. Filtrate added sodium oxalate(Na₂C₂O₄) as much as 1 g, mixed until evenly distributed and precipitate formed filtered using paper filter whatman so that it is declared as a sample.

RESULT AND DISCUSSION

In this study, we will know the chemical characteristics of *Eucheuma cottonii* seaweed kombucha tea with different sugar concentrations which can be seen from the results of total ash content analysis, sugar content analysis, polyphenol analysis, and tannin analysis. The results of chemical characteristics can be seen in Table 1. it shows that variety analysis shows a very real difference in the chemical characteristics of kombucha tea seaweed *Eucheuma cottonii*.

Ash Content Analysis

Table 1. Chemical Characteristics of *Eucheuma cottonii* Seaweed Kombucha Tea With Different Sugar Concentrations

Treatment	Total Ash Content (%)	Total Sugar Content (%)	Polyphenol Content (%)	Tannin Content (%)
A (9%)	0,113±0,006 ^a	11,17±0,58 ^a	0,140±0,001 ^a	0,118±0,007 ^a
B (10%)	0,144±0,006 ^b	10,33±0,29 ^a	0,154±0,006 ^b	0,145±0,006 ^b
C (11%)	0,165±0,004 ^c	9,0±0,50 ^b	0,163±0,001 ^c	0,162±0,002 ^c
D (12%)	0,773±0,006 ^d	7,67±0,29 ^c	0,177±0,002 ^d	0,202±0,003 ^d

Total Ash Content Analysis

The average total ash content produced in *Eucheuma cottonii* seaweed kombucha tea in treatment A (9%) was 0.113%, in treatment B (10%) by 0.144%, in treatment C (11%) by 0.165% and in treatment D (12%) by 0.770%. Analysis of the variety of total ash content of kombucha tea had a significant effect ($\alpha=0.05$). The highest total ash content value in treatment D (12%) was 0.770% and the lowest total ash content value in treatment A (9%) was 0.113%. This is because the difference in the concentration of sugar given affects the value of the total ash content produced. Based on the quality requirements of dry tea in packaging according to SNI 3836:2013, the maximum total ash content is 8%. So the results of this study are still in accordance with SNI 3836:2013. This is because the concentration of sugar itself has its own ash content value, so that the more sugar added to kombucha tea, it will increase the minerals it contains (Naufal et al., 2022). The determination of ash content is closely related to the mineral content contained in a material, the purity and cleanliness of the material (Husna et al., 2014).

Total Sugar Content Analysis

The average total sugar content produced by *Eucheuma cottonii* seaweed kombucha tea in treatment A (9%) was 11.17%, in treatment B (10%) by 10.33%, in treatment C by 9.0% and treatment D (12%) by 7.67%. With the highest value of the highest total sugar content in treatment A (9%) of 10.33% and the lowest value in treatment D (12%) of 7.67%. Analysis of the variety of total sugar content had a significant effect ($\alpha=0.05$) on the kombucha tea seaweed *Eucheuma cottonii*. Based on the quality standards of tea drinks in packaging according to SNI 3143: 1992, the minimum total sugar content is 6%, so the results of this study are still in accordance with SNI 3143: 1992. The decrease in total sugar content produced in seaweed kombucha tea *Eucheuma cottonii* is due to that the SCOBY in kombucha tea makes sugar as a source of carbon and as a nutrient for these microbes to multiply and the sugar will be converted into alcohol and CO₂ during the fermentation process (Pratiwi et al., 2012). The CO₂ gas then reacts with water vapor and forms carbonic acid. In this sugar fermentation process, yeast plays an active role in the decomposition of sugar into CO₂ and organic acids and other components (Simanjuntak, and Siahaan, 2011).

Polyphenol Level Analysis

The average polyphenol content produced by *Eucheuma cottonii* seaweed kombucha tea in treatment A (9%) was 0.140%, treatment B (10%) was 0.154%, treatment C (11%) was 0.163% and treatment D (12%) was 0.177%. With the highest value of the highest polyphenol content in treatment D (12%) of 0.177% and the lowest value in treatment A (9%) of 0.140%. Analysis of various polyphenol levels had a significant effect ($\alpha=0.05$) on kombucha tea seaweed *Eucheuma cottonii*. Polyphenol levels in kombucha tea have increased due to the raw material of seaweed *Eucheuma cottonii* and the addition of sugar concentration in kombucha tea. Polyphenols are one of the bioactive compounds produced by seaweed or algae and can be used to protect from sunlight (Cahyaningrum et al., 2016). Polyphenols in seaweed have antioxidant activity so that they can prevent various

degenerative diseases and diseases due to oxidative stress including cancer, aging, narrowing of blood vessels. Polyphenol group compounds that act as natural antioxidants in red seaweed include catechins (gallo catechin, epicatechin, epigallocatechin gallate), gallic acid, flavonols, flavonol glycosides, caffeic acid, hesperidin, myricetin (Ananda, 2019).

Tannin Level Analysis

The average tannin content produced by *Eucheuma cottonii* seaweed kombucha tea in treatment A (9%) was 0.118%, treatment B (10%) was 0.145%, treatment C (11%) was 0.162% and treatment D (12%) was 0.202%. With the highest value of the highest tannin content in treatment D (12%) of 0.202% and the lowest value in treatment A (9%) of 0.118%. Analysis of various tannin levels had a significant effect ($\alpha=0.05$) on kombucha tea seaweed *Eucheuma cottonii*. The concentration of *Eucheuma cottonii* seaweed and the length of fermentation time for 12 days resulted in a change in the value of tannin content in kombucha tea. This is because tannins oxidize into tehafavin, tehaurubigin and tehnaphtoquinone compounds. This also occurs due to the activity of *Acetobacter xylinum* bacteria which can polymerize catechins contained in tea as a medium for the fermentation process (Sulistiaway and Imas, 2022). The results of this study are still in accordance with the quality standards of tea drinks in packaging according to SNI 3143: 1992, namely tannin levels in tea are positive or present.

CONCLUSION

Based on the treatment used are different sugar concentrations, namely in treatment A (9%), treatment B (10%), treatment C (11%) and treatment D (12%) in making seaweed kombucha tea drink *Eucheuma cottonii* and carried out 3 repetitions. The best results were obtained in treatment D (12%) with a total sugar content value of 7.67%, total ash content of 0.773%, polyphenol content of 0.177% and tannin content of 0.202%.

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