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Research Article

Chemical Characteristic, Antioxidant Activity, and Consumer Acceptance Level of Kombucha from *Sargassum cristaefolium* Seaweed Tea

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Abstract
Seaweed infusion is a nutritious beverage rich in bioactive compounds, which is essential for human health. Enhancing the functional properties of seaweed tea can be achieved through the fermentation process to create kombucha. This research aims to explore the impact of fermentation duration on the chemical attributes, antioxidant capacity, and consumer preference of kombucha derived from *Sargassum cristaefolium* tea. The experimental approach involved varying fermentation periods, including no fermentation, 4, 8, 12, and 16 days of fermentation. Tests carried out included yield, water content, total phenol content, the antioxidant activity (through DPPH and FRAP methods), pH levels, total acidity, total reducing sugars, and sensory evaluation. The findings revealed that fermentation duration significantly influenced the chemical composition, antioxidant activity, and consumer acceptance. Notably, the 12-day fermentation period exhibited superior antioxidant activity compared to other treatments, showcasing a total phenolic content of 25.14±0.07 mg GAE/100 g, DPPH antioxidant activity of 83.00±0.83%, and FRAP antioxidant activity of 4.40±0.07 mM/g. Conversely, the optimal treatment in terms of chemical attributes and consumer preference for *S. cristaefolium* kombucha was observed with a 4-day fermentation period, yielding a pH of 3.07±0.02, total acidity of 1.20±0.07%, total reducing sugars of 2.86±0.34%, and hedonic ratings for color, aroma, taste, appearance, and overall quality at 4.18±0.76, 4.11±0.78, 4.40±0.72, 4.35±0.71, and 4.26±0.14, respectively.

Keywords: antioxidant, kombucha, *Sargassum cristaefolium,* seaweed tea

1. INTRODUCTION

Seaweed is one of the abundant biological resources in Indonesia, including brown seaweed [1]. Brown seaweed contains potential bio-active compounds such as polyphenols, flavonoids, chlorophylls, fucoxanthins, showing antioxidant activity [2]-[5]. One of the brown seaweeds that is widely found in Indonesia is *Sargassum cristaefolium*. This seaweed shows potential bio-active compounds that can be further developed as a source of pharmaceuticals, nutraceuticals, and functional food ingredients [6]. This seaweed has the potential as a source of natural antioxidants because it contains active ingredients that function as antioxidants [7].

Tea stands as a favored beverage across diverse demographics. Brown seaweed tea contains a variety of secondary metabolite compounds, including steroids, saponins, tannins, terpenoids, glycosides, flavonoids, and phenolics, known for their potential as potent antioxidants [8]. Nonetheless, tea derived from Sargassum sp. seaweed faces challenges due to the undesirable fishy scent it emits, leading to reduced consumer preference [9]. The fishy aroma emanates from the presence of trimethylamine compounds, fatty acids, ammonia, and fatty acid oxidation products [10]. To address these issues, the transformation of seaweed tea into kombucha beverages through fermentation presents a promising solution.

Kombucha is a mixture of tea with sugar fermented with the help of symbiosis between acetic acid bacteria and yeast that will form a plate on the surface known as symbiotic culture of bacteria and yeast (SCOBY) [11]. Kombucha is one of the drinks that has antioxidant activity that can prevent cellular oxidation by neutralizing, reducing, and inhibiting the formation of new free radicals in the body by donating electrons to free radicals. This mechanism prevents damage in the body due to oxidative stress [12]. The antioxidant activity of kombucha tea is influenced by several factors, one of which is the fermentation period, where the fermentation period plays a role in increasing the

antioxidant activity of kombucha tea [13]. The fermentation process of bacteria and yeast will increase the amount of phenol in tea which can increase antioxidant activity. The higher the phenolic compounds contained in tea, the higher the antioxidant activity [14]. The fermentation period has a significant effect on the total phenolics and antioxidant activity of fermented red galangal kombucha drinks [15]. Apart from time, temperature also affects the antioxidant activity of Porphyra dentata seaweed kombucha [16]. In addition to increasing antioxidant activity, fermentation can also improve the aroma of seaweed tea because during the fermentation process volatile compounds such as alcohol, acetic acid, and organic acids are produced which produce a distinctive sour aroma [17]. Pratiwi et al. has conducted a study related to the effect of fermentation time (0, 4, 8, 12, and 16 days) on the physical and chemical properties of kombucha drinks from *Sargassum sp* seaweed where the results of the study showed that fermentation time affects the physical and chemical properties of Sargassum sp kombucha drinks [18]. Therefore, by carrying out the fermentation process, it is expected to improve the aroma of seaweed tea. This study aims to determine the effect of the best fermentation time of kombucha tea in making *S. cristaefolium* seaweed kombucha tea on chemical characteristics, antioxidant activity, and consumer acceptance levels.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study include S. cristaefolium seaweed, commercial kombucha tea (Kombucha Artisan Tea), lime, SCOBY (Natsilver), ethanol (Sigma Aldrich), Folin-Ciocalteu reagent (Merck USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma Aldrich), gallic acid (Merck KGaA Germany), sodium carbonate (Merck Germany), acetic acid (Merck USA), vitamin C, sodium acetate trihydrate (Merck KGaA Germany), sodium hydroxide, 2,4,6 tri-pyridyl-s-triazine (TPTZ) (Merck USA), iron(III) chloride hexahydrate (Merck KGaA, Germany), iron(II) sulfate heptahydrate (Merck USA), sodium hydroxide (Merck KGaA, Germany), and 3,5-dinitrosalicylic acid (DNS, Sigma Aldrich).

2.2. Methods

2.2.1. Collection, Identification, and Sample Preparation

Sample collection and identification utilized the methodology outlined in previous work [19] with certain adaptations. Specimens of *Sargassum sp.* were harvested from Teluk Awur Beach, Jepara. The seaweed was carefully obtained by severing the base of the thallus with a sharp blade. Subsequently, the specimen was placed in a resealable plastic bag and stored in a chilled container with ice. Next, the sample is washed until clean with running water. Following this, the seaweed was weighed (initial weight) and subjected to further sample preparation. Several fresh seaweed samples were preserved in 70% alcohol to uphold their morphological integrity, ensuring they remain intact for identification purposes at the Pharmacognosy Laboratory, Faculty of Pharmacy, Universirtas Gadjah Mada for taxonomical classification.

2.2.2. Making Tea from Sargassum cristaefolium Seaweed

The seaweed S. cristaefolium underwent a meticulous washing process with flowing water to eliminate any impurities. A seaweed specimen weighing up to 200 g was then immersed in 2 L of lime solution at a pH of 5, with an initial temperature of 85 °C, for a duration of 16 min. Subsequently, the seaweed was drained and air-dried at ambient temperature for a period of 24 h. Following this, the seaweed was finely sliced and subjected to roasting for a duration of 10–15 min. If the seaweed was not utilized for the preparation of seaweed tea, it was stored in a refrigerator at a temperature of 4 °C [20]. The final *S. cristaefolium* seaweed tea was meticulously weighed to ascertain the yield. Upon yield calculation, the moisture content of the seaweed tea was assessed utilizing a moisture content analyzer.

2.2.3. Making Kombucha from Sargassum cristaefolium Seaweed Tea

The *S. cristaefolium* seaweed was weighed as much as 50 g, then put into a sterile glass jar and brewed with 1 L of water at 80 °C for 15 min. The

Table 1. Impact of fermentation duration on yield, water content, pH, total acid content, and concentration of reducing sugars in *S. cristaefolium* seaweed tea kombucha.

Fermentation Time	Yields (%)	Water (%)	pH	Total Acid (%)	Reducing Sugar (%)
No fermentation	16.20±0.40^{a}	3.48±0.19^{a}	7.46±0.08^{a}	0.02±0.01^{d}	0.64±0.01^{f}
4 days	16.28±0.58^{a}	3.51±0.16^{a}	3.07±0.02^{b}	1.20±0.07^{c}	2.86±0.03^{b}
8 days	16.33±0.38^{a}	3.73±0.10^{a}	3.03±0.01^{b}	1.33±0.02^{c}	2.34±0.01^{c}
12 days	16.55±0.96^{a}	3.59±0.16^{a}	2.97±0.02^{bc}	1.54±0.07^{b}	1.76±0.02^{d}
16 days	16.78±1.58^{a}	3.39±0.10^{a}	2.90±0.03^{c}	1.94±0.14^{a}	1.58±0.01^{e}
Commercial kombucha			2.97±0.01^{bc}	1.17±0.08^{c}	3.68±0.03^{a}

Note: Each value is expressed as mean±SD in triplicate experiments. Values a-f with different letters in the same column indicate significant differences between treatments ($p < 0.05$), analyzed using Tukey HSD.

brewed water was filtered to separate the dregs of S. cristaefolium seaweed tea and tea water, then 10% sugar (b/v) was added and dissolved. The tea solution was cooled to a temperature of ±25 °C then 10% original kombucha water and 1 piece of nata (SCOBY) aged 1–2 months with a diameter of ±10–12 cm with a thickness of ±0.5 cm were added. The jar was covered with cotton cloth and tied with rubber, then fermented at room temperature and should not be exposed to direct sunlight [21][22]. Each sample was given different fermentation treatments, namely without fermentation, and fermentation for 4, 8, 12, and 16 days.

2.2.4. Serving Kombucha from Sargassum cristaefolium Seaweed Tea

After fermentation, *S. cristaefolium* seaweed tea kombucha was stored at 4 °C when not directly used for research. *S. cristaefolium* seaweed tea kombucha was tested for total phenol, antioxidant activity, pH, total acid, reducing sugar, and consumer acceptance level test (hedonic test). For the hedonic test, *S. cristaefolium* tea kombucha was served into test glasses that were randomly coded.

2.2.5. Total Phenol Analysis

Total phenol testing refers to research conducted by Sinurat and Suryaningrum [9] with a modification. To make a 5% Na_2CO_3 solution, 5 g of Na_2CO_3 are needed and distilled water is added to a volume of 100 mL. Then homogenized by vortexing. A standard gallic acid solution is made with a stock solution concentration of 1000 ppm. A 1 mg of gallic acid is put into a bottle and distilled water is added to 1 mL. Then, homogenized by vortexing. Furthermore, a series of gallic acid dilutions are made with concentrations of 0, 20, 40, 60, 80, 100, 120, and 140 ppm. Test samples are prepared with a concentration of 100 mg/mL including *S. cristaefolium* seaweed tea infusion, S. cristaefolium seaweed kombucha tea and commercial kombucha tea. A 1 g of *S. cristaefolium* seaweed tea was dissolved in 10 mL of distilled water then vortexed until homogeneous and filtered using filter paper to separate the tea dregs and tea solution. *S. cristaefolium* seaweed tea kombucha and commercial kombucha tea were diluted to a concentration of 100 mg/mL. A 1 mL of kombucha sample was added in 10 mL of distilled water and homogenized by vortexing. *S. cristaefolium* seaweed, *S. cristaefolium* seaweed tea kombucha, commercial kombucha tea and standard gallic acid solution, each taken as much as 1 mL. Next, 1 mL of 96% ethanol, 5 mL of distilled water and 0.5 mL of 50% Folin-Ciocalteu reagent were added. After that, it was left for 5 min and then 1 mL of 5% Na_2CO_3 was added. Then the mixture was homogenized and incubated in the dark for one hour. The absorbance of gallic acid solution and sample were measured using a UV-Vis spectrophotometer at 725 nm. Gallic acid solution was used as a standard curve by making a line equation from the absorbance value of the gallic acid solution. Furthermore, the sample absorbance data was entered into the regression equation and the phenol content value was obtained which was expressed in mg GAE/g. The calculation of total phenol content uses the following Formula 1.

$$\text{Total phenol}\left(\text{mg}\frac{\text{GAE}}{\text{g}}\right) = \text{C x}\frac{\text{v}}{\text{m}} \quad \textbf{(1)}$$

where v = volume of test solution (mL), m = mass of test solution (g), and C = concentration of test solution (mg/mL).

2.2.6. Antioxidant Activity

2.2.6.1. DPPH Free Radical Scavenging Activity

Antioxidant activity testing using the DPPH method was carried out based on research by Muthia et al. [23] with some modifications. A 3.9 mg of DPPH powder was dissolved in ethanol up to 100 mL, and homogenized with a vortex to obtain a DPPH solution with a concentration of 0.1 mM. A 1 g of vitamin C was dissolved in 100 mL of distilled water and vortexed until the solution is homogeneous. The *S. cristaefolium* seaweed tea kombucha, commercial kombucha, and *S. cristaefolium* seaweed tea with a concentration of 1000 mg/mL were also prepared. The solution was put into a test tube with each: blank solution (1.0 mL ethanol + 0.7 mL DPPH solution), test solution (1.0 mL ethanol + 0.7 mL DPPH solution + 0.1 mL test solution), positive control (1.0 mL ethanol + 0.7 mL DPPH solution + 0.1 mL vitamin C solution), then incubated in a dark room for 15 min. The absorbance value was measured using a UV-Vis spectrophotometer at 515 nm. The DPPH inhibition rate was calculated using the following Formula 2.

$$\text{DPPH } scavenging\ activity\ (\%) = \left[\frac{(\text{A control} - \text{A sample})}{\text{A control}}\right] \text{x } 100\% \quad \textbf{(2)}$$

2.2.6.2. Ferric Reducing Antioxidant Power (FRAP)

Antioxidant activity testing using the FRAP method was carried out based on research conducted by Suhaila et al. [24]. The acetate buffer solution having pH 3.6 was determined by mixing 0.775 g of sodium acetate trihydrate with 4 mL of concentrated acetic acid and dissolved using distilled water to obtain an exact volume of 250 mL, with the yield is stored as a stock solution at 4 °C. After this, 10 mM mL-1 of the TPTZ solution was prepared by mixing 0.15 g of TPTZ in 40 mM/L of HCl, to complete an exact volume of 50 mL. Although the 40 mM/L solution was arranged by dissolution of 0.828 mL of concentrated HCl in 250 mL of distilled water, the process was still continuously carried out. The extracted TPTZ solution was further stored at 4 °C, for use within a period of 24 h. Furthermore, 0.54 g $FeCl_3.6H_2O$ were dissolved in distilled water and prepared to approximately 100 mL, to provide a solution of 20 mM/L, which was stored for 24 h at 4°C. The FRAP reagent was also defined by mixing 2.5, 25, and 2.5 mL of TPTZ, acetate buffer, and $FeCl_3 \cdot 6H_2O$ solutions (1:10:1), which were approximately prepared to 100 mL using distilled water. Standard solutions (10,000 µM/L) were then provided by dissolving 2.78 g of $FeSO_4 \cdot 7H_2O$ in 1 L of distilled water and serially diluted to obtain concentrations of 50, 100, 150, 200, 250 and 300 ppm, respectively. The preparation of seaweed tea *S. cristaefolium*, kombucha seaweed tea *S. cristaefolium*, and commercial kombucha, as well as vitamin C, was carried out by dissolving 1 g of each material in 100 mL of distilled water. FRAP reagent of 900 µL was also mixed with 120 µL of each sample solution, the mixture was homogenized using a vortex, and allowed to rest for 15 min. The absorbance of the solution was assayed at 595 nm, as the data provided was further handled using Microsoft Excel. In addition, the standard solution of $FeSO_4 \cdot 7H_2O$ was used as an ideal curve, by making a line equation for its absorbance value. The data from the sample were also enrolled in the line formula, to provide the FRAP value (in µM/g).

2.2.7. Analysis of pH

The pH measurement was done using a pH meter. Before the pH meter was used, the electrode was rinsed using distilled water, then dried using tissue. The sample was placed in a container with a certain volume until the electrode is immersed in the sample. Before being used to measure again, the electrode was rinsed with distilled water and dried using tissue to prevent cross-contamination or data bias. This was done the same for all samples.

2.2.8. Total Acid Analysis

The total acid test through the titration method as detailed by Zubaidah et al. [25] with adaptations. A 10 g sample of kombucha was placed in a volumetric flask, then diluted with distilled water up to the 100 mL mark. Subsequently, 50 mL of the solution was transferred to an Erlenmeyer flask and combined with 1% phenolphthalein indicator in a quantity of 2–3 drops. The mixture was then titrated with 0.1 M NaOH until the color transitioned to pink. Following the color alteration, a waiting period of 15–30 seconds ensued, and if the pink hue

persisted without further change, the volume of NaOH titrant was recorded. The total acid titration was then computed utilizing the prescribed Formula 3.

$$Total\ acid\ content = \frac{V1 . M . B}{V1}\ x\ 100 \quad (3)$$

where V1 = volume of 0.1 M NaOH solution (mL), V2 = sample volume (mL), M = molarity of NaOH solution (0.1 M), and B = molecular weight of lactic acid (90 g/mol).

2.2.9. Reducing Sugar Analysis

Reducing sugar analysis utilizing the 3,5-dinitrosalicyilic acid (DNS) method as described in Jain et al research [26] with modifications. A solution of 8 g of NaOH was dissolved in 100 mL of distilled water and thoroughly mixed by vortexing. The DNS reagent was prepared by dissolving 1 g of DNS in 20 mL of 2 M NaOH. Additionally, a K-Na tartrate solution was created by dissolving 30 g of K-Na tartrate in 50 mL of distilled water and homogenizing it. The DNS solution was agitated using a homogenizer at a speed of 10,000 rpm until complete dissolution (resulting in an orange hue), following which the K-Na tartrate solution was added and stirred until fully dissolved using a homogenizer at 10,000 rpm. Once dissolved, distilled water was added to reach a final volume of 100 mL and mixed by vortexing. A glucose solution was prepared by dissolving 200 mg of glucose in 100 mL of distilled water in a measuring flask to create a glucose stock solution with a concentration of 2000 ppm. Subsequently, the stock solution underwent a series of dilutions to achieve concentrations of 0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, and 0.50 mg/mL. Sample solutions of *S. cristaefolium* seaweed tea, *S. cristaefolium* seaweed kombucha tea, and commercial kombucha tea were prepared at a concentration of 10 mg/mL. A sample of 1 mL was taken and placed into a test tube, followed by the addition of 1 mL of DNS reagent and vortexing. The mixture was then heated in a water bath at 92 ° C for 5 min, cooled to room temperature, supplemented with 1 mL of distilled water, and vortexed. Subsequently, the absorbance was measured at 540 nm. The standard curve was established by measuring the absorbance of standard glucose at various concentrations and deriving a regression line equation. The reducing sugar content was determined using the standard curve equation, where the absorbance value serves as the y-axis and the reducing sugar value (mg/mL) as the x-axis. The calculation of reducing sugar content was then performed using the designated Formula 4.

$$Reducing\ sugar\ content\ (\%) = \frac{X}{m}\ x\ 100 \quad (4)$$

where X is reducing sugar (mg) and m is sample mass (mg).

2.2.10. Hedonic Evaluation Test

In order to assess the degree of consumer acceptance towards *S. cristaefolium* kombucha tea, we refer to the study conducted by Suryono et al. [27], with certain modifications. This evaluation involved 80 individuals as untrained assessors, aged between 19 to 25, and from diverse backgrounds. The assessment took place in the Organoleptic Room at the Department of Fisheries, Gadjah Mada University. The hedonic test was carried out using samples of *S. cristaefolium* seaweed tea prepared through different treatments: non-fermented (control), fermented for 4, 8, 12, and 16 days [18]. The various kombucha tea samples were served in 10 mL cups, each labeled with a random 3-digit code for data tracking purposes. Subsequently, the assessors were provided with hedonic evaluation sheets [28] and instructed to taste the kombucha samples directly, evaluating them based on color, aroma, taste, and appearance. The sensory scale utilized ranged from 1 to 5, where 5 indicated 'very like', 4 'like', 3 'somewhat like', 2 'dislike', and 1 'very dislike'.

2.2.11. Data Aanalysis

This research employed a completely randomized design (CRD) research framework. The independent variable under scrutiny was the fermentation duration of *S. cristaefolium* seaweed kombucha tea, while the dependent variables encompassed total phenol, antioxidant activity, pH levels, total acid concentration, reducing sugar content, and sensory attributes of *S. cristaefolium* seaweed kombucha tea. The data collected underwent meticulous processing utilizing

Microsoft Excel and IBM SPSS Statistics 25. The distribution of data from the water content, yield, total phenol, antioxidant, pH, total acid, reducing sugar content, and hedonic evaluations was ascertained through the One-Sample Kolmogorov-Smirnov Test. Should the data exhibit a normal distribution ($p > 0.05$), subsequent analysis entailed ANOVA, whereas non-normally distributed data underwent further scrutiny via the Kruskal-Wallis Test. The analyses pertaining to the yield test, water content test, antioxidant activity assessment utilizing the DPPH and FRAP methodologies, total phenol quantification, pH measurements, total acid content determination, and reducing sugar tests were conducted through ANOVA. In cases where the ANOVA results revealed a notable discrepancy ($p < 0.05$), the Tukey post hoc test was conducted to delineate variances among treatments. The data derived from the hedonic assessment of the samples were subject to the Kruskal-Wallis Test, followed by the Mann-Whitney test to discern distinctions among treatments. The identification of the optimal product was achieved through the exponential comparison method.

3. RESULTS AND DISCUSSIONS

3.1. Yield

Yield was calculated to assess the efficacy of the *S. cristaefolium* seaweed tea production process. Table 1 exhibits the yield data for the treatments sans fermentation, with fermentation durations of 4, 8, 12, and 16 days correspondingly, namely 16.20±0.40, 16.28±0.58, 16.33±0.38, 16.55±0.96, and 16.78±1.58%, where the yield across treatments exhibited no significant variance. This outcome surpasses the findings of the research conducted by Pratiwi and Husni [20], which reported a range of 11.75–13.74%. The disparity in yield is believed to stem from variations in moisture content within the substance and discrepancies in ambient temperature during the drying process. As indicated by Yamin et al. [29], the disparity in high or low yield of a food component is predominantly influenced by the moisture content present in said food component [30].

3.2. Water Content

Water content is the predominant factor that impacts the deterioration of food quality, with the quality of food products declining as water content increases. Elevated water content in tea products can lead to a shortened shelf life, susceptibility to mold growth, and a loss of the distinct aroma and flavor of the brewed tea, often replaced by the scent of mold growth [31]. Hence, it can be inferred that the water content plays a pivotal role in determining the quality of a food product. In the production of *S. cristaefolium* seaweed tea, the seaweed is utilized in a moist state with a water content of 79.05%. Analysis of Table 1 reveals that the water content of *S. cristaefolium* seaweed tea for non-fermentation, fermentation for 4, 8, 12, and 16 days respectively, ranges from 3.48±0.19 to 3.39±0.10%. There is no significant variance in water content among the different treatments. In accordance with the SNI (SNI 3836:2013) regulations, the maximum allowable water content for packaged dry tea is 8%. The recorded water content of dry *S. cristaefolium* seaweed tea samples falls within the stipulated range of 3.39–3.73%. A study by Kartikaningsih et al. [32] concerning *S. cristaefolium* seaweed tea soaked in lime water ($CaCO_3$) with a pH of 6 for 6 h and subsequently ovened at 80 °C for 20 min exhibited a water content of 14.12%. Maintaining low water content levels can effectively restrict microbial proliferation and chemical reactions. Tea is known to be hygroscopic, readily absorbing moisture from the surrounding environment, thus necessitating proper storage conditions to uphold its quality.

3.3. Acidity

Acidity (pH) stands as a crucial environmental factor influencing the fermentation process of kombucha, leading to the production of various organic acids such as acetic and gluconic acids. Each microorganism thrives within a specific pH range, impacting the antioxidant properties of kombucha [33][34]. Monitoring pH levels is essential not only for tracking the fermentation progress but also for determining its completion [35]. The pH values of *S. cristaefolium* seaweed tea kombucha ranged from 7.46±0.08 for non-fermentation to 2.90±0.03 after 16 days of fermentation, while commercial kombucha exhibited a pH of 2.97±0.01 (Table 1). The results illustrate a clear trend of decreasing pH with

prolonged fermentation duration. Particularly, the 16-day fermented *S. cristaefolium* seaweed tea kombucha displayed the lowest pH value of 2.90, whereas the pH of the commercial counterpart closely resembled that of the 12-day fermented *S. cristaefolium* seaweed tea kombucha (pH 2.97). The non-fermented seaweed tea kombucha with *S. cristaefolium* maintained a neutral pH of 7.46, indicating the absence of fermentation due to the lack of original kombucha starter and SCOBY. Research by Aung and Eun [16] on *Porphyra dentata* seaweed kombucha revealed a gradual decrease in pH from 3.13 to 2.88 throughout the fermentation process, ensuring its safety for consumption. It is noted by Cardoso et al. [36] that pH levels below 2.5 pose health risks due to excessive acetic acid content, while values above 4.2 jeopardize microbiological safety. The initial stages of fermentation witness a slow decline in pH as yeast adapts, delaying the formation of organic acids influencing pH. As fermentation progresses, the increase in protons (H^+) from organic acids, derived from glucose conversion by *Acetobacter xylinum* bacteria, contributes to pH reduction [37]. The metabolic activity of *A. xylinum* bacteria, facilitated by the dissociation of acetic acid, further lowers the pH of kombucha. Additionally, the conversion of yeast-produced ethanol into organic acids by acetic acid bacteria leads to a pH decrease, accentuated during the stationary phase where ethanol is utilized for acetic acid production, ultimately reducing the pH of kombucha [35].

3.4. Total Acid Content

Total titrated acid (TAT) refers to the complete concentration of acid present in a substance or product. The measurement of TAT holds more significance than the pH level in determining the quantity of organic acid within a product [38]. In food analysis, TAT is assessed through acid-base titration to approximate the total acid concentration. Various acids, particularly organic acids, can influence the taste, color, microbial stability, and overall quality of food products [39]. As highlighted by Hafsari et al. [35], the assessment of total acid is a crucial parameter, serving as an indicator of the fermentation process where organic acids are generated, marking the culmination of fermentation. The data presented in Table 1 illustrates the total acid content of *S. cristaefolium* seaweed tea kombucha across different fermentation durations, ranging from no fermentation to 16 days. Specifically, the total acid content was found to be 0.02±0.01% for no fermentation, gradually increasing to 1.20±0.07%, 1.33±0.02%, 1.54±0.07%, and 1.94±0.14% for fermentation periods of 4, 8, 12, and 16 days, respectively. In comparison, commercial kombucha exhibited a total acid content of 1.17±0.08%. The research outcomes indicate a positive correlation between fermentation duration and total acid levels. Notably, *S. cristaefolium* seaweed tea kombucha subjected to a 16-day fermentation period demonstrated the highest total acid content at 1.94%, which significantly varied ($p < 0.05$) from the no fermentation group and the 12-day fermentation group. Conversely, the total acid content of commercial kombucha did not exhibit a significant difference ($p > 0.05$) when compared to *S. cristaefolium* seaweed tea kombucha fermented for 4 and 8 days. The negligible total acid content in *S. cristaefolium* seaweed tea kombucha without fermentation can be attributed to the absence of original kombucha starter and SCOBY, hindering the fermentation process and subsequent organic acid production that influences total acid levels.

From the test results, it is known that the longer the fermentation time, the total acid of *S. cristaefolium* seaweed tea kombucha increases, this is in line with the decrease in pH value. This is in accordance with the statement of Kamaludin and Handayani [40] which states that total acid is closely related to pH value, where the longer the fermentation shows an increase in total acid and a decrease in pH, this is due to the decomposition of glucose into acid so that there is an increase in total acid and a decrease in pH. In addition, research conducted by Pratiwi et al. [21] related to the effect of fermentation time on the physical and chemical properties of kombucha drinks from *Sargassum sp*. seaweed has a pH value that decreases and a total acid value that increases with the length of fermentation time (0–16 days of fermentation), where the pH value ranges from 3.09–4.89 and the total acid value ranges from 0.24–2.98%. During the fermentation process, the total acid will increase because the acetic acid bacteria in kombucha have experienced a logarithmic phase, where the acetic

acid bacteria will synthesize alcohol into more organic acids so that the total acid produced is also higher [21]. The longer the fermentation, the more acetic acid is formed as a result of the metabolism of *A. xylinum* bacteria and the fermentation results will be more acidic. Acetic acid produced during the fermentation process affects the taste and aroma of kombucha, where the concentration of acid formed will tend to increase, but at certain times the acid concentration can decrease if the sugar and ethanol sources have run out because the bacteria use it as a carbon source to produce organic acids. This will cause the bacteria to experience a death phase [41]. There are two stages of oxidation during the formation of acetic acid by *A. xylinum* bacteria, the first stage is converting alcohol into acetaldehyde which is catalyzed by the enzyme alcohol dehydrogenase. Continued in the second stage, namely the acetylaldehyde compound forms acetic acid with the help of acetylaldehyde dehydrogenase, so that the main products produced are acetic acid, gluconic acid, and cellulose [35].

3.5. Reducing Sugar Content

Measurement of reducing sugar is conducted to ascertain the quantity of carbon within sugar that has been metabolized by microbes, thus making reducing sugar a pivotal parameter in evaluating the efficacy of the kombucha fermentation process [42]. The assessment of reducing sugar through the DNS method necessitates a glucose standard curve yielded the equation y = 1.8015x + 0.0219 with a coefficient (R^2) of 0.9986. Determining reducing sugar entails inputting the absorbance value into the equation and subsequently utilizing it in the formula for computing the reducing sugar content. The findings from the analysis of reducing sugar in kombucha brewed with *S. cristaefolium* seaweed tea are detailed in Table 1.

The reducing sugar content of *S. cristaefolium* seaweed tea kombucha in the treatment without fermentation, fermentation for 4, 8, 12, and 16 days were 0.64±0.01, 2.86±0.34, 2.34±0.01, 1.76±0.02, and 1.58±0.01%, respectively, while for commercial kombucha 3.68±0.03%. The results showed that the longer the fermentation have lower reducing sugar content. Commercial kombucha had the highest reducing sugar content, while the lowest reducing sugar content was in *S. cristaefolium* seaweed tea without fermentation. The length of fermentation significantly affected the reducing sugar content of all treatments and commercial kombucha ($p < 0.05$). The control had the lowest reducing sugar content because it only used *S. cristaefolium* seaweed tea infusion without added sugar, however, the reducing sugar content formed was 0.64% which indicated that *S. cristaefolium* seaweed tea contained reducing sugar, although in low levels. Research conducted by Nagarajan et al. [43] related to the effect of the duration of the acid hydrolysis process on the reducing sugar content of brown algae *S. cristaefolium* had a reducing sugar content of 3.69±0.07%. Meanwhile, Pratiwi et al. [21] reported on the effect of fermentation time on the physical and chemical properties of kombucha drinks from *Sargassum sp*. seaweed had a total sugar content that decreased along with the longer fermentation time where the total sugar content

Table 2. Effect of fermentation time on total phenol content and antioxidant activity (DPPH inhibition and FRAP) of *S. cristaefolium* seaweed tea kombucha.

Fermentation Time	Total Phenol (mg GAE/100 g)	DPPH (%)	FRAP (mM/g)
No fermentation	11.55±0.14^{f}	61.10±0.30^{g}	1.70±0.05^{g}
4 days	16.06±0.13^{e}	75.86±0.91^{f}	2.75±0.05^{f}
8 days	20.49±0.33^{d}	80.33±0.34^{d}	3.40±0.05^{d}
12 days	25.14±0.07^{b}	83.00±0.83^{c}	4.40±0.07^{c}
16 days	23.32±0.19^{c}	78.68±0.32^{c}	3.61±0.07^{e}
Commercial kombucha	84.33±1.50^{a}	86.84±0.75^{a}	7.46±0.11^{b}
Vitamin C	-	84.84±0.13^{b}	789.39±63.63^{a}

Note: Each value is expressed as mean±SD in triplicate experiments. Values a-f with different letters in the same column indicate significant differences between treatments ($p < 0.05$), analyzed using Tukey HSD.

without fermentation was 17.07% and the total sugar continued to decrease until 16 days of fermentation to 12.53%. This study is also in line with the study of Aung and Eun [16] regarding the effect of time and temperature on *Porphyra dentata* seaweed kombucha where the reducing sugar content decreased significantly in the last days of fermentation (13–18 days). The sugar content that decreases with the length of fermentation is because during the fermentation process, yeast will break down sucrose in the medium into alcohol which is continued with the oxidation of alcohol into acetic acid by acetic acid bacteria. Microorganisms need energy to survive, cell reproduction, and the movement of motile microorganisms, so the substrate that is easy to use is reducing sugar such as glucose and fructose which are used as a carbon source. SCOBY contains a symbiotic culture of bacteria and yeast that require carbon as a nutrient, where the carbon source in kombucha fermentation is obtained from the addition of sugar. Yeast needs carbon to produce alcohol and acetic acid bacteria need carbon to convert alcohol into acetic acid [42]. During the fermentation process, alcohol will be formed and at the end of fermentation, organic acids will accumulate so that the sugar content will continue to decrease. Sucrose will be converted into glucose and fructose which have simpler forms so that they are easily used by microorganisms to produce alcohol. Glucose will be converted into gluconic acid in the secondary biochemical activity of Acetobacter bacteria, while fructose is only used a little by microorganisms so that it will remain part of the kombucha solution which will be detected when testing the reducing sugar content. The main products produced from the biochemical process of yeast and acetic acid bacteria are alcohol, gluconic acid, acetic acid and cellulose [35].

3.6. Total Phenol Content

The determination of total phenol content was carried out by making a standard curve of gallic acid [44]. The regression equation y = 0079x – 0.0149 and R^2 of 0.9988 were obtained from standard curve of gallic acid. Table 2 shows that the total phenol content of *S. cristaefolium* tea kombucha in the treatment without fermentation, fermentation for 4, 8, 12, and 16 days were 11.55±0.14, 16.06±0.13, 20.49±0.33, 25.14±0.07, 23.32±0.19, and 84.33±1.50 mg GAE/100 g, respectively. The total phenol content of *S. cristaefolium* seaweed tea kombucha and commercial kombucha were significantly different ($p < 0.05$). The highest total phenol content in commercial kombucha was 84.33±1.50 mg GAE/100 g and the lowest total phenol content in the unfermented sample (11.55±0.14 mg GAE/100 g). The total phenol content of commercial kombucha is higher than that of *S. cristaefolium* seaweed tea kombucha due to differences in the basic ingredients used [45]. Tea contains flavonoid compounds that function as antioxidants, one of which is the most abundant and active catechin compound, so it is often used as an indicator of the quality of tea products [46]. Based on the results of this study, it is known that the longer the fermentation, the total phenol content of *S. cristaefolium* seaweed tea kombucha increases, starting from the treatment without fermentation to 12 days of fermentation. Yeast and bacterial metabolism can increase phenol compounds, this is thought to be due to the biotransformation process that utilizes the enzymes of a plant cell to increase certain biological activities [47]. Chlorogenic acid is one of the phenolic acids found in *Sargassum muticum* [48]. *Sargassum cristaefolium* is thought to contain chlorogenic acid because it is still in the same family, namely Sargassceae. Microbes will degrade chlorogenic acid into caffeic acid and caffeic acid will break down into cinnamic acid [49]. *Saccharomyces cerevisiae* in kombucha culture during fermentation is able to produce vinyl phenol reductase enzyme, where the enzyme with ferulic acid reductase enzyme will decarboxylate cinnamic acid into trans-4-hydroxymethoxycinnamic acid (ferulic acid) and trans-4-hydroxycinnamic acid (*p*-coumaric acid) which will undergo decarboxylation to form phenol compounds, namely vinylguaiacol (4-VG) and 4-vinylphenol (4-VP) [50]. In addition, the increase in phenolic compounds is thought to be due to the metabolism of bacteria and yeast that produce flavonoid compounds through enzymatic reactions that degrade other matrix components to form phenolic compounds [47]. However, in 16 days of fermentation, the total phenol content decreased, thought to be due to an increase in the amount of organic acids and a decrease in pH, indicating that

S. cristaefolium seaweed kombucha was very acidic due to the activity of yeast and bacteria. According to Hassmy et al. [51], an acidic environment causes phenolic compounds in kombucha to become more stable and difficult to release protons that can bind to free radicals, so that antioxidant activity decreases. The decrease in antioxidant activity is directly proportional to the decrease in phenolics. The longer the fermentation, the higher the total phenol content in *S. cristaefolium* tea kombucha, but at the end of fermentation, the total phenol content decreased. This is in line with the research of Aung and Eun [16] regarding the effect of time and temperature on *Porphyra denata* seaweed kombucha which has a total phenol content that increases during fermentation but after 22 days of fermentation there is a significant decrease in total phenol content, the average amount of total phenol with an incubation temperature of 25 °C is 27.08–33.43 mg GAE/100 mL and an incubation temperature of 30 °C is 27.08–37.00 mg GAE/100 mL. This is because higher temperatures accelerate bacterial growth which causes faster consumption and the formation of trans polyphenols which will reduce phenolic content. When compared to this study, *S. cristaefolium* seaweed tea kombucha has a lower average total phenol (16.06–25.14 mg GAE/100 g). The decrease in phenolic content of kombucha from *S. cristaefolium* seaweed tea at 16 days of fermentation is likely due to a combination of chemical degradation of phenolic compounds and microbial dynamics, particularly influenced by acidic pH. Phenolic compounds are not always stable under extended fermentation. Over time, phenolic degradation can occur due to enzymatic transformation: Microorganisms in kombucha (e.g., *Acetobacter, Lactobacillus, Saccharomyces*) produce enzymes like polyphenol oxidase or peroxidase that can transform or degrade phenolics [52]. Microbial activity in kombucha becomes significantly inhibited below pH 3, particularly affecting yeast hydrolytic enzymes responsible for phenolic liberation [53].

3.7. DPPH Radical Scavenging Activity

The antioxidant activity of *S. cristaefolium* seaweed tea kombucha in inhibiting DPPH free radicals can be seen in Table 2. The DPPH inhibitory activity of *S. cristaefolium* seaweed tea kombucha in the treatment without fermentation, fermentation for 4, 8, 12, and 16 days were 61.10±0.30, 75.86±0.91, 80.33±0.34, 83.00±0.83, and 78.68±0.32%, respectively, while the antioxidant activity of commercial kombucha and vitamin C was 86.84±0.75 and 84.84±0.13%, respectively. The DPPH inhibitory activity of *S. cristaefolium* seaweed tea kombucha, commercial kombucha, and vitamin C were significantly different ($p < 0.05$). The highest antioxidant activity was in commercial kombucha (86.84±0.75%) and the lowest in the non-fermentation treatment (61.10±0.30%). According to Wibowo et al. [46] the tea production process (eg during the heating process) can affect antioxidant levels. The inhibition of DPPH free radicals by vitamin C is greater than that of *S. cristaefolium* seaweed tea kombucha. Vitamin C is included in a very pure natural antioxidant, and has the ability to ward off extracellular free radicals because it has a free hydroxyl group that acts as a free radical scavenger [54].

Based on the results of the antioxidant activity test, it can be seen that the longer the fermentation, the percentage of DPPH free radical inhibition of *S. cristaefolium* tea kombucha increased from the non-fermentation treatment to 12 days of fermentation. In addition, this study shows a tendency for an increase in the percentage of inhibition of DPPH free radicals to be positively correlated with an increase in total phenol during fermentation. The increase in antioxidant activity can be caused by *S. cristaefolium* containing phenolic compounds that can increase in number during fermentation. The fermentation process carried out by bacteria and yeast will increase the amount of phenol in kombucha so that it can increase antioxidant activity. The increase in antioxidant activity is due to the presence of free phenolics produced during fermentation [51].

Fermentation time can affect antioxidant levels, where there is induction of damage to the cell wall structure so that the phenolic components bound to the cell wall will be released then through an enzymatic reaction into free phenolics or can be synthesized into bioactive components that can increase antioxidant activity [13][33]. Phenolic compounds have hydroxyl groups bound to aromatic rings, making them easy to oxidize by

donating hydrogen atoms to free radicals, and phenolic compounds have the ability to form stable phenoxy radicals during oxidation reactions, so they have high antioxidant activity [55].

Factors that affect the increase in antioxidant activity in fermented products are the type of microorganisms involved, fermentation time, temperature, solvent, pH, water content, type of product and aerobic conditions. However, in 16 days of fermentation, the percentage of DPPH radical inhibition decreased, allegedly due to an increase in the amount of organic acids and a decrease in pH indicating that *S. cristaefolium* seaweed kombucha is already very acidic due to the activity of yeast and bacteria. According to Hur et al. [33] and Fadilah et al. [34] pH affects kombucha fermentation which will form organic acids such as acetic and gluconic acids, and pH is closely related to microbial growth and changes in the structure of phytochemical compounds because each microorganism has an optimal pH for its living environment which can affect antioxidant activity. A decrease in pH can interfere with bacterial growth because the environment is not suitable for survival and metabolism [35]. According to Hassmy et al. [51] acidic conditions cause phenolic compounds in kombucha to become more stable and difficult to release protons that can bind to DPPH free radicals so that antioxidant activity decreases. The longer the fermentation, the DPPH free radical inhibition activity in *S. cristaefolium* seaweed tea kombucha increases but decreases at the end of fermentation.

The DPPH free radical inhibition activity of *S. cristaefolium* seaweed tea kombucha (75.86–83.00%) is higher than that of black tea kombucha (70.40–78.62%) [56]. The high antioxidant activity of *S. cristaefolium* seaweed tea kombucha is because it contains active ingredients that function as antioxidants such as steroids, saponins, tannins, terpenoids, glycosides, flavonoids, and phenols [7]. In addition, dry black tea in the manufacturing process undergoes an enzymatic oxidation process carried out by the polyphenol oxidase enzyme so that it can reduce the levels of catechins in black tea [57]. In addition, the processing of dry black tea includes the stages of withering, rolling, and enzymatic polyphenol oxidation, drying, sorting, and packaging. These stages that are too long can be a factor in damaging the polyphenol components, namely catechins.

3.8. Ferric Reduction Antioxidant Power (FRAP)

The FRAP method is a method for determining the antioxidant activity content of a material based on the ability of antioxidant compounds to reduce the ferric (Fe^{3+}) complex from TPTZ to ferrous (Fe^{2+}) which is indicated by a color change to blue and can be measured at 595 nm [58]. This study used $FeSO_4.7H_2O$ as a standard curve. The results of determining the $FeSO_4 \cdot 7H_2O$ standard curve obtained a regression equation of y = 0.0052x + 0.0461 with a R^2 of 0.9965. Determination of the antioxidant activity value by entering the sample absorbance into the regression equation. Table 2 shows that the antioxidant activity (FRAP) of *S. cristaefolium* seaweed tea kombucha in the treatment without fermentation, fermentation for 4, 8, 12, and 16 days were 1.70±0.05, 2.75±0.05, 3.40±0.05, 4.40±0.07, 3.61±0.07, and 7.46±0.11 mM/g, respectively. The antioxidant activity (FRAP) of *S. cristaefolium* seaweed tea kombucha was significantly different ($p < 0.05$) from commercial kombucha. The highest antioxidant activity was found in commercial kombucha (7.46±0.11 mM/g) and the lowest in samples without fermentation (1.70±0.05 mM/g). The antioxidant activity of *S. cristaefolium* seaweed tea kombucha is lower than commercial kombucha, presumably because during the process of making dry *S. cristaefolium* seaweed tea, the steps of soaking at a temperature of 85 °C, drying for 24 hours at room temperature, and roasting are carried out which can damage the polyphenol components. According to Wibowo et al. [46] during the tea leaf production process, antioxidant levels can be affected, for example the heating process which can reduce antioxidant levels. Heating at high temperatures can damage the flavonoid structure which causes damage to the natural function of flavonoids [59]. In this study, vitamin C was used as a comparison with a concentration of 10 ppm which had an antioxidant activity value of 789.39±63.63 mM/g. The antioxidant activity of vitamin C is greater than that of *S. cristaefolium* seaweed tea kombucha. According to Isnindar et al. [54], vitamin C is included in the very pure natural antioxidants, where vitamin C has the ability to

ward off extracellular free radicals because it has free hydroxyl groups which act as free radical scavengers.

Based on the results of the antioxidant activity test, it can be seen that the longer the fermentation, the antioxidant activity of *S. cristaefolium* seaweed tea kombucha increased, starting from the treatment without fermentation to 12 days of fermentation. The tendency for increasing antioxidant activity is positively correlated with the increase in total phenolics during fermentation. The increase in antioxidant activity is due to the presence of free phenolics produced during fermentation [54]. Previous studies have stated that during kombucha fermentation, microbes will carry out metabolism which will produce phenolic compounds, flavonoids, and beneficial metabolites (organic acids, vitamins, minerals, and enzymes) which have an important role in the antioxidant activity of kombucha drinks [60]. The 16-day fermentation, antioxidant activity decreased, allegedly due to a decrease in pH which is increasingly acidic so that an accumulation of organic acids is formed in *S. cristaefolium* seaweed tea kombucha. The high accumulation of organic acids can affect microbial metabolism in producing bioactive compounds that are antioxidants. Hafsari et al. [35] stated that a decrease in pH can interfere with bacterial growth due to an unsuitable environment for survival and metabolism. In addition, increasingly acidic kombucha can reduce the ability of phenolic compounds to reduce Fe^{3+} ions to Fe^{2+}. Hassmy et al. [51] reported that an acidic environment causes phenolic compounds in kombucha to become more stable and difficult to donate hydrogen atoms or electrons so that free radical compounds become unstable so that antioxidant activity decreases. The FRAP value of *S. cristaefolium* seaweed tea kombucha ranges from 1.70–4.40 mM/g, where these results fall into the very strong category [61]. Based on the FRAP value, the antioxidant activity of S. cristaefolium tea kombucha (4.40 mM/g) was relatively higher than that of black tea kombucha (2.73 mM/g), and relatively similar to the antioxidant activity of green tea kombucha (4.80 mM/g) [56].

3.9. Consumer Evaluation of Kombucha S. cristaefolium Seaweed Tea

Hedonic tests are conducted to determine the level of consumer acceptance by asking for personal responses from panelists about liking or disliking the product being assessed. Consumer responses are measured using a hedonic scale: very dislike, dislike, somewhat like, like, and very like [27][62]. Product attributes tested hedonically include color, aroma, taste and appearance.

3.9.1. Color Hedonic

The color preference value of *S. cristaefolium* seaweed tea kombucha ranged from 2.85±1.01–4.18±0.76. The highest color preference value was in the 4-day fermentation treatment (4.18±0.76; like), and the lowest was in the non-fermentation treatment (2.85±1.01; dislike). The length of fermentation time of *S. cristaefolium* seaweed tea kombucha had a significant effect ($p < 0.5$) on the level of color preference. The longer the fermentation, the color of the seaweed tea kombucha *S. cristaefolium* shows a fading color. This is in accordance with the results of the hedonic test of color parameters conducted by Wistiana and Zubaidah [22] which stated that the longer the fermentation, the level of preference for the color of kombucha decreased, which was caused by the longer the fermentation, the color of the kombucha produced faded due to the decomposition of components in kombucha. In addition, research conducted by Pratiwi et al. [21] also stated that *Sargassum sp*. kombucha was dark brown at the beginning of fermentation, but the longer the fermentation time, the dark brown color of the kombucha became brighter, which was caused by the ability of the microbial consortium to degrade the color so that the color faded. One of the factors that causes changes in the color of kombucha is that the longer the fermentation, the more kombucha microbes increase and the microbes will degrade the compounds in kombucha which causes the color of kombucha to fade [63].

3.9.2. Aroma Hedonic

Aroma is one of the determining factors for the quality of a food product. Aroma arises because aroma has volatile properties, is slightly soluble in water, and fat [64]. The level of preference for the aroma of *S. cristaefolium* seaweed tea kombucha ranges from 2.16±0.96–4.11±0.78 (Table 3). The

Table 3. Hedonic value of kombucha *Sargassum cristaefolium* seaweed tea.

Fermentation Time	Hedonic				
	Color	Aroma	Taste	Appearance	Overall
No fermentation	2.85±1.01^{e}	2.16±0.96^{e}	2.40±1.03^{e}	2.65±1.17^{e}	2.52±0.30^{e}
4 days	4.18±0.76^{a}	4.11±0.77^{a}	4.40±0.72^{a}	4.35±0.71^{a}	4.26±0.14^{a}
8 days	4.05±0.81^{a}	3.79±0.88^{b}	3.91±0.79^{b}	4.13±0.83^{ab}	3.97±0.15^{b}
12 days	3.81±0.77^{bc}	3.28±0.90^{c}	3.36±0.75^{c}	3.75±0.70^{c}	3.55±0.27^{c}
16 days	3.58±0.89^{cd}	2.81±0.90^{d}	2.98±0.90^{d}	3.41±0.87^{d}	3.20±0.36^{dc}

Note: Values a-e with different letters in the same column indicate significant differences between treatments ($p < 0.05$), analyzed using Mann-Whitney.

length of fermentation time of *S. cristaefolium* seaweed tea kombucha has a significant effect ($p < 0.5$) on the aroma preference value. The highest aroma preference value was in the 4-day fermentation treatment (4.11±0.78; like) which produced a slightly sour aroma and no fishy smell. The lowest preference value was in the non-fermented treatment (2.16±0.96; dislike) because there was a fishy aroma in *S. cristaefolium* seaweed tea. According to Purnami et al. [17] during the fermentation process, volatile compounds such as alcohol, acetic acid, and organic acids will be produced which produce a distinctive sour aroma that can cover the fishy aroma of seaweed. The longer the fermentation, the more sour the aroma of *S. cristaefolium* seaweed tea kombucha will be, so it is less preferred by the panelists. This was also reported by Simanjuntak et al. [65] longer the fermentation, the more sour the apu-apu kombucha will produce. The stronger sour aroma is because the longer the fermentation, the accumulation of organic acids will occur and volatile compounds will be formed. The longer the fermentation, the more chemical compounds such as acetic acid, a volatile metabolic product of Acetobacter bacteria, increase [63]. The metabolic products of bacteria and yeast are in the form of organic acids such as acetic acid, gluconic acid, and gluconic acid, as well as alcohol, which gives the distinctive sour aroma of kombucha.

3.9.3. Taste Heconic

Taste is a panelist's perception that is assessed based on the stimulation of the sense of taste including salty, sweet, sour, and bitter tastes [63]. Table 3 shows the level of taste preference for *S. cristaefolium* seaweed tea kombucha. The fermentation time of *S. cristaefolium* seaweed tea kombucha had a significant effect ($p < 0.5$) on taste. The level of taste preference for *S. cristaefolium* seaweed tea kombucha was highest in the 4-day fermentation treatment (4.40±0.72; like) where the resulting taste was predominantly sweet and slightly sour. Meanwhile, the lowest taste preference value was shown in the treatment without fermentation (2.40±1.03; dislike), because the taste of *S. cristaefolium* seaweed tea was bland. In this study, unfermented *S. cristaefolium* seaweed tea cannot be compared with each treatment, because it is not given added sugar while each treatment of the fermentation time of *S. cristaefolium* seaweed tea kombucha is given added sugar. Based on these differences, it can affect the level of panelist acceptance of samples without fermentation and *S. cristaefolium* tea kombucha, where samples without fermentation tend to be disliked by panelists when compared to *S. cristaefolium* seaweed tea kombucha with added sugar. The longer the fermentation, the taste of *S. cristaefolium* seaweed tea kombucha becomes more sour so that it is less preferred by panelists. These results are in accordance with research conducted by Febriella et al. [66] regarding the innovation of fermented herbal drinks where 3-day fermentation has a value of 3.56 (liked) and 9-day fermentation has a value of 2.24 (less preferred). Simanjuntak et al. [65] reported that kombucha apu-apu (*Pistia stratiotes*) fermented for 4 days had a specific sour taste, while kombucha fermented for 12 days had a very sour taste. The longer the fermentation, the lower the pH, resulting in a more sour taste. The stronger sour

taste tends to be disliked by consumers because it is something new for consumers to taste kombucha drinks that have a sour taste [18].

3.9.4. Appearance Hedonic

The level of preference for the appearance of *S. cristaefolium* seaweed tea kombucha ranged from 2.65±1.17–4.35±0.71 (Table 3). The highest appearance preference value was in *S. cristaefolium* seaweed tea kombucha fermented for 4 days (4.35±0.71; like), while the lowest was in the treatment without fermentation (2.65±1.17; panelists did not like). The length of fermentation had a significant effect ($p < 0.5$) on the level of panelists' preference for the appearance of *S. cristaefolium* seaweed tea kombucha. The longer the fermentation, the less the appearance of *S. cristaefolium* seaweed tea kombucha was preferred by the panelists. Seaweed tea kombucha *S. cristaefolium* fermented for 4 days has a more cloudy appearance, while 16-day fermentation has a less cloudy appearance with clear brown specifications. It can be said that the longer the fermentation, the lower the turbidity level of kombucha. This is in accordance with research conducted by Nurhayati et al. [67] which stated that panelists considered 2% Cascara kombucha to have a more cloudy appearance compared to 1% Cascara kombucha and the longer the fermentation, the lower the turbidity level of Cascara kombucha. Turbidity in kombucha is caused by the total dissolved solids contained in kombucha. Basically, the total dissolved solids of kombucha are dominated by reducing sugars, non-reducing sugars such as sucrose, in addition to organic acids, pectin, proteins, pigments, vitamins, and minerals also produce total dissolved solids [17]. The longer the fermentation, the more the microbial activity that degrades the sucrose substrate (sugar) and the dissolved content in *S. cristaefolium* seaweed tea used in microbial metabolism causes the total solids to decrease [68].

3.9.5. Overall Hedonic

The overall hedonic level for *S. cristaefolium* seaweed tea kombucha can be seen in Table 3. The length of fermentation time had a significant effect ($p < 0.05$) on the overall hedonic value of *S. cristaefolium* seaweed tea kombucha. The overall liking value of *S. cristaefolium* seaweed tea kombucha in the treatment without fermentation, fermentation for 4, 8, 12, and 16 days, respectively, were 2.52±0.30; 4.26±0.14; 3.97±0.15; 3.55±0.27; and 3.20±0.36. The 4-day fermentation was the most preferred by the panelists, while the *S. cristaefolium* seaweed tea kombucha without fermentation treatment was the least preferred. The fermentation period affected the aroma, color, taste, and appearance of the *S. cristaefolium* seaweed tea kombucha, where as the fermentation period increased, the overall preference level decreased. The aroma and taste of the *S. cristaefolium* tea kombucha produced were too sour, the color faded, and the appearance was less cloudy or tended to be clear brown. All of this was caused by the metabolism of the microbes in it which utilized the components in kombucha to survive, cell proliferation, and the movement of motile microorganisms. In addition, fermentation can also improve the aroma of *S. cristaefolium* seaweed tea which has a fishy aroma that is not liked by consumers. During the fermentation process, volatile compounds such as alcohol, acetic acid, and organic acids will be produced which produce a distinctive sour aroma that can cover the fishy aroma of seaweed [17].

The 16-day fermentation process of kombucha from *S. cristaefolium* seaweed tea generally decreases the preference level due to several main factors. First, during fermentation, yeast converts sugar into ethanol, which is then oxidized by acetic and lactic acid bacteria into organic acids (such as acetic and lactic acids). Second, kombucha with a normal pH ranges from 2.5–3.5. When it drops below 2.5, the taste becomes very sharp, with the intensity of astringent and vinagery flavors increasing drastically [69]. Scaling up the production of kombucha using *S. cristaefolium* seaweed tea is feasible but presents both opportunities and challenges. Kombucha generally has a good shelf-life due to its acidity and microbial stability. However, *S. cristaefolium* contains bioactive compounds (e.g., polyphenols, fucoidans) that may be sensitive to oxidation and microbial degradation, possibly affecting flavor and functional properties over time. Proper pasteurization (or cold-chain distribution for raw kombucha), packaging, and antioxidant stabilization

can extend shelf-life to 2–4 months. Scaling up is technically feasible with moderate capital and process adjustments. Success depends on managing raw material variability, optimizing fermentation, and ensuring product stability and safety [70].

4. CONCLUSIONS

The length of fermentation affects the chemical characteristics of *S. cristaefolium* tea kombucha including pH, total acid, and total reducing sugar, where the longer the fermentation, the pH and reducing sugar content decreases, while the total acid continues to increase. In terms of chemical characteristics, the best treatment of *S. cristaefolium* tea kombucha is 4 days fermentation with a pH value of 3.07±0.02; total acid 1.20±0.07%; and total reducing sugar 2.86±0.34. In addition, the length of fermentation also affects antioxidant activity, where the longer the fermentation, the total phenol content, antioxidant activity (DPPH and FRAP methods) increases even though at the end of fermentation it decreases. The 12-day fermentation treatment had the highest antioxidant activity compared to other treatments with a total phenol content of 25.14±0.07 mg GAE/100 g, DPPH antioxidant activity of 83.00±0.83%; and FRAP antioxidant activity of 4.40±0.07 mM/g. The length of fermentation also affects the consumer acceptance level of *S. cristaefolium* tea kombucha, where the consumer acceptance level continues to decrease with increasing fermentation time. The most preferred seaweed kombucha *S. cristaefolium* is a 4-day fermentation time with hedonic values of color, aroma, taste, appearance, and overall parameters in sequence, namely 4.18±0.76, 4.11±0.78, 4.40±0.72, 4.35±0.71, and 4.26±0.14.

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Conflicts of Interest

The author(s) declare no conflict of interest.

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