Application of Check-All-That-Apply (CATA) for formulating and characterizing sargassum seaweed-based kombucha drink: effects of different sugar types and fermentation times

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Abstract. One approach to refining the taste of *Sargassum* tea involves subjecting it to fermentation to produce Kombucha tea. This study was designed to assess the impact of fermentation duration and various types of sugars on the sensory attributes of Seaweed Kombucha, alongside the physicochemical properties and flavor profile associated with the optimal sugar type and a fermentation duration. Seaweed Kombucha samples were subjected to treatments involving different sugars (sucrose, sorbitol, and steviol) and fermentation duration of 9 days, with sampling intervals at days 0, 3, 5, 7, and 9. The characteristics of Seaweed Kombucha were evaluated based on parameters such as pH, alcohol content, color, total sugar content, and sensory analysis. Utilizing hedonic sensory assessment, the most favorable Seaweed Kombucha sample was identified as the one treated with steviol sugar and fermented for 3 days. The sensory flavor profile of Seaweed Kombucha was elucidated through Check-All-That-Apply (CATA) and Gas Chromatography-Mass Spectrometry (GC-MS) methodologies. The optimal Seaweed Kombucha sample exhibited a volatile compound profile comprising acids, alcohols, ketones, heterocyclic compounds, and other constituents.

1 Introduction

Herbal beverages are widely appreciated for their distinctive flavor profiles, which are typically derived from a diverse array of natural ingredients. Kombucha tea, a traditional

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fermented beverage, is created by fermenting a tea and sugar solution using a Symbiotic Colony of Bacteria and Yeast (SCOBY) [1]. This fermentation process produces various volatile compounds, including alcohols, aldehydes, ketones, acids, esters, and benzenoids, contributing to its unique flavor, which combines sweet and sour notes [2].

One of the underutilized natural resources in food science is *Sargassum* sp., a type of brown seaweed rich in bioactive compounds such as fucoidan and fucoxanthin. Recent studies have highlighted its potential in enhancing the nutritional and sensory qualities of food products [3]. Despite its nutritional potential, *Sargassum* sp. is often overlooked due to its strong odor and undesirable taste [4, 5]. However, various processing techniques, such as fermentation and the incorporation of complementary flavors, have been investigated to improve its palatability and consumer acceptability [6]. This study explores the transformation of *Sargassum* sp. into a seaweed-based Kombucha beverage, evaluating the effects of varying fermentation durations and sugar types on its sensory properties to enhance its flavor profile and appeal.

The primary objective of this research is to assess how different fermentation times and sugar sources influence the sensory attributes of Seaweed Kombucha. This study seeks to determine the optimal conditions that improve its physicochemical properties and taste profile, thereby promoting the use of *Sargassum* sp. in functional beverage formulations. By employing Check All That Apply (CATA) analysis, a well-established method in sensory evaluation, the study provides a detailed sensory profile of Seaweed Kombucha, offering insights that could lead to the development of innovative variants distinct from conventional tea-based Kombucha.

CATA analysis has proven to be an effective method for sensory evaluation, particularly in the assessment of tea beverages. Previous studies, such as Tong, Hunaefi, and Liou, [7, 8, 9] have demonstrated the value of CATA in characterizing sensory attributes and understanding consumer preferences. In related research by [10] CATA analysis was employed to evaluate the sensory attributes of sustainable seaweed stick snacks made from the by-products of milkfish broth combined with *Gracilaria* sp., identifying key attributes such as smoothness, firmness, and taste enhancement due to the inclusion of amino acids from the broth. This method was also applied to seaweed-based analog rice, demonstrating the influence of microwave cooking on the product’s texture and sensory properties, thereby optimizing its appeal as a functional food. These studies indicate that optimizing the sensory attributes of *Sargassum* sp. and other seaweed-based products can significantly enhance their marketability and consumer acceptance, while contributing to sustainable food development and promoting health and well-being [11].

To the best of our knowledge, no previous research has explored the potential of using different sugar types to mask the strong flavor of *Sargassum* during the fermentation of Seaweed Kombucha. This study aims to fill this gap, providing a comprehensive assessment of sensory attributes to contribute valuable data to the development of novel herbal beverages. Understanding how to optimize the sensory attributes of Seaweed Kombucha improve its marketability and enhance its potential as a mood-enhancing beverage. Hence, this study leverages the benefits of CATA analysis to evaluate the sensory attributes of Seaweed Kombucha formulation with different type of sugar and fermentation time. This study also aims to contribute to the broader dialogue on innovative herbal beverages, highlighting their potential to enhance consumer well-being and create enjoyable, health-promoting experiences.

2 Methods

2.1 Sampling

The primary materials utilized in this study include *Sargassum* sp. sourced from Pandan Beach, Ujung Genteng, Sukabumi Regency, West Java, Indonesia. The seaweed was washed using freshwater with flowing water. *Sargassum* seaweed was thoroughly washed to remove impurities such as sand and stones. Additional materials employed comprise SCOBY culture (fermentation media) which contained lactic acid bacteria (*Acetobacer* and *Lactobacillus*) and yeast (*Saccharomyces*) [12], to produce Seaweed Kombucha, water, sucrose sugar (gulaku, Lampung, Indonesia), sorbitol sugar (tropicana slim, Bogor, Indonesia), steviol sugar (tropicana slim, Bogor, Indonesia), and distilled water. The equipment consists of a stove, containers, stirrers, sieves, glass jars, aluminium foil, volumetric flasks, pH meter, alcohol meter, analytical balance (Mettler Toledo ME, USA), measuring glasses, blender (Philips), basin, ladle, funnel, tea bags, and Gas Chromatography Mass Spectrometry (GC-MS) (Agilent 7890B, USA).

2.2 Production of *Sargassum* Herbal Tea

The process of making Seaweed Kombucha followed the method by Harrison [13]. Seaweed was grounded using a blender. The dried *Sargassum* was weighed and placed into tea bags, 60 g/bag. Then, the tea bags were steeped in 1 L of boiling water for 5 minutes. The tea solution was filtered into sterilized jars. Sugar (sucrose, sorbitol, steviol) was added, 200 g each, to the respective treatments and mixed until dissolved.

2.3 Production of Seaweed Kombucha

The cooled *Sargassum* tea solution was inoculated with Seaweed Kombucha starter (SCOBY culture) with a SCOBY diameter of 9 cm. The solution was covered with a clean cloth to allow some air to enter. The cloth was tied with string or rubber band and stored in a room with good air circulation or in an incubator. The fermentation process lasts for 9 days at room temperature, and the container should not be moved or shaken during fermentation [14]. Treatments used in the research include different fermentation times of Seaweed Kombucha on days 0, 3, 5, 7, and 9. These samples were labelled according to the sugar type and day of fermentation duration. Samples with sucrose sugar were labelled as "suk", samples with sorbitol sugar as "sor", and samples with steviol sugar as "stev", followed by the fermentation time treatment. For example, Seaweed Kombucha with sucrose sugar fermented from day 0 to day 9 is labelled as suk0, suk3, suk5, suk7, suk9, and so forth. The samples underwent sensory hedonic and CATA analysis to determine the best treatment. The sample with the selected characteristic was analyzed for pH, alcohol content, color, sensory properties, total sugar, and flavor using GC-MS.

2.4 Analysis Procedure

2.4.1 Hedonic Sensory Analysis (Modified from [13])

Hedonic sensory testing was conducted using human senses as the primary tool to evaluate a product. This testing involved 30 panelysts (16 females and 14 males). The evaluation encompassed parameters of appearance, color, aroma, taste, aftertaste, and overall impression.

Hedonic testing employed a scaling method ranging from 1 to 9, including extremely dislike (1), very dislike (2), dislike (3), somewhat dislike (4), neutral (5), somewhat like (6), like (7), very like (8), and extremely like (9).

2.4.2 Check-All-That-Apply (CATA) Sensory Analysis [15]

CATA sensory analysis was conducted using a list of sensory attributes consisting of terms or phrases related to the product. The sensory attribute list utilized in this study was derived from previous research findings [2]. Panelists were instructed to select applicable characteristics of Seaweed Kombucha from the attribute list. The sensory attribute list describing Seaweed Kombucha comprised 42 attributes, categorized into appearance by visual inspection (amber, yellow, green, brown, black), aroma by olfaction (citrus, tea, sweet, beer/fermented, alcoholic, sour, bitter, unfamiliar, floral, milky, nutty, sweet, fire/animal, fruit, marine, mineral, earthy, and plants/herbaceous), and taste/flavor by tasting (citrus, tea, sweet, beer/fermented, alcoholic, sour, bitter, unfamiliar, floral, milky, nutty, sweet, fire/animal, fruit, marine, mineral, earthy, and plants/herbaceous). Data analysis was performed using XLSTAT 2023 software, which included Cochran's Q test, correspondence analysis, and principal coordinate analysis.

2.4.3 pH Analysis

The pH analysis of Seaweed Kombucha was conducted using a pH meter. Prior to measurement, the pH meter was calibrated using pH 4 and pH 7 buffer solutions. The pH meter electrode was immersed in the Seaweed Kombucha sample, and the measurement results were recorded.

2.4.4 Color Analysis [16]

Color analysis was conducted using image processing method with a smartphone camera (Samsung SM-A715F/DS) for photography and Microsoft PowerPoint 2019 software for processing. The processed results from Microsoft PowerPoint 2019 were expressed in red, green, blue (RGB), then converted to L* (lightness/darkness), a* (redness/greenness), and b* (yellowness/blueness) using the colormine.org website.

2.4.5 Total Sugar Analysis

Total sugar analysis was performed using a Brix refractometer. The testing began with calibrating the instrument by dropping distilled water solution and then calibrating it. The refractometer was then cleaned from distilled water and ready for testing. Total sugar testing was conducted by adding a few drops of sample solution to the prism part. Observe the instrument through the eyepiece in a well-lit area, where the scale can be automatically visible and readable.

2.4.6 Flavor Analysis (GC-MS) [17]

Flavor testing by GC-MS. The flavor compounds in Seaweed Kombucha tea were determined using Head Space-Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS), a method that employed 2-Octanol as an internal standard. The concentration of each flavor compound was calculated based on the relative peak area of each compound relative to the internal standard (µg/L).

2.4.7 Research Design and Data Analysis

The research was conducted using a complete randomized design (CRD) with one factor. The treatment factor used was Seaweed Kombucha with different fermentation times. Replications were carried out for each treatment three times. Data with normal distribution were analyzed using analysis of variance (ANOVA).

3 Result and Discussion

3.1 Sensory Hedonic

Hedonic testing was conducted to measure panelist's overall liking of the product. Liking assessment of the product was measured across 6 parameters: appearance, color, aroma, taste, aftertaste, and overall impression. This sensory evaluation was performed on 30 untrained panelists, comprising 53% females and 47% males. The panelists ranged in age from 20 to 23 years. They came from various ethnic groups, comprising Javanese (34%), Sundanese (34%), Betawi (17%), Minangkabau (3%), Bugis (3%), Batak (3%), Malay (3%), and Banjar (3%). Additionally, 3% of the panelists were active smokers. The tested samples consisted of Seaweed Kombucha with three different sugar treatments: sucrose, sorbitol, and steviol, which were then fermented for 9 days. Samples of Seaweed Kombucha were taken for testing on days 0, 3, 5, 7, and 9 during the fermentation period. The results of hedonic sensory evaluation of Seaweed Kombucha can be seen in Table 1.

Appearance is one of the crucial parameters in determining consumer acceptance levels [18]. The analysis of variance results in Table 1 indicates that the appearance of Seaweed Kombucha does not exhibit significant differences ($p>0.05$). Table 1 illustrates that the average liking scores of panelists towards the appearance of Seaweed Kombucha range from 5.00 (neutral) to 5.60 (somewhat like). The highest values for the appearance parameter are observed in samples stev3 with a value of 5.60, while the lowest average values are found in sample suk7 with a value of 5.00.

Color in a product is considered as an important factor by consumers [19]. The analysis of variance indicates that the color of Seaweed Kombucha does not exhibit significant differences ($p>0.05$). The average liking scores of panelists towards the color of Seaweed Kombucha range from 4.97 (neutral) to 5.87 (somewhat like). The highest average values are obtained from samples stev5 and sor5 with a value of 5.87. The lowest average values are obtained from sample stev0 with a value of 4.97. The color observed in Seaweed Kombucha can be attributed to the presence of natural pigments in *Sargassum*, which impart a reddish-brown color [20]. It is speculated that the brownish color is preferred by the panelists.

Aroma in a product is generated by volatile compounds released during the cooking process [21]. The analysis of variance indicates that the aroma of Seaweed Kombucha does not exhibit significant differences ($p>0.05$). The average liking scores of panelists towards the aroma of Seaweed Kombucha range from 3.40 (dislike) to 4.93 (neutral). The highest average values are obtained from sample Seaweed Kombucha suk9 (4.93). The lowest average values are obtained from sample Seaweed Kombucha suk0 (3.40). Seaweed Kombucha beverages have a sour aroma created by acidic components resulting from the fermentation process. The longer the fermentation time, the higher the chemical compounds such as acetic acid that evaporate, thus creating a strong and pungent aroma [22]. It is this fairly strong sour aroma in Seaweed Kombucha that is less favored by the panelists.

Taste in sensory testing is another important factor in consumer product acceptance [19]. The analysis of variance indicates that taste attributes in Seaweed Kombucha exhibit significant differences ($p<0.05$). The average liking scores of panelists towards the taste of Seaweed Kombucha range from 2.70 (dislike) to 5.17 (neutral). The highest average values are obtained from samples Seaweed Kombucha Sor0 (5.17) and Stev3 (5.17). The lowest average values are obtained from sample Seaweed Kombucha suk9 (2.70). *Sargassum* tea beverages generally have a bitter taste caused by tannin compounds present in *Sargassum* seaweed [23]. Seaweed Kombucha beverages have a distinctive taste. The acidic taste formed in Seaweed Kombucha is derived from acetic acid, giving Seaweed Kombucha its sour aroma and vinegar-like taste [22]. It is this sour taste in Seaweed Kombucha that is less favored by some panelists.

Table 1. Hedonic sensory evaluation of seaweed kombucha with varying sugars and fermentation durations.

Sample	Parameter					
	Appearance	Color	Aroma	Taste	Aftertaste	Overall
Suk0	5.03 ± 2.01^{a}	5.50 ± 2.06^{a}	3.40 ± 2.19^{a}	3.33 ± 1.79^{a}	3.87 ± 2.19^{a}	3.63 ± 1.90^{a}
Sor0	5.23 ± 1.98^{a}	5.03 ± 2.03^{a}	4.13 ± 2.15^{a}	5.17 ± 1.91^{b}	5.07 ± 2.12^{b}	4.70 ± 1.74^{b}
Stev0	5.23 ± 1.91^{a}	4.97 ± 1.92^{a}	4.10 ± 2.09^{a}	4.77 ± 2.33^{b}	5.10 ± 2.09^{b}	4.47 ± 1.70^{ab}
Suk3	5.30 ± 1.62^{a}	5.23 ± 1.91^{a}	4.03 ± 2.17^{a}	2.77 ± 1.77^{a}	3.57 ± 2.18^{a}	3.40 ± 1.61^{a}
Sor3	5.33 ± 1.56^{a}	5.50 ± 1.61^{a}	4.03 ± 2.04^{a}	4.83 ± 2.20^{b}	5.00 ± 2.13^{ab}	5.13 ± 1.59^{b}
Stev3	5.60 ± 1.50^{a}	5.50 ± 1.70^{a}	4.43 ± 1.98^{a}	5.17 ± 2.09^{b}	5.03 ± 2.13^{b}	5.07 ± 1.51^{b}
Suk5	5.30 ± 1.70^{a}	5.53 ± 1.91^{a}	4.33 ± 1.65^{a}	2.77 ± 2.14^{a}	2.83 ± 2.26^{a}	3.33 ± 1.83^{a}
Sor5	5.37 ± 1.61^{a}	5.87 ± 1.70^{a}	4.67 ± 1.81^{a}	4.40 ± 1.83^{ab}	4.53 ± 2.24^{ab}	4.93 ± 1.55^{b}
Stev5	5.03 ± 1.75^{a}	5.87 ± 1.59^{a}	4.60 ± 1.90^{a}	4.67 ± 2.04^{b}	4.63 ± 2.39^{ab}	4.73 ± 1.74^{b}
Suk7	5.00 ± 1.98^{a}	5.53 ± 2.08^{a}	4.73 ± 1.76^{a}	2.77 ± 1.98^{a}	2.80 ± 1.85^{a}	3.30 ± 1.86^{a}
Sor7	5.53 ± 1.66^{a}	5.77 ± 1.72^{a}	4.60 ± 1.63^{a}	4.53 ± 1.70^{b}	4.47 ± 2.06^{ab}	5.03 ± 1.69^{b}
Stev7	5.07 ± 1.95^{a}	5.77 ± 1.61^{a}	4.70 ± 1.53^{a}	4.93 ± 1.95^{b}	4.53 ± 1.80^{ab}	5.00 ± 1.51^{b}
Suk9	5.07 ± 2.02^{a}	5.63 ± 1.90^{a}	4.83 ± 2.12^{a}	2.70 ± 1.86^{a}	3.53 ± 2.19^{a}	3.17 ± 1.78^{a}
Sor9	5.20 ± 2.16^{a}	5.23 ± 2.05^{a}	4.93 ± 1.55^{a}	4.63 ± 1.97^{b}	5.10 ± 1.97^{b}	4.87 ± 1.66^{b}
Stev9	5.33 ± 2.25^{a}	5.47 ± 1.81^{a}	4.67 ± 2.01^{a}	4.77 ± 1.99^{b}	5.03 ± 2.22^{ab}	5.17 ± 1.66^{b}

Note: Different superscript letter notations within the same column for each fermentation day indicate significant differences ($p < 0.05$).

The analysis of variance indicates that the aftertaste attribute in Seaweed Kombucha exhibits significant differences ($p<0.05$). The average liking scores for the aftertaste attribute in Seaweed Kombucha range from 2.80 (dislike) to 5.10 (neutral). The highest average aftertaste scores are obtained from samples Seaweed Kombucha stev0 (5.10) and sor9 (5.10). The lowest average liking scores are obtained from sample Seaweed Kombucha suk7 (2.80). Panelists perceive Seaweed Kombucha beverages to have a bitter aftertaste. This bitter taste is less favored by the panelists. The presence of a bitter taste after consuming Seaweed Kombucha is speculated to be less favored by some panelists.

The analysis of variance indicates that the overall attribute in Seaweed Kombucha exhibits significant differences ($p<0.05$). The average liking scores of panelists towards the overall impression of Seaweed Kombucha range from 3.30 (dislike) to 5.17 (neutral). The highest average liking scores for overall impression are obtained from sample Seaweed Kombucha stev9 (5.17). The lowest average liking scores are obtained from sample Seaweed Kombucha suk7 (3.30).

3.2 Sensory Profile of Seaweed Kombucha using the CATA Method

The CATA method is a sensory profile analysis technique that can be quickly performed by consumer-based panelists [24]. According to Oliver [25], the advantages of this method are its simplicity and ease of application by both trained and untrained panelists. CATA analysis allows respondents to select multiple applicable options, providing deeper insights into diverse consumer preferences and perceptions. This flexibility makes CATA a powerful tool for understanding consumer attitudes and refining product formulations based on detailed feedback, ultimately supporting the optimization and promotion of Seaweed Kombucha sargassum tea [24]. The sensory profile evaluation is conducted for appearance (by looking at), aroma (by sniffing), and taste/flavor (by tasting) aspects of Seaweed Kombucha samples. The sensory profile of Seaweed Kombucha will be interpreted through three analyses: Cochran's Q test, correspondence analysis, and principal coordinate analysis (PCoA). The CATA sensory evaluation included 30 untrained panelists (53% women, 47% men). Panelists were asked to select sensory attributes they believed were present in the product. The list of attributes used in this study was based on literature and determined by the panelists. The CATA testing involved 30 panelists with a total of 41 attributes. The results of Cochran's Q test on Seaweed Kombucha can be seen in Table 2.

Cochran's Q test analysis with multiple pairwise comparisons using the McNemar procedure was conducted to identify significant differences among samples for each tested attribute [26]. The results of the Cochran's Q test indicate that the 15 Seaweed Kombucha samples with different sugar treatments and fermentation durations exhibit both significantly different and non-significantly different attributes. Attributes deemed significantly different have p-values less than the 5% significance level ($p<0.05$), including sweet (aroma), beer/fermented (aroma), alcoholic (aroma), sour (aroma), tea (taste/flavor), sweet (taste/flavor), beer/fermented (taste/flavor), alcoholic (taste/flavor), sour (taste/flavor), bitter (taste/flavor), and unfamiliar (taste/flavor). Attributes deemed non-significantly different have p-values greater than the 5% significance level ($p>0.05$). This aligns with [27], indicating that data with p-values greater than the 0.05 significance level do not exhibit significant differences among samples. Subsequently, the Seaweed Kombucha samples will be represented using correspondence analysis (CA).

Table 2. Result of Cochran's Q on Seaweed Kombucha

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Attributes					
***Appearance* (*by looking at*)**	**p-value**	**Aroma (*by sniffing*)**	**p-value**	***Taste/Flavour* (*by tasting*)**	**p-value**
Amber	0.412	*Citrus*	0.745	*Citrus*	0.077
Yellow	0.346	*Tea*	0.067	*Tea*	0.029*
Green	0.924	*Sweet*	0.039*	*Sweet*	<0.0001*
Brown	0.245	*Beer/fermented*	0.017*	*Beer/fermented*	<0.0001*
Black	0.450	*Alcoholic*	0.000*	*Alcoholic*	0.017*
		Sour	0.000*	*Sour*	<0.0001*
		Bitter	0.391	*Bitter*	0.001*
		Unfamiliar	0.500	*Unfamiliar*	0.005*
		Floral	0.425	*Floral*	0.347
		Milky	0.527	*Milky*	0.527
		Nutty	0.606	*Nutty*	1.000
		Sweet	1.000	*Sweet*	1.000
		Fire/animal	0.558	*Fire/animal*	0.717
		Fruit	0.842	*Fruit*	0.744
		Marine	0.094	*Marine*	0.527
		Mineral	0.133	*Mineral*	0.008*
		Earthy	0.450	*Earthy*	0.450
		Plants/herbaceous	0.633	*Plants/herbaceous*	0.664

Note: *Significance value ($p < 0.05$), significant difference in sensory attributes.

Correspondence analysis (CA) serves to analyze the pattern of relationships between products and attributes [26]. The results of correspondence analysis are represented in a biplot map that focuses on the angles between products and attributes [28]. This analysis provides a summary of dynamic sensory characteristics [29]. The results of correspondence analysis on the biplot map can be seen in Figure 2.

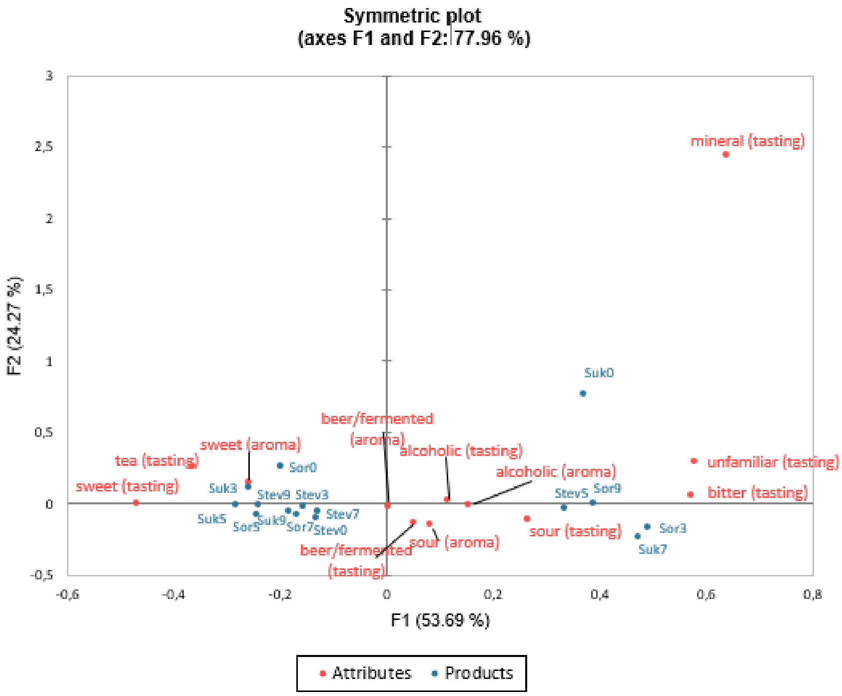

Figure 2. *Symmetric plot* profile of Seaweed Kombucha sensory

The results of correspondence analysis are presented in a symmetric plot graph, consisting of F1 on the x-axis representing 53.69% of the data and F2 on the y-axis representing 24.27% of the data. F1 and F2 together represent 77.96% of the data variance. Based on the symmetric plot graph, there are four quadrants with relatively owned sensory attributes for each product. Quadrant 1 contains samples suk0 and sor9 with attributes including mineral (tasting), unfamiliar (tasting), bitter (tasting), alcoholic (tasting), and alcoholic (aroma). Quadrant II contains samples suk3 and sor0 with attributes such as sweet (tasting), tea (tasting), and sweet (aroma). Quadrant III contains samples suk5, stev9, sor5, suk9, stev3, sor7, stev0, and stev7 with attributes mainly consisting of sweet (tasting). Quadrant IV contains samples stev5, suk7, and sor3 with attributes including beer/fermented (aroma), beer/fermented (tasting), sour (aroma), alcoholic (aroma), and sour (tasting). The Seaweed Kombucha samples will be further represented based on the results of Principal Coordinate Analysis (PCoA) through a graph mapping panelists' preferences to sample attributes.

Principal Coordinate Analysis (PCoA) aims to identify sensory attributes preferred by panelists or consumers [30]. The results of the PCoA graph in Figure 3 indicate that the liking points are in Quadrant I with attributes such as mineral (tasting). Additionally, attributes closest to the liking points include beer/fermented (tasting), beer/fermented (aroma), and alcoholic (tasting). This suggests that the preferred attributes by panelists are mineral (tasting), beer/fermented (tasting), beer/fermented (aroma), and alcoholic (tasting).

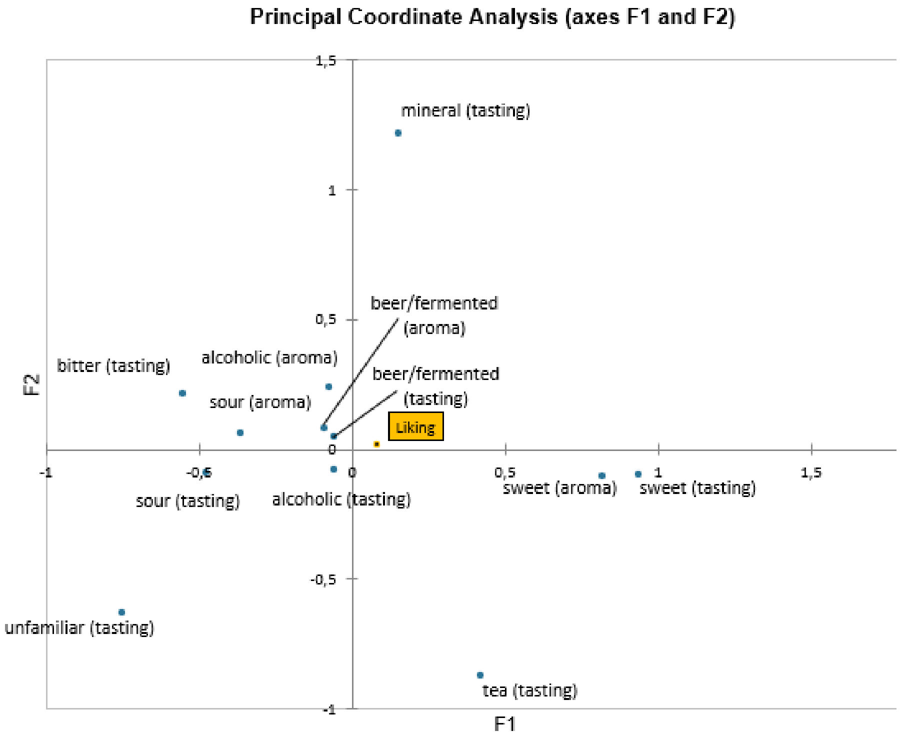

Figure 3 The PCoA plot illustrates the correlation between sensory attributes and panelists' preferences.

3.3 Selection of the best Seaweed Kombucha

The selection of the best Seaweed Kombucha sample for further analysis with GC-MS is determined based on the results of the sensory hedonics and CATA tests. The sensory test results indicate that the best sugar treatment is found in Seaweed Kombucha with steviol sugar (Table 1). Subsequently, the fermentation duration is chosen based on the abundance of best samples using steviol sugar, which appears to be on the 3rd day of fermentation. The best Seaweed Kombucha sample is the one treated with steviol sugar and fermented for 3 days (stev3).

3.4 Characteristics of Seaweed Kombucha

Seaweed Kombucha is a fermented tea beverage made using SCOBY. The fermentation process results in characteristic features of the Seaweed Kombucha beverage. The physical characteristics of Seaweed Kombucha will be assessed through testing of pH, total sugar content, and color to understand the properties present in a Seaweed Kombucha beverage. The characteristics of Seaweed Kombucha can be observed in Table 3.

Table 3. Characteristics of Seaweed Kombucha with Steviol Addition with 0 and 3 Days of Fermentation

Fermentation Duration (day)		**Stev0**	**Stev3**
pH		2.93 ± 0.02	2.79 ± 0.01
Sugar content		3.00 % ± 0.00	3.10 % ± 0.00
Color (*CIE Lab*)	**L***	46.38 ± 1.22	54.93 ± 1.71
	a*	4.59 ± 1.41	2.91 ± 1.20
	b*	47.58 ± 0.90	46.62 ± 1.72
Color Difference	**ΔL***	-	8.55 ± 4.87
	Δa*	-	-1.68 ± 1.49
	Δb*	-	-0.95 ± 1.33
ΔE*		-	8.76

3.5 Acidity Level (pH)

The acidity level (pH) denotes the acidic or basic nature of a solution, measured on a scale from 0 to 14. Analyzing pH values is crucial for controlling Seaweed Kombucha products as it can influence their taste. The pH values of Seaweed Kombucha on day 0 with different sugar treatments ranged from ≤3.00. The results of pH measurements for Seaweed Kombucha. The standard pH value for Seaweed Kombucha Brewers International ranges from 2.20 to 3.80. Analysis of variance with a t-test in Appendix 4 shows that fermentation time significantly affects the pH of Seaweed Kombucha ($p < 0.05$). The pH value produced in the best-treated Seaweed Kombucha meets this standard with a value of 2.79. The decrease in pH during fermentation is caused by bacteria and yeast producing acetic acid and gluconate during the fermentation process. Acetobacter bacteria in Seaweed Kombucha grow at pH 3.5-8.5 and can survive at pH below 3. Yeasts in Seaweed Kombucha can grow at the optimum pH of 4.5-6.0.

3.6 Color

The CATA method is a sensory profile analysis technique that can be quickly performed by consumer-based panelists [24]. According to Oliver [25], the advantages of this method are its simplicity and ease of application by both trained and untrained panelists. CATA analysis allows respondents to select multiple applicable options, providing deeper insights into diverse consumer preferences and perceptions. This flexibility makes CATA a powerful tool for understanding consumer attitudes and refining product formulations based on detailed feedback, ultimately supporting the optimization and promotion of Seaweed Kombucha sargassum tea [24]. The sensory profile evaluation is conducted for appearance (by looking at), aroma (by sniffing), and taste/flavor (by tasting) aspects of Seaweed Kombucha samples. The sensory profile of Seaweed Kombucha will be interpreted through three analyses: Cochran's Q test, correspondence analysis, and principal coordinate analysis (PCoA Color index in food products is one of the important parameters determining the product's quality.

Color analysis in this research uses RGB color features, which will be converted into L, a, b. The *L, a, b* color segmentation functions to identify colors digitally. Fermentation time can cause differences in Seaweed Kombucha color analyzed with *CIE Lab** coordinates. Absolute color coordinate differences are referred to as Delta. This formula serves to calculate the difference between two colors to identify inconsistencies and help control product color more effectively. Color measurement of Seaweed Kombucha is performed three times for each parameter. The color analysis results of Seaweed Kombucha can be seen in Table 3.

The Δb value indicates the level of change in yellow-blue color. A positive value indicates that the product's color tends to yellow, while a negative value indicates that the product's color tends to blue. The best Seaweed Kombucha sample (stev3) has a Δb value of -1.69, indicating a change in color towards blue.

The ΔE value in the color analysis of Seaweed Kombucha indicates different color changes. The analysis of variance in Appendix 4 shows that fermentation time has no significant effect on the color of Seaweed Kombucha ($p > 0.05$). Seaweed Kombucha samples with steviol sugar show fluctuating color value changes. The highest change in value in steviol sugar Seaweed Kombucha occurs on the 7th fermentation day with a value of 10.22. The lowest change in value in steviol sugar Seaweed Kombucha occurs on the 5th fermentation day with a value of 1.77. The best Seaweed Kombucha sample (stev3) has a color change value of 8.76.

Color changes in Seaweed Kombucha are caused by fermentation time. Research conducted by [31] states that as fermentation time increases, Seaweed Kombucha color will fade. This is because the SCOBY bacteria's ability to degrade certain compounds. Color degradation in Seaweed Kombucha occurs because microbes utilize total soluble solids as energy, so as fermentation progresses, the solvent in the medium will be depleted, and the liquid will become clearer or colorless.

3.7 Total Sugar

Total sugar in food is referred to as total sugar content. Total sugar content generally exists in the form of monosaccharides or oligosaccharides. The results of total sugar content testing in Seaweed Kombucha can be seen in Table 3. The test results show fluctuations in total sugar content. The total sugar content in the best Seaweed Kombucha sample (stev3) is 3.10%. The total sugar content in Seaweed Kombucha is influenced by the type of sugar used. The total sugar content in Seaweed Kombucha during the fermentation process will increase according to the sucrose content of the sugar used. This is because during the fermentation process, the number of bacteria will increase, resulting in increasing sugar compounds [32]. According to [33], microbes require sugar as a carbon source. The total sugar value will decrease because yeast (*Saccharomyces cerevisiae*) breaks down sucrose into alcohol, increasing the alcohol content.

3.8 Flavor Profile of Seaweed Kombucha using GC-MS

Flavor is an attribute of foods, beverages, and spices that arises due to stimulation from all senses when food or drink passes through the digestive and respiratory tracts, especially taste and aroma [34]. These sensations arise due to volatile and non-volatile chemical components that emerge when eating or drinking. Volatile components provide aroma sensations, evaporate quickly, and provide initial impressions. Non-volatile components do not provide aroma sensations but contribute to taste sensations such as sweet, bitter, sour, and salty. Non-volatile components serve as a medium for volatile components and help retain the evaporation of volatile components [35]. Volatile components in a food material significantly influence the quality of the resulting flavor. Volatile components can be observed through GC-MS analysis. Flavor profile testing is performed using samples before fermentation (stev0) and the best sample from sensory testing (stev3). The results of GC-MS analysis of Seaweed Kombucha can be seen in Table 4.

Table 4. Flavor Profile of Seaweed Kombucha with GC-MS Analysis

Sample	*Retention time* (min)	Category	Compound name	Odor	Probability (%)
Stev0	2.981	Heterocyclic components and others	Methanamine	Fish oil [36]	28.5%
		Acid	N-hydroxy-N-methyl-	Acidic (aroma) [37]	
	3.449	Alcohol	(S)-(+)-2-Amino-3-methyl-1-butanol	bitter, floral (aroma) [37]	15.3%
	3.864	Ketone	2-Cyclopenten-1-one	-	71.1%
		Acid	2-hydroxy-	Acidic (aroma) [37]	
Stev3	2.973	Heterocyclic components and others	Methanamine	Fish oil [36]	52.0%
		Acid	N-hydroxy-N-methyl-	Acidic (aroma) [37]	
	3.434	Alcohol	(S)-(+)-2-Amino-3-methyl-1-butanol	Bitter, floral (aroma) [37]	32.4%
	3.856	Ketone	2-Cyclopenten-1-one	-	69.8%
		Acid	2-hydroxy-	Acidic (aroma) [37]	

The GC-MS analysis results of Seaweed Kombucha, as presented in Table 4, revealed fluctuations in the probability of volatile components. The volatile component profile of the Seaweed Kombucha samples encompassed several volatile compounds, including Methanamine, N-hydroxy-N-methyl-, (S)-(+)-2-Amino-3-methyl-1-butanol, 2-Cyclopenten-1-one, and 2-hydroxy-. These volatile compounds belong to various groups, such as acids, alcohols, ketones, heterocyclic components, and others.

The analysis indicated changes in the probability between the initial Seaweed Kombucha sample (stev0) and the best sample fermented for 3 days (stev3) using steviol sugar. Volatile components like Methanamine and N-hydroxy-N-methyl- increased in the stev3 sample to 52.0%. Additionally, the volatile component (S)-(+)-2-Amino-3-methyl-1-butanol, classified as an alcohol compound, exhibited an increase in the stev3 sample to 32.4%. However, volatile components such as 2-Cyclopenten-1-one and 2-hydroxy- decreased in the stev3 sample to 69.8%.

These findings align with the research by Zhao [2], which demonstrated that volatile components in Seaweed Kombucha typically belong to groups such as alcohols, aldehydes, ketones, acids, esters, benzenoids, and others. Alcohol and acid groups tend to increase significantly during the fermentation process.

The detected volatile components, primarily belonging to the alcohol and acid groups, contribute to the bitter and acidic taste in Seaweed Kombucha. These flavors are characteristic and favored by consumers in tea or tisane-based beverages. Studies by Ehoche [38] indicated that components like tannins, catechins, and amino acids influence tea flavor. Catechins, for instance, contribute to approximately 70-75% of the bitter and astringent taste. The sweet taste of tea is attributed to amino acids. These distinctive flavors are preferred aspects in tea beverages.

4 Conclusions

The physical characteristics of Seaweed Kombucha were influenced by the duration of fermentation employed. Factors affecting these characteristics include pH, alcohol content, and total sugar content. Seaweed Kombucha treated with steviol sugar during a 3-day fermentation period represents the optimal sample. The finest Seaweed Kombucha exhibits physical attributes such as a pH of 2.79, 0% alcohol content, a ΔE value of 7.66, and 3.10 g of total sugar. The flavor profile of Seaweed Kombucha indicates the presence of various compounds such as acids, alcohols, ketones, heterocyclic components, and others.

5 Recommendations

Future research is advised to evaluate the nutritional value and compare it with commercial products. Additionally, conducting flavor profile testing on *Sargassum* tea would provide valuable insights into its sensory attributes and potential as a beverage product.

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