Synergistic Formulation and Shelf Stability Evaluation of Brown Seaweed (*Sargassum* sp.) Infused Tisane Enhanced with Butterfly Pea Flower and Sappan Wood

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Abstract. Conventional seaweed beverages often concentrate solely on individual compounds, lacking the integration of complementary herbs. This study aimed to develop a functional tisane using *Sargassum* sp., butterfly pea flowers, and sappan wood, resulting in an antioxidant-rich tisane with favourable sensory attributes and storage stability. The research consisted of two phases: formulating the functional tisane and evaluating its quality stability. *Sargassum* sp. tisane contained flavonoids, saponins, phenols, and tannins. Hedonic testing revealed that the composition of *Sargassum* sp. significantly influenced taste and aroma preferences, with Formula F1 (ginger) achieving the highest taste score of 3.87. Formula F1 included sterols, saponins, and tannins, with an antioxidant content of 9.93 ppm. While the tisane's pH slightly decreased during storage, importantly antioxidant activity remained consistently high until the end of storage. Microbial and other shelf-life parameters were evaluated using a combination of low-temperature storage and standard food preservatives in sterilized bottles. In conclusion, this study highlighted the potential of formulating a functional tisane using *Sargassum* sp., butterfly pea flowers, and sappan wood. These findings contributed to an improved comprehension of how to create appealing, stable, and bioactive-rich tisanes by utilizing a combination of marine herbs.

1 Introduction

Currently, the beverage market is segmented into both conventional and functional beverages, with the demand for functional herb beverages expected to experience sustained growth through 2028. The majority of the functional beverages available in Indonesia market are imported from other countries, particularly China, Japan, and other East Asian nations. However, beverages from these countries typically contain a single active ingredient and

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frequently require brewing; there is a notable absence of ready-to-drink functional beverages. In fact, today, consumers tend to prefer products that offer convenient packaging and presentation, mainly because they require less preparation time [1]. Functional beverages are defined as beverages that contain active components that provide health advantages beyond their basic nutritional content [2,3]. An instance of a functional beverage is tisane, which consists of dried leaves, flowers, and bark that are prepared to provide both flavour and potential health advantages [4]. Continuing attempts have been initiated to develop functional beverages, with a particular focus on incorporating natural components.

Seaweed is considered one of the most beneficial compounds derived from marine resources [5]. According to Husni [6], the seaweed species *Sargassum* sp. has emerged as a promising candidate for creating functional beverages. In our previous study, we explored the preparation of a slimming beverage using *Sargassum* sp. as the primary ingredient. Although the beverage was continue tested *in vivo* and revealed antidiabetic activity, its taste was not well-received by the panelists. This unfavorable sensory parameter was attributed to the beverage being composed solely of *Sargassum* sp. and sugar, without any additional formulation.

Sargassum sp. is a brown seaweed abundant in secondary metabolites, such as alkaloids, glycosides, tannins, phenolics, flavonoids, and steroids, which are widely used in the pharmaceutical and medical industries [7]. With an IC_{50} value of 68.89 ppm, *Sargassum* sp. possesses a high concentration of active compounds that exhibit strong antioxidant activity. Considering its bioactive content, *Sargassum* sp. seaweed possesses enormous potential for the development of functional beverage products that promote healthy consumption [8]. Previous research on seaweed beverages tended to focus on one specific seaweed component, omitting the addition of local herbs, such as butterfly pea flowers and sappan wood.

Butterfly pea flowers, plants rich in anthocyanin pigments, show promise for development as local natural colorants across various food industries [9,10]. They can be brewed directly upon harvesting or after drying, subsequently steeped in warm water. Anthocyanins, members of the flavonoid family, act as bioactive compounds owing to their antioxidant properties [11,12]. Beverage product development based on butterfly pea flowers has also been introduced by Kiranawati [13] with the inclusion of lemon.

Sappan wood (*Caesalpinia sappan* L) possesses vibrant pigments due to the brazilin compound, presenting colorations ranging from dark orange to deep red. Traditional medicinal applications in certain regions recognize sappan wood by its pink hue. Historically, sappan wood has been utilized both as a natural colorant and traditional medicine, cherished for its organoleptically agreeable taste [14]. One of the flavonoid groups present in sappan wood is anthocyanin. Beverage formulations based on sappan wood were previously explored by Nirmagustina [15], which included ginger and lemongrass in the mix. The significant health benefits and rich bioactive compounds inherent in *Sargassum* sp., butterfly pea flowers, and sappan wood suggest potential for the development of a ready-to-drink functional beverage combining marine and local herbs ingredients. Interestingly, the production process of such functional beverages is comparatively new, hence necessitating quality stability tests to ensure commercial viability and public or sensory acceptance.

The aim of this study is to assess the most effective composition for a functional herbal drink using seaweed (*Sargassum* sp.), butterfly pea flower (*Clitoria ternatea*), and sappan wood (*Caesalpinia sappan*). The objective is to generate a functional beverage that not only contains a significant number of antioxidants but also receives positive sensory feedback. Furthermore, this study aims to evaluate the stability of the functional beverage when exposed to different storage temperatures and preservation conditions.

2 Materials and methods

2.1 Materials

The primary materials utilized in this study include *Sargassum* sp., harvested from the Sayang Heulang Beach area, Garut, Indonesia in December, dried butterfly pea flowers acquired from Tasikmalaya, Indonesia, and sappan wood obtained from Tangerang, Indonesia. Other ingredients utilized comprise honey, sorbitol sweetener (Tropicanaslim, Indonesia), ginger, lemon, and lemongrass. Materials employed for testing include aquades and methanol (Merck, Germany), as well as DPPH (Merck, Germany) for antioxidant testing.

The equipment utilized in this research includes a blender (Philips), Pyrex funnel, basin, spoon, spatula, gloves, mask, plastic, measuring glass, and scale. Chemical composition analysis employs various instruments such as porcelain crucibles, spatulas, analytical balance (Mettler Toledo ME, USA), desiccator, oven (Memmert, USA), Erlenmeyer flask, Kjeldahl flask, reflux condenser, furnace, measuring glass, funnel, clamp, beaker, pipette, and filter paper. TPC analysis utilizes equipment including petri dishes, test tubes, an incubator set at 35°C (Memmert, USA), autoclave (Hirayama, Japan), stomacher (Seward, UK), vortex mixer (IKA, UK), and colony counter (Quebec, USA).

2.2 The fabrication of *Sargassum tisane*

The research consisted of four stages: raw material profiling, pre-formulation with six treatments involving concentrations of *Sargassum* sp. ranging from 0.05% to 0.5%, formulation and characterization of selected treatments with three variations in flavour using ginger extract (F1), lemongrass (F2), and lemon (F3), as well as evaluation of the quality stability of the functional beverage during storage. Analysis in the raw material profiling stage included proximate and phytochemical analyses. Pre-formulation stage analysis consisted of viscosity, colour analysis, and hedonic sensory evaluation. The formulation stage involved descriptive and hedonic sensory analysis, phytochemical, and antioxidant analyses. The quality stability analysis stage included pH stability, antioxidant, and Total Plate Count (TPC) analysis.

The production of the functional beverage, involving several steps: weighing of *Sargassum* sp., butterfly pea flowers, and sappan wood according to predetermined compositions; boiling water to extract compounds from the materials (*Sargassum* sp., butterfly pea flowers, and sappan wood). Boiling was maintained at 100°C for 10 minutes to ensure optimal flavour extraction. The mixing process involved combining *Sargassum* sp., butterfly pea flowers, and sappan wood in a pot and stirring evenly. The filtration process

Table 1. Sargassum seaweed tisane functional drink formulation.

Ingredients	**F1**	**F2**	**F3**
Sargassum sp. (% b/v)	0.05	0.05	0.05
Water (L)	1	1	1
Butterfly pea flowers (% b/v)	0.15	0.15	0.15
Sappan wood (% b/v)	0.05	0.05	0.05
Sorbitol (% b/v)	1.0	1.0	1.0
Honey (% v/v)	3.0	3.0	3.0
Ginger (% b/v)	1.0	-	-
Lemongrass (% b/v)	-	1.0	-
Lemon (% v/v)	-	-	3.0

Note: F1: Ginger flavour variant, F2: Lemongrass variant, F3: Lemon variant

employed a 20-mesh filter to obtain a maximum final product yield with minimal precipitation. Subsequently, sorbitol, honey, and predetermined flavour variations were added post-filtration. The composition of each ingredient was detailed in Table 1.

Subsequently, the long-term preservation of the functional beverage's quality was assessed by examining characteristics such as pH stability, antioxidant capacity, and total microbial count. The beverage was test under two different temperature conditions, specifically room temperature and cold temperature, along with the inclusion of two preservatives: Sodium Benzoate and Sodium Metabisulfite. The functional beverage was supplemented with Sodium Benzoate preservative at a concentration of 0.1% w/w, and Sodium Metabisulfite was added at a concentration of 0.01% w/w. The pH stability was monitored for a duration of 24 days, with measurements recorded at intervals of 4 days. whereas the antioxidant capacity and total microbial count were simply assessed on day 0 and day 24.

2.2 The evaluation of fabrication of *Sargassum tisane*

2.2.1 Proximate content

The proximate analysis, consisting of moisture content, ash content, protein content, fat content, and carbohydrate content, was conducted according to AOAC standards [16,17].

2.2.2 Viscosity

The viscosity analysis assessed the resistance of a filtrate to flow, with higher viscosity indicating greater resistance. The viscosity test aimed to evaluate the consistency of a prepared formulation. The viscosity of the functional tisane beverage was measured using a Brookfield Viscometer with spindle number 2 at a speed of 100 rpm. The testing was conducted in triplicate, and the values displayed on the screen were recorded in cPs unit.

2.2.3 Colour profile

The colour analysis was conducted using the CIE L* a* b* model. The colour analysis was performed using a Minolta CR-400 Chromameter. Prior to measurement, the instrument was calibrated using a white plate standard. The samples were poured into uniformly sized containers, and measurements were then taken on the L*, a*, and b* value scales. The L* value indicated the brightness parameter, ranging from 0 (black) to 100 (white). The a* value or chroma indicated colour intensity. The b* value or hue represented the dominant wavelength that determined whether the colour was red, green, or yellow. Colour testing was conducted in triplicate during the pre-formulation stage.

2.2.4 pH

The pH analysis was conducted using a pH meter. pH measurement, or acidity level, was performed to indicate the acidity and alkalinity levels of a solution, often expressed as pH values. The pH value indicates the concentration of H+ ions (hydrogen) dissolved in a solution. pH values, particularly during storage of functional tisane beverage, were observed.

2.2.5 Antioxidant

The antioxidant activity test was conducted using the DPPH (1-diphenyl-2-picrylhydrazyl) method with a concentration of 0.1 mM. The functional beverage samples were dissolved in methanol solvent with concentrations of 25, 50, 75, and 100 ppm. Each concentration sample was mixed with the DPPH solution, then incubated for 30 minutes and measured for absorbance using a UV-Vis spectrophotometer at a wavelength of 517 nm. The antioxidant activity of each sample was expressed as the percentage inhibition of free radicals, calculated using the formula:

$$Percentage\ inhibition\ (\%) = 1 - \frac{Absorbance\ of\ sample}{Absobance\ of\ control} x100\% \quad (1)$$

2.2.6 Phytochemical profile

The phytochemical test was a preliminary test conducted to determine the presence of active compounds such as alkaloids, flavonoids, hydroquinone phenols, steroids, triterpenoids, saponins, and tannins qualitatively. The purpose of phytochemical testing was to identify active compounds in *Sargassum* sp. seaweed. Phytochemical testing included alkaloid, flavonoid, steroid, saponin, phenol, and tannin tests, referring to Altemimi [18].

2.2.7 Sensory evaluation

The sensory evaluation was conducted using affective and descriptive methods. The affective method employed in this research was hedonic sensory evaluation, while the descriptive method used was Flash profile. Flash profile (FP) is an alternative sensory analysis technique adapted from Free Choice Profiling to understand product sensory positioning. Trained panelists chose their own terms to describe and evaluate a series of products simultaneously, then rated the products for each attribute they created individually. Testing was carried out during both the pre-formulation and formulation stages of the functional beverage. The evaluation was done subjectively with the assistance of 30 trained panelists, comprising 11 males and 19 females, aged 20-24 years, from the Jakarta, Bogor, Bekasi and Tangerang area, that comprised from West Java, Central Java, East Java, and Sumatra Tribes. The testing was conducted from March 30 to April 10, 2022, in Bogor, West Java, Indonesia. Panelists provided numerical scores based on their preference. Since it was a new product, a hedonic scale of 1 to 5 was used, where 5 indicated " like extremely " and 1 indicated "disliked extremely".

2.2.8 Total Plate Count (TPC)

The Total Plate Count (TPC) test was conducted to determine the microbial load. The determination of this value was crucial because microbial growth and activity can lead to physical changes in the product, such as the formation of mucus, gas, foam, different or deviant colours, acid formation, unpleasant and pungent odors, and even poisoning. A sample of 1 mL was taken and added to 9 mL of diluent solution. Subsequently, the solution was homogenized by vertexing. Dilution and inoculation were performed up to a dilution of 10^2. From each dilution, 1 mL was aseptically pipetted into duplicate sterilized petri dishes and supplemented with 15-20 mL of sterile Plate Count Agar (PCA) media.

The petri dishes were incubated upside down in an incubator at 37°C for 48 hours. The total microbial count was calculated using the Standards Plate Count (SPC) method. The

number of colonies observed was compared to the specified requirement. According to the Indonesian National Standard (SNI 14.1.4.8-7388-2009), the microbial contamination requirement for non-carbonated flavoured beverage products is 2×10^2 colonies/mL.

2.3 Data analysis

The chemical composition analysis, viscosity, colour, antioxidant, and pH stability analyses were conducted using One-way ANOVA. The data were processed with the assistance of SPSS 25.0 and Microsoft Excel 2013. If the data analysis indicated a significant effect ($p<0.05$), further testing was conducted using Duncan's Multiple Range Test with a confidence interval of 95%. Sensory data were analysed using the Kruskal-Wallis test with SPSS 25.0 software, followed by post-hoc testing using Dunn's test.

3 Results and discussions

3.1 The chemical characteristics of the raw materials

The raw materials consist of *Sargassum* sp. seaweed, butterfly pea flowers, and sappan wood. The results of the chemical composition analysis can be seen in Table 2. *Sargassum* sp. seaweed had a moisture content of 17.82%. The moisture content obtained for Sargassum sp. seaweed was the highest compared to the moisture content of butterfly pea flowers and sappan wood. The moisture content value in this study was lower than the results obtained by Diachanty [19], who found a moisture content of 26.25%. The higher moisture content was caused by environmental humidity due to the entry of water from the environment into the material [20]. Dried *Sargassum* sp. seaweed had a higher ash content compared to the ash content of butterfly pea flowers and sappan wood. The ash content value of *Sargassum* sp. seaweed in this study was 16.11%, which was lower compared to the ash content found in the previous study [19], which resulted in an ash content of 31.52%. The high ash content in seaweed was influenced by the presence of salt and other minerals that adhered to it, such as Na, Ca, K, and Mg. The more mineral content, the higher the ash content.

The analysis results of the protein content showed that dried butterfly pea flowers had the highest protein content. The protein content in butterfly pea flowers was 18.94%. The protein content value of *Sargassum* sp. in this study was higher compared to the previous study, which had a protein content value of 3.64% [19]. Protein is an organic component that is easily denaturised during processing or post-handling. The analysis of the fat content in the three materials showed low fat content values, ranging from 0.07% to 2.02%. The fat content of *Sargassum* sp. seaweed was 1.25%. Fat content could be influenced by the moisture content of the material. The higher the moisture content resulting from the drying process, the lower the fat content obtained, and vice versa.

The highest carbohydrate content was found in dried sappan wood, with a value of 88.15%. *Sargassum* sp. seaweed and butterfly pea flowers had almost the same carbohydrate content, which was 56.31% and 56.41%, respectively. Marine macroalgae generally stored their food reserves in the form of carbohydrates, especially polysaccharides. Carbohydrates generally had a relationship with fibre in a material. The high fibre content could be caused by the high polysaccharides in seaweed cells [21,22].

Table 2. The chemical composition of raw materials of functional beverage.

Component (%)	*Sargassum* sp.	Butterfly pea flowers	Sappan wood
Moisture content	17.82±0.12[b]	17.56±0.11[b]	10.2±0.06[a]
Ash	16.11±0.02[c]	5.08±0.06[b]	0.97±0.01[a]
Protein	8.53±0.13[b]	18.94±0.07[c]	0.61±0.01[a]
Fat	1.25±0.01[b]	2.02±0.02[c]	0.07±0.00[a]
Carbohydrate	56.31±0.02[a]	56.41±0.01[b]	88.15±0.03[c]

Different letters following the numbers in the same column indicate differences at the 5% significance level.

Table 3. Pre-formulation Sensory Hedonic Analysis.

Sample	Taste	Aroma	Colour	Viscosity
S0.05	3.87±0.94[b]	3.60±0.72[c]	3.53±0.73[ab]	4.00±0.64[b]
S0.1	3.97±1.19[a]	3.10±0.96[b]	3.90±0.80[b]	3.67±0.61[a]
S0.2	3.17±1.15[a]	2.97±0.80[b]	3.73±0.79[ab]	3.63±0.56[a]
S0.3	2.73±0.82[a]	3.20±0.61[b]	3.80±0.66[ab]	3.53±0.63[a]
S0.4	2.93±1.02[a]	3.03±0.81[b]	3.50±0,63[a]	3.63±0.62[a]
S0.5	2.70±1.02[a]	2.43±0.85[a]	3.50±0.63[a]	3.57±0.62[a]

*The numbers followed by different letters in the same row indicate statistically differences at the 5% significance level.
**Hedonic score: 1-dislike extremely; 2-dislike slightly; 3-neither like nor dislike; 4-like slightly; 5-like extremely

The phytochemical test results indicate that the *Sargassum* sp. extract contains flavonoids, saponins, phenols, and tannins. The methanol extract of butterfly pea flowers contains steroids, flavonoids, saponins, hydroquinone phenols, and tannins. Pyo [23] revealed that the phytochemical content of a material is influenced by several factors, including species, varieties, growth conditions, seasonal variations, processing methods, and storage.

3.2 Pre-formulation evaluation

Viscosity measurements were conducted on pre-formulation samples using a Brookfield Viscometer to determine the product's level of viscosity. The viscosity values of the functional beverage in the pre-formulation stage ranged from 10.8 to 11.2 cP.

The results of colour spectrum testing indicate that higher values of lightness correspond to brighter colours in the samples. The highest lightness value was observed in treatment S0.1, while the lowest lightness value was observed in treatment S0.5. Increasing the amount of Sargassum sp. treatment in the sample resulted in decreased lightness values. This is consistent with the findings of Pakaya [24], who demonstrated that increasing seaweed concentration leads to darker-colored products. Analysis of variance results indicate that the concentration of *Sargassum* sp. seaweed treatment significantly affects the L*, a*, and b* values.

Then, sensory analysis was conducted. Sensory analysis conducted at the pre-formulation stage aimed to determine the panelists' acceptance levels of different concentrations of *Sargassum* sp. seaweed. Six samples were tested, including S0.05 (*Sargassum* sp. 0.05%), S0.1 (*Sargassum* sp. 0.1%), S0.2 (*Sargassum* sp. 0.2%), S0.03 (*Sargassum* sp. 0.3%), S0.4 (*Sargassum* sp. 0.4%), and S0.5 (*Sargassum* sp. 0.5%). The sensory hedonic analysis measured four aspects: taste, aroma, color, and viscosity. The results of the sensory hedonic analysis at the pre-formulation stage are presented in Table 3.

In general, the sensory evaluation scores on the hedonic parameters tended to decrease with an increase in the amount of *Sargassum* sp. added. The decreasing sensory scores indicated that the sensory appeal of the functional beverage decreased. The decline in hedonic scores for the taste of the functional beverage could be attributed to the increasing presence of *Sargassum* sp. extract, which imparted a bitter or astringent taste [6]. Sinurat and Suryaningrum [25] reported that the astringent taste in seaweed tea was caused by the presence of tannins abundant in brown seaweed Sargassum sp. Therefore, Sample S0.05 was chosen in this initial pre-formulation.

3.3 Formulation and characterization of selected treatments

3.3.1 Descriptive and hedonic sensory evaluation at the formulation stage

Descriptive sensory analysis was conducted to understand the profiling description of the functional beverages. The descriptive sensory analysis was performed using the Word Clouds web tool to display the description results for the three beverages with attributes of taste, aroma, and aftertaste. Generally, each treatment F1, F2, and F3 provided different attributes of taste, aroma, and aftertaste. Sweet taste was associated with sugar, while sour taste emerged from lemon and was accompanied by bitterness. Meanwhile, the aroma of ginger, lemongrass, and lemon was dominant in each product, as well as the aftertaste, where sweet taste dominated in the mouth after swallowing, along with sourness, and some panelists also indicated flavors associated with fruits and acidity. Overall, F1 was associated with sweetness and ginger, F2 with sweetness and lemongrass, while F3 was associated with acidity, fruity notes, and lemon with a hint of sweetness attribute.

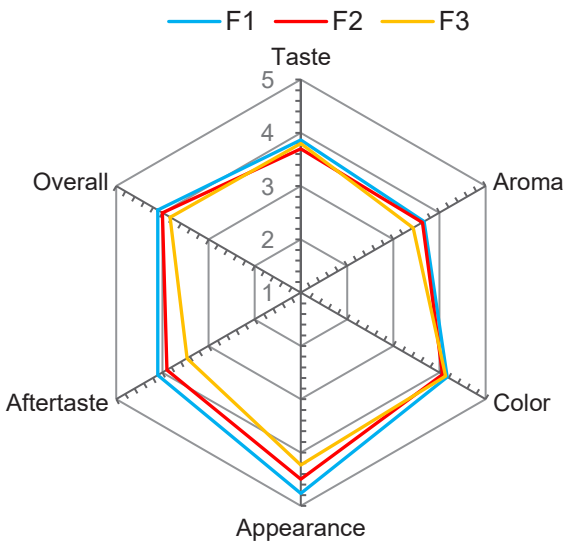

Fig. 1. Sensory Profile of the Functional Beverages. (F1: Ginger variant), (F2: Lemongrass variant), dan (F3: Lemon variant).

The attributes tested in the hedonic sensory evaluation included taste, aroma, color, appearance, aftertaste, and overall impression. Taste was the most important parameter in determining the panelists' acceptance level of the functional beverage product. Taste was influenced by several factors, including chemical compounds, temperature, concentration,

and interactions with other taste components [26,27]. The functional beverage was made with three flavor variants: ginger (F1), lemongrass (F2), and lemon (F3). Aroma was the smell of a food product, which was the response to volatile compounds from a food or beverage that entered the nasal cavity and were perceived by the olfactory system. The more volatile components present in a substance, the sharper the aroma formed. The aroma of the three beverages had distinct characteristics corresponding to the treatment's flavor variants. The results of the hedonic sensory analysis could be seen in Figure 1.

The functional beverage's taste had an average hedonic score of 3.79 (like). The highest hedonic score was found in formula F1 (ginger) with a score of 3.87. The Kruskal-Wallis analysis showed that the variation in taste among the functional beverages did not significantly affect the panelists' liking for the taste of the functional beverages. This indicates that all three formulas of the functional beverage can be used as they have nearly the same level of liking. The ginger variant was the most preferred functional beverage based on the taste parameter. The ginger variant exhibited a sweet and spicy taste. The spicy taste is due to the presence of strong volatile compounds in ginger. The formation of this spicy taste comes from compounds like gingerol, shagaol, and zingerone found in ginger [28]. The hedonic test results for the aroma parameter showed that the panelists' liking ranged from 3.43 to 3.67. This indicates that panelists liked formulas F1 and F2, while formula F3 showed neutral liking. The results indicated that the variation in taste among the functional beverages did not significantly affect the panelists' liking preferences for the aroma of the functional beverages.

Color is a result of visual perception and can be a consideration in evaluating a product. Color is an important indicator for food and beverages, representing freshness and liking level. The hedonic test results for the color parameter showed that the panelists' liking ranged from 4.07 to 4.17. This indicates that the color of all three treatments of the functional beverage was liked by the panelists. The variation in taste among the functional beverages did not significantly affect the color of the functional beverages. Formulas F1 and F2 had a blue color, which is the natural color of butterfly pea flower. Formula F3 had a purple color due to pH changes caused by the addition of lemon to the functional beverage.

Appearance is a primary factor in consumer judgment of a food product. Consumers tend to choose products with attractive appearances. The color appearance produced in the functional beverages was blue in samples F1 and F2, while sample F3 had a purple appearance. All three treatments had average scores ranging from 4.23 to 4.77. The variation in taste among the functional beverages significantly affected the appearance of the functional beverages. Aftertaste is the taste that remains after swallowing a liquid. The perception of whether an aftertaste is pleasant or not is usually based on subjective judgment, indicating that everyone preserve have different preferences. The variation in taste among the functional beverages showed significantly different results in the aftertaste of the functional beverages. Overall is a combination of the previous parameters that evaluate the entire functional beverage. The hedonic test results for the overall liking of the functional beverages showed that the panelists liked the beverages. The average overall scores ranged from 3.83 to 4.10.

3.3.2 Phytochemicals

The phytochemical analysis has been conducted on the three formulations of functional beverages. Compounds tested include alkaloids, steroids, flavonoids, saponins, phenols, and tannins. These phytochemical compounds, as chemical constituents found in plants, play a crucial role in health, including their function in preventing degenerative diseases. The results of phytochemical analysis on the functional beverages are presented in Table 4.

Tabel 4. Phytochemical Analysis of Functional Beverages.

Phytochemical Analysis	Treatments			Positive Results
	F1.	F2	F3	
Alkaloid				
Dragendorff	-	+	+	Red Sediment
Meyer	-	+	+	White Sediment
Wagner	-	+	+	Brown Sediment
Steroid	+	+	+	Blue and green
Flavonoid	-	+	+	Red/yellow/orange
Saponin	+	+	+	Formation of foam
Fenol Hidrokuinon	-	-	+	Green and blue-green
Tanin	+	+	+	Dark red

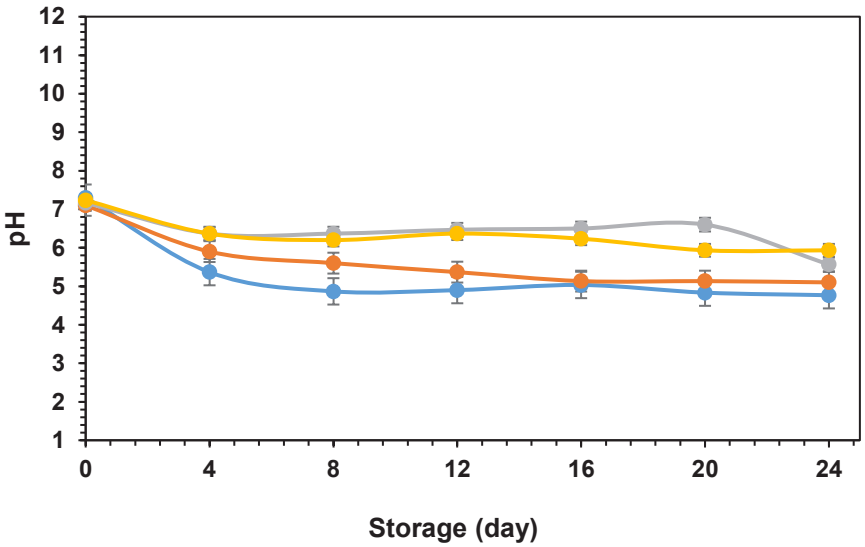

Fig. 2. Graph of beverage pH stability during storage. Note: Sample was stored in; (RTS: Room Temperature with the addition of Sodium Benzoate), (RTSS: Room Temperature with the addition of Sodium Benzoate and Sodium Metabisulfite), (CTS: Chilling Temperature with the addition of Sodium Benzoate), (CTSS: Chilling Temperature with the addition of Sodium Benzoate and Sodium Metabisulfite).

The phytochemical analysis results indicate that formulas F2 and F3 tested positive for alkaloids with the Dragendorff, Meyer, and Wagner reagents, while formula F1 did not contain alkaloids. Alkaloids are a group of organic compounds predominantly found in nature, mainly derived from plants. Nearly all alkaloids contain nitrogen atoms, which confer basic properties and are part of heterocyclic rings. In Fact, alkaloids are widely used in medicine [29].

The flavonoid analysis yielded positive results for formulas F2 and F3, characterized by yellow-orange coloration. Flavonoids represent the largest group of natural phenolic compounds. Flavonoids act as antioxidants and antibiotics. They also protect cell structures, enhance the effectiveness of vitamin C, and prevent osteoporosis. Saponin testing on all three formulas produced positive results, evidenced by the formation of stable foam during testing. Saponins are chemical compounds that impart bitterness to food items. They are non-toxic

as they cannot be absorbed by the intestines [30]. The functional beverage exhibited positive results for steroids, as indicated by the blue coloration. Steroids function as anti-allergic agents, inhibit asthma, reduce skin inflammation, and enhance immune function [31]. Formula F3 tested positive for hydroquinone phenol compounds. Hydroquinone phenol and its derivatives act as oxidative inhibitors, binding free radicals and reacting with Reactive Oxygen Species (ROS) to form more stable compounds [32].

All three formulas of the functional beverage tested positive for tannins, evidenced by the dark red coloration. Ojo [33] reported that tannins serve as antioxidants, binding free radicals to protect cells and prevent various diseases. Tannins impart a bitter taste to food items. They prevent the oxidation of LDL cholesterol in the blood, thus reducing the risk of stroke. However, excessive consumption of foods or beverages containing tannins is not advisable as tannins have the ability to bind with proteins and iron. Tannins can precipitate the mucosa proteins in the small intestine, reducing nutrient absorption.

3.3.3 Antioxidants

The antioxidant content in the three formulas showed varying results. The functional beverage exhibited antioxidant content of 9.93 ppm in the ginger variant, 55.98 ppm in the lemongrass variant, and 20.62 ppm in the lemon variant. The ginger (F1) and lemon (F3) variants fall into the category of very strong antioxidants, while the lemongrass variant has strong antioxidant properties. Antioxidants are categorized as very strong if the IC50 value is less than 50 ppm, strong if the IC50 value is 50-100 ppm, moderate if the IC50 value is 100-150 ppm, and weak if the IC50 value is 150-200 ppm [34].

This functional beverage has a relatively high antioxidant content due to the high antioxidant content of the raw materials used. Sedjati [35] reported that *Sargassum* sp. extract has an IC50 value of 69.274 µg/ml, indicating its potential as an antioxidant due to its phenolic compound and polysaccharide content. The antioxidant activity values in butterfly pea flower and sappan wood are also classified as strong, as reported by Palimbong and Pariama [36], with butterfly pea flower having an IC50 value of 53.61 µg/ml and sappan wood having an IC50 value of 83.33 µg/ml [28]. Consumption of foods or beverages containing antioxidants is known to enhance immune status and inhibit the onset of degenerative diseases.

3.4 Quality (shelf-life) stability during storage

3.4.1 pH stability

The pH value of a food product was one of the crucial factors determining the resistance level against the growth of spoilage microorganisms during processing, distribution, and storage. The functional beverage subjected to pH value stability testing consisted of four types: beverages with the addition of sodium benzoate stored at room temperature (RTS), beverages with the addition of sodium benzoate and sodium metabisulfite stored at room temperature (RTSS), beverages with the addition of sodium benzoate stored at chilling temperature (CTS), and beverages with the addition of sodium benzoate and sodium metabisulfite stored at chilling temperature (CTSS). The Chilling Temperature used for storage ranged from 5-10°C. pH measurements during storage were conducted over a period of 4 weeks and observed every four days. This testing aimed to determine the pH stability of the functional beverage during storage. The graph depicting the pH stability of the beverage during storage is presented in Figure 2.

The pH testing of functional beverages on day-0 revealed pH levels ranging from 7.10 to 7.30. Then, observations on day-12 showed a slight decrease in pH for samples stored at room temperature, while the pH of samples stored at chilling temperature increased. By day-16, the pH values of all four samples underwent changes: the RTS sample increased to 5.03, the RTSS sample to 5.13, the CTS sample to 6.50, and the CTSS sample to 6.23. Overall, a decrease in pH during storage was observed. Factors contributing to pH reduction may have included the potential growth of microorganisms such as lactic acid bacteria, followed by the breakdown of sugar components resulting in acid components. Significant changes in pH could alter the taste of a product [37].

3.4.2 Antioxidant stability

The antioxidant levels in the beverage, which had been stored for 24 days, increased. Storage for 24 days, whether at chilling or room temperature, did not cause significant changes ($p>0.05$) in the antioxidant activity of the functional beverage. Antioxidant stability could be influenced by pH and storage temperature. Antioxidant testing was conducted on beverages with different storage conditions and the addition of Sodium Benzoate and Sodium Metabisulfite. The antioxidant test results after storage could be seen in Table 5.

The findings demonstrate a highly favorable outcome for the functional beverage, even during a storage period of approximately 24 days. The presence of a small amount of antioxidants indicates a greater level of antioxidant effectiveness. The acids generated during storage will enhance the antioxidant activity in a product [38]. The sample held at a low temperature with the addition of sodium benzoate and sodium metabisulfite showed the highest level of antioxidant activity, measuring at 4.47 ppm.

Table 5. Antioxidant of Seaweed Tisane beverage activity after 24 days of storage.

Treatment	Antioxidant activity (ppm)
RTS	17.19 ± 3.25^{b}
RTSS	5.07 ± 0.09^{ab}
CTS	6.24 ± 0.24^{a}
CTSS	4.47 ± 3.25^{c}

Note: Sample was stored in; RTS: Room Temperature with Sodium Benzoate Addition, RTSS: Room Temperature with Sodium Benzoate and Sodium Metabisulfite Addition, CTS: Chilling Temperature with Sodium Benzoate Addition, CTSS: Chilling Temperature with Sodium Benzoate and Sodium Metabisulfite Addition.

Table 6. Total Plate Count (TPC) after 24 days of storage.

Treatment	$\times10^2$ colony/mL
RTS	149.02 ± 12.31^{b}
RTSS	90.50 ± 10.82^{b}
CTS	2.01 ± 1.41^{a}
CTSS	1.31 ± 1.85^{a}

Note: Sample was stored in;
RTS : Room temperature with the addition of Sodium Benzoate
RTSS : Room temperature with the addition of Sodium Benzoate and Sodium Metabisulfite
CTS : Chilling temperature with the addition of Sodium Benzoate
CTSS : Chilling temperature with the addition of Sodium Benzoate and Sodium Metabisulfite

3.4.3 Total plate count

The microbial contamination analysis based on the Total Plate Count (TPC) of the functional beverage showed low microbial contamination levels on day 0 of storage, specifically $<0.1x10^2$ colonies/mL. This result complies with the microbial contamination standard for non-carbonated flavored beverage products according to SNI 14.1.4.8-7388-2009, which is 2×10^2 colonies/mL. Microbial contamination in the functional beverage, which underwent storage for 24 days with different treatments, experienced a significant increase. The results of the Total Plate Count (TPC) test after 24 days of storage can be seen in Table 6.

The statistical analysis of variance conducted on microbiological contamination testing during storage, including various temperature and preservative treatments, revealed notable disparities in the outcomes. Functional beverages maintained at a low temperature for 24 days and treated with Sodium Benzoate (CTS) showed the most favorable outcomes, meeting the microbiological contamination criteria for non-carbonated flavoured beverages as specified in SNI 7388-2009. The treatment maintained at room temperature with the addition of Sodium Benzoate and Sodium Metabisulfite exhibited the greatest average colony count. Environmental conditions have a substantial impact on the growth and activity of microorganisms. While the results shown above are currently in the early stages for commercial beverages, they serve as a demonstration or proof of concept of how the combination of preservatives, colour stabilizers, and low temperatures can effectively preserve the quality of beverages from a bacteriological perspective. Furthermore, the packaging procedure and sterilization of packaging bottles have a significant impact on reducing microorganisms in the food industry. Conducting this study in the future is essential due to the lack of natural-based functional beverages that can maintain a long shelf life while also being highly accepted by consumers in terms of taste or sensory experience.

4 Conclusions

Sargassum sp., butterfly pea flower, and sappan wood have high nutritive, non-nutritive (fibre), and antioxidant content, making them a successful synergistic blend for formulating functional beverages with new combined ingredients. In the pre-formulation stage, the S0.05 treatment emerged as the most favourable option based on panelist evaluations, incorporating 0.5 g of *Sargassum* sp. All three variants of the functional beverage were well-received by the panelists, with the ginger variant (F1) receiving the highest acceptance score. The different storage processes, including room temperature and chilling temperature, as well as the addition of Sodium Benzoate and Metabisulfite, influenced the quality stability of the functional beverage during storage.

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References

1. D. Granato, G. F. Branco, F. Nazzaro, A. G. Cruz, and J. A. F. Faria, Compr. Rev. Food Sci. Food Saf. **9**, 292 (2010)
2. A. Tolun and Z. Altintas, in *Funct. Med. Beverages* (Elsevier, 2019), pp. 235–284
3. I. Dini, in *Funct. Med. Beverages* (Elsevier, 2019), pp. 1–40
4. C. Ravikumar, J. Pharm. Sci. & Res. 6(5), 4 (2018)
5. W. Ramadhan, W. V. Krisnawan, A. N. Firdaos, and B. Riyanto, OP Conf. Ser. Earth Environ. Sci. 1 (2023)

6. A. Husni, D. Ariani, and S. A. Budhiyanti, J. Agritech **35**, 368 (2015)
7. S. Jeeva, J. M. @ Antonisamy, C. Domettila, B. Anantham, and M. Mahesh, Asian Pac. J. Trop. Biomed. **2**, S30 (2012)
8. M. Gazali, N. Nurjanah, and N. P. Zamani, J. Pengolah. Has. Perikan. Indones. **21**, 167 (2018)
9. R. Singh, C.-C. Yu, G.-W. Chen, C.-H. Chen, N. Y. Sinaki, J. Lin, and F. Koksel, Foods **11**, 3447 (2022)
10. C. Chusak, J. A. Y. Ying, J. L. Zhien, P. Pasukamonset, C. J. Henry, S. Ngamukote, and S. Adisakwattana, Food Chem. **295**, 646 (2019)
11. N. N. Hasanah, E. M. Mohamad Azman, A. Rozzamri, N. H. Z. Zainal Abedin, and M. R. Ismail-Fitry, Polymers (Basel). **15**, 2541 (2023)
12. I. W. Suarna and I. M. S. Wijaya, J. Trop. Biodivers. Biotechnol. **6**, 63013 (2021)
13. T. M. Kiranawati, R. R. Mariana, and N. Efrinasari, J. Agroindustri **12**, 29 (2022)
14. Miksusanti, Elfita, and H. S, J. Penelit. Sains **15**, 60 (2012)
15. D. E. Nirmagustina, Zulfahmi, and Oktafrina, J. Teknol. Ind. Dan Has. Pertan. **16**, 22 (2011)
16. [AOAC] Association of Official Analytical Chemists, *Official Methods of Analysis of AOAC International (17th Ed.)*, 17th ed. (AOAC International Press, Maryland, 2000)
17. [AOAC], *Official Method of Analysis of The Association of Official Analytical Chemists.* (2005)
18. A. Altemimi, N. Lakhssassi, A. Baharlouei, D. Watson, and D. Lightfoot, Plants **6**, 42 (2017)
19. S. Diachanty, Nurjanah, and A. Asadatun, JPHPI, **20**, (2017)
20. N. K. I. Swari, R. Wirawan, N. Qomariyah, and K. Al Hadi, Konstan - J. Fis. dan Pendidik. Fis. **4**, 1 (2019)
21. S. Purwaningsih, J. Santoso, E. Handharyani, N. P. Setiawati, and E. Deskawati, IOP Conf. Ser. Earth Environ. Sci. **414**, 0 (2020)
22. A. Leandro, D. Pacheco, J. Cotas, J. C. Marques, L. Pereira, and A. M. M. Gonçalves, *Seaweed's Bioactive Candidate Compounds to Food Industry and Global Food Security* (2020)
23. Y.-H. Pyo, Y.-J. Jin, and J.-Y. Hwang, Prev. Nutr. Food Sci. **19**, 108 (2014)
24. S. T. Pakaya, N. Yusuf, and L. Mile, J. Ilm. Perikan. Dan Kelaut. **2**, 174 (2014)
25. E. Sinurat and T. D. Suryaningrum, J. Pengolah. Has. Perikan. Indones. **22**, 581 (2019)
26. A. T. Omidiran, O. P. Sobukola, A. Sanni, A. A. Adebowale, O. A. Obadina, L. O. Sanni, K. Tomlins, and T. Wolfgang, Food Sci. Nutr. **4**, 80 (2016)
27. C. Ruiz-Capillas and A. M. Herrero, Foods **10**, 582 (2021)
28. M. R. Palupi and T. D. Widyaningsih, J. Pangan Dan Agroindustri **3**, 1458 (2015)
29. M. Heinrich, J. Mah, and V. Amirkia, Molecules **26**, 1836 (2021)
30. Y. P. Timilsena, A. Phosanam, and R. Stockmann, Int. J. Mol. Sci. **24**, 13538 (2023)
31. W. Ericson-Neilsen and A. D. Kaye, Ochsner J. **14**, 203 (2014)
32. A. Schieber, J. Agric. Food Chem. **66**, 13051 (2018)
33. M. A. Ojo, Prev. Nutr. Food Sci. **27**, 14 (2022)
34. I. G. Munteanu and C. Apetrei, Int. J. Mol. Sci. **22**, 3380 (2021)
35. S. Sedjati, E. Supriyantini, A. Ridlo, N. Soenardjo, and V. Y. Santi, J. Kelaut. Trop. **21**,

137 (2018)
36. S. Palimbong and A. S. Pariama, J. Sains Dan Kesehat. **2**, 228 (2020)
37. D. Renate, F. Pratama, K. Yuliati, and G. Priyanto, J. Agritech **34**, 330 (2014)
38. A. P. Purba, B. Dwiloka, and H. Rizqiati, J. Teknol. Pangan **2**, 49 (2018)