Antioxidant Activity and Consumer Preference of Brown Algae *Sargassum hystrix* Juice as a Functional Drink

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ABSTRACT

Sargassum hystrix juice is a functional beverage made from *S. hystrix* through the immersion process, crushing, filtering, and pasteurization. Therefore, this study aims to determine the effect of *S. hystrix* concentration on antioxidant activity, level of consumer acceptance, and chemical composition of the juice. The fresh *S. hystrix* used were washed, immersed for three days, and blended at a concentration of 10, 20, 30, 40, and 50%, respectively. Meanwhile, the qualities of juice that were observed include yield, antioxidant activity, total phenol, water, ash, protein, and fiber content, formol number, total sugar, total plate count, and sensory analysis. The results showed that the *S. hystrix* juice had a value of yield 43.00-85.67%, pH 7.5, antioxidant activity 20.50-44.57%, total phenolic 32.78-293.11 mg GAE/g, water 93.03-95.39%, ash 0.36-6.34%, protein 0.88-4.96%, crude fiber 0.41-5.04%, formol number 15-21.67 mL N NaOH/100 mL, total sugar 3.10-4.61%, total plate count $2.3x10^3$-$4.3x10^3$CFU/mL, sensory of color 1.83-2.95, flavor 2.49-3.29, and taste 2.29-3.50.

Keywords: Antioxidant; functional drink; juice; *Sargassum hystrix*

INTRODUCTION

The consumption patterns of Indonesians, especially in urban communities, have a change of eating habits from natural into fast foods that are rich in fat and free radicals (Fagbemi *et al*., 2013). This condition was one of the factors that caused an increase in the prevalence of degenerative diseases such as coronary heart disease, diabetes mellitus, hypertension, heart disease, stroke, and some types of cancer (Barhe and Tchouya, 2016). Meanwhile, the presence of free radicals in the body leads to degenerative diseases such as diabetes mellitus, narrowing of blood vessels, coronary heart disease, stroke, and cancer (Kang *et al*., 2010).

Disease prevention is carried out by setting nutrient consumption such as natural antioxidant that is capable to fight degenerative diseases. These antioxidants delay or prevent the occurrence of free radical auto-oxidation reactions (Farag *et al*., 2003). Currently, the antioxidant activity of various food is one of the most widely studied topics due to its implications on health, especially the potential as a functional ingredient (Balboa *et al*., 2013). Therefore, the attention on the use of natural antioxidants is increasing and one source of these is algae. Budhiyanti *et al*. (2011) have studied

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the antioxidant activity of the cytoplasm and membrane fractions of *Sargassum hystrix.* However, the utilization of antioxidant compounds from brown algae *S.hystrix* in food is limited, therefore, more studies are required on its application in food.

Functional beverages contain ingredients that improve health status or prevent certain diseases (Tangkeallo and Widyaningsih, 2014). Juice is a beverage derived from fruit or vegetables and smoothies (Caswell, 2009), the ones from algae are very rare. Furthermore, Juice is usually served cold, hence, it is easy to maintain and minimize the damage of the antioxidant compounds contained in *S. hystrix.* Therefore, this study was conducted to produce functional drinks such as *S. hystrix* juice that contains natural antioxidants.

MATERIALS AND METHODS

Materials

The primary equipment used for *Sargassum hystrix* juice processing includes homogenizer (Philips, Tokyo, Japan), analytical balance (Denver Instrument Company AA-200, New York, USA), Kjeldahl flask, electric stove (Maspion, Indonesia), Spectrophotometer Microlab 300 (Elitech Group, France), ELISA reader (Microplate Reader, Japan), and incubator (Isuzu Incubator, Japan). The main materials used for the processing include *S. hystrix* sample from Sepanjang Beach, Gunung Kidul, Folin-Ciocalteu, and DPPH from Sigma-Aldrich (St Louis, MO, USA), Na_2CO_3, and HNO_3 from DifcoFisher Scientific (Ottawa, ON, Canada).

Sargassum hystrix Preparation and Immersion

The samples of brown algae *S. hystrix* were collected at Pantai Sepanjang, Gunung Kidul, from January to June 2016 and washed using clean water to remove impurities and residual seawater. Meanwhile, the immersion method based on Supirman *et al.*, (2013) with modification was conducted by soaking 250 g of *S. hystrix* into 750 mL of water and adding lemon juice until a pH value of 3 is reached. Furthermore, 100g of fresh pandan leaves were for three days to eliminate the fishy smell and every 12 hours, water used was changed to avoid saturation of dirt and salt. In addition, the fresh and soaked samples were tested for antioxidant activity, total phenol content, and proximate analysis.

Sargassum hystrix Juice Processing

The materials required needed to make the juice were brown alga *S. hystrix*, sugar, and water. After immersion, the sample was blended with water and

Tabel 1. Formulation ratio of materials for making *Sargassum hystrix* juice.

Materials	Concentration of *S. hystrix* (%)				
	10	20	30	40	50
S. hystrix (%, w/v)	20	40	60	80	100
Water (mL)	170	150	130	110	90
Sugar (%, w/v)	10	10	10	10	10
Total volume (mL)	200	200	200	200	200

sugar by the concentration based on threshold test and *S. hystrix* juice was stored in a freezer (-18°C) before further analysis. Meanwhile, the juice was processed based on Iriani *et al*. (2005) with modifications. The formula of *S. hystrix* juice is shown in Table 1.

Sample Analysis

The sample was analyzed to obtain the value of yield (Magro *et al*., 2015), pH (Zainoldin, 2009), antioxidant activity (Zubia *et al*., 2009), total phenol (Kang *et al*., 2010), the content of water, ash, protein, crude fiber, total sugar, formol number, total plate count (AOAC, 2019), and the level of consumer acceptance (Setyaningsih *et al*., 2010). Meanwhile, a consumer acceptance analysis was conducted by 80 untrained panelists to assess the parameter of color, flavor, and taste of each juice treatment. This assessment was conducted by giving a score from 1 to 5 (1 = strongly dislike, 2 = dislike, 3 = neutral, 4 = like, and 5 = strongly like).

Statistical Analysis

Chemical analysis data were analyzed using ANOVA with a 95% confidence level and the results showed a significant difference which was followed by the Tukey-HSD test. Meanwhile, consumer acceptance data were analyzed using Kruskal-Wallis and when there was a significant difference, it was followed by Mann-Whitney test. This data analysis was carried out with SPSS 20.0.

RESULTS AND DISCUSSION

Yield

The yield of *S. hystrix* juice is shown in Table 2 and the result showed that its higher concentration had a lower value of the yield. Meanwhile, Magro *et al*. (2016) stated that the grape juice with and without the addition of enzyme pectinase produced 69.85 and 75.83% of yield, respectively. Iriani *et al*. (2005) also stated that the yield of kuini mango juice with the addition of pectinase enzymes had a value of 78.10 to 84.92%. Therefore, the high-water content in the processing of the juice made *S. hystrix* more soluble in water which led to higher yield, while more solvent produced a higher volume of extract (Kamaluddin *et al*., 2014).

pH

The pH of each *S. hystrix* concentration had the same value which was 7.5 (Table 2). This showed that the concentration of *S. hystrix* in the juice did not affect the pH which tends to be neutral. In this study, the destruction process and pasteurization time, as well as the amount of sugar used in each treatment were the same, therefore, there was no real effect on the juice produced. Moreover, Harjantini and Rustanti (2015) stated that the functional beverage of srikaya yogurt jelly with the addition of carrageenan has a pH of 4.33 to 5.74. Furthermore, Pamungkas *et al*. (2014) stated that jelly drinks of hantap leaf extract have a pH value of 7.87.

Antioxidant Activity

The antioxidant activity of this juice depends on the concentration of *S. hystrix* as shown in Table 2, where the highest activity was 43.91% for 40% concentration. Moreover, Husni *et al*. (2015b) stated that fast drinks with the addition of *S. polycystum* ethanolic extract have an antioxidant activity of 25.7 to 65.41% while yogurt has 72.34 ± 0.01% at a concentration of 6000 ppm (Husni *et al*., 2015a). Similarly, Pamungkas *et al*. (2014) stated that jelly drinks of hantap leaf extract have an antioxidant activity of 20.93%. Furthermore, the heat process applied to the food processing and pH value of food also influenced the stability of the product's antioxidant activity (Tensiska *et al*., 2003).

Total Phenol Content

Total phenol content as shown in Table 2 indicated that *S. hystrix* concentrations influenced its content in the juice. Meanwhile, the concentration of 50% had the highest value of total phenol content, while the lowest was at 10 and 20% concentrations. The correlation analysis showed that there was a strong and positive correlation between total antioxidant activity and total phenol content. Moreover, Husni *et al*. (2015b) stated that the greater the addition of *S. polycystum* extract in fast drinks, the more the antioxidant activity and positively correlated with total phenol. The relationships between total phenol and antioxidant activity of *S. hystrix* juice are shown in Figure 1.

Brown macroalgae compounds have potential as antioxidants, namely, polysaccharides, phenolic compounds, lipids, pigments, vitamins, terpenoids, and sterols (Balboa *et al*., 2013). Meanwhile, fast drinks with the addition of *S. polycystum* ethanolic extract had a total phenol content from 27.74 to 100.36 mg GAE/g (Husni *et al*., 2015b). Similarly, Pamungkas *et al*. (2014) stated that jelly drinks of hantap leaf extract had a total phenol content of 10.6 GAE/100 g. When compared to

Tabel 2. Parameter values of *S. hystrix* juice

Parameters	The concentration of *Sargassum hystrix*				
	10%	20%	30%	40%	50%
Yield	85.67[d]	72.33[c]	64.33[b]	57.67[b]	43.00[a]
pH	7.50	7.50	7.50	7.50	7.50
Antioxidant Activity	20.50[a]	27.29[b]	37.09[c]	43.91[d]	44.57[d]
Total Phenol Content	32.78[a]	39.78[a]	131.51[b]	179.05[b]	293.11[c]
Water Content	93.03[a]	95.39[b]	95.05[b]	95.12[b]	94.77[b]
Ash Content	0.79[ab]	0.36[a]	5.58[ab]	5.68[ab]	6.34[b]
Protein Content	0.88[a]	2.33[ab]	2.63[ab]	3.79[ab]	4.96[b]
Crude Fiber Content	0.41[a]	0.79[a]	2.85[b]	5.04[c]	4.97[c]
Total Sugar Content	4.61[b]	4.13[b]	4.11[b]	3.18[a]	3.10[a]
Total Plate Count	$2.3x10^3$	$3.0x10^3$	$2.8x10^3$	$4.3x10^3$	$3.1x10^3$
Formol Number	15.00[a]	15.67[ab]	18.00[ab]	21.33[b]	21.67[b]
Color Preference	2.95[b]	2.95[b]	2.79[b]	2.95[b]	1.83[a]
Flavour Preference	2.83[ab]	3.29[c]	3.01[bc]	2.89[b]	2.49[a]
Taste Preference	3.50[c]	3.09[b]	3.34[bc]	3.15[bc]	2.29[a]

Values in the same line with different letters are significantly different at $p < 0.05$

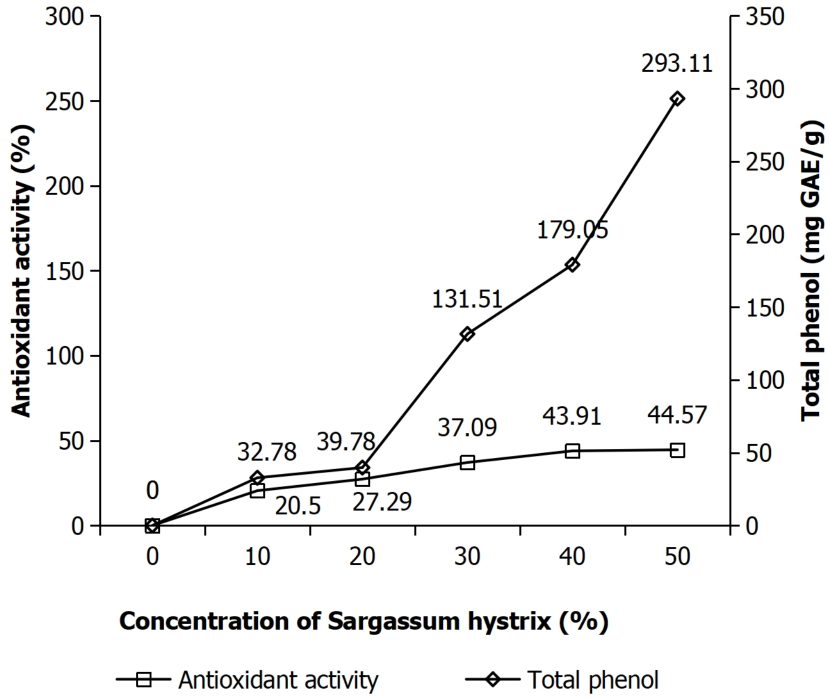

Figure 1. Relationships between total phenol and antioxidant activity of *Sargassum hystrix* juice

some of these studies, the total phenolic content of the *S. hystrix* juice was significantly high.

Proximate Analysis

The water content of *S. hystrix* juice varied from 93.03 to 95.39% as shown in Table 2. Meanwhile, Pamungkas *et al*. (2014) stated that the water content of jelly drinks from hantap leaf extract was 87.37% and yogurt with the addition of *S. polycystum* ethanolic extract varied from 77.69 to 85.76% (Husni *et al*., 2015a). Therefore, *S. hystrix* juice had a higher water content because it was liquid.

Table 2 showed that the concentration of *S. hystrix* juice affected ash content, therefore, a greater concentration of *S. hystrix* produced higher ash content. Moreover, Husni *et al*. (2015b) stated that fast drinks with the addition of *S. polycystum* ethanolic extract had ash content from 0.06 to 11.54%. Furthermore, Wibowo and Fitriyani (2012) stated that the ash content of fast drinks *Eucheuma cottonii* were18 to 25%, while yogurt with the addition of *S. polycystum* ethanolic extract had an ash content of 0.84 to 1.17% (Husni *et al*., 2015a). When compared to previous studies, the ash content values of *S. hystrix* juice were fairly small ranging from 0.36 to 6.34 ppm.

The protein content of the juice was affected by the concentration of *S. hystrix* and the range was 0.88 to 4.96% as shown in Table 2. A previous study by Pamungkas *et al*. (2014) stated that jelly drinks of hantap leaf extract had a protein content of 0.10%, while yogurt with the addition of *S. polycystum* ethanolic extract had 3.22 to 6.53% (Husni *et al*., 2015a). Therefore, the protein content of *S. hystrix* juice was not significantly different from a previous study which stated that a higher concentration of *S. hystrix* in the juice leads to more protein content.

Crude Fiber Content

The crude fiber content of *S. hystrix* juice was 0.41 to 4.97% as shown in Table 2. and is not significantly different from Wibowo and Fitriyani (2012) which stated that the *Eucheuma cottonii* fast drinks had a fiber content of 5.25 to 11.83%. Similarly, Harjantini and Rustanti (2015) stated that the functional beverage srikaya yogurt jelly with the addition of carrageenan had a fiber content of 2.14 to 3.40%.

Total Sugar Content

The effect of *S. hystrix* concentration on the total sugar content of juice is shown in Table 2. Meanwhile, the amount of sugar used in juicing was 10%, however, not all was soluble, some were filtered or lost during the process. There was an insignificant difference between the total sugar content of *S. hystrix* juice of 10%, 20%, and 30%, and also between 40%

and 50% concentrations. The total sugar content was affected by the amount of sugar in the product. Furthermore, the total sugar content of *S. hystrix* juice (3.10 to 4.61%) was very small compared to the fast drinks with the addition of *S. polycystum* ethanolic extract (76.13 to 87.18%) (Husni *et al*., 2015b), due to the 10% (w/v) that was used. Therefore, a higher concentration of *S. hystrix* in juice reduces the total sugar content of the juice.

Total Plate Count

Table 2 showed that there was no influence of *S. hystrix* concentration in the juice on the total plate count. The total plates count at 10, 20, 30, 40, and 50% were 2.3×10^3, 3.0×10^3, 2.8×10^3, 4.3×10^3, and 3.1×10^3 CFU/mL, respectively. This value was not significantly different when compared to pineapple juice fortified by chitosan. Meanwhile, Husniati and Oktarina (2012) stated that the addition of chitosan on pineapple juice had a total plate count of 8.40×10^3– 9.19×10^3 CFU/mL.

Formol Number

The influence of *S. hystrix* concentration against the formol number of juice is shown in Table 2 which indicated that its concentration affected the formol number of juice ($p < 0.05$). This showed that a higher concentration of *S. hystrix* increased the formol number of juice which ranges from 15.00 to 21.67 mL N NaOH/100 mL. Meanwhile, increased formol numbers indicated their proteolytic activity in degrading proteins into amino acids, peptides, and ammonia. This number had fulfilled SNI 01-3719-1995, where the minimum requirement formol number of fruit juice was 15 mL N NaOH/100 mL. Furthermore, Su *et al*. (2005) stated that the ratio of formol number to total protein contributes to the delicious flavor.

Sensory Properties of *S. hystrix* Juice

Color

Table 2 showed that there was an insignificant difference between the juice color and *S. hystrix* at a concentration of 10, 20, 30, and 40%, respectively. Meanwhile, the *S. hystrix* concentration of 50% was significantly different from other treatments, therefore, higher concentration reduced the levels of juice color that was dominated by the green and ranges from 1.83 to 2.95. Husni *et al*. (2015b) stated that fast drinks with the addition of *S. polycystum* ethanolic extract had color values from 2.4 to 3.2. Similarly, the color value of yogurt with the addition of *S. polycystum* ethanolic extract was 2.67 to 3.08 (Husni *et al*., 2015a).

Flavor

The flavor of juice was affected by *S. hystrix* concentration as shown in Table 2. Meanwhile, the flavor of *S. hystrix* juice at the concentration of 10, 20, 30, 40, and 50 were 2.83, 3.29, 3.01, 2.89, and 2.49, respectively. This value is approximately equal to instant drink with the addition of *S. polycystum* ethanolic extract that had flavor values from 1.29 to 3.66 (Husni *et al*., 2015b), while yogurt had flavor values from 2.42 to 3.10 (Husni *et al*., 2015a).

Taste

The taste of the juice was affected by *S. hystrix* concentration as shown in Table 2. Meanwhile, the taste of *S. hystrix* juice for the concentration of 10, 20, 30, 40, and 50 were 3.50, 3.09, 3.34, 3.15, and 2.29, respectively. The taste of *S. hystrix* juice was higher compared to fast drinks of *S. polycystum* ethanolic extract that had a taste of 1.32 to 3.59 (Husni *et al*., 2015b). Similarly, yogurt with the addition of *S. polycystum* ethanolic extract had a taste of 2.15 to 2.76 (Husni *et al*., 2015a). Meanwhile, Supirman *et al*. (2013) stated that the addition of lime in *S. filipendula* drink can not eliminate the fishy smell, therefore, preference level panelists to the drink are low.

CONCLUSION

Based on the results, the selection of appropriate formulation for *S. hystrix* juice depends on treatment with a high value of the antioxidant activity, total phenol content, and high sensory hedonic value. In this study, the best formulation was the treatment of *S. hystrix* juice concentration by 40% that has high antioxidant activity and sensory value. Therefore, a higher concentration of *S. hystrix* in the juice leads to higher antioxidant activity and positively correlated with total phenol content.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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