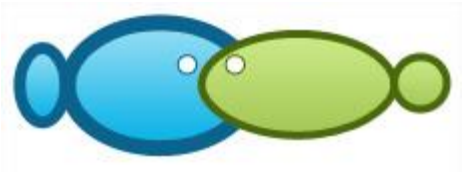

The impact of immersion time in lime solution on antioxidant and antidiabetic properties and consumer evaluation of *Sargassum hystrix* seaweed tea

Damayanti Rahmatika Pratiwi, Amir Husni

Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Bulaksumur, Yogyakarta 55281, Indonesia. Corresponding author: A. Husni, a-husni@ugm.ac.id

Abstract. *Sargassum hystrix* is a brown seaweed that contains bioactive substances, such as antioxidant and antidiabetic compounds, and is used as a functional ingredient in the food industry. They are mostly found in Indonesian waters, growing along rocky shorelines. Since the beverages extracted from seaweed are found to be less preferred by consumers due to the fishy smell, efforts are needed to curtail this problem. Based on this condition, the lime solution immersion method is observed as a suitable approach to reduce the pungent smell. Therefore, this study aims to determine the effect of the immersion time of the lime solution on antioxidant and antidiabetic properties, as well as on consumers' preference for *S. hystrix* tea. In this research, the variation of seaweed immersion time at 0, 4, 8, 12, and 16 min, was used in a lime solution of pH 5 at 85°C. Multiple analyses were further carried out on the beverage, including moisture and total phenol content, hedonic teat, and antioxidant property, such as inhibitions of DPPH (1,1-diphenyl-2-picrylhydrazyl), FRAP (ferric reduction antioxidant power), and α-glucosidase. The results indicated that the immersion time had no effect ($p > 0.05$) on the water content, although it significantly affected total phenol levels, antioxidant, and antidiabetic activities, as well as the consumer preference for *S. hystrix* tea ($p < 0.05$). The best quality was obtained at 16 min, which indicated water and total phenol contents at 3.10 ± 0.33% and 76.81±0.23 mg GAE g^{-1}, with DPPH, FRAP, and α-glucosidase inhibition activities at 53.86±1.27%, 121.82±0.91 μM g^{-1}, and 52.86±0.89%, respectively. It also showed consumer evaluations for appearance, color, flavor, taste, and overall quality, at 4.39±0.91, 4.33±0.78, 4.08±1.23, 4.25±1.08, and 4.26+0.13, respectively. Furthermore, immersion time in lime solution improved the quality and consumer evaluation of *S. hystrix* tea.
Key Words: Bioactive, brown seaweed, consumer preference, fishy smell, functional food.

Introduction. Seaweeds are important economic plants often found in Indonesian waters and have different varieties, such as *Sargassum hystrix* (Kadi 2005). They are found mainly growing along rocky shorelines and possess bioactive substances, such as antidiabetic (Husni et al 2018; Gotama et al 2018; Azizi et al 2019; Azizah et al 2019; Husni et al 2020; Nurkhanifah et al 2020; Nurfahmi et al 2018), anticancer (Husni et al 2021), antistress (Nur'aini et al 2018; Husni et al 2019), and antioxidant compounds (Lailatussifa et al 2017; Suhaila et al 2019; Ardiana & Husni 2020). They are also used as functional ingredients in the food industry.

One of *S. hystrix* uses in the food industry is its processing as tea, because it is a healthy drink that contains useful nutrients. For example, China has long developed *Sargassum* tea as a drink to eliminate phlegm (Alura et al 2016). One of the obstacles in this development is the presence of a fishy smell, which makes it less preferred by consumers (Sinurat & Suryaningrum 2019). To eliminate this pungent odor, the immersion in hot water method was considered. However, Sinurat & Suryaningrum reported that although the method was applied, the level of consumer preference was still low, and the addition of ingredients to remove the fishy smell was necessary. According to Supirman et al (2012), immersion of seaweed in a lime solution of pH 5 eliminated the odor and increased the consumers preference for the tea. However, the use of this treatment for a

long time affected the active ingredients of this product. Although research on the effect of immersion time in lime solution on *S. hystrix* tea has never been conducted, several studies have previously been carried out involving the production of seaweed drink. This includes Supirman et al (2012), which focused on the effect of acid immersion lime (*Citrus auratifolia*) pH differences, and sun drying on the chemical quality of brown algae (*Sargassum fillipendula*) tea. Sinurat & Suryaningrum (2019) studied the antioxidant activity and sensory properties of *Sargassum sp*., under various time immersion and Seng et al (2017) focused on the production of tea from Malaysian brown seaweed (*Sargassum binderi*). Therefore, this study aims to determine the effect of lime solution immersion time on antioxidant and antidiabetic activities, as well as consumer evaluation of *S. hystrix* tea.

Material and Method

Materials. The materials used in this study included *Sargassum hystrix*, which was obtained in August 2020, from the coast of Teluk Awur, Jepara, Central Java, Indonesia. The commercial seaweed tea used for comparison was also obtained from SMEs in Bali. Lime (*Citrus aurantifolia*) was purchased from the Kranggan Traditional Market Yogyakarta. Other materials such as Folin Ciocalteu reagent, Na_2CO_3, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethanol, gallic acid, K_2SO_4, 2,4,6-tri (2-pyridyl)–s triazine (TPTZ), $FeCl_3$, and $FeSO_4$, were purchased from Merck, USA; acarbose, α-glucosidase from *Saccharomyces cerevisiae*, and p-nitrophenyl-α-D-glucopyranoside (PNPG), were obtained from Sigma-Aldrich, Germany.

Preparation of the sample. Seaweed samples from *S. hystrix* were collected by cutting the lower thallus with scissors. The samples were then cleaned and divided for morphological identification. Other samples were further deposited within a cool box and stored in the refrigerator, to be delivered to the laboratory.

Manufacture of seaweed tea and dose of immersion time. The production of seaweed tea was in line with the method described by Sinurat & Suryaningrum (2019), which involved several modifications. Furthermore, 200 g of *S. hystrix* were cleaned and immersed in 2000 mL of pH 5 lime solution, which was prepared by mixing 2000 and 6.4 mL of water and lime solvent at 85°C. The immersion time options used in the experiment were 0 (without immersion), 4, 8, 12, and 16 min., respectively. The seaweed was drained and dried at room temperature for 24 hours after the immersion process. A roasting process was also applied to the seaweed in a frying pan for 15-20 min, and the product was divided into pieces of 0.5 ± 0.1 cm using a knife. Based on these processes, seaweed tea was then extracted into a sealed standing pouch, and stored at 4°C.

Serving of seaweed tea. Approximately 1 g of *S. hystrix* tea was placed in a beverage bag and used to prepare a tea by immersing it in 100 mL of hot water for 6 min. During the brewing process, the tea bags were repeatedly lifted and dropped (5 times) into the steam, as well as shaken 2-3 times. After this, the bags were discarded from the tea solution (Seng et al 2017). The seaweed solvent was further extracted for the analysis of total phenol content, antioxidant and α-glucosidase inhibitory activities, as well as consumer acceptance test.

Analysis of water content. The water content of the raw material and seaweed tea was investigated using a moisture analyzer (Ohaus MB120), which contained a pan where the 0.5 g sample was placed for analysis. After the sample was fully dried for 2-20 min, the moisture content was displayed on the analyzer screen and recorded.

Analysis of the total phenol content. Analysis of total phenol content was performed according to the method described by Sinurat & Suryaningrum (2019). Approximately 1 mL of tea solution was placed in a Falcon tube and mixed with 5, 1, and 0.5 mL of distilled water, 96% ethanol, and 50% Folin Ciocalteu reagent, respectively. This mixture was left

to rest for 5 min and further mixed with 1 mL of 5% Na_2CO_3. The compound was shaken with a vortex and placed in a dark room for 1 h. After this, standard solutions were prepared by making gallic acid solvents, for concentrations of 0, 20, 40, 60, 80, and 100 ppm (Sinurat & Suryaningrum 2019). Furthermore, 1 mL of this solution for each concentration was placed in a Falcon tube and mixed with 5, 1 and 0.5 mL of distilled water, 96% ethanol, and 50% Folin Ciocalteu reagent, respectively. This compound was also left for 5 min, then mixed with 1 mL of 5% Na_2CO_3. After this, the mixture was shaken with a vortex and placed in a dark room for 1 h. The absorbance of this sample and standard solution were then recorded using a UV-Vis spectrophotometer (Lamda 25, PerkinElmer), at a wave length of 725 nm. Furthermore, the method for calculating the total phenol content is as follows:

$$\text{Total fenol (mg GAE/g)} = \text{x} \,.\, \frac{\text{v}}{\text{m}}$$

Where:
x = the test solution concentration (mg mL^{-1})
v = the test solution volume (mL)
m = the test solution mass (g)

DPPH free radical scavenging assay. The scavenging capacity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was predicted to evaluate the antioxidant property of seaweed tea, through the use of the method described by Muthia et al (2019). This involved creating a homogenized *S. hystrix* beverage, lime, and vitamin C solutions with distilled water. Accordingly, 3 mg of DPPH powder were dissolved in 10 mL distilled water to create a solution (0.76 mM), which was stored at 4°C, for use within the maximum of 24 h. Furthermore, the absorbance of the control and sample solutions were assessed using the UV-VIS spectrophotometer (Multiply Go), at 517 nm wavelength. The percent inhibition was also used to estimate the antioxidant capacity, as shown in the following formula,

$$\text{Inhibitoryion activity (\%)} = \frac{(C-D)-(A-B)}{(\text{C}-\text{D})} X\ 100$$

where: A = sample (160 µL of sample + 40 µL of 0.76 mM DPPH);
B = sample control (160 µL of sample + 40 µL of distilled water);
C = negative control (160 µL of distilled water + 40 µL of 0.76 mM DPPH);
D = blank (200 µL of distilled water).

Ferric reduction antioxidant power (FRAP) assay. This estimation required the method described by Suhaila et al (2019), where Fe^{3+} was decreased to Fe^{2+}. The spectrophotometer was used to quota iron (III) chloride, modified into Fe^{2+} compound at 595 nm wavelength. These modifications were potentially detected with a solution color conversion to blue. Furthermore, the acetate buffer solution having pH 3.6 was defined by mixing 0.775 g of $CH_3COONa.3H_20$ (sodium acetate trihydrate) with 4 mL of concentrated acetic acid and dissolved using distilled water to obtain an exact volume of 250 mL, with the yield stored as a stock solution at 4 ° C. After this, 10 mM mL^{-1} of the TPTZ solution was prepared by mixing 0.15 g of TPTZ in 40 mM L^{-1} of HCl, to complete an exact volume of 50 mL. Although the 40 mM L^{-1} solution was arranged by dissolution of 0.828 mL of concentrated HCl in 250 mL of distilled water, the process was still continuously carried out. The extracted TPTZ solution was further stored at 4°C, for use within a period of 24 h. Furthermore, 0.54 g $FeCl_3.6H_2O$ were dissolved in distilled water and prepared to approximately 100 mL, to provide a solution of 20 mM L-1, which was stored for 24 h at 4 ° C. The FRAP reagent was also defined by mixing 2.5, 25, and 2.5 mL of TPTZ, acetate buffer, and $FeCl_3.6H_2O$ solutions (1:10:1), which were approximately prepared to 100 mL using distilled water. Standard solutions (10,000 µM L^{-1}) were then provided by dissolving 2.78 g of $FeSO_4.7H_2O$ in 1000 mL of distilled water and serially diluted to obtain

concentrations of 50, 100, 150, 200, 250 and 300 ppm, respectively (Suhaila et al 2019). The preparation of *S. hystrix* and commercial tea solutions, as well as vitamin C, was carried out by dissolving 1 g of each material in 100 mL of distilled water. Meanwhile, the pH 5 solution was produced by adding lime juice to distilled water at 85 ° C. FRAP reagent of 900 µL was also mixed with 120 µL of each sample solution, the mixture was homogenized using a vortex, and allowed to rest for 15 min. The absorbance of the solution was assayed at a wavelength of 595 nm, as the provided data were further handled using Microsoft Excel. In addition, the standard solution of $FeSO_4.7H_2O$ was used as an ideal curve, by making a line equation for its absorbance value. The data from the sample were also enrolled in the line formula, to provide the FRAP value (in µM g^{-1}).

Inhibitory activity of α-glucosidase. The analysis of α-glucosidase inhibitory activity was carried out by follwoing to the method explained by Azizi et al (2019). The lime solution and acarbose (an antidiabetic drug) were used as standard solvents, as the mixture contained 25, 50, 10 and 25 mL of 0.5 mM p-nitrophenyl-α-D-glucopyranoside (PNP-G), 0.1 M phosphate buffer pH 7, the sample extract and 0.2 U mL^{-1} α-glucosidase, respectively. Furthermore, the sample was mixed and incubated at 37 ° C for 30 min, as the reaction stopped after applying 100 mL of 0.2 M Na_2CO_3. Enzyme activity was determined by recording the absorbance of p-nitrophenol formed in a microplate reader at 405 nm, using a UV-VIS spectrophotometer (Multiply Go). In addition, absorbance values were used to analyze the percentage inhibition of the enzymes.

$$\text{Percentage inhibition} = [(K-(S1-S0))/K] \times 100\%$$

Where K, S1, and S0 denote the absorbance of the control-blank, as well as a sample with and without enzyme.

***Analysis of consumer evaluation of seaweed tea*.** The analysis of consumer evaluation was conducted using the hedonic test, as described by Suryono et al (2018). This process used 80 untrained panelists, who lived near the Universitas Gadjah Mada campus, with an age between 16 and 54 years, with work backgrounds as students (elementary, high, and university), housewives, entrepreneurs, casual daily laborers, and civil servants. The steps of tea preparation included soaking one tea bag in 100 mL of hot water for 6 min. During the brewing process, these bags were repeatedly lifted and dropped (5 times) into hot water and shaken 2-3 times before being discarded from the solution (Seng et al 2017). Approximately 25 mL of *S. hystrix* tea solution was placed in the cup, and each sample was further coded. The panelists were asked to taste the samples and to assess the appearance, color, flavor, and taste on a 5 point scale (5, 4, 3, 2, 1 = like it very much, like it, somewhat like it, dislike it, dislike it very much) (Putri & Mardesci, 2018).

Data analysis. Data collected were used to calculate the mean ± standard deviation (n = 3), using the Statistical Package for Social Sciences (SPSS) and Microsoft Excel. Furthermore, Kolmogorov-Smirnov (to assess normality) and real difference tests were conducted, using the method of multiple comparisons.

Results and Discussion

The water content of Sargassum hystrix seaweed tea. The effect of the immersion time of lime solution (pH of 5) immersion time on the water content of *S. hystrix* tea is presented in Figure 1. Furthermore, the moisturization of the seaweed (*S. hystrix*) and tea utilized was 78.78% and 2.86 ± 0.61 ~ 3.21 ± 0.67%, respectively. This indicated that the immersion time of *S. hystrix* in a lime solution of pH 5 had no significant effect ($p > 0.05$) on the water content of the tea. The result of this seaweed beverage was in line with the Indonesian National Standard (SNI) for dry packaged tea, with a maximum value of 8%

(National Standardization Agency 2013). In addition, the water content level of the *S. hystrix* tea was quite similar, compared to that of *Sargassum sp*. (2.14±0.21%), which was immersed in boiling water for 5 min (Sinurat & Suryaningrum 2019). However, this level was lower than the water content (9.67%) of *S. filipendula* tea (Supirman et al 2012). Water content is a factor that is very important in the shelf life and deterioration of food products, and the moisturization of dry materials should be less than 10%, in order to protect enzymatic processes and the developments of microorganisms (Sinurat & Suryaningrum 2019).

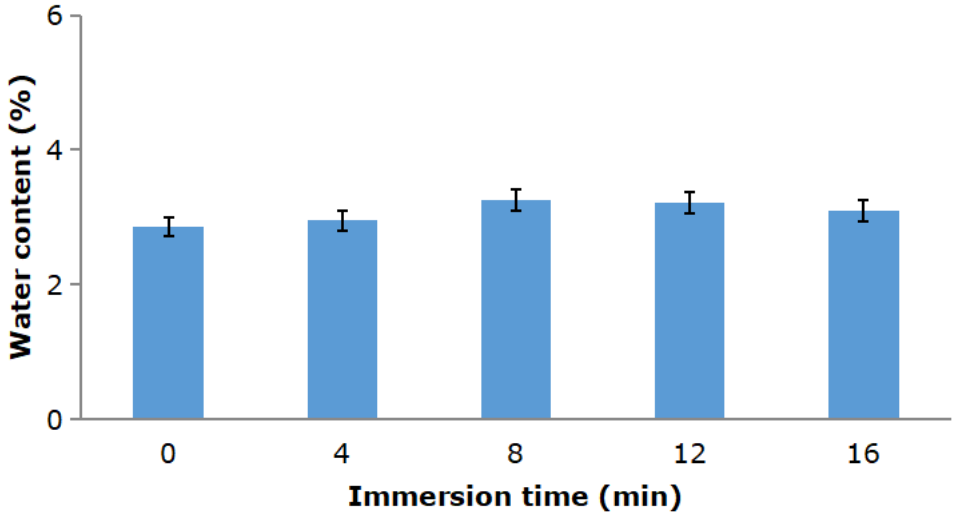

Figure 1. The effect of immersion time in a lime solution pH 5 on the water content of *S. hystrix* seaweed tea.

Total phenol content. The effect of lime solution (pH 5) immersion time on the total phenol content of *S. hystrix* tea is presented in Figure 2. Based on the results, the total content of this beverage at 0, 4, 8, 12, and 16 min was 16.01 ± 0.17, 26.45 ± 0.56, 37.10 ± 1.09, 56.05 ± 1.16, 76.81 ± 0.23, 10.56 ± 0.71, and 56.49 ± 0.66 mg GAE g^{-1}, respectively. This indicated that immersion time had a significant effect ($p < 0.05$) on the increase in the total phenol level of *S. hystrix* tea. Furthermore, Pujimulyani et al (2010), reported that immersion increased the overall contents, by using an acid solution at 100°C. In addition, immersion minimized the damage of polyphenol compounds by using hot water, due to increasing the phenolic levels in the products (Nurhayati et al 2018).

Sinurat & Suryaningrum (2019), also reported that the total phenol content of *Sargassum sp.* tea immersed in hot water for 0 ~ 5 min, ranged from 1.80 ~ 2.22 mg GAE g^{-1}. Other studies showed that the overall level of *S. filipendula* soaked in a lime solution of pH 5 for 6 h was 6.48 ± 0.03 mg GAE g^{-1} (Supirman et al 2012). This indicated that the phenolic content of *S. hystrix* tea was higher than that of the *Sargassum sp.* and *S. filipendula* beverages. These results occurred due to the effect of temperature and time when immersing and roasting the seaweed tea. Dewata et al (2017) reported that the use of high temperature had a greater effect on the total phenol content, due to increased release of phenolic compounds in cell walls. However, the delay of immersion time caused a decrease in phenol levels, due to damage to phenolic compounds in cell components (Ibrahim et al 2015). Additionally, immersion of tea for a very short period caused improper dissolution, due to lower levels of phenol (Tambun et al 2016). Furthermore, the optimal temperatures for the immersion of seaweed tea were 0 ~ 90°C (Putri et al 2014).

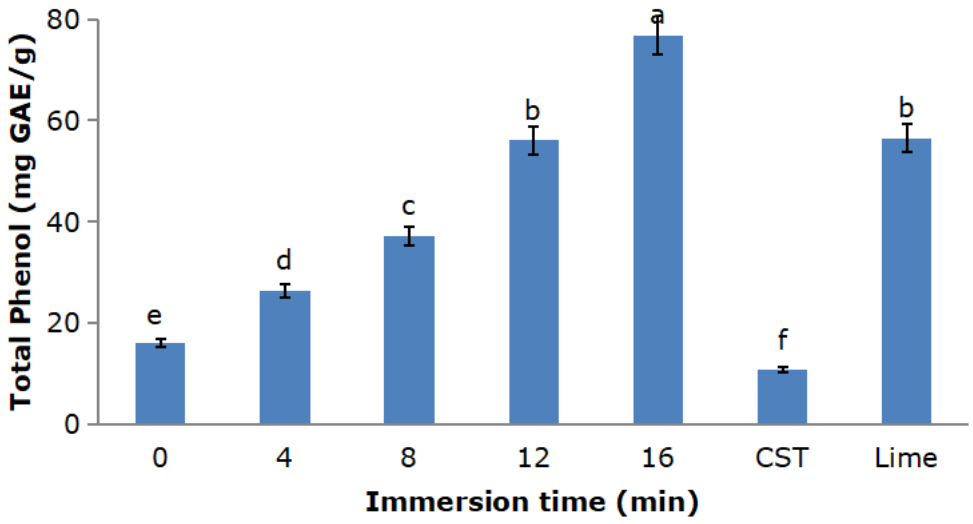

Figure 2. The effect of immersion time in lime solution pH 5 on the total phenol content of *S. hystrix* seaweed tea. Note: CST = commercial seaweed tea. Bars with different letters indicate a significant difference between groups ($p<0.05$).

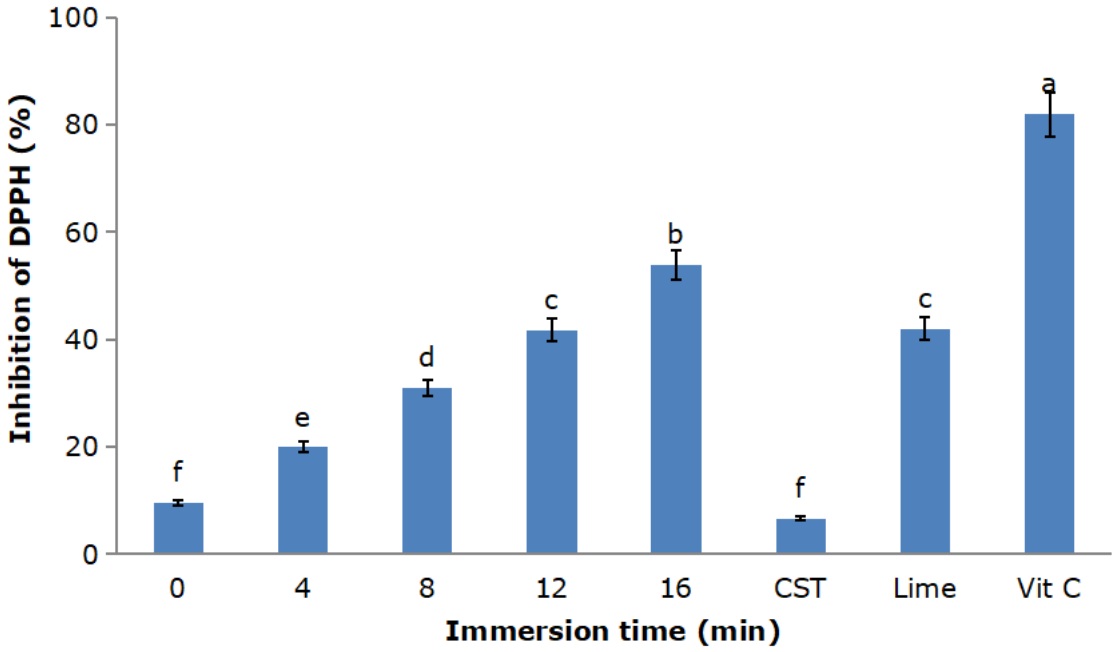

Figure 3. Effect of immersion time in lime solution pH 5 on inhibition activity of DPPH of *S. hystrix* seaweed tea. Note: CST = commercial seaweed tea. Bars with different letters indicate significant differences between groups ($p<0.05$).

DPPH radical scavenging activity. The DPPH inhibition activity values of *S. hystrix* tea at immersion periods of 0, 4, 8, 12, and 16 min were 9.64 ± 1.36, 19.93 ± 1.57, 30.99 ± 1.68, 41.72 ± 1.02, and 53.86 ± 1.27%, respectively. This indicated that immersion time had a significant effect ($p<0.05$) on antioxidant property. Furthermore, the DPPH activity of *S. hystrix* tea was higher and lower than that of *Sargassum sp*. (4.00 ± 1.34 ~ 18.00 ±

2.01%) and *S. filipendula* (64.13 ± 0.40%) beverages, respectively (Sinurat & Suryaningrum, 2019; Supirman et al 2012). This was due to the longer immersion time of *S. filipendula* tea, at 6 h.

Based on Figure 3, the antioxidant property of *S. hysfrix* tea was higher. Generally, the heating and immersion treatments decreased and increased the antioxidant capacity of products, respectively (Halvorsen et al 2006). Furthermore, Sinurat & Suryaningrum (2019), reported that the heating process by immersion increased the antioxidant capacity. Pujimulyani et al (2010) also reported that hot water immersion had the capability to release antioxidant compounds from within cells, in order to increase this capacity.

Ferric reduction antioxidant power (FRAP). The FRAP values of *S. hystrix* tea at immersion periods of 0, 4, 8, 12, and 16 min were 168.79 ± 1.39, 157.58 ± 1.39, 146.67 ± 1.39, 137.27 ± 0.91, and 121.82 ± 0.91 μM g^{-1}, respectively. Furthermore, the highest and lowest antioxidant capacities were found in 16 (121.82 ± 0.91 μM g^{-1}) and 0 (168.79 ± 1.39 μM g^{-1}) min, respectively. The capacity was higher when the FRAP value was lower, due to the decreased sample concentration needed to reach the absorbance produced by the $FeSO_4$ standard solution (Clarke et al 2013). The antioxidant property (FRAP value) of the results of this study (121.82 ± 0.91 M/g) was higher compared to the antioxidant property of the new sago baruk (269.96 mol/100 g) as reported by Momuat & Suryanto (2016). This is because the soak time for *S. hystrix* seaweed tea (16 minutes) is shorter than the new pith sample (24 hours). An immersion process that is too long can reduce antioxidant property (Wicaksono & Zubaidah 2014).

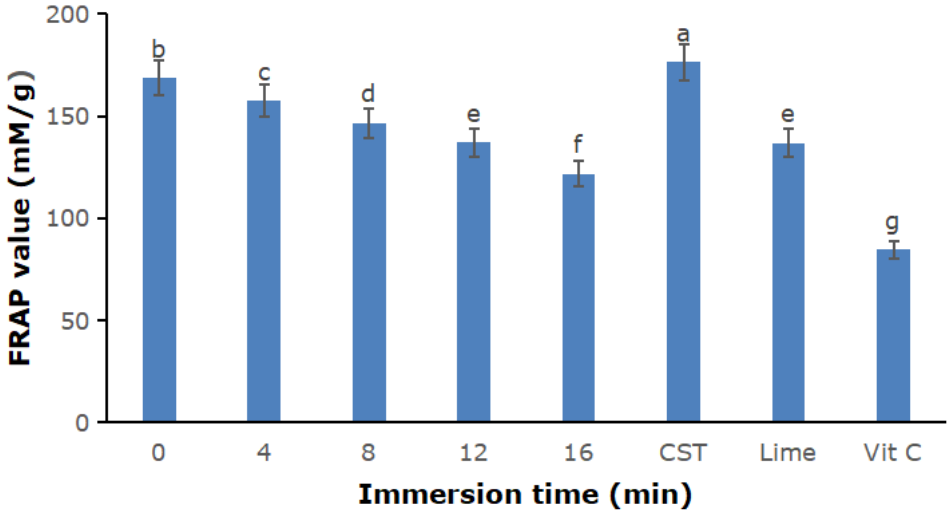

Figure 4. The effect of immersion time in lime solution pH 5 on the FRAP value of seaweed tea from *S. hystrix*. Note: CST = commercial seaweed tea. Bars with different letters indicate significant differences between groups ($p<0.05$).

The antioxidant capacity of *S. hystrix* tea was further enhanced by increasing the immersion time. This indicated that immersion in lime solution pH 5 at 85°C elevated the capacity of this seaweed beverage. The increase was assumed to happen due to changes in seaweed compounds, such as inactive to active flavonoids (Pujimulyani et al 2010). Furthermore, the immersion process under acidic conditions led to flavonoid compounds in the form of glycosides, which were degraded to aglycones and sugars, therefore increasing antioxidant capacity (Perdana et al 2018). The higher polyphenol content of the flavonoid

group also helped the OH classes donate H^+ atoms, therefore, increasing this capacity (Supriyanto et al 2014).

Inhibition activity of α-glucosidase. The α-glucosidase activity values of *S. hystrix* tea for immersion periods of 0, 4, 8, 12, and 16 min, were 12.67 ± 1.69, 22.66 ± 1.66, 33.55 ± 0.83, 43.70 ± 1.10, and 52.86 ± 0.89%, respectively. This indicated that immersion time had a significant effect ($p<0.05$) on the activity of α-glucosidase. The highest and lowest activities were found at 16 (52.86 ± 0.89%) and 0 (12.67 ± 1.69%) min, respectively. This was due to flavonoids being one of the main compounds in lime (Prastiwi & Ferdiansyah 2017). Flavonoids are α-glucosidase inhibitors in carbohydrates breakdown, before being absorbed as monosaccharides (Permatasari et al 2019). Furthermore, Serang & Bani (2017) reported that lime contained phenolic substances, such as flavonoids, glycosides, tannins, and saponins, which affected the inhibition activity of α-glucosidase. The α-glucosidase inhibitory activity of this study (52.86 ± 0.89%) was lower than that of *S. hystrix* seaweed tea (83.98 ± 2.37%) substituted with 5% cinnamon (Setiyawan 2020). This is because cinnamon contains phenolic or polyphenolic compounds such as flavonoids, glycosides, tannins and saponins that have an effect on α-glucosidase inhibitory activity (Apriani 2012).

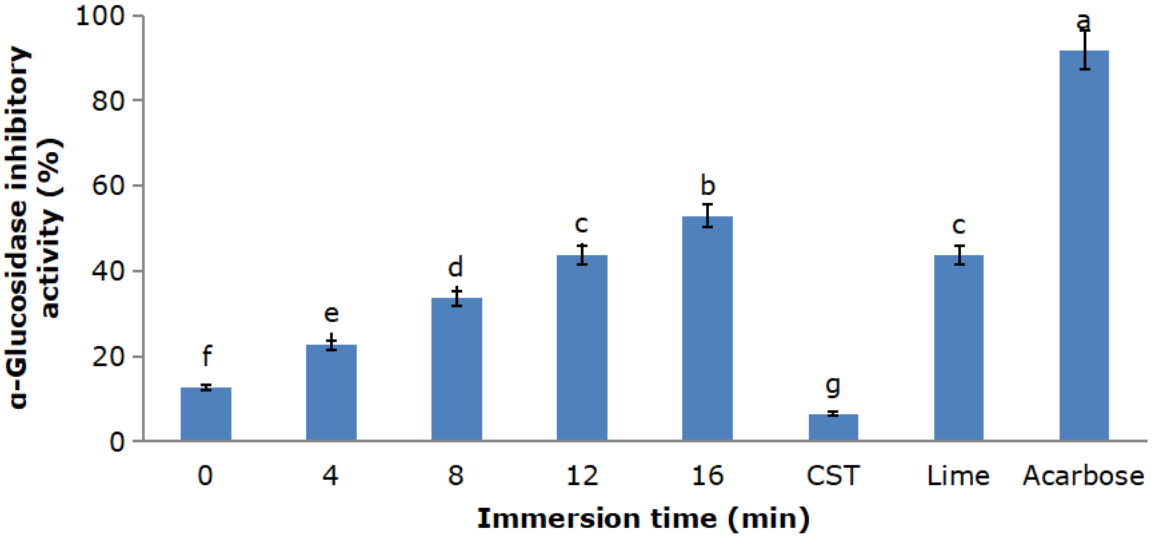

Figure 5. Effect of immersion time in lime solution pH 5 on inhibition activity of α-glucosidase of seaweed tea from *S. hystrix*. Note: CST = commercial seaweed tea. Bars with different letters indicate significant differences between groups ($p<0.05$).

This antioxidant activity was lower compared to acarbose, which is a popular antidiabetic drug often used as α-glucosidase inhibitors. Acarbose worked competitively to inhibit α-glucosidase actions within cells, to decrease glucose absorption and postprandial hyperglycemia. In diabetic patients, it decreases postprandial hyperglycemia and HbA1C by 30-50% and 0.5-1%, respectively (Dinicolantonio et al 2015). Furthermore, acarbose was able to completely inhibit the hydrolysis of carbohydrates with α-glucosidase, in order to increase repression and protect higher blood sugar levels. However, this drug reportedly had various gastrointestinal side effects, such as induced diarrhea and flatulence (Rosak & Mertes 2012).

***Consumer evaluation of Sargassum hystrix seaweed tea*.** Consumer evaluations of *S. hystrix* tea in terms of appearance, color, flavor and taste, were obtained using the hedonic test. Darmawan et al (2014) reported that appearance was a major factor that often affected product quality perception. This was because consumers preferred products

with attractive appearances. Furthermore, the value of consumers' evaluation on the appearance of this seaweed tea was 2.35 ± 1.43 to 4.39 ± 0.91, with the highest and lowest levels observed in the 16 (4.39, "like it" and "like it very much") and 0 (2.35, "somewhat like it" and "like it") min of immersion treatments. Sinurat & Suryaningrum (2019), reported appearance evaluations for *Sargassum sp.* of 4.7 points ("like it" and "like it very much"), for immersion in boiling water during 1 min. Luthfiyana et al (2016) also explained that a good appearance increased the evaluations of other parameters, such as color, flavor, and taste.

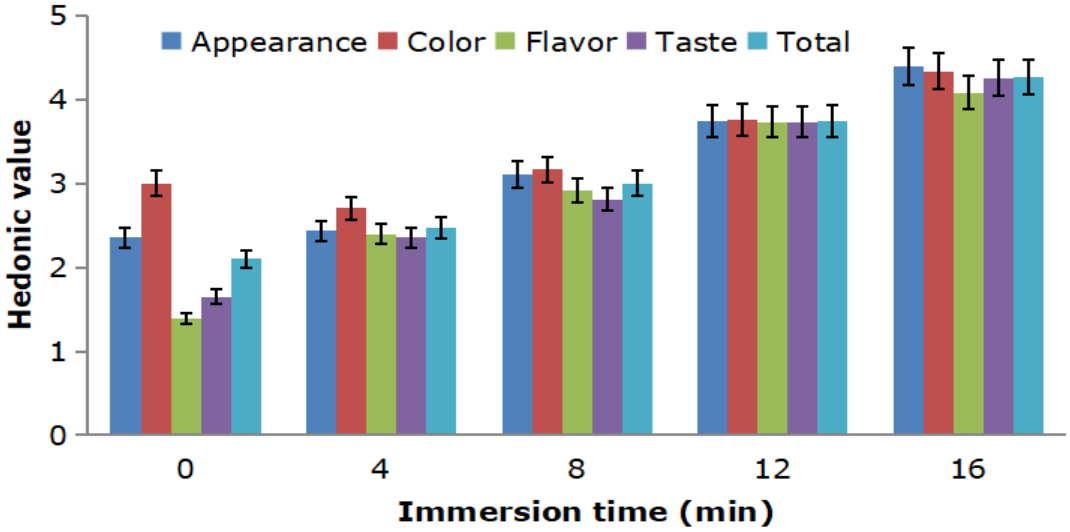

Figure 6. The effect of immersion time in lime solution pH 5 on the evaluation of *S. hystrix* seaweed tea characteristics.

According to Apandi et al (2016), consumer acceptance level of food products was often altered by product color. The evaluations of *S. hystrix* tea color were between 2.70 ± 0.60 and 4.33 ± 0.78, with the highest and lowest levels observed in 16 (4.33, "like it" and "like it very much") and 4 (2.70, "dislike it" and "somewhat like it") min of immersion treatment. These values were higher and lower, respectively, than those of *S. filipendula* (4.10) and *Sargassum sp*. (4.65) beverages, which were immersed in lime solution pH 5 and boiling water for 6 h and 1 min, respectively (Supirman et al 2012, Sinurat & Suryaningrum, 2019). These phenomena occurred due to differences in the treatment and samples used in each study.

Consumers evaluated the *S. hystrix* tea flavor with values between 1.39 ± 0.65 and 4.08 ± 1.23, with the highest level observed at 16 min (4.08, "like it" and "like it very much") of immersion treatment. These evaluations were higher than those of *S. filipendula* (2.50) and *Sargassum sp*. (3.71) beverages, which were immersed in lime solution pH 5 and boiling water for 6 h and 1 min, respectively (Supirman et al 2012, Sinurat & Suryaningrum 2019).

The effect of immersion on the taste consumer preference of *S. hystrix* seaweed tea is presented in Figure 6. The taste of the seaweed tea was evaluated with 1.65 ± 1.01 to 4.25 ± 1.08 points. This indicated that immersion time had a significant effect ($p<0.05$) on the taste evaluation of *S. hystrix* tea. The longer the time, the stronger the lime taste of the beverage. In addition, the hot water immersion process induced a change in the main polymers of seaweed, based on becoming oligomeric compounds which consumers

preferred (Sinurat & Suryaningrum 2019). The taste of seaweed tea was also induced by tannins in *Sargassum sp*. Meanwhile, the hot lime solution immersion process decreased the tart taste of *S. hystrix* tea, as the highest value of consumer preference was obtained at 16 min (4.25, like and very like). This value was higher compared to *S. filipendula* and lower compared to *Sargassum sp.* beverages, at 3.30 and 4.65 points, respectively.

Consumers also evaluated the overall quality of *S. hystrix* tea (Fig. 6) and the values were 2.10 ± 0.73 ~ 4,26 ± 0.13. The highest and lowest evaluations were observed at 16 and 0 min of immersion treatment. This indicated that time significantly affected consumer evaluations. The longer the immersion period, the higher the consumers' overall assessment of *S. hystrix* tea.

Conclusions. The lime solution immersion time affected the antioxidant and antidiabetic activities, as well as the consumer evaluation of *S. hystrix* tea. This study indicated that immersion of seaweed in lime solution for 16 min produced the best characteristics of the *S. hystrix* beverage, including the water content, total phenolic composition, DPPH, FRAP, and α-glucosidase activities, at 3.10 ± 0.33%, 76.81 ± 0.23 mg GAE g^{-1}, 53.86 ± 1.27%, 121.82 ± 0.91 μM g^{-1}, and 52.86 ± 0.89%, respectively. It also showed that consumers' evaluations for appearance, color, flavor, taste, and overall quality were 4.39 ± 0.91, 4.33 ± 0.78, 4.08 ± 1.23, 4.25 ± 1.08, and 4.26 ± 0.13 points. Therefore, immersion treatment in lime solution improved the quality and consumer evaluation of *S. hystrix* tea.

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Authors:
Damayanti Rahmatika Pratiwi, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Bulaksumur, Yogyakarta 55281, Indonesia, e-mail: damayantirp@mail.ugm.ac.id
Amir Husni, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Bulaksumur, Yogyakarta 55281, Indonesia, e-mail: a-husni@ugm.ac.id

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