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Soaking Time in Lime Solution Increases the Antioxidant Activity, Antidiabetic Activity, and Consumer Acceptance Level of *Sargassum Polycystum* Seaweed Tea

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Abstract: The brown seaweed *Sargassum polycystum* contains bioactive compounds, including antioxidants and antidiabetes, letting it be a functional food ingredient such as seaweed tea. However, seaweed tea is less preferred by consumers because of its fishy smell, which must be reduced using various methods, including soaking in a lime solution. This study aims to determine the effect of the soaking time in lime solution on the antioxidant activity, antidiabetic activity, and consumer acceptance of *S. polycystum* seaweed tea. In this study, the seaweed was soaked in a lime solution at pH 5 at 85°C for 0, 4, 8, 12, and 16 minutes. Analysis was conducted on *S. polycystum* seaweed tea to obtain the moisture content, total phenol, antioxidant activity (inhibition of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reduction antioxidant power (FRAP)), the inhibitory activity of α-glucosidase, and consumer preference test using hedonic. The results show that the soaking time in the lime solution at pH 5 had no effect on the water content but had a significant effect ($p<0.05$) on the total phenol, antioxidant and antidiabetic activities and consumer preference of *S. polycystum* seaweed tea. The best treatment was obtained at 16 minutes of soaking time with a water content of 4.68 ± 0.12%, a total phenol content of 74.53 ± 0.59 mg GAE/g, a DPPH inhibitory activity of 52.70 ± 0.86%, a FRAP value of 123.94 ± 1.39 μM/g, an α-glucosidase inhibitory activity 51.56 ± 0.70%, and consumer preference levels for appearance, color, flavor, taste and overall of 4.63 ± 0.74, 4.49 ± 0.75, 4.39 ± 1.07, 4.43 ± 1.03, and 4.49 ± 0.11, respectively.

Keywords: lime, *Sargassum polycystum*, seaweed tea, soaking.

在石灰溶液中浸泡时间可提高马尾藻多囊海藻茶的抗氧化活性、抗糖尿病活性和消费者接受度

摘要：棕色海藻马尾藻多囊含有生物活性化合物，包括抗氧化剂和抗糖尿病剂，使其成为海藻茶等功能性食品成分。然而，消费者不太喜欢海藻茶，因为它有鱼腥味，必须使用各种方法来减少这种气味，包括浸泡在石灰溶液中。本研究旨在确定在石灰溶液中的浸泡时间对多囊海藻茶的抗氧化活性、抗糖尿病活性和消费者接受度的影响。在这项研究中，海藻在85°C、酸碱度值为5的石灰溶液中浸泡0、4、8、12和16分钟。对多囊海藻茶进行水分含量、总酚、抗氧化活性（1,1-二苯基-2-苦基肼的抑制和铁还原抗氧化能力的抑制）、α-葡萄糖苷酶，以及使用享乐的消费者偏好测试。结果表明，在氢的潜力值为5的石灰溶液中浸泡时间对含水量没有影响，但对多囊海藻茶的总酚、抗氧化和抗糖尿病活性以及消费者偏好有显着影响（磷值<0.05）。最佳处理为浸泡时间16分钟，含水量为4.68±0.12%，总酚含量为74.53±0.59毫克每克没食子酸当量，二苯基-2-苦基肼抑制活性为52.70±0.86%，铁还原抗氧化能力值为123.94±1.39微米/克，α-葡萄糖苷酶抑制活性51.56 ± 0.70%，消费者对外观、颜色、风味、味道和总体的偏好水平为4.63 ± 0.74、4.49 ± 0.75、4.39 ± 1.07、4.43、± 4.03分别为 0.11。

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关键词：生石灰、海藻多囊、紫菜茶，浸泡。

1. Introduction

Seaweed is a very abundant biological resource, including brown seaweed. One of the abundant brown seaweed species in the world is *the Sargassum polycystum.* Many studies show that *S. polycystum* has bioactivity, including antioxidant [1], antibacterial [2], anti-inflammatory [3], antistress [4], anticoagulant [5], antidiabetic [6], and anticancer activities [7]. Thus, *S. polycystum* has a high potential to be used as a food and industrial ingredient.

The use of seaweed in the industrial sector includes the food, beverage, medicine, cosmetics, paper, detergent, paint, and textile industries [8]. One of the uses of seaweed in the beverage industry is the manufacture of seaweed tea. Seaweed tea is a health drink that contains nutrients needed by the body, including antioxidants [9]. However, seaweed tea has limitations: the flavor and taste of seaweed tea are generally not preferred by consumers because of the fishy smell. One alternative to reduce the fishy smell is soaking in boiling water [10]. However, consumer preference remained low, so it was necessary to add other natural ingredients, such as lime solution. Soaking in lime solution at pH 5 for 6 hours could increase *Sargassum filipendula* seaweed tea [9].

Additionally, a soaking time that is too long can reduce the antioxidant activity of a food product [11]. Until now, research on the manufacture of seaweed tea from *S. polycystum* is still very rare. Therefore, this study aims to determine the effect of the soaking time in lime solution on the antioxidant activities, antidiabetic activities, and consumer acceptance of *S. polycystum* seaweed tea.

2. Material and Methods

2.1. Materials

The materials in this research are brown seaweed *S. polycystum* obtained from Sepanjang Beach, Gunungkidul Yogyakarta. The commercial seaweed tea for comparison was obtained from SMEs in Bali. Lime (*Citrus aurantifolia*) was obtained from the Kranggan Market Yogyakarta. Other materials were Folin Ciocalteu reagent (produced by Merck, USA), Na2CO3 (produced by Merck, USA), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (produced by Merck, USA), ethanol (produced by Merck, USA), gallic acid (produced by Merck, USA), K2SO4 (produced by Merck, USA), 2,4,6-tri (2-pyridyl)–s triazine (TPTZ) (produced by Merck, USA), FeCl3 (produced by Merck, USA), FeSO4 (produced by Merck, USA), vitamins C (produced by Merck, USA), acarbose (produced by Sigma-Aldrich, Germany), α-glucosidase from *Saccharomyces cerevisiae* (produced by Sigma-Aldrich, Germany), and p-nitrophenyl-α-D-glucopyranoside (PNPG) (produced by Sigma Aldrich).

2.2. Preparation of Seaweed *Sargassum Polycystum*

Seaweed samples of *S. polycystum* were taken from Sepanjang Beach, Gunungkidul Yogyakarta, in August 2020. Seaweed was collected by cutting the lower thallus near holdfast using scissors. Then, the samples were washed and separated for morphological identification. The other samples were stored in an icebox to be brought to the laboratory and stored in the refrigerator.

2.3. Manufacture of *Sargassum Polycystum* Seaweed Tea

The manufacture of seaweed tea refers to the research of Sinurat & Suryaningrum [10] with several modifications. 200 g of seaweed *S. polycystum* was washed using clean water and subsequently soaked in 2000 mL of pH 5 lime solution. The pH 5 lime solution was prepared by mixing 2000 mL of water at 85°C and approximately 6.4 mL of lime water. The initial temperature of the water was 85°C, and the temperature decreased during the soaking process. The variations in soaking time were 0 (without soaking), 4, 8, 12, and 16 minutes. After soaking, the seaweed was drained and dried for 24 hours in a baking sheet placed on a terrace and not exposed to direct sunlight. Then, the seaweed was roasted in a frying pan for 15-20 minutes and cut with a knife into pieces 0.5 ± 0.1 cm in size. Before use, seaweed tea was placed in a sealed standing pouch and stored at 4°C.

2.4. Serving of *Sargassum Polycystum* Seaweed Tea

As much as 1 g of seaweed tea was put in a teabag. Brewing was done by placing 1 tea bag in 100 mL of boiling water for 6 minutes. During the brewing process, the teabags were raised and lowered into boiling water 5 times and stirred 2-3 times; then, the teabags were removed from the solution [12]. The seaweed tea solution was used for the total phenol analysis, antioxidant activity analysis, α-glucosidase inhibition test, and consumer preference tests.

2.5. Analysis of the Water Content

The water content was analyzed using a moisture analyzer Ohaus MB120. A sample of 0.5 g was placed on the plate in the moisture content analyzer; then, the appliance was closed. Next, the cells were incubated for 2-20 minutes until the drying process was complete. The displayed results on the moisture content analyzer screen were recorded.

2.6. Total Phenol Analysis

The total phenol analysis refers to the research of Sinurat & Suryaningrum [10]. In total, 1 mL of tea solution was put into a Falcon tube, and 1 mL of 96% ethanol, 5 mL of distilled water, and 0.5 mL of 50% Folin Ciocalteu reagent were added. The mixture was allowed to stand for 5 minutes, and 1 mL of 5% Na2CO3 was subsequently added. The mixture was homogenized with vortexing and incubated in the dark for 1 hour. Standard solutions were prepared by making gallic acid solutions with 0, 20, 40, 60, 80, and 100 ppm concentrations. Then, 1 mL of gallic acid solution was taken for each concentration and put into a Falcon tube, 1 mL of 96% ethanol, 5 mL of distilled water, and 0.5 mL of 50% Folin Ciocalteu reagent were added. The mixture was allowed to stand for 5 minutes, and 1 mL of 5% Na_2CO_3 was subsequently added. The mixture was homogenized with vortexing and incubated in the dark for 1 hour. The sample solution's absorbance and standard solution were measured using a UV-Vis spectrophotometer (Lamda 25, PerkinElmer) at a wavelength of 725 nm. The formula to calculate the total phenol content is as follows:

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

where:
x = concentration of the test solution (mg/mL)
v = volume of test solution (mL)
m = mass of the test solution (g)

2.7. DPPH Free Radical Scavenging Assay

The scavenging ability against DPPH free radicals was estimated to evaluate the antioxidant activity of seaweed tea using the modified method of Muthia et al. [13]. The DPPH solution was prepared by dissolving 0.39 mg DPPH powder into 100 mL ethanol. Then, the solution mixture was incubated for 30 minutes at 4°C. Tea samples were prepared by dissolving 1 g of seaweed tea for each treatment and commercial seaweed tea in 100 mL of water. Then, 0.1 mL of the tea sample was placed in a Falcon tube wrapped in aluminum foil, and 1 mL of ethanol and 0.7 mL of DPPH solution were added. A vitamin C sample was prepared by dissolving 1 g of vitamin C in 100 mL of water. Then, 0.1 mL of vitamin C solution was taken and put into a Falcon tube wrapped in aluminum foil, and 1 mL of ethanol and 0.7 ml of DPPH solution were added. The lime solution was prepared by making a solution of lime pH 5 at 85°C. A volume of 0.1 mL of the lime solution at pH 5 was placed in a Falcon tube wrapped in aluminum foil, and 1 mL of ethanol and 0.7 ml of DPPH solution were added. The blanks were 0.7 ml of DPPH and 1 ml of ethanol. The solution mixture (tea sample, vitamin C, lime solution, and blank) was homogenized with vortexing and incubated for 15 minutes at room temperature. The absorbance was measured at a wavelength of 515 nm using a UV-Vis spectrophotometer. The percent inhibition was calculated using the following formula:

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

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); and D = blank (200 µl of aqua dest).

2.8. Ferric Reduction Antioxidant Power (FRAP) Assay

This assessment required the method described by Suhaila et al. [14], where Fe^{3+} was reduced to Fe^{2+}. A spectrophotometer was used to measure the iron(III) chloride modified into Fe^{2+} complexes at 595 nm wavelength. These changes were observed with a solution color transformation to blue. In addition, an acetate buffer solution with pH 3.6 was formulated by adding 0.775 g of sodium acetate trihydrate ($CH_3COON.3H_2O$) to 4 mL of concentrated acetic acid, followed by dissolution with distilled water to obtain an exact volume of 250 mL. The yield was stored at 4°C as a stock solution. Subsequently, 10 mM/mL 2,4,6-tripyridil-s-triazine (TPTZ) solution was formulated by dissolving 0.15 g of TPTZ in 40 mM/L HCl to achieve an exact volume of 50 mL. In contrast, the 40 mM/L HCl solution was prepared by dissolving 0.828 mL concentrated HCl in 250 mL distilled water. Therefore, the generated TPTZ solution was reserved at 4°C for use within 24 hours. Additionally, 0.54 g $FeCl_3.6H_2O$ was dissolved in distilled water and made approximately 100 mL to produce a 20-mM/L $FeCl_3.6H_2O$ solution, which was stored for up to 24 hours at 4°C. The FRAP reagent was formulated by combining 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3.6H_2O$ solution (10:1:1) before making approximately 100 mL with distilled water. Furthermore, standard $FeSO_4.7H_2O$ (10,000 µM/L) solutions were produced by dissolving 2.78 g $FeSO_4.7H_2O$ in 1000 mL distilled water before serial dilution to optimal concentrations of 50, 100, 150, 200, 250, and 300 ppm. The *S. polycystum* seaweed tea solution, commercial seaweed tea, and vitamin C were prepared by dissolving 1 g of each ingredient in 100 mL of distilled water. The pH 5 lime solution was made by adding lime juice to distilled water at 85°C to the desired pH. In total, 900 µL of FRAP reagent was mixed with 120 µL of each sample solution. Then, the solution mixture was vortexed and incubated for 15 minutes. The absorbance of the solution was measured at a wavelength of 595 nm. Then, the absorbance data were processed using Microsoft Excel. The standard solution of $FeSO_4.7H_2O$ was used as a standard curve by making a line equation for the absorbance value of the $FeSO_4.7H_2O$ solution. The absorbance data of the sample were input into the equation of the line to obtain the FRAP value in µM/g.

2.9. Inhibition of the α-Glucosidase Activity

An inhibition test of α-glucosidase was performed using the method described by Azizi et al. [15]. Acarbose, an antidiabetic drug, and lime solution were used as the standards. The test consists of 50 mL of 0.1 M phosphate buffer (KH_2PO_4) pH 7, 25 mL of 0.5 mM p-nitrophenyl-α-D-glucopyranoside (PNP-G, as the substrate), 10 mL of the sample extract (1 g/100 mL) or standard (1 g/100 mL), and 0.2 U/mL α-glucosidase of 25 mL. The sample was also mixed and incubated at 37°C for approximately 30 min, and the reaction was stopped using 100 mL of 0.2 M Na_2CO_3. The p-nitrophenol that was formed using a microplate reader was used to determine the enzyme activity, which was inhibited at a wavelength of 405 nm. Furthermore, the absorbance values were used to analyze the percentage inhibition of the enzyme.

Percentage inhibition = [(K−(S1−S0))/K] x100%

where K, S1, and S0 are the absorbance of the control blank, a sample with enzyme and a sample without enzyme.

2.10. Analysis of the Consumer Acceptance

The level of consumer acceptance was analyzed using the hedonic test based on Suryono et al. [16]. This analysis used 80 untrained panelists. The steps were as follows: brew a sample of *S. polycystum* seaweed tea for each treatment by soaking 1 bag (1 gram) of the sample in 100 mL of boiling water for 6 minutes while raising and lowering 5 times and stirring 2-3 times; then, remove the teabag from the solution. In total, 25 mL of *S. polycystum* seaweed tea solution was put into the cup. Each sample was coded, and the panelists were asked to provide a sample response by directly tasting the tested sample and giving an assessment (score 1-5) of the sample, including the parameters of appearance, color, flavor, and taste [17]. The level of consumer acceptance of *S. polycystum* seaweed tea was obtained from the score given by the panelists with the following values: 5 = very like, 4 = like, 3 = somewhat like, 2 = dislike, 1 = very dislike.

2.11. Statistical Analysis

The analysis phase was conducted using 3 replications (n = 3). Data analysis was performed using SPSS (developed by SPSS Inc., USA). Data analysis included a normality test using the one-sample Kolmogorov-Smirnov test followed by a real difference test using multiple comparisons.

3. Results and Discussion

3.1. Water Content of *Sargassum Polycystum* Seaweed Tea

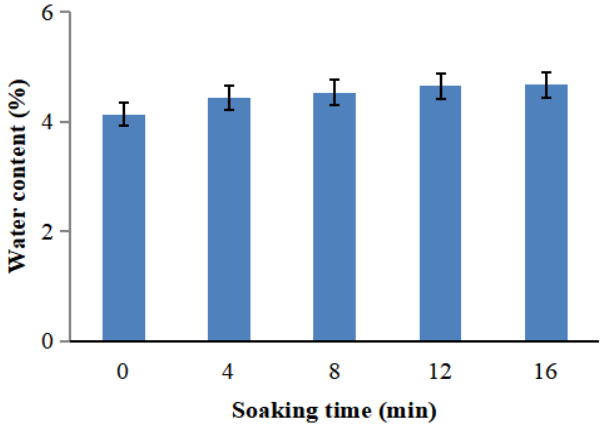

Fig. 1 Effect of the soaking time in lime solution at pH 5 on the water content of *S. polycystum* seaweed tea

The effect of the soaking time in lime solution at pH 5 on the water content of *S. polycystum* seaweed tea is shown in Fig. 1. The water content of *S. polycystum* seaweed as raw material was 92.96%. After the seaweed tea was made by soaking in the pH 5 lime solution for 0 ~ 16 minutes, the water content of *S. polycystum* seaweed tea was 4.14 ± 0.11 ~ 4.68 ± 0.12%. The analysis indicates that the soaking time in lime solution at pH 5 had no significant effect ($p>0.05$) on the water content of seaweed tea. The water content of *S. polycystum* seaweed tea was consistent with the Indonesian National Standard (SNI) for dry packaged tea with a maximum water content of 8% [18]. Sinurat & Suryaningrum [10] reported that *Sargassum sp.* soaked in boiling water for 0 ~ 5 minutes were 2 ~ 6%, while Supirman et al. [9] reported that the water content of *S. filipendula* seaweed tea was 9.67%. The water content plays a major role in the deterioration and shelf life of food products. The moisture content of dry ingredients should be below 10% to prevent enzymatic processes and the growth of microbes [10]. The reason is that dry materials are generally stored for a long time; if an enzymatic process occurs, it will change its stored chemical composition, so the effects of the active compounds therein can also change.

3.2. Total Phenol Content

The total phenol content of *S. polycystum* seaweed tea is shown in Fig 2. *S. polycystum* seaweed tea that was soaked for 0, 4, 8, 12, and 16 minutes had total phenol contents of 15.26 ± 0, 38.25.47 ± 0.59, 35.54 ± 0.58, 55.75 ± 0.75, and 74.53 ± 0.59 mg GAE/g, respectively. The soaking time had a significant effect ($p<0.05$) on the increase in total phenol content of seaweed tea because soaking with an acid solution at 100°C can increase the total phenol content [19]. Soaking using hot water can minimize the damage to polyphenol compounds to increase the total phenol in ingredients [20].

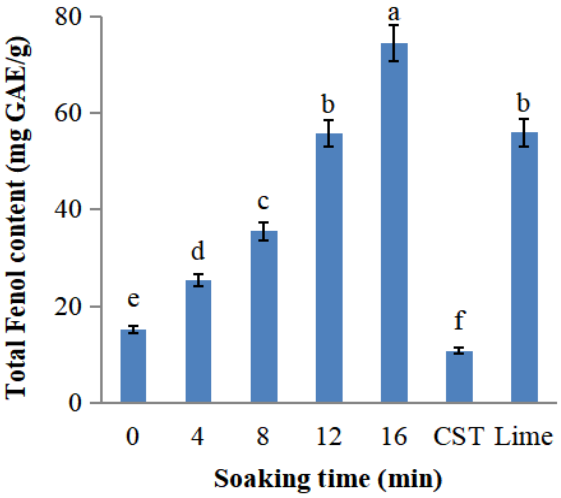

Fig. 2 Effect of the soaking time in lime solution at pH 5 on the total phenol content of *S. polycystum* seaweed tea. Note: CST = commercial seaweed tea

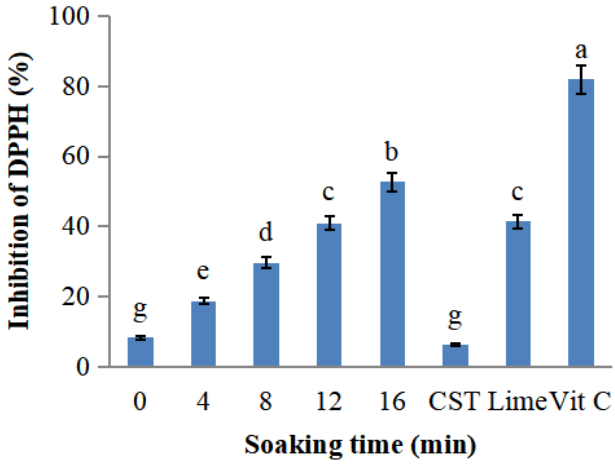

Fig. 3 Effect of the soaking time in lime solution at pH 5 on the DPPH inhibitory activity of *S. polycystum* seaweed tea. Note: CST = commercial seaweed tea

Supirman et al. [9] reported that the total phenol content of *S. filipendula* seaweed tea soaked in lime solution at pH 5 for 6 hours was 6.48 ± 0.03 mg GAE/g. Meanwhile, the total phenol content of seaweed tea from *Sargassum sp*. that was soaked in hot water for 0, 1, 3, and 5 minutes was 1.80 ~ 2.22 mg GAE/g [10]. The *S. polycystum* seaweed tea had a higher total phenol content than *Sargassum sp*. and *S. filipendula* seaweed tea. This result can occur due to temperature and time when one soaks and roasts seaweed tea. According to Dewata et al. [21], high temperatures will increase the total phenol levels because high temperatures can increase the release of phenol compounds in the cell walls.

Additionally, a brewing time that is too long will decrease the total phenol levels because it can damage phenolic compounds in cell components [22]. Brewing tea for too short a time will make the compounds in the tea sample not properly dissolve, so the total phenol content decreases [23]. During brewing, phenol compounds are damaged at high temperatures, with an optimal temperature range of 0 ~ 90°C [22].

3.3. DPPH Radical Scavenging Activity

The antioxidant activity of *S. polycystum* seaweed tea using the DPPH method is shown in Fig. 3. The DPPH inhibitory activities of *S. polycystum* seaweed tea that was soaked for 0, 4, 8, 12, and 16 minutes were 8.35 ± 0.71, 18.88 ± 1.15, 29.75 ± 0.87, 40.99 ± 1.08, and 52.70 ± 0.86%, respectively. The data analysis results show that the soaking time significantly affected ($p<0.05$) the DPPH inhibitory activity. The *S. polycystum* seaweed tea had higher antioxidant activity than *Sargassum sp*. seaweed tea (4.00 ± 1.34 ~ 18.00 ± 2.01%) [10] but a lower antioxidant activity than *S. filipendula* seaweed tea (64.13 ± 0.40%) soaked in solution lime at pH 5 [9]. The reason is that the *S. filipendula* seaweed in the pH 5 lime solution was soaked for a longer time, i.e., for 6 hours.

Fig. 3 shows that a longer soaking time in the pH 5 lime solution corresponds to a higher antioxidant activity of *S. polycystum* seaweed tea. In general, the heating treatment of ingredients can reduce the antioxidant activity, but heating by soaking can increase the antioxidant activity [24]. According to Suri et al. [11], a longer soaking time can reduce antioxidant activity. Sinurat & Suryaningrum [10] reported that the heating process with the right time by soaking could increase the antioxidant activity. Boonkorn [19] explained that soaking in hot water could release antioxidant components from within the cells to increase their antioxidant activity.

3.4. Ferric Reduction Antioxidant Power (FRAP)

The effect of the soaking time in lime solution at pH 5 on the FRAP value of *S. polycystum* seaweed tea is shown in Fig. 4. The FRAP values of *S. polycystum* seaweed tea that was soaked for 0, 4, 8, 12, and 16 minutes were 170.91 ± 0.91, 159.70 ± 1.89, 149.09 ± 0.91, 138.79 ± 1.89, and 123.94 ± 1.39 µM/g, respectively. The highest antioxidant activity was found in *S. polycystum* seaweed tea that was soaked for 16 minutes (123.94 ± 1.39 µM/g), while the lowest antioxidant activity was found in unsoaked seaweed tea (170.91 ± 0.91 µM/g). The antioxidant activity is higher if the FRAP value is lower because a lower sample concentration is required to achieve the absorbance produced by the $FeSO_4$ standard solution to convert Fe^{3+} to Fe^{2+} [25]. Based on FRAP value, *S. polycystum* seaweed tea's antioxidant activity was higher than *S. muticum* fucoidan [26].

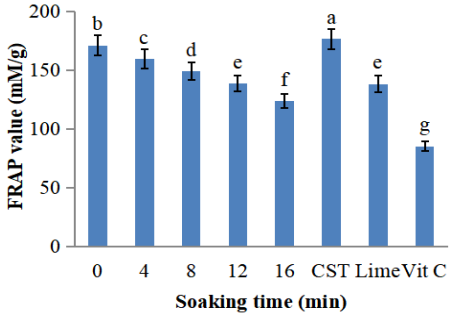

Fig. 4 Effect of the soaking time in lime solution at pH 5 on the FRAP value of *S. polycystum* seaweed tea. Note: CST = commercial seaweed tea

The antioxidant activity of *S. polycystum* seaweed tea increased with increasing soaking time. Thus, soaking in lime solution at pH 5 and 85°C can increase the antioxidant activity of *S. polycystum* seaweed tea. The increase in antioxidant activity during soaking was thought to occur because the compounds in seaweed, such as flavonoids, become more active [19]. In addition, the soaking process under acidic conditions can result in flavonoid compounds in the form of glycosides, which will be degraded into aglycones and sugars, which increase the antioxidant activity [27]. More OH groups donate H+ atoms with a higher polyphenol content of the flavonoid group, more OH groups donate H^+ atoms, so the antioxidant activity increases [28].

3.5. Inhibitory Activity of α-Glucosidase

Data on the effect of the soaking time in the pH 5 lime solution on the α-glucosidase inhibitory activity of *S. polycystum* seaweed tea are shown in Fig. 5. *S. polycystum* seaweed tea-soaked for 0, 4, 8, 12, and 16 minutes had inhibitory activities of 11.47 ± 0.82, 20.71 ± 1.02, 32.34 ± 0.68, 41.59 ± 0.60, and 51.56 ± 0.70%, respectively. The data show that a longer soaking time resulted in a higher α-glucosidase inhibitory activity. The highest α-glucosidase inhibitory activity was produced in the 16-minute soaking treatment (51.56 ± 0.70%), while the lowest was produced without soaking (11.47 ± 0.82%). This result can occur because one of the main ingredients of lime is flavonoids [29]. Flavonoids inhibit α-glucosidase in the breakdown of carbohydrates before being absorbed as monosaccharides [30].

Based on Fig. 5, the highest α-glucosidase inhibitory activity was obtained after soaking in the pH 5 lime solution for 16 minutes (51.56 ± 0.70%), while the lowest was obtained without soaking (11.47 ± 0.82%). The inhibitory activity of α-glucosidase by lime solution at pH 5 was quite high (42.82 ± 0.31%); thus, soaking seaweed in lime solution at pH 5 affects the α-glucosidase inhibitory activity of *S. polycystum* seaweed tea. Lime contains phenolic or polyphenol compounds such as flavonoids, glycosides, tannins, and saponins, which affect the inhibitory activity of α-glucosidase [31].

The *S. polycystum* seaweed tea had higher α-glucosidase inhibitory activity than commercial seaweed tea but lower than acarbose. Acarbose is often used for α-glucosidase inhibitors. Acarbose works competitively to inhibit the action of α-glucosidase within cells from reducing glucose absorption and postprandial hyperglycemia. In diabetic patients, acarbose can reduce postprandial hyperglycemia 30-50% and HbA1c 0.5-1% [32]. Acarbose can also competitively inhibit the hydrolysis of carbohydrates with α-glucosidase to inhibit the hydrolysis process and prevents blood sugar levels from increasing. However, this drug was reported to cause gastrointestinal side effects such as diarrhea and flatulence [33].

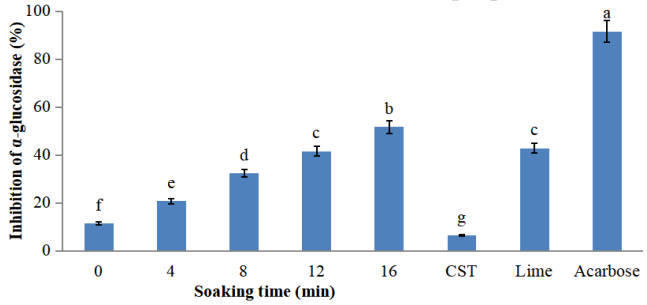

Fig. 5 Effect of immersion time in lime solution at pH 5 on the α-glucosidase inhibitory activity of *S. polycystum* seaweed tea. Note: CST = commercial seaweed tea

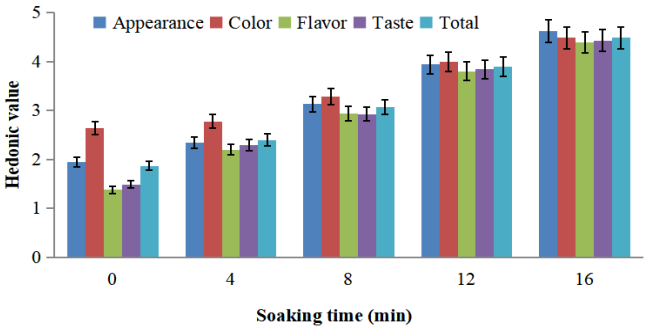

Fig. 6 Effect of the soaking time in lime solution at pH 5 on the hedonic value of *S. polycystum* seaweed tea

3.6. Level of Consumer Acceptance

Hedonic testing was conducted to determine the level of consumer acceptance of *S. polycystum* seaweed tea in terms of appearance, color, flavor, and taste. Appearance greatly affects the quality of a product [34]. Products with an attractive appearance can increase consumer attractiveness. The effect of soaking in lime solution at pH 5 on the color preference of *S. polycystum* seaweed tea is shown in Figure 6. The consumer preference value for the appearance of seaweed tea was 1.95-4.63. The sample without soaking had the lowest preference value (1.95), i.e., between very dislike and dislike, while the 16-minute soaking treatment had the most preferred appearance (4.63), i.e., between like and very like. Sinurat & Suryaningrum [10] reported that the level of preference for the appearance was 4.7 for *Sargassum sp*. that was soaked in boiling water for 1 minute, i.e., "like it until you truly like it". A good appearance will increase the value of other parameters such as color, flavor, and taste [35].

Color visually determines the level of acceptance of the product by consumers. The level of consumer acceptance of food products is influenced by color attributes [36]. The consumer acceptance of *S. polycystum* seaweed tea color preferences is presented in Fig. 6. The level of consumer acceptance of tea colors was 2.64-4.49. The sample immersed in the pH 5 lime solution for 16 minutes had the highest color value (4.49), i.e., between like and very like. The one

without soaking had the lowest color preference value (2.64), i.e., between dislike and somewhat like. The hedonic value of color in this study was higher than that of *S. filipendula* seaweed tea that was soaked in a pH 5 lime solution for 6 hours (4.10) [9] but still lower than that of *Sargassum sp*. seaweed tea that was immersed in boiling water for 1 minute (4.65) [10]. A possible reason is the different treatments and samples.

The consumer acceptance of the flavor value of S. polycystum seaweed tea is presented in Fig. 6. The level of consumer acceptance of flavor tea was 1.38-4.39. The sample soaked in the pH 5 lime solution for 16 minutes had the highest value of flavor preference (4.39), i.e., between like and very like. The results of this study were higher than the level of preference for the tea flavor of *S. filipendula* (2.50) soaked in pH 5 lime solution for 6 hours [9] and *Sargassum sp*. tea (3.71) soaked in boiling water for 1 minute [10].

The level of taste preference determines whether consumers can accept a product. Products that have many functions for health but unacceptable taste by consumers do not sell [17]. The effect of immersion on the taste preference level of *S. polycystum* seaweed tea is presented in Figure 6. The level of consumer preference for the taste of the tea was 1.49-4.43. The soaking time had a significant effect ($p < 0.05$) on the taste preference level of *S. polycystum* seaweed tea. A longer soaking time corresponds to a stronger lime taste in *S. polycystum* seaweed tea. In addition, according to Sinurat & Suryaningrum [10], the soaking process with hot water made the most dominant polymer compounds in seaweed change to oligomeric compounds, which the panelists preferred. The tannins caused the taste of seaweed tea in the brown seaweed *Sargassum sp*. Soaking in a hot lime solution can reduce the tartness of *S. polycystum* seaweed tea. The highest level of taste preference (4.43) was produced by soaking in a pH 5 lime solution for 16 minutes, i.e., between like and very like. The results of this study were higher than the taste preference level of *S. filipendula* seaweed tea (3.30) soaked in pH 5 lime solution for 6 hours [9]. Still, it was lower than the taste preferences of *Sargassum sp*. seaweed tea (4.65) that was immersed in boiling water for 1 minute [10].

Fig. 6 also shows the overall consumer preference of *S. polycystum* seaweed tea. The level of consumer acceptance of tea was 1.87-4.49. Based on statistical tests, the most preferred *S. polycystum* seaweed tea was seaweed tea soaked in the pH 5 lime solution for 16 minutes, and the least preferred tea by consumers was the one without soaking. The soaking time in lime solution at pH 5 affects the level of consumer acceptance. With a longer soaking time, the *S. polycystum* seaweed tea products were more preferred. S. polycystum seaweed tea's overall consumer preference was higher compared to *Sargassum sp*. seaweed tea [10].

S. polycystum seaweed can be made into functional beverage products in the form of tea through the following steps: 1) Seaweed *S. polycystum* was washed using clean water and then soaked pH 5 lime solution for 16 minutes. 2) After soaking, the seaweed was drained and dried for 24 hours in a baking sheet, which was placed on a terrace and not exposed to direct sunlight. 3) The seaweed was roasted in a frying pan for 15-20 minutes and cut with a knife into pieces 0.5 ± 0.1 cm in size. 4) Seaweed tea was placed in a sealed standing pouch and ready to use.

4. Conclusion

In conclusion, the soaking time in lime solution at pH 5 affects the antioxidant and antidiabetic activities and the consumer acceptance of *S. polycystum* seaweed tea. Soaking seaweed in lime solution pH 5 for 16 minutes was the best treatment for *S. polycystum* seaweed tea with the following characteristics: water content $4.68 \pm 0.12\%$; total phenol 74.53 ± 0.59 mg GAE/g; inhibition of DPPH $52.70 \pm 0.86\%$; FRAP value 123.94 ± 1.39 μM/g; α-glucosidase inhibitory activity $51.56 \pm 0.70\%$; hedonic values for appearance, color, taste, flavor and overall: 4.63 ± 0.74, 4.49 ± 0.75, 4.39 ± 1.07, 4.43 ± 1.03, and 4.49 ± 0.11, respectively. In producing *S. polycystum* seaweed tea, it is strongly influenced by the manufacturing process, one of which is the length of immersion in lime. Therefore, it is necessary to optimize the optimum time to get the best quality and the most preferred by consumers in the future.

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