INTRODUCTION

Seaweed is a component of the ecosystem along the coasts of Indonesia [1]. It can be divided into green seaweed or Chlorophyta, brown seaweed or phaeophyta, and red seaweed or Rhodophyta [2]. Brown seaweed has a higher level of bioactive compounds than the two other types [3]. One type of brown seaweed found along the coast of Indonesia is Sargassum spp., such as Sargassum plagiophyllum.

Among many bioactive compounds contained in brown seaweed including Sargassum sp., antioxidants are particularly interesting because they are key compounds in treating various degenerative diseases and for preventing aging. Natural antioxidants in seaweed come from phlorotannin compounds, ascorbic acid, tocopherol, and carotenoid. Various studies have also revealed that phlorotannin is an antioxidant compound that plays the most important role in photoprotection in seaweed [4].

Phlorotannin is a polyphenol compound that is produced by the polymerization of a phloroglucinol monomer (1,3,5-trihydroxy benzene). As an antioxidant, phlorotannin has 10–100 times better activity against free radicals than flavonoids found in terrestrial plants [5]. However, the antioxidant activity of phlorotannin compounds and their use as food dyes are strongly influenced by their stability. Phlorotannin stability, such as that of the compounds polyphenols, is affected by pH, metal ions, light, temperature, oxygen, and enzyme activity [6-9]. Therefore, to maintain the antioxidant levels and physiological effects of phlorotannin compounds during the drying and storage process, a stabilizer is used, a commonly used example of which is maltodextrin.

Maltodextrin is a polymer composed of d-glucose units with dextrose equivalent (DE) <20, which is used as a coating agent [10]. Maltodextrin is known to have low permeability to oxygen, thereby preventing oxidation of the active substance and increasing its stability during storage. The use of maltodextrin could, thus, have a positive effect on the stability of phlorotannin compounds during storage, preventing or minimizing their oxidation, which may affect the decrease in phlorotannin content and antioxidant activity.

Besides using maltodextrin as a stabilizer, freeze-drying is used in the drying process to improve the stability of phlorotannins. This is because the use of a drying temperature below room temperature is suitable for heat-sensitive compounds such as polyphenol compounds [11]. Phlorotannins are members of the polyphenol compounds, so their conversion into powder using freeze-drying techniques can prevent their degradation by drying at high temperatures, as in the dry spray and oven method.

Therefore, to maintain phlorotannin stability against a high temperature in the drying process and against oxidation during storage, this study used S. plagiophyllum extract powder with a freeze-drying technique using maltodextrin as a stabilizer. Then, the stability and characteristics of S. plagiophyllum extract powder were investigated for 8 weeks of storage.

MATERIALS AND METHODS

Research method

S. plagiophyllum simplicia setup

Brown seaweed S. plagiophyllum (Mertens) J.G. Agardh. was taken directly from Binuangun Beach, Lebak, Banten. The samples were then washed and dried by aeration for about 4 days [12]. The simplicia was then identified at the Oceanographic Institute of the Indonesian Institute of Sciences, Ancol, North Jakarta.

S. plagiophyllum (Mertens) J.G. Agardh. extraction

The simplicia were cut into small pieces, weighed to 600 g, put into a brown bottle, and extracted by maceration for 24 h, followed by stirring at room temperature [13]. The extract was filtered with filter paper,
after which the filtrate was obtained and the residual residue was discarded. The filtrates obtained from four bottles were then combined.

**Characterization of *S. plagyophyllum* liquid extract**

Liquid extracts were identified organoleptically by visual/olfactory analysis, including of their color and odor. The pH of the liquid extract was measured using a pH meter.

**Determination of phlorotannin *S. plagyophyllum* liquid extract levels**

Determination of phlorotannins content in the liquid extract was performed by reacting the extract filtrate with Folin–Ciocalteu solution and 7.5% sodium carbonate solution, followed by incubation at room temperature in the dark for 70 min. The absorption of the solution was measured using ultraviolet-visible spectrophotometry at a wavelength of 707 nm. The levels were calculated by comparing the uptake of the extract with the standard uptake of phloroglucinol [13].

**Determination of *S. plagyophyllum* liquid extract antioxidant activity**

The determination of antioxidant activity was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical. An extract weighing a total of 5 g was put into a 10-mL volumetric flask, ethanol p.a. was added until it reached the limit marks of the volumetric flask, forming the main solution with a concentration of 500,000 ppm. The formed solution was sonicated for 60 min, introduced into a centrifugation tube, and centrifuged at 1500 rpm for 4 min [14]. The main solution was then diluted to six different concentrations. Subsequently, each solution was incubated in the dark at a temperature of 28°C (room temperature) for 30 min, followed by measurement at a wavelength of 516 nm. Then, its inhibition concentration (IC$_{50}$) was measured using the following formula of a linear regression equation: $y=ax+b$, with y being the average percent inhibition and x being the concentration of each test solution. Ascorbic acid was used for comparison, with the same treatment applied to the samples. The resulting linear regression equation was used to calculate the IC$_{50}$ value, namely, the sample concentration required to inhibit 50% of the concentration of free radical DPPH. The percentage DPPH inhibition of each sample series solution was calculated by dividing (blank uptake-sample uptake) with blank uptake then times 100%.

While the antioxidant activity represented by the IC$_{50}$ values was calculated using the obtained linear regression equation, the y value is 50% inhibition so that y is changed to 50.

**Making *S. plagyophyllum* extract powder**

The powder consisted of four different formulations, namely without maltodextrin (F1), as well as with maltodextrin DE 10–15 2.5% (F2), 5% (F3), and 10% (F4). Each formulation was dried by multilevel drying as shown in Table 1. First, it was inserted into a freezer at −20°C fitted to the FDU 1200 Eleya freeze-drying tool and dried to form a stable solid mass for about 72 h at −45°C under pressure of 1.53 p.a. The dried powder that was still present in the drying container was then taken and dredged. Subsequently, each powder formulation was disrupted with a blender, weighed, and stored in a glass container that was tightly sealed [15].

**Physical characterization of powdered *S. plagyophyllum* liquid extract**

Organoleptic powders can be identified by their odor, color, and texture. Here, the morphology of the powder was observed by scanning electron microscopy (EVO MA 10; Carl Zeiss, United States) at 14 KV EHT and an secondary electron (SE) detector SE. The particle size distribution of *Sargassum* powder was measured using a 2000 Mastersizer tool from Malvern. The water level of the powder was measured using a moisture analyzer at 105°C. The pH of the powder was measured using a pH meter for 1 g of powder dispersion in 10 mL of aquadest.

**Test of phlorotannin levels in powder *S. plagyophyllum* extract**

Phlorotannin levels were tested using the Folin–Ciocalteu method. Powder of each of F1, F2, F3, and F4 was weighed and put into a 10-mL volumetric flask, aquadest was added to adjust the volume until limit marks of the volumetric flask, followed by shaking to homogeneity. The determination of phlorotannin content was performed as for the liquid extract.

**Antioxidant activity test of powdered *S. plagyophyllum* liquid extract**

The antioxidant activity of the extract was tested by measuring the preventive activity against the free radical DPPH. A number of powders in each formulation of F1, F2, F3, or F4 were weighed and added with volume of ethanol p.a. to the limit of volumetric flask until the desired concentration was obtained, followed by performance of the antioxidant activity test the same as for the liquid extract.

**Powder stability test of liquid *S. plagyophyllum* extract**

Stability tests including phlorotannin and water levels were performed fortnightly for 8 weeks. These tests were carried out at three temperature storage conditions: 42°C, 282°C, and 402°C. The test was performed on F4, which has the highest phlorotannin content. F1 was also included for comparison between powders without and with maltodextrin.

**RESULTS**

**Simplicia setup**

Physical comparison of fresh *S. plagyophyllum* (Mertens) J.G. Agardh. and its dried form are shown in Fig. 1. From about 30 kg of fresh *S. plagyophyllum* (Mertens) J.G. Agardh. after drying, only ~3 kg of simplicia was obtained, giving a yield of 10%.

The *S. plagyophyllum* (Mertens) J.G. Agardh. was further extracted, resulting in extraction filtrate which was brown. The liquid extract had a dark brown color and a distinctive smell as shown in Fig. 2.

**Test of the acidity of liquid *S. plagyophyllum* extract**

The test results showed that the pH of the liquid extract was about 7 or neutral.

**Determination of phlorotannin levels of liquid *S. plagyophyllum* extract**

Regarding the optimization of the incubation time, it was found that 70 min was the optimal period for incubation because that duration was associated with higher absorption of standard phloroglucinol solution than the other times (10, 20, 30, 40, 50, 60, 80, and 90 min). In the calibration curve, a linear regression equation of $y=-0.0936+0.0043x$ was obtained, with an $r=0.99939$ to be used for the determination of phlorotannin levels in liquid extract and powder. Thereafter, the fluoroethene content in the liquid extract was determined as mg of phloroglucinol per gram of simplicia and mg of phloroglucinol per gram of powder.

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**Table 1: Extracted *S. plagyophyllum* powder formulation**

| Material            | Formula                  | F1 (without maltodextrin) | F2 (MD 2.5%) | F3 (MD 5%) | F4 (MD 10%) |
|---------------------|--------------------------|---------------------------|--------------|------------|-------------|
| Extract (g)         |                          | 400                       | 400          | 400        | 400         |
| Maltodextrin DE 10–15 (g) | -                        | 10                        | 20           | 40         |
| Coating ratio: Extract | -                      | 1.40                      | 1.20         | 1.10       |

DE: Dextrose equivalent

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gram of liquid extract. The average liquid extract level of the three tests was 2390.02 mg phloroglucinol per gram of simplicia or 0.24 mg of phloroglucinol per gram of liquid extract.

**Determination of antioxidant activity of S. plagyophyllum liquid extract**

The test on the antioxidant activity of the extract was performed by making a solution of DPPH using ethanol solvent p.a. that generated maximum wavelength at 516 nm. This test used ascorbic acid as a positive control for comparison with the activities of antioxidant liquid extract and dried powder. The ascorbic acid antioxidant activity test was performed in duplicate, and an IC$_{50}$ of ascorbic acid of 3.11±0.02 ppm was obtained. The antioxidant test of liquid extract was carried out at six selected concentrations of 500, 1000, 1500, 2000, 2500, and 3000 ppm; the results showed that only limited inhibition occurred, which did not reach 50%, but at higher concentration extracts, namely 5000%, 25,000%, and 50,000%, the inhibition of DPPH by the extract was lower than at the concentration of 3000 ppm. Therefore, for the liquid extract, IC$_{50}$ calculation cannot be performed because the obtained results are not relevant to the test.

**Production of S. plagyophyllum extract powder**

The largest amount of powder was produced for F4, followed by F3, F2, and F1 in this order (Table 2).

The S. plagyophyllum extract powder in each formulation has a distinctive odor, as in the extract. The morphological view of the powder is shown in Fig. 3.

**Size distribution of particle powder test of S. plagyophyllum liquid extract**

The results of the measurement show that the greater the maltodextrin percentage used in the powder formulation, the larger the particle size of the powder. However, there are exceptions, in that the result for F4 was smaller than for F3, although with no significant difference between them (Table 3).

**Water level test of powdered S. plagyophyllum liquid extract**

The resulting water level has been identified in the requirements of powdered water content or not. For natural materials, the water level that fulfills the requirements is not >10%. The water levels of the four formulas are shown in Table 4.

**Tests of acidity of powdered S. plagyophyllum extract**

Each powder solution of the four different formulations as used for the pH assays could be completely dissolved. This test was performed in triplicate (Table 5).

**Test of phlorotannin powder levels of liquid S. plagyophyllum extract**

The concentrations of phlorotannin in the four powder formulas are shown in Table 6. Of the four formulas, the highest phlorotannin content was identified in F4.

**Antioxidant activity test of powdered S. plagyophyllum liquid extract**

The percentage inhibition obtained from F2, F3, and F4 did not reach 50%, so IC$_{50}$ could not be calculated. As the percentage inhibition of each formula differed, to compare antioxidant activities, the percentage inhibition of each formulation at a concentration of 5000 ppm was used, as shown in Table 7.

There was a test for level stability of powdered S. plagyophyllum liquid extract. F1 and F4 moisture tests showed that F1 had a higher water level than F4 at all lead temperatures. In both F1 and F4, the highest water level occurred during storage at a temperature of 4°C, followed by temperatures of 28°C and 40°C (Table 8).
Test of phlorotannin level stability of powdered liquid S. plagyophyllum extract

The results of the stability test showed that, in F1, on 8 weeks of storage, stability had decreased significantly compared with that for F4. The phlorotannin levels of F1 and F4 powders are given in two different units, namely mg phloroglucinol per number of powders produced and percentage (%), as shown in Tables 9 and 10, respectively.

DISCUSSION

Simplicia setup

Drying was performed by aeration. This method was chosen because this drying method can produce phlorotannin compounds for seaweed better than exposure to direct sunlight or use of an oven. This is because heat can affect the level of phlorotannin produced. The best method of drying to optimize the stability of seaweed phlorotannin compounds is by aeration, followed by use of an oven and least effectively exposure to direct sunlight [9]. About 30 kg of fresh S. plagyophyllum (Mertens) J.G. Agardh. only produced 3 kg of simplicia, so the yield was 10% (Fig. 1).

S. plagyophyllum (Mertens) J.G. Agardh. extraction

The extraction filtrate was brown. This filtrate was used for the evaluation of the extract and the creation of powder.

Organoleptic test of liquid S. plagyophyllum extract

The liquid extract was dark brown in color and had a distinctive (fishy) smell. The brown color was obtained because of the seaweed S. plagyophyllum (Mertens) J.G. Agardh. being a brown seaweed with a brown pigment [1].

Test of acidity of liquid S. plagyophyllum extract

This test was performed to determine whether there was any change of pH between the liquid extract before drying and the powder obtained after drying using the freeze-dryer tool, for the four established formulas. The test results showed that the pH of the liquid extract was around 7 or neutral. This is because liquid extracted in aqua demineralisation solvent has a pH of 7 (according to CoA), resulting in an average pH obtained from three tests of 7.82±0.08.

Table 4: Water levels of freeze-dried powders

| Formulation | Water level (%) |
|-------------|-----------------|
| F1          | 6.81            |
| F2          | 6.34            |
| F3          | 5.81            |
| F4          | 5.16            |

Table 5: Acidity of powder and liquid extracts

| Formula | Acidity (pH) |
|---------|--------------|
| F1      | 10.66±0.01   |
| F2      | 9.93±0.01    |
| F3      | 9.59±0.01    |
| F4      | 9.41±0.02    |
| Extract | 7.82±0.02    |

Table 6: The concentrations of phlorotannin in four powder formulas

| Formula | Amount of extract (g) | Amount of maltodextrin (g) | Amount in 400 g of dried extract powder (mg) | Phlorotannin level per g of powder (mg phloroglucinol per g of liquid extract) | Phlorotannin level in 400 g of dried liquid extract (mg phloroglucinol) | Level (%) |
|---------|-----------------------|---------------------------|-------------------------------------------|-----------------------------------------------------------------|------------------------------------------------------------------------|-----------|
| F1      | 400                   | 0                         | 5.91                                      | 6.30                                                            | 37.10±0.12                                                            | 0.63      |
| F2      | 400                   | 10                        | 15.13                                     | 4.02                                                            | 60.96±2.01                                                            | 0.40      |
| F3      | 400                   | 20                        | 25.39                                     | 3.20                                                            | 80.20±1.22                                                            | 0.32      |
| F4      | 400                   | 40                        | 44.58                                     | 2.54                                                            | 113.06±1.36                                                           | 0.25      |

The best method of drying to optimize the stability of seaweed phlorotannin compounds is by aeration, followed by use of an oven and least effectively exposure to direct sunlight [9]. About 30 kg of fresh S. plagyophyllum (Mertens) J.G. Agardh. only produced 3 kg of simplicia, so the yield was 10% (Fig. 1).

Determining phlorotannin level of liquid S. plagyophyllum extract

From the optimization of the incubation time, it was found that 70 min was the optimum time for incubation. Subsequently, a calibration curve was produced, which was used to determine the phlorotannin content in the liquid extract calculated as mg phloroglucinol per gram of simplicia and mg phloroglucinol per gram of liquid extract. The average liquid extract level of the three tests was 2.39±0.02 mg phloroglucinol per gram of simplicia or 0.24 mg of phloroglucinol per gram of liquid extract. This latter value is low because liquid extract has not been used to attempt to separate the phlorotannin compound from other compounds in the simplicia, which also occurs during the extraction process, so, the extract contains phlorotannin. Brown seaweed is known to contain not only polar compounds in the form of phlorotannins but also other polar compounds, which can be carried by aquadest (solvent used for extraction). This leads to the result of the test containing only a small amount of phlorotannin.

Making S. plagyophyllum extract powder

The largest amount of powder was produced by F4, followed by F3, F2, and F1 in this order. If the same amount of extract is used, then the amount of powder produced is proportional to the amount of maltodextrin used in the formulation. An increase in the amount of maltodextrin used in the formula will increase the amount of solids or powders produced. An increase in the amount of powder in line with an increase in the amount of maltodextrin used relates to the nature of maltodextrin as a drying agent and may increase the amount of solids in the resulting powder [15].

Organoleptic test of powdered liquid S. plagyophyllum extract

The powder in each formulation has a distinctive odor, as in the extract. The less maltodextrin used, the more distinctive the odor. Meanwhile, the color of the powder appeared to become more faded with the increasing amount of maltodextrin used.

Test of the water level of powdered S. plagyophyllum liquid extract

The measurements showed that the powder with the highest water level was F1, followed by F2, F3, and F4. The water level was higher in formulations with less maltodextrin. This is because the use of maltodextrin in the formulation can decrease the water level as well as the hygroscopicity of the dried powder in the freezing process. Maltodextrin is a drying agent that can increase the amount of solid (powder) and decrease the amount of free water during the drying process so that the resulting powder has less water than the dried powder without maltodextrin [15].

The powdered water level of natural substances is not > 10%, and since all powder formulations of F1, F2, F3, and F4 had a water level of > 10%, all formulas meet the water level condition.

Test of acidity of powdered S. plagyophyllum liquid extract

Each powder solution of the four different formulations for pH assays could be completely dissolved. When compared with the pH of the extract solution before the drying process, all solutions derived from the four different formulas had a more basic pH than the extract solution. This is because the drying process attracts acidic substances into the solution to be dried, so the dried powder will have more alkaline properties than the solution. The pH test results showed that the less maltodextrin used...
Test of phlorotannin level of powdered *S. plagyophyllum* liquid extract

Among the four formulas, the highest phlorotannin content was obtained in F4 because F4 has the largest amount of maltodextrin, stabilizing the phlorotannin content of 400 g of liquid extract, higher than the levels presented in F1, F2, and F3. This corresponds to maltodextrin functioning as a stabilizer in the drying process.

Test of antioxidant activity of powdered *S. plagyophyllum* liquid extract

The greater the amount of maltodextrin used in the formulation, the higher the levels of powder and phlorotannin in the powder used and the higher the percentage inhibition that can be detected. However, there was no case reaching the 50% percent inhibition obtained from F2, F3, and F4, which mean that IC<sub>50</sub> could not be calculated. In F4, the highest detectable powder IC<sub>50</sub> value percent was 12.05%, followed by F3 of 8.78% and F2 of 6.31%, while F1 did not exhibit antioxidant activity. Since the percentag inhibition of each formula differs, the antioxidant activities as reflected in the percentage inhibition of each formulation at a concentration of 5000 ppm were compared, as shown in Table 7.

Test of water level stability of powdered *S. plagyophyllum* liquid extract

F1 and F4 water level tests showed that F1 had a higher water level than F4 at all temperatures. This was due to the absence of maltodextrin from the powder. In F4, maltodextrin serves to maintain the water level of the powder during storage. Besides lowering the hygroscopicity of powder of the drying product, the use of maltodextrin may also reduce hygroscopicity during storage [15].

Test of phlorotannin stability of powdered *S. plagyophyllum* liquid extract

The results of the stability test showed that 8 weeks of storage significantly decreased stability in F1 compared with that in F4 (Tables 9 and 10). The highest phlorotannin levels for F1 and F4 occurred on storage at 4°C, followed by those at a temperature of 40°C and at a temperature of 28°C. For F4, the levels obtained were more stable due to maltodextrin protecting the phlorotannin compounds against environmental conditions (temperature, light, moisture, and oxygen).

The stability tests regarding the water level and the phlorotannin level in powder showed that the formulation with the lowest water level and the most stable phlorotannin level at 8 weeks of storage was F4 stored at 4°C. Therefore, the powder dried with maltodextrin was better during storage than the powder dried without maltodextrin.

CONCLUSION

Powder of *S. plagyophyllum* liquid extract with and without maltodextrin can be prepared by freeze-drying. The powder formulation with dried maltodextrin obtained with this technique can improve the stability of phlorotannin compounds during drying but does not produce good antioxidant activity. The maltodextrin-dried powder has higher phlorotannin content and lower water content during storage at 4°C (optimal storage temperature) than a dried powder without maltodextrin.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

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