



PROTEIN CONTENT AND COLOR OF GREEN MACROALGAE *Ulva lactuca* (L.) ON SOAKING TIME AND DRYING METHOD

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ABSTRACT

The green macroalgae *Ulva lactuca* (L.) is a potential alternative protein source. This study investigates the effect of soaking and different drying methods on the protein and color quality of *U. lactuca*. Moisture and ash content, color, and protein content, i.e., crude protein, in vitro protein digestibility, and protein solubility of the seaweed were determined. In its conduct, fresh *U. lactuca* was soaked in fresh water for various soaking times (0, 1, 2, and 3 h). Soaking *U. lactuca* with the highest protein digestibility was selected for further drying method investigation, i.e., sun-drying, shade-drying, and oven-drying at 40°C. The results reveal that soaking treatment did not affect protein quality significantly, but there was a significant effect of soaking treatment on the ash content and color quality of *U. lactuca*. Among the drying method, sun drying and oven drying resulted in higher in-vitro protein digestibility and protein solubility compared to shade drying.

Keywords: *Ulva lactuca*; macroalga; soaking; drying; in-vitro protein digestibility

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INTRODUCTION

Indonesia has protein-rich food potential, especially those from the sea. One of the marine resources that have not been widely utilized as a source of protein was macroalgae. Macroalgae have the potential as a source of protein and amino acids. There were three types of macroalgae on the coast, red, green, and brown macroalgae. Red and green macroalgae contain more protein than brown macroalgae. The protein composition of green or red macroalgae ranges from 10% to 47% (Joël Fleurence and Levine, 2016). This suggests that macroalgae could be used as an alternative protein source.

Ulva lactuca (L.) is one of the green macroalgae that is found abundant in Sepanjang Beach Gunungkidul Yogyakarta. It contains proteins in varying concentrations ranging from 10% to 23%, Calcium in the

range of 0.8-5.6 percent, and magnesium in the range of 2-5.2 percent (Joël Fleurence and Levine, 2016). However, the nutrient levels in *U. lactuca* (L.) tend to vary depending on the season, the geographic location where it grows, harvest age, and nutrients in the waters (Ortiz, 2006). It is also known for its relatively quick growth rate, which makes these macroalgae promising to be developed as an alternative source of protein to prevent stunting issues. Vegetable protein sources, on the other hand, have poor protein digestibility due to the presence of anti-nutrients. Seaweed's antinutrient components were polysaccharides or trypsin inhibitors (J. Fleurence et al., 2018). Soaking was one method of reducing antinutrient compounds in ingredients, which was intended to improve protein digestibility in vegetable protein sources. Immersion in sodium bicarbonate for 8 hours has been

reported to reduce tannin antinutrient factors by more than 90%, phytic acid by approximately 22%, and protein digestibility by around 4% in white lupines (El-Adawy et al., 2000).

Since the rigidity of the seaweed cell walls hinders protein extraction, various methods to break the cell walls before protein extraction have been used, including mechanical forces, osmotic disruption, and the use of cell wall-acting enzymes (J. Fleurence et al., 1995; Vilg and Undeland, 2017; BjarnadÚttir et al., 2018; (Robin et al., 2018; V·squez et al., 2019). The use of freshwater can induce an osmotic shock, which disrupts the seaweed cells (Liot et al., 1993). Cell disruption destroys the polysaccharides that were expected to increase protein digestibility. Soaking in freshwater could hence be a quick alternative to increase the protein content in seaweed by partially removing minerals and potential other non-protein compounds.

In addition to immersion, good drying practices for algae allow for higher quality and performance of its protein content. Theoretically, green macroalgae is a potential source of proteins and essential amino acids which need proper preservation for longer shelf life. Traditionally preservation method usually performed by farmers was drying under the sun. It was a simple and inexpensive way to maintain the quality of macroalgae and longer its shelf life. Uribe et al. (2018) investigated the effects of various drying methods of phytochemicals in *Ulva spp.* Convective drying showed better antioxidant capacity and physicochemical content of *Ulva spp.* Moreover, Naz et al. (2011) reported that the shade-drying method could retain the green color of mako (*Solanum nigrum*) until 5 months. The precise role of immersion time and drying method on *Ulva* protein quality, on the other hand, has not been thoroughly investigated. Therefore, the protein digestibility of *Ulva lactuca* administered using various soaking and drying methods will be evaluated in this study.

MATERIALS AND METHODS

Materials

Fresh *Ulva lactuca* above 2 cm collected from Sepanjang Beach, Gunungkidul (8°8'12"S 110°34'0" E) from October until January. Soaking treatment using fresh water from Local Water Company (PDAM). Seaweed was stored dry at room temperature with non-translucent plastic bags until further analysis.

For protein content analysis, Kjeldahl Tablet (110958, Merck Germany), sulfuric acid (7664-93-9, Mallinckrodt Ireland), sodium hydroxide (106462, Merck Germany), boric acid (100165, Merck Germany), and hydrochloric acid (10957, Merck Germany). For *in vitro* protein digestibility test, pepsin was purchased from Sigma Life Science P7000 (Germany), trichloroacetic acid (TCA) (100807, Merck Germany). Meanwhile, for protein solubility analyses, bovine serum albumin (BSA) was purchased from (12657, Merck Germany), Folin-Ciocalteu reagent (109001, Merck Germany). All other chemicals and solvents in this study were of analytical grade, and solutions were prepared with distilled water.

Preparation of Samples

The research is divided into two steps. The first step was to find out the influence of soaking time and the next step was to find out the influence of various drying methods on the moisture content, ash content, color, and quality of proteins including protein content, protein digestibility, and protein solubility. The best result on the first step then using for drying treatments. All treatments are done with triplicate sampling.

The effect of soaking time

Four kilograms of fresh *U. lactuca* were rinsed on the spot with seawater, drained, and immediately brought to the laboratory. At the laboratory, the algae were sorted to guarantee they were free from epiphyte, foreign biota, sand, and other contaminants. The clean sample was drained and divided into 4 groups for the following treatment. Clean *U. lactuca* were soaked in tap water for 0, 1, 2, and 3 h at room temperature. The alga was

soaked in water with a ratio of 1:2. The soaked samples were drained and dried at 40°C for 10 h, RH 29% using a cabinet dryer. The dried samples were packed in non-translucent plastic bags and stored at room temperature.

The effect of the drying method

Dry *U. lactuca* with the best soaking treatment was then selected for further investigation. The fresh and clean *U. lactuca* was dried by different drying methods, i.e., oven-drying, sun-drying, and shade-drying. The sun-drying process was performed by spreading the samples on a bamboo tray (“*para-para*”) and drying them under direct sunlight for 10 h (approximately at 40°C, RH 30%, 62.700 lux). Meanwhile, shade-drying was held by spreading the samples on bamboo trays and placing them in a shaded place for 1 d (approximately 30°C, RH 46 %, 330 lux).

Protein Content

Protein content determined with Kjeldahl methods. 500 mg of the samples were mixed with 1 g Kjeldahl Tablet and 10 mL concentrated H₂SO₄ in the Kjeldahl flask and heated in a hot plate until the solution became clear. The solution was then distilled with NaOH 30% and 10 mL of boric acid 2% with PP indicator caught the distillate. Titration with HCl 0.02 N until the color became clear red. The volume of HCl is equivalent to nitrogen content on the sample then multiple by 5 to determine the crude protein content (Angell et al., 2015).

$$\% \text{Protein content} = \frac{(\text{VHCl sample} - \text{VHCl blanko}) \times \text{N HCl} \times 14.008 \times 5 \times 100\%}{\text{mgs of sample}}$$

In-vitro Protein Digestibility

In-vitro protein digestibility measured using a vitro digestion test (Tanaka et al., 1978). Two hundred milligrams of samples were suspended in 9 ml walphole buffer (HCl.NH₃COONa) 0,1 N pH 2 and 1 ml of 2% pepsin then incubated at 37°C and 100 rpm for 5 hours using orbital incubator (Stuart S1500). Samples centrifuge with centrifugal (PLC-05) for 20 minutes at 3000 rpm at room temperature and add 5 ml of solution with 5 ml of 20% trichloroacetic acid (TCA) solution. Samples were incubated for 15 hours at room temperature and filtered

with Whatman No. 541 paper. Crude protein content in the filtrate was then analyzed with kjeldahl methods.

$$\% \text{vitro protein digestibility} = \frac{\% \text{ filtrate crude protein content}}{\% \text{ sample crude protein content}}$$

Protein Solubility

Total protein on filtrate tested with lowry-follin to determined the protein solubility. BSA standard and samples pipetted 100µl in a microplate. Two hundred microlite of alkaline solution consisting of NaOH 0,1 N and Na₂CO₃ 2% were mixed with CuSO₄.5H₂O 1% solution and Na₂Tartrate.2(H₂O) 2% with v/v ratio 100:1:1 mixed with samples or standard. Mixtures were incubated mixture for 10-15 minutes. Twenty microlites of Folin 0.1N were added and incubated for 30 minutes, then were measured the absorbance at wavelength 650 nm (Lowry et al., 1951).

Moisture and ash content

Samples were analyzed for moisture content using a moisture analyzer (Ohaus MB120) by adding 1 gram of sample to the pan and then drying at 105°C using a halogen dryer.

Ash content measurement using thermogravimetric methods according to (Standar Nasional Indonesia, 1992). One gram sample put in crucible porcelain and then was burnt in a 600°C furnace (Thermolyne F48058) until 5 hours and put in an oven (Mettler U-40) at 105°C for twelve hours or until constant weight.

Color

Color of macroalgae measured using hunter scale with Chromameter Konica Minolta Spectrophotometer CM-5. Approximately 2 grams of samples were put in a clear Petri-dish and color was measured with the notation L*, a*, and b*. The color scale was estimated in figure 1 (Harrysson, 2019).

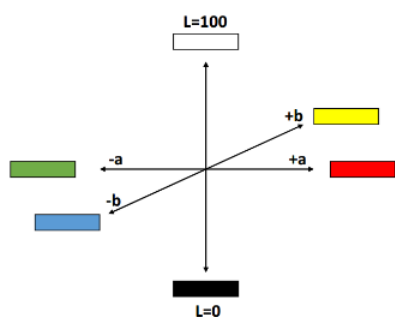


Figure 1: Hunter color

The total color difference is estimated with the equation:

$$\Delta E = \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2} *$$

*1 represents zero soaking time and fresh *U. lactuca* and 2 represents the value at the respective analysis point.

Statistical Analysis

Tests were done in triplicate experiments. The data was viewed as a mean value with a standard deviation. One-way ANOVA was used for statistical analysis, followed by Duncan for comparison of means using R program series 4.0.4. At a 5% level of significance, different alphabetic letters in the column are statistically different (R Core Team, 2018).

RESULT AND DISCUSSION

Protein Content

The quality of foods depends on the quality of the nutrients. The protein content is one of the parameters for food quality. The protein content of *Ulva sp* is higher than terrestrial plants (Kim et al., 2011). Protein content, in-vitro digestibility, and solubility after soaking and drying treatment will be discussed.

Effect of Soaking Time on *Ulva lactuca* (L.) Protein Content

Soaking in freshwater could increase the total protein of *U. lactuca* (Harrysson, 2019). But in this research, soaking time had no significant different on protein content. According to Adebayo S. F. (2014), soaking treated lima bean with de-ionized water at 12, 24, 36, and 48 hours does not affect on protein content. In this study, soaking for 1 hour increased the protein content but was not significant (Table 1). Soaking *U. lactuca* for 2-3 hours showed a reduction of protein content but no significant difference ($P < 0.05$) with unsoaking sample. Siah et al. (2014) reported that some important water-soluble nutrients in macroalga like minerals and protein will leach out during soaking in water.

Soaking treatment also not affected the protein digestibility. Soaking was expected to disrupt the seaweed cells (Liot et al., 1993), that could increase the protein digestibility. In this study, during the soaking time until 3 hours, there was no increase the protein digestibility and tend to lower percentage of protein digestibility. Polysaccharides can inhibit the absorption of nutrients (J. Fleurence et al., 2018), however, soaking treatment until 3 hours cannot crush the *Ulva sp.* body, which means that the polysaccharides cannot be destroyed so that the protein digestibility does not increase through immersion.

Protein solubility is affected by total protein in the filtrate. Soaking in 3 hours had the lowest protein solubility. Immersion treatment could cause water soluble protein had been dissolved into immersion water (Siah et al., 2014). Soaking treatment with tap water gave no positive effect on *Ulva lactuca* protein quality.

Table 1 Protein Quality of *Ulva lactuca* (L.) after soaking treatment

Treatment	Protein content* (g/100g)	Protein digestibility (%)	Protein Solubility (mg/ml)
No soaking	8.8±0.48 ^{ab}	43.56±0.61 ^a	41.44±1.89 ^a
Soaking in 1 h	9.31±0.05 ^a	41.35±3.80 ^a	38.46±0.23 ^a
Soaking in 2 h	8.46±0.30 ^b	38.64±4.19 ^a	34.03±3.10 ^b
Soaking in 3 h	8.55±0.08 ^b	39.43±3.01 ^a	24.71±0.66 ^c

Data are the mean values ± SD; n = 3; ANOVA analysis followed by Duncan with $P < 0,05$

*Dry basis (db)

Effect of Drying Method on *Ulva lactuca* (L.) Protein Content

Uribe et al. (2018) said that the protein content of fresh *Ulva* spp. was about 10.78%. Drying treatment made changes to *Ulva*'s protein content concentration. Oven drying retained protein content better than sun and shade drying. Treatment using oven and sun drying methods made the concentration of protein content more than shade drying method because removal of moisture by heat generally improves concentration of nutrients (Hassan et al., 2007). Vegetables such as mint, coriander, curry leaves, and bitter melon which were dried with solar drying, shade drying, red-light drying, oven 60°C, and 90°C showed that the highest percentage of crude protein reached by using oven 60°C (Naikwade, 2015).

In-vitro protein digestibility of seaweeds with sun drying and oven drying is not significantly different. The drying temperature could affect protein digestibility. Higher temperature causes higher protein digestibility because of the denaturation of green pea protein (Gonzalez et al., 2020). But denaturation did not happen to the seaweed because the drying temperature on oven and sun drying were 40°C which did not reach the denaturation temperature. Protein digestibility of oven and sun drying methods not significantly different because used the same drying temperature.

Higher moisture content lower protein digestibility of *U. lactuca*. Higher amount of processing moisture in brown rice and pinto bean composite flour affected lower protein digestibility (Pinem, 2007). Shade drying (21.58% moisture content) had the lowest protein digestibility because of it had the highest moisture content compared with oven and sun drying. Anti-nutrient in seaweed includes antioxidants components. Low temperature retains antioxidants components (Badmus et al., 2019). The lowest digestibility by shade drying could be caused

by the existence anti-nutrient component in seaweeds.

Oven and sun drying not significantly different on protein solubility but shade drying had the lowest and significantly different on protein solubility. It was same trends with protein digestibility. Total protein determined in shade drying filtrate was the lowest than oven and sun drying treatment. Low dry temperature retains *U. lactuca* polysaccharides. It's an anti-nutritional compound in seaweeds (J. Fleurence et al., 2018). The polysaccharides could inhibit protein solubility in filtrate.

Moisture and Ash Content

Moisture and ash content are important elements that affect the quality of *Ulva lactuca*. It could change because of post-harvest treatment. Lower ash content causes an increase in the protein content. Soaking in freshwater will remove some of the minerals and other potential non-protein compounds (Harrysson, 2019).

Effect of Soaking Time on Moisture and Ash Content of *Ulva lactuca* (L.)

Soaking affects the moisture and ash content of *Ulva lactuca*. The longer soaking time would increase the moisture content of the material. The moisture content increases significantly when compared to the moisture content of the material without immersion. Same result with (Adebayo S. F., 2014; Gana I. M. et al., 2014), soaking increased the moisture content of lima beans and grains. During the soaking time, water enters the material and can be bound inside the material matrix. The soaking process can increase the amount of water tied to the material so the moisture content of the dried material increases. An increase in moisture content in dried *Ulva lactuca* was not expected because it will reduce protein quality. However, no significant difference ($P < 0,05$) of moisture content at 1-3 hours of soaking time.

Table 2 Protein Quality of *Ulva lactuca* (L.) after drying treatment

Treatment	Protein content* (g/100g)	In-vitro Protein digestibility (%)	Protein Solubility (mg/ml)
Oven drying	11.00±0,61 ^a	34.85±0,49 ^a	53±1,54 ^a
Sun drying	9.14±1,01 ^b	37.68±2,85 ^a	53.38±0,38 ^a
Shade drying	8.89±0,58 ^b	31.77±1,14 ^b	39.92±0,77 ^b

Data are the mean values ± SD; n = 3; ANOVA analysis followed by Duncan with P<0,05

*Dry basis (db)

Opposite to moisture content, ash content decrease during soaking time. It was expected to increase protein content. Harrysson (2019) reported that dried *U. lactuca* with fresh water soaking treatment and dried in an oven dryer had lower ash content but higher protein content than *U. lactuca* dried using oven-dried without soaking in fresh water. Minerals would dissolve during the soaking process and soaking time for 3 hours had the lowest ash content. Same with the result of Poeloengasih et al. (2019), the longer soaking time of *U. lactuca* in tap water would decrease the ash content. Decrease in ash content in *Ulva sp.* but not proven to increase the protein content since there was no significance difference of protein content with immersion treatment until 3 hours (Table 1).

Effect of Drying Method on *Ulva lactuca* (L.) Moisture and Ash Content

Drying methods affect the moisture content of the seaweeds as consequence of different temperatures and RH various drying methods. The equilibrium moisture content of drying methods could affect protein content, protein digestibility, and protein solubility which lower moisture content had a positive impact on the protein quality of *U. lactuca* (Table 2).

The temperature and RH of oven drying at (40°C, 29%) sun drying at around (40°C,

30%), and shade drying (at 30°C, 46%). Higher drying temperature made lower equilibrium moisture content. The equilibrium moisture content of seaweeds rises as the fade of drying temperature. Prasetyo et al. (2018) reported that *Ulva sp.* that dried in an oven at temperatures 40°, 50°, and 60°C had equilibrium moisture content of about 14,10; 9,89; 6,03. Low relative humidity (RH) is expected to increase the drying rate (Sabudin et al., 2014). Oven and sun drying had the same temperature and slightly different RH conditions. It was affected by moisture content but not significantly different.

Drying *Ulva lactuca* using a freeze dryer and oven dryer result in the same ash content (Harrysson, 2019). Uribe et al. (2018) reported that the ash content of dried *Ulva spp* using oven convective dryer (oven) and solar drying no significant difference (MRT, P<0.05). The result of this research matched with a previous study, there was no significant effect (P<0,05) on the ash content using different drying methods. Sun and shade drying are done at an outdoor place. Higher ash content at sun and shade drying could cause by impurities during drying, but it was not necessary since it was not significantly different with oven drying's ash content.

Table 3 Moisture and ash of *Ulva lactuca* (L.) after soaking treatment

Treatment	Moisture content (g/100 g)	Ash content* (g/100 g)
No soaking	11.44±0.11 ^b	37.79±0.07 ^a
Soaking in 1 h	19.52±0.10 ^a	30.06±0.01 ^b
Soaking in 2 h	19.7±0.085 ^a	29.32±0.26 ^c
Soaking in 3 h	19.62±0.32 ^a	29.23±0.38 ^c

Data are the mean values ± SD; n = 3; ANOVA analysis followed by Duncan with P<0,05

*Dry basis (db)

Table 4 Moisture and Ash Content of *Ulva lactuca* (L.) after drying treatment

Treatment	Moisture content (gr/100 gr)	Ash content* (gr/100 gr)
Oven drying	16.79±1,01 ^b	37.79±0,07 ^a
Sun drying	19.48±1,99 ^{ab}	38±0,97 ^a
Shade drying	21.58±0,35 ^a	38.12±0,52 ^a

Data are the mean values ± SD; n = 3; ANOVA analysis followed by Duncan with P<0,05

*Dry basis (db)

Color

Color is one of the most important appearance attributes of food materials since it influences consumer acceptability (Akoy et al., 2008), it also showed product quality changes. Changes in the color surface caused by the thermal process may occur due to enzymatic/non-enzymatic processes (Silva et al., 2019). In this study, color examination by chromameter with the scale of L*, a*, and b*. L* represented brightness/lightness, a* represented positive values for reddish colors and negative values for greenish ones, and b* represented positive values for yellowish colors and negative values for bluish ones (Granato and Masson, 2010). This study were showed color changes of *U. lactuca* affected by time soaking and drying methods.

Effect of Soaking Time on the Color of *Ulva lactuca* (L.)

Soaking treatment showed a negative effect on *U. lactuca* color because it faded the brightness of *U. lactuca*. In the **Table 5**, the L* value of unsoaking *U. lactuca* that lower than the soaking samples. Siah et al., (2014) reported that during soaking macroalga with water, some important water-soluble nutrients in macroalga like minerals and protein will leach out. This also including with the macroalga color pigment. Suryani et al. (2015) said that the bleaching technique is a process of removing pigment. It will lead to discoloration in macroalgae. The value of unsoaking *U. lactuca* was lower significantly than soaking samples. It means

that soaking *U. lactuca* in tap water could decrease the green color.

The b* value represents the yellow to blue color. Soaking treatment increase the yellow color of *U. lactuca*. It caused a dull color in the samples. Soaking in one hour showed the highest lightness (L*) and yellow (b*) values and caused significant color differences with control (ΔE). It showed the phenomenon that the color leaching process is optimum at one hour. After one hour, the color became Table again which showed in the number of ΔE at two and three hours.

Effect of Drying Method on the Color of *Ulva lactuca* (L.)

The brightness (L* scale) of *Ulva lactuca* is significance different in various methods of drying. Drying affected to increase the brightness of samples shown on the L* value of fresh *U. lactuca* that lower than drying samples. Shade drying had the lowest brightness according to another drying method because of low temperature and low intensity of sunlight. The temperature of shade drying is below 30°C, so it could retain the dark green color of seaweeds. According to the green to red color represent on a* scale, the green color of oven drying no significant difference with sun drying while shade drying had the lowest green. The b* scale of oven drying highest significance which means the yellow color of oven drying samples was the highest intensity. Shade drying retains the color of *U. lactuca* seen from the lowest ΔE value, compared to the fresh sample.

Table 5 Color of *Ulva lactuca* (L.) after soaking treatment

Treatment	L	A	B	ΔE
No soaking	37.15±0.18 ^c	-7.12±0.17 ^c	19.44±0.54 ^d	
Soaking in 1 h	44.84±0.46 ^a	-6.12±0.1 ^b	25.17±0.46 ^a	9.65±0.48 ^a
Soaking in 2 h	42.03±0.34 ^b	-5.27±0.1 ^a	21±0.80 ^c	5.48±0.51 ^b
Soaking in 3 h	41.99±0.36 ^b	-6.05±0.13 ^b	22.75±0.61 ^b	6±0.61 ^b

Data are the mean values ± SD; n = 3; ANOVA analysis followed by Data are the mean values ± SD; n = 3; ANOVA analysis followed by Duncan with P<0,05

ΔE compared unsoaking - soaking *U. lactuca*

Table 6 Color of *Ulva lactuca* (L.) after drying treatment

Treatment	L*	a*	b*	ΔE
Fresh	21.2±0.61 ^d	-5.54±0.14 ^b	9.79±0.28 ^d	-
Oven drying	37.15±0.18 ^b	-7.12±0.17 ^c	19.44±0.54 ^a	18.71±0.41 ^a
sun drying	38.46±0.59 ^a	-6.82±0.25 ^c	15.69±0.77 ^b	18.29±0.68 ^a
shade drying	28.41±0.35 ^c	-3.95±0.16 ^a	13.87±1.02 ^c	8.46±0.74 ^b

Data are the mean values ± SD; n = 3; ANOVA analysis followed by Duncan with P<0,05
 ΔE compared drying-fresh *U. lactuca*

CONCLUSION

Post-harvest treatment without soaking and drying with oven or sun drying could be used for retaining protein content and in-vitro digestibility of *U. lactuca*.

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