e-ISSN:2528-0759; p-ISSN:2085-5842
Available online at https://e-journal.unair.ac.id/JIPK

JIPK
(JURNAL ILMIAH PERIKANAN DAN KELAUTAN)
Scientific Journal of Fisheries and Marine

Short Communication

Effect of Cooking Methods on Nutritional Quality of Sea Lettuce (*Ulva lactuca*) Flakes

Ardiba R. Sefrienda[1]*, Jasmadi[1], Hilda Novianty[1], Indyaswan T. Suryaningtyas[1,2], and Rachma Wikandari[3]

[1]Research Center for Food Technology and Processing, National Research and Innovation Agency, Yogyakarta, 55861. Indonesia
[2]Department of Food and Life Science, Pukyong National University, Busan. South Korea
[3]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University Yogyakarta. Indonesia

OPEN ACCESS

ARTICLE INFO

Received: May 24, 2022
Accepted: July 08, 2022
Published: July 28, 2022
Available online: January 29, 2023

*) Corresponding author:
E-mail: ardiba2@gmail.com

Keywords:
Green Seaweed
Roasting
Boiling-Roasting
In-vitro Protein Digestibility
Amino Acid

Abstract

Plant-based protein has been increasingly demanded as a sustainable protein source. Sea lettuce (*Ulva lactuca*) is one of the potential sources as plant protein due to its high protein content. During processing, the sea lettuce is exposed to heat which might affect its nutrition, particularly the protein quality. This study aimed to evaluate two different cooking processes on the nutritional quality of the sea lettuce based on the proximate and protein quality analyses. The samples were raw, roasted, and boiled-roasted sea lettuce. All treatment using temperature 100°C. The protein quality was assessed by *in-vitro* protein digestibility, solubility, and amino acid profiles. The result showed that boiling treatment followed by roasting treatment had significantly higher protein content, amino acid score and essential amino acid index, and predicted-protein efficiency ratio compared with the value of roasting treatment only. It resulted in protein content of 18.87% (dry basis), amino acid score of 37.96%, essential amino acid index of 79.41% and predicted-protein efficiency ratio of 2.58. Therefore, boiling followed with roasting process is recommended to maintain the nutrition quality of sea lettuce.

Cite this as: Sefrienda, A. R., Jasmadi, Novianty, H., Suryaningtyas, I. T., & Wikandari, R. (2023). Effect of Cooking Methods on Nutritional Quality of Sea Lettuce (*Ulva lactuca*) Flakes. *Jurnal Ilmiah Perikanan dan Kelautan*, 15(1):142–151. http://doi.org/10.20473/jipk.v15i1.36078

1. Introduction

Stunting is one major nutritional problem faced until today in many countries including Indonesia. About 30.8% of Indonesian children suffers from stunting (Kementerian Kesehatan Republik Indonesia, 2018). On the other hand, Indonesia is rich in diversity of high protein marine products such as algae. Algae are an abundant, inexpensive, and attractive resource for food or food ingredients with high-quality protein, dietary fiber, polysaccharides, polyunsaturated fatty acids (PUFAs), minerals, vitamins, pigments, and phytochemicals such as polyphenols (Bayomy, 2021). Sea lettuce (*Ulva lactuca*) is among macroalgae species that contains a considerable amount of protein. It has been reported that *U. lactuca* contains 13-26% protein in dry basis (Kim *et al*., 2011; Pangestuti and Kim, 2015; Rasyid, 2017; Shpigel *et al*., 2017; El-Din, 2019).

It is a fast growing, cosmopolitan species, which enables it to live everywhere. Kazir *et al*. (2019) reported that *Ulva* sp. grows at a rate of more than 20 g of dry weight.m^{-2}.day^{-1} in the Israeli Mediterranean Sea intertidal zone, it ranked among the highest within photosynthesizing organisms. No reports about *U. lactuca* growing in Indonesia, but it is abundant in Sepanjang Beach Gunungkidul in October to January. *U. lactuca* is also rich in amino acids, proteins, vitamins, and a promising species for commercial uses (Calheiros *et al*., 2019). It has higher protein content than brown seaweed (Tiwari and Troy, 2015). Therefore, it has the potential to be developed as a protein alternative. The complex polysaccharide structure and the presence of antinutritive substances such as phytic acids, phenolic compounds, and protease inhibitors may decrease protein accessibility of *U. lactuca* to gastrointestinal enzymes (Kim *et al*., 2011). Food processing is necessary to increase protein accessibility to human gastrointestinal system. Food processing using heat treatment causes partial or complete denaturation of the original protein structure, making the protein more accessible to gastrointestinal enzymes for better utilization (Maehre *et al*., 2016).

In Indonesia, *U. lactuca* is not cultivated but harvested. It is usually sold locally nearby the sea and consumed as flakes, in which it is fried at high temperature. Culinary processes greatly affects the color, flavor, and nutrients content of foods (Yong *et al*., 2019). Food processing, such as boiling, steaming, and vacuum cooking soften the *Ulva rigida* tissues, so it improves its odor like cooked fish, salted and dried fish and crustacean aromas (Sánchez-García *et al*., 2021). Frying could reduce the amount of protein and destroy some amino acids, changing the quality of protein composition in food. In addition, frying increase the fat content of the product. High temperature in cooking seaweeds can cause a high level of oxidative reactions since the morphology of *Ulva* sp. makes it highly sensitive to heat (Chen and Roca, 2018). Therefore, an alternative processing of sea lettuce is needed. Boiling using temperature at 100°C and roasting in 100°C is lower temperature than frying (165-185°C) (Oke *et al*., 2017). It can be expected to cause less negative impact on the protein quality. However, there is no study reported effect of boil-roast and roast treatment on protein quality of the *U. lactuca*. Therefore, this study aims to investigate the effect of boiling and roasting on the protein digestibility, protein solubility, and amino acid composition of *U. lactuca*.

2. Material and methods

2.1 Material

Fresh *Ulva lactuca* was collected from their natural habitat at Sepanjang Beach, Gunungkidul (8°8'12"S 110°34'0"E) in December 2020 until February 2021 (intertidal zone) (Figure 1). The samples were rinsed using clean sea water to minimize dirt, epiphyte, rubble, animal, and sand. The sample (10 kg) was sun dried for about 12 hours. The samples were stored at room temperature (29°C, RH 56%) in non-translucent plastic bags until further analysis no longer than 2 weeks. Three distinct treatments (uncooked, boiled-roasted, and roasted) were applied to the samples to determine protein digestibility, protein solubility, and amino acid composition.

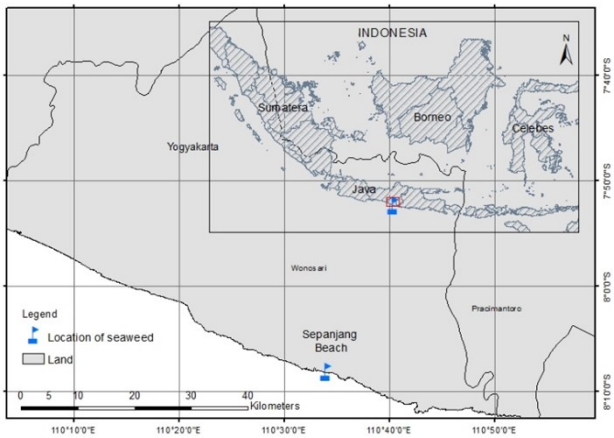

Figure 1. The location where *Ulva lactuca* was collected in this study

Chemical and reagents were used such as LC–MS grade acetonitrile, BSA standard, folin, cupric sulphate, potassium tartrate, and sodium carbonate were supplied by Merck, Germany. Analytical grade AccQ-Tag kit and Pierce Amino Acid Standard H (2.5 mM amino acid and 1.25 mM cysteine) from Waters

(Millford, MA, USA). L-glutamic acid, α-aminobutyric acid (AABA), γ-aminobutyric acid (GABA), trans-4-hydroxy-l-proline, L-cysteic acid monohydrate, L-methionine sulfone, cell free 13C–15N-labeled amino acid mixture, and pepsin enzyme were purchased from Sigma-Aldrich, Singapore.

2.2 Methods

2.2.1 Cooking treatments

Sun dried *U. lactuca* was divided into 3 groups of treatments, i.e., raw, roast treatment, and boil-roast treatment. Each group contained 100 gr of dried *U. lactuca*. Roast samples were roasted using electric oven (Kirin, Indonesia) at 100°C, 10 min. boil-roast samples were boiled in boiling water (100°C) for 30 minutes according to the best heat treatment time of *Palmaria pamata* by Maehre *et al.* (2016) with ratio of sun-dried *U. lactuca*: water (1:2), and then roasted at 100°C for 10 min. All samples were crushed into flakes using a blender to a size of approximately 1-3 mm and done in triplicate.

2.2.2 Proximate composition

The proximate composition was analyzed in triplicate according to (AOAC, 2000). In which, 2 gr per replicate for moisture and ash content by using thermogravimetric methods, 2 gr per replicate for crude fat content by using Soxhlet extraction method, 500 mg for each replicate for crude protein content using Kjeldahl methods, and carbohydrate content by difference.

2.2.3 In-vitro protein digestibility

In-vitro digestion tests are used to determine protein digestibility (Tanaka *et al.*, 1978). About 200 mg of raw and treated samples were suspended in 9 ml of Walpole buffer (HCl.NH_3COONa; 0.1 N; pH 2) and 1 ml of 2% pepsin enzyme, then incubated for 5 hours at 37°C, with continuous shaking at 100 RPM in an orbital incubator (Stuart S1500, UK). Subsequently, samples were centrifuged for 20 minutes with PLC Series centrifuge, South Korea. Five milliliters of the supernatant were combined with 5 ml of a 20% trichloroacetic acid (TCA) solution, incubated for 15 hours at room temperature and filtered with Whatman No. 541 paper. Kjeldahl methods were used to determine the crude proteins in the filtrate. The protein digestibility was then calculated based on Equation 1.

$$\%\text{Protein digestibility} = \frac{\%\text{ filtrate protein content}}{\%\text{ sample protein content}} \quad \ldots\text{Eq. 1}$$

2.2.4 Protein solubility

One hundred microliters of filtrate from *in-vitro* digestibility test (2.4) then measured the solubility of total protein using Lowry-Folin methods. In a microplate, 100µl of BSA standard and samples were pipped then mixed with 200 µl alkaline solution consist of NaOH 0,1 N and Na_2CO_3 2% are mixed with $CuSO_4.5H_2O$ 1% solution and Na_2Tartrate.2(H2O) 2% with a v/v ratio of 100:1:1. Incubated mixture within 10-15 minutes. Then 20 µl Folin-ciocalteu 0.1N was added, and the mixture was incubated for 30 minutes. At a wavelength of 650 nm, the absorbance was measured (Lowry *et al.*, 1951).

2.2.5 Amino acids composition

Amino acids composition was evaluated in the accredited and certified laboratory of Saraswanti Indo Genetech (SIG), Bogor, West Java, Indonesia. The method uses LC-MSMS and UPLC according to (Dahl-Lassen *et al.*, 2018). Methionine and cystine determined using LC-MSMS. L-cystic acid and L-methionine sulfone 1000 mg. L^{-1} used as standard injected 7 spots using LC-MSMS with a range 0.05-1.07 mg. L^{-1} in L-methionine and 5-107 mg. L^{-1} L-cystic acid. Samples were prepared by freeze-thawing and addition of natrium bisulfite, adjust the pH to 2.2±0.05, centrifugation (1400 RPM, 3 min) and filtering with RC 0,2 µm filter membrane. As much as 2µl sample was injected to LC-MSMS coupled with Imtakt Intrada Amino Acid column, mobile phase formic acid 0.1% in acetonitrile and ammonium formate 100mM, flow rate 0.4 $ml.min^{-1}$.

Histidine, isoleucine, leucine, phenylalanine, threonine, valine, lysine, serine, glutamic acid, alanine, arginine, glycine, aspartic acid, tyrosine, and proline determined with UPLC (Waters, Milford, USA). Samples (0.1-1 gr) hydrolyzed with HCl, filtrated with syringe filter 0.2 µl, added standard internals and derivatized. Samples were then injected into UPLC using column C18 at 49°C column temperature. Eluent accq. tag ultra and aqua bides with gradient pump system used as mobile phase. Samples were detected using PDA detector. Tryptophan could not be identified because of its sensitivity to acid hydrolysis (Motta *et al.*, 2019).

2.2.6 Estimation of amino acids quality

2.2.6.1 Amino acid score (AAS)

Amino acid score calculated according to WHO (2007) using Equation 2. Whereby, a ratio between amino acid in the sample and in the required pattern was calculated:

$$AAS = \frac{\text{mg of amino acid in 1 gr test protein}}{\text{mg of amino acid in requirement pattern}} \quad \text{...Eq. 2}$$

2.2.6.2 Essential amino acid index (EAAI)

Essential Amino Acid Index indicated the quality of amino acid composition in samples. It was calculated using the Equation 3:

$$EAAI = 10^{\log EAA} \quad \text{...Eq. 3}$$

$$EAA = 0.1\,[\log(a1.a1s^{-1}.100) + (a2.a2s^{-1}.100) + \cdots + (an.ans^{-1}.100) \quad \text{...Eq 4}$$

where:

a_1, a_2, and a_n are the content of amino acids in the sample, while $a_{1s,}$ $a_{2s,}$ and a_{ns} are essentials amino acid requirements in the protein standard (WHO, 2007).

2.2.6.3 Predicted PER (P-PER)

The predicted PER was calculated by considering Leu and Tyr amino acid as mentioned in Equation 5 adapted from (Oluwaniyi *et al*., 2010).

$$P - PER = -0.468 + 0.454(Leu) - 0.105(Tyr) \quad \text{...Eq 5}$$

2.2.7. Statistical analysis

The triplicate data was interpreted as a mean with a standard deviation. For statistical analysis, ANOVA was used, followed by Duncan for mean comparison. Different letters in the column are statistically different at a significance level 95%.

3. Result and Discussion

3.1 Result

3.1.1 Proximate composition

The result showed that cooking method affect the proximate composition of the *U. lactuca* flakes (Table 1). ANOVA analysis showed that raw, boiled-roasted, and roasted *U. lactuca* flakes had statistically significant difference ($P < 0.05$) in crude protein, ash, crude fat, and moisture content. The carbohydrate content was calculated using by-difference method. Statically analysis revealed that roasting did not affect the carbohydrate content of *U. lactuca* while boiling-roasting treatment did.

3.1.2 In-vitro protein digestibility and protein solubility

In-vitro protein digestibility of raw sample was 53.05% (Figure 2). Meanwhile, the *in-vitro* protein digestibility of boiled-roasted and roasted samples reach 77.31% and 78.3%, respectively, which were significantly higher ($P < 0.05$) than the raw sample. Both boiled-roasted and roasted samples showed no significant difference ($P < 0.05$).

Boiling-roasting and roasting treatments increase the total protein solubility of the samples from 69.92% to 78.77% in boiled-roasted samples and 84.54% in roasted samples. Cooking treatments increase total protein solubility significantly ($P < 0.05$) and roasted treatment showed the highest total protein solubility compared to raw and boiled-roasted samples.

3.1.3 Amino acid composition

The total amino acid of boiled-roasted samples was 818.25 mg/gr of *U. lactuca* protein and roasted U. lactuca was 804.30 mg/gr of *U. lactuca* protein. These amounts were lower than raw *U. lactuca*, which contained 899.42 mg/gr of *U. lactuca* protein. However, cooking treatment increase % EAA (essential amino acid) significantly ($P < 0.05$).

Estimation of amino acids quality could be identified by calculating the essential amino acid scores (EAAS), essential amino acid index (EAAI), and predicted-protein efficiency ratio (P-PER). Essential amino acid scores were calculated using standard and equation from WHO (2007). The limiting of amino acids is marked by bold letters (Table 3).

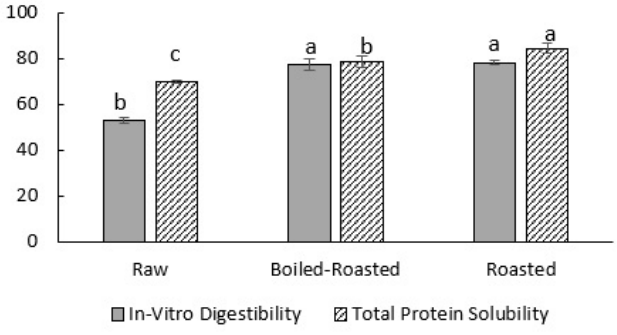

Figure 2. Mean values of triplicates of *In-vitro* protein digestibility and protein solubility of raw, boiled-roasted, and roasted *U. lactuca*, different letters indicate significant differences in each measurement, n= 3; ANOVA analysis followed by Duncan with $P < 0.05$

Table 1. Proximate composition of *U. lactuca* flakes after cooking treatment

Treatment	Raw (control)	Boiled-Roasted	Roasted
Crude protein (gr (100 gr DM)$^{-1}$)	16.29+0.24^{c}	18.87±0.32^{a}	17.22+0.18^{b}
Ash (gr (100 gr DM)$^{-1}$)	39.34±0.49^{a}	27.53+0.11^{c}	38.58±0.30^{b}
Crude Fat (gr (100 gr DM)$^{-1}$)	1.24±0.02^{b}	1.43±0.01^{a}	1.00+<0.01^{c}
Moisture (gr (100 gr DM)$^{-1}$)	19.64±0.08^{a}	12.39±0.06^{b}	4.41±0.02^{c}
Carbohydrate* (gr (100 gr DM)$^{-1}$)	43.14±0.32^{b}	52.18±0.37^{a}	43.21±0.50^{b}

Data are the mean values ± SD; n = 3; ANOVA analysis followed by Duncan with $P < 0.05$; Different superscript letters in the same column indicate significant difference ($P < 0.05$).
*by difference, DM= dry matter

Table 2. *Ulva lactuca* amino acid composition of raw, roasted, and boiled-roasted *U. lactuca* (mg/gr of *U. lactuca* protein)[x]

Amino acids	Raw	Boiled-Roasted	Roasted	WHO standard[y]
His*	17.81±0.04^{a}	5.69±0.02^{c}	16.29±<0.01^{b}	15
Ile*	37.87±0.01^{b}	38.76±0.01^{a}	35.90±0.02^{c}	30
Leu*	72.20±0.04^{b}	72.71±0.03^{a}	67.38±0.02^{c}	61
Met*	6.20±<0.01^{b}	7.44±<0.01^{a}	4.91±<0.01^{c}	22
Phe*	69.65±0.02^{a}	55.97±0.02^{c}	59.21±0.07^{b}	41
Thr*	56.89±0.04^{a}	53.83±0.01^{b}	53.68±<0.01^{c}	25
Val*	55.15±0.04^{b}	55.57±<0.01^{a}	51.81±<0.01^{c}	40
Lys*	39.64±0.01^{a}	37.5±0.01^{b}	33.83±0.02^{c}	48
Ser	61.02±0.01^{a}	58.47±0.04^{b}	58.11±0.01^{c}	n.e.
Glu	100.55±0.12^{a}	88.24±0.09^{b}	86.04±0.05^{c}	n.e.
Ala	68.27±0.09^{b}	69.15±0.07^{a}	63.24±0.1^{c}	n.e.
Arg	74.29±0.03^{a}	60.46±0.04^{c}	64.02±0.02^{b}	n.e.
Gly	65.56±0.01^{a}	61.85±0.02^{c}	62.33±0.02^{b}	n.e.
Asp	93.36±0.1^{a}	85.63±0.08^{b}	77.61±0.04^{c}	n.e.
Tyr	37.78±0.03^{a}	23.78±0.02^{c}	29.96±0.02^{b}	n.e.
Pro	43.17±0.01^{a}	43.2±<0.01^{b}	39.96±0.02^{c}	n.e.
Cys	n.d.	3.54±<0.01	n.d.	n.e.
Σ TAA	899.42±0.11^{a}	818.25±0.09^{b}	804.30±0.12^{c}	-
Σ EAA	355.41±0.02^{a}	327.47±0.08^{b}	323.02±0.21^{c}	-
% EAA	39.52±0.01^{c}	40.02±0.02^{b}	40.16±0.02^{a}	-
EAA/NEAA	0.65±<0.01^{b}	0.67±<0.01^{a}	0.67±<0.01^{a}	-

- n.d.: not detectable (<0.88 mg/gr of *U. lactuca* protein); n.e.: not established
- different letters between columns/treatments indicate significant differences, n= 3; ANOVA analysis followed by Duncan with $P < 0.05$
- * Essential amino acid.
- [x]Values presented are means of triplicates ± SD; ANOVA analysis followed by Duncan with $P < 0.05$
- [y](WHO, 2007)

Table 3. Essential amino acid scores (%), Essential amino acid index (EAAI), and P-PER of raw, roasted, and boiled-roasted *U. lactuca*

Amino acids	Raw	Boiled-Roasted	Roasted	WHO Standard[y]
His*	118.76	**37.96**	108.6	15
Ile*	126.24	129.21	119.67	30
Leu*	122.37	123.24	114.21	61
Met+cys	**28.16**	49.93	**22.34**	22
Phe+Tyr*	262.01	194.52	217.49	41
Thr*	227.57	215.31	214.72	25
Val*	137.88	138.92	129.53	40
Lys*	88.09	83.33	75.18	48
Try*	n.d.	n.d.	n.d.	-
EAAI	46.02	51.92	51.76	
P-PER	2.41	2.58	2.28	

n.d.: Not determined

[y](WHO, 2007)

3.2 Discussion

In general, macroalgae (or seaweeds) have a high protein content (10–47%) (Dominguez and Loret, 2019). Cooking treatment was required before consumption. Roasted and boiled-roasted treatment were two of the most used methods to produce ready-to-eat *U. lactuca* flakes. This cooking treatment may influence the protein quality of *U. lactuca* flakes. Therefore, to determine the protein quality of *U. lactuca* flakes, various characteristics such as proximate, *in-vitro* protein digestibility, protein solubility, and amino acid profile were evaluated.

3.2.1 Proximate composition

The crude protein content of boiled-roasted and roasted increased by 2.58% and 0.93% compared to raw sea lettuce, respectively. This result was in accordance to Bayomy (2021) who reported that heat treatments on *Ulva lactuca* could increase or decrease the nutrition depending on method preparations and heat exposure duration. Adu *et al.* (2015) showed that thermal cooking process significantly increased crude protein of Indian almond seed for about 2.83%. The increase was attributed to the destruction of antinutritional factors resulting in release of nutrients.

Cooking affected the ash content of the sample. The lowest ash content was found in boiled-roasted samples. One explanation could be during boiling, some soluble compound including micro and macro elements would dissolve or leach out in water thus reduce the ash content (Bayomy, 2021). The decrease of ash content during washing and boiling was also reported by several studies of *Ulva lactuca* from Sepanjang Beach Yogyakarta that decrease from 35.32% to 13.63% and *Caulerpa* sp. from Tual Maluku that decrease from 1.16% to 0.98% (Nurjanah *et al.*, 2018; Poeloengasih *et al.*, 2019).

Furthermore, the decrease of ash content in cooking treatment affected the elevation of others proximate parameters namely crude protein, crude fat, and carbohydrate. Our result demonstrated that the fat content of the boiled-roasted sample was higher than that of the raw sample. Nwafor *et al.* (2017) reported there was a significant effect of the thermal cooking process (boiling/roasting) which increase crude fat of legume seed compared to untreated samples. The application of hot water to U. lactuca sample might be enabling the fat content to be released from the matrices of samples. Okibe *et al.* (2015) suggested that increasing trend of fat was the result of thermal treatments due to heat breaking the bond between lipoprotein and polysaccharides which cause lipids to be released and solubilized in the extracting solvent. Thermal processing decreased the moisture content. It caused water to evaporate during roasting, which affects the moisture content. Boiling treatment could increase the in-bound water in the samples that could not be evaporated by heating treatment at 100° C for 10 minutes, so that the moisture content of boiled-roasted samples are higher than roasted samples. Boiled-roasted samples showed the highest carbohydrate content because of the leaching of ash content in boiling treatment affected the total percentage of carbohydrate that calculated by difference.

3.2.2 Protein quality

3.2.2.1 In-vitro protein digestibility and protein solubility

Protein digestibility was one of the parameters determining the protein quality. Most seaweeds, especially green and red algae, seem to be an interesting and suitable source of proteins for human and animal food but their digestibility is limited by anti-nutrients compounds such as polysaccharides (Fleurence *et al*., 2018). This might be because heat treatment not only improve the taste and texture, increase the bioavailability of certain nutrients but also decrease the anti-nutrients in seaweed as stated by Maehre *et al*. (2016).

The *in-vitro* protein digestibility of both the boiled-roasted and roasted treatments were not significantly different. It can be concluded that both boiling and roasting treatment were recommended to increase *in-vitro* protein digestibility of *U. lactuca.* Córdova-Ramos *et al*. (2020) articulated that, generally applying heat and water weakens the original structure of the plants, resulting in a softer, less solid texture, which probably influences the higher *in-vitro* protein digestibility of the treatment samples.

Protein solubility was one of the parameters used to assess the protein quality in samples. It was related to the interaction between water molecules and peptide bond, hydrogen bond or the side. Roasting can modify the dietary protein in raw form, wherein hydrophobic groups are oriented inward and hydrophilic groups are oriented outward (Yan *et al*., 2021). Polysaccharides can block the nutrition (Fleurence *et al*., 2018), and cooking treatment may destroy the polysaccharide structure. Cooking treatment does not always increases the protein solubility of samples.

The smaller particle size cause higher protein solubility (Pereira *et al*., 2016). Flakes size that produced from roasted samples was small, so it increases the protein solubility. Roasting *U. lactuca* flakes only 10 minutes may optimally retains protein solubility of *U. lactuca*. In line with Yan *et al*. (2021) prolonged roasting time of cashew nuts from 8 to 12 min decreases the protein solubility. Boiled-roasted samples were high in crude protein content but low in protein solubility because of the flake size being bigger than that of roasted flakes size so it lowers the protein solubility.

3.2.2.2 Amino acid composition

Amino acid composition was one parameter to evaluate the quality of food protein. The amino acid content was affected by roasting and boiling-roasting treatments. Heat treatment was responsible for reductions of protein value in meat-based foods as result of the destruction or unavailability of the constituent amino acids. Heat treatment duration contributed to the change in amino acid contents of products (Bi *et al*., 2021). In comparison to a prior study, the Total Amino Acid (TAA) of all samples in this investigation is still lower than the value reported by Bikker *et al*. (2016) for *U. lactuca* 998.86 mg/g of seaweed protein. The source and handling of Bikker's seaweeds may have contributed to the higher TAA levels because they collected and preserved the seaweed in freeze dried condition prior to analysis. Freeze drying can prevent the loss of critical chemical constituents in seaweed during the drying process when compared to conventional approaches (Badmus *et al*., 2019).

3.3.2.2.1 Non-essential amino acids (NEAAs)

Glutamic acid of *U. lactuca* is the most prevalent amino acid composition in all samples. It elicits umami taste by stimulating the umami receptor (Mouritsen *et al*., 2019). Boiling-roasting and roasting treatment decrease the glutamic acid content because of released amino acid during the process. Similar phenomenon is reported in jackfruit seeds, in which the glutamic acid of boiled jackfruit seeds was lower compared to the raw (Amechi and Ngozi, 2017). Serine, arginine, aspartic acid, and tyrosine declined during cooking treatment. Boiling-roasting treatment increased alanine, proline, and cystine. Roasting treatment did not increase the non-essential amino acids in *U. lactuca*.

3.3.2.2.2 Essential amino acids (EAAs)

Boiling-roasting treatment significantly increase Isoleucine, Leucine, and Valine content in *U. lactuca* flake, while roasting treatment decrease the essential amino acids (Table 2). Total essential amino acids in raw sample were 355.41 mg.gr^{-1} protein of *U. lactuca*, while it decreased below 10% in the boiled-roasted and roasted samples. Nevertheless, the relative (%) of essential amino acids and ratio of EAA/NEAA of samples after cooking treatment were higher than raw samples because of the non-essential amino acids were lower for the cooked samples. Similar findings were reported in the study of Maehre *et al*. (2016), where the relative of EAA of *Palmaria palmata* and *Alaria esculenta* increased after the boiling process for 30 minutes.

3.3.2.2.3 Estimation of amino acids quality

Estimation of amino acids quality identified by calculating essential amino acid scores and comparing with WHO standard for essential amino acid.

Roasting decreases histidine although the amount was still above WHO standard on amino acid requirement (WHO, 2007). The boiled-roasted sample had lower histidine than that of roasted sample. This cooking process decreases the histidine to a lower level than that of WHO standards from 118.76 % (raw seaweed) to 108.6 % (roasted seaweed) and 37.96 % (boiled-roasted seaweed). This reveals that histidine content of boiled-roasted seaweed was lower than recommended by the World Health Organization.

The content of isoleucine, leucine, phenylalanine, threonine, and valine of *U. lactuca* varies in all samples, but the level of amino acids was still above WHO requirement. Methionine-cystine and lysine of *U. lactuca* were below WHO requirement. AAS of boiled-roasted samples based on methionine-cystine content amounted to 49%, two times higher than raw and roasted sample which made up 28.16% and 22.34%, respectively. Maehre *et al*. (2016) also had same finding that boiling treatment significantly increased methionine and cystine of *Palmaria palmata*. Tryptophan was not detected in all samples.

High quality of amino acids profile could be determined by high essential amino acid index (EAAI) and P-PER values. The EAAI value was considered to be high, moderate, and low if the values scores above 90%, in the range of 70–89% and below 70%, respectively (Brown and Jeffrey, 1992). The EAAI of all the samples of *U. lactuca* were below 70%, which reveals that cooking process did not increase the amino acid quality. The P-PER of all samples of *U. lactuca* obtained in this study were considered high. The value was close to P-PER of casein in milk with the value of 2.5 as standard reference (Munro, 2002).

Boiled-roasted treatment increased the EEAI 12.82% and P-PER 7% of *U. lactuca* compared with raw samples while roasted *U. lactuca* increased the EEAI 12.47% and decreased the P-PER 5.4% compared with raw samples. Boiled-roasted treatment in temperature 100°C had the good impact to increase the protein quality of *U. lactuca.*

4. Conclusion

Boiling-roasting treatment of *U. lactuca* in flakes production increases its protein quality as indicated by its protein content, *in-vitro* protein digestibility, protein solubility, relative percentage (%) Essential Amino Acid, Essential and Non-Essential Amino Acid Ratio, and Essential Amino Acid Index compared to the raw one. This study offers an alternative cooking method to enhance the nutritional quality of seaweed-derived food products.

Acknowledgment

This research was funded by Finance Ministry LPDP Priority National Research Program number 29/E1/II/PRN/2020.

Authors' Contributions

All authors have contributed to the final manuscript. The contribution of each author is as follows, Ardiba R. Sefrienda; designed concept, analyzed data, drafted manuscript. Hilda Novianty: designed concept, drafted, and revised manuscript. Jasmadi; designed concept, analyzed data, drafted manuscript. Indyaswan T. Suryaningtyas; drafted and revised manuscript. Rachma Wikandari; checked and revised manuscript. All authors discussed the results and contributed to the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding Information

This research Project was fully sponsored by Finance Ministry LPDP Priority National Research Program with grant number 29/E1/II/PRN/2020.

References

Adu, O. B., Ogundeko, T. O., Ogunrinola, O. O., Saibu, G. M., & Elemo, B. O. (2015). The effect of thermal processing on protein quality and free amino acid profile of *Terminalia catappa* (Indian Almond) seed. *Journal of Food Science and Technology*, 52(7):4637-4641.

Amechi, N. C., & Ngozi, E. O. (2017). Effect of boiling on amino acid composition of jackfruit (*Artocarpus heterophyllus*) seed from South East Nigeria. *Journal of Advances in Food Science & Technology*, 3(4):175-181.

AOAC. (2000). Official methods of analysis of the association of official analysis chemists (17th ed.). Washington D.C.: AOAC International.

Badmus, U. O., Taggart, M. A., & Boyd, K. G. (2019). The effect of different drying methods on certain nutritionally important chemical constituents in edible brown seaweeds. *Journal of Applied Phy-*

cology, 31(6):3883-3897.

Bayomy, H. M. (2021). Effects of culinary treatments on the physicochemical properties of *Ulva lactuca* collected from Tabuk coast of Red sea in Saudi Arabia. *Saudi Journal of Biological Sciences*, 29(4):2355-2362.

Bi, S., Wang, A., Lao, F., Shen, Q., Liao, X., Zhang, P., & Wu, J. (2021). Effects of frying, roasting and boiling on aroma profiles of adzuki beans (*Vigna angularis*) and potential of adzuki bean and millet flours to improve flavor and sensory characteristics of biscuits. *Food Chemistry*, 339:127878.

Bikker, P., van Krimpen, M. M., van Wikselaar, P., Houweling-Tan, B., Scaccia, N., van Hal, J. W., Huijgen, W. J. J., Cone, J. W., & López-Contreras, A. M. (2016). Biorefinery of the green seaweed *Ulva lactuca* to produce animal feed, chemicals and biofuels. *Journal of Applied Phycology*, 28(6):3511-3525.

Brown, M. R., & Jeffrey, S. W. (1992). Biochemical Composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae. 1. Amino acids, sugars and pigments. *Journal of Experimental Marine Biology and Ecology*, 161(1):91-113.

Calheiros, A. C., Reis, R. P., Castelar, B., Cavalcanti, D. N., & Teixeira, V. L. (2019). *Ulva* spp. as a natural source of phenylalanine and tryptophan to be used as anxiolytics in fish farming. *Aquaculture*, 509:171-177.

Chen, K., & Roca, M. (2018). Cooking effects on chlorophyll profile of the main edible seaweeds. *Food Chemistry*, 266:368–374.

Córdova-Ramos, J. S., Glorio-Paulet, P., Camarena, F., Brandolini, A., & Hidalgo, A. (2020). Andean lupin (*Lupinus mutabilis* sweet): processing effects on chemical composition, heat damage, and in vitro protein digestibility. *Cereal Chemistry*, 97(4):827-835.

Dahl-Lassen, R., van Hecke, J., Jørgensen, H., Bukh, C., Andersen, B., & Schjoerring, J. K. (2018). High-throughput analysis of amino acids in plant materials by single quadrupole mass spectrometry. *Plant Methods*, 14(8):1-10.

Dominguez, H., & Loret, E. P. (2019). *Ulva lactuca*, a source of troubles and potential riches. *Marine Drugs*, 17(6):1-20.

El-Din, S. M. M. (2019). Temporal variation in chemical composition of *Ulva Lactuca* and *Corallina mediterranea*. *International Journal of Environmental Science and Technology*, 16(10):5783-5796.

Fleurence, J., Morançais, M., & Dumay, J. (2018). Seaweed proteins. In R. Y. Yada (Ed.), Proteins in food processing. (pp. 245-262). Cambridge: Elsevier Ltd.

Tiwari, B. K., & Troy, D. J. (2015). Seaweed sustainability: food and non-food applications processing of seaweeds. Cambridge: Academic Press.

Kazir, M., Abuhassira, Y., Robin, A., Nahor, O., Luo, J., Israel, A., Golberg, A., & Livney, Y. D. (2019). Extraction of proteins from two marine macroalgae, *Ulva* sp. and *Gracilaria* sp., for food application, and evaluating digestibility, amino acid composition and antioxidant properties of the protein concentrates. *Food Hydrocolloids*, 87:194-203.

Kementerian Kesehatan Republik Indonesia. (2018). Hasil Utama Riskesdas 2018. Jakarta: Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan RI.

Kim, S. K., Pangestuti, R., & Rahmadi, P. (2011). Chapter 5 – Sea lettuces: culinary uses and nutritional value. *Advances in Food and Nutrition Research*, 64:57-70.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein Measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193(1):265-275.

Maehre, H. K., Edvinsen, G. K., Eilertsen, K. E., & Elvevoll, E. O. (2016). Heat treatment increases the protein bioaccessibility in the red seaweed dulse (*Palmaria palmata*), but not in the brown seaweed winged kelp (*Alaria esculenta*). *Journal of Applied Phycology*, 28(1):581-590.

Motta, C., Castanheira, I., Gonzales, G. B., Delgado, I., Torres, D., Santos, M., & Matos, A. S. (2019). Impact of cooking methods and malting on amino acids content in amaranth, buckwheat and quinoa. *Journal of Food Composition and Analysis*, 76:58-65.

Mouritsen, O. G., Duelund, L., Petersen, M. A., Hartmann, A. L., & Frøst, M. B. (2019). Umami taste,

free amino acid composition, and volatile compounds of brown seaweeds. *Journal of Applied Phycology*, 31(2):1213-1232.

Munro, P. A. (2002). Milk proteins: Caseins, functional properties and food uses. In H. Roginski (Ed.), Encyclopedia of dairy sciences. (pp. 1909-1915). Oxford: Elsevier.

Nurjanah, Jacoeb, A. M., Hidayat, T., & Chrystiawan, R. (2018). The change in fiber components of *Caulerpa* sp. seaweed (from Tual Maluku) due to boiling process. *Jurnal Ilmu dan Teknologi Kelautan Tropis*, 10(1):35-48.

Nwafor, F. I., Egonu, S. N., Nweze, N. O., & Ohabuenyi, S. N. (2017). Effect of processing methods on the nutritional values and anti-nutritive factors of *Adenanthera pavonina* L. (Fabaceae) seeds. *African Journal of Biotechnology*, 16(3):106-112.

Oke, E. K., Idowu, M. A., Sabukola, O. P., Adeyeye, S. A. O., & Akinsola, A. O. (2017). Frying of food: A critical review. *Journal of Culinary Science & Technology*, 16(2):1-21.

Okibe, F. G., Jubril, B., Paul, E. D., Shallangwa, G. A., & Dallatu, Y. A. (2015). Effect of cooking methods on proximate and mineral composition of fluted pumpkin (*Telfairia occidentalis*) leaves. *International Journal of Biochemistry Research & Review*, 9(2):1-7.

Oluwaniyi, O. O., Dosumu, O. O., & Awolola, G. V. (2010). Effect of local processing methods (boiling, frying and roasting) on the amino acid composition of four marine fishes commonly consumed in Nigeria. *Food Chemistry*, 123(4):1000-1006.

Pangestuti, R., & Kim, S. K. (2015). Seaweed proteins, peptides, and amino acids. *Seaweed Sustainability*, 125-140.

Pereira, R. N., Rodrigues, R. M., Ramos, O. L., Malcata, F. X., Teixeira, J. A., & Vicente, A. A. (2016). Production of whey protein-based aggregates under ohmic heating. *Food and Bioprocess Technology*, 9(4):576-587.

Poeloengasih, C. D., Srianisah, M., Jatmiko, T. H., & Prasetyo, D. J. (2019). Postharvest handling of the edible green seaweed *Ulva lactuca*: mineral content, microstructure, and appearance associated with rinsing water and drying methods. IOP Conference Series: *Earth and Environmental Science*, 253(1):012006.

Rasyid, A. (2017). Evaluation of nutritional composition of the dried seaweed *Ulva lactuca* from Pameungpeuk waters, Indonesia. *Tropical Life Sciences Research*, 28(2):119-125.

Sánchez-García, F., Mirzayeva, A., Roldán, A., Castro, R., Palacios, V., Barroso, C. G., & Durán-Guerrero, E. (2021). Effect of different cooking methods on sea lettuce (*Ulva rigida*) volatile compounds and sensory properties. *Journal of the Science of Food and Agriculture*, 101(3):970-980.

Shpigel, M., Guttman, L., Shauli, L., Odintsov, V., Ben-Ezra, D., & Harpaz, S. (2017). *Ulva lactuca* from an Integrated Multi-Trophic Aquaculture (IMTA) biofilter system as a protein supplement in gilthead seabream (*Sparus aurata*) diet. *Aquaculture*, 481:112-118.

Tanaka, Y., Adoracion, R., Juliano, B. O., & Donald, B. (1978). Properties of whole and undigested fraction of protein bodies of milled rice. *Agricultural and Biological Chemistry*, 42(11):2015-2023.

WHO (World Health Organization). (2007). Protein and amino acid requirements in human nutrition: report of a Joint FAO/WHO/UNU Expert Consultation. Geneva: World Health Organization.

Yan, X., Wang, Y., Chen, Y., Xie, J., & Yu, Q. (2021). Effect of roasting duration on the solubility, structure, and IgE-binding capacity of cashew nut proteins. *Innovative Food Science and Emerging Technologies*, 68:102635.

Yong, W., Amin, L., & Dongpo, C. (2019). Status and prospects of nutritional cooking. *Food Quality and Safety*, 3(3):137-143.