

Characterization of *Ulva reticulata* Seaweed Salt: Amino Acid Profile, Chemical Composition, and Water Activity

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ABSTRACT

Seaweeds are one of the most abundant forms of biodiversity in Indonesian waters. *Ulva reticulata* is a potential seaweed. *U. reticulata* was developed as a salt preparation since it contains complete amino acids, especially glutamate and aspartic acid. These two amino acids contribute to the salty and umami flavors. Therefore, the objective of this study was to assess the chemical composition, water activity, and amino acid profile of *U. reticulata* seaweed salt preparations. This study consisted of three treatments, including treatment without the addition of maltodextrin (P0), addition of 2% maltodextrin (P1), and addition of 4% maltodextrin (P2), which was repeated four times. The data obtained were analyzed using ANOVA with SPSS. The results showed that *U. reticulata* seaweed salt has an average moisture, ash, fat, protein, and carbohydrate content of 4.60-6.83%, 29.39-62.15%, 0.96-1.45%, 2.03-3.87%, 26.17-61.16%, respectively. Furthermore, the resulting water activity values averaged 0.33-0.39. For the amino acid profile, 16 types of amino acids were confirmed and among the highest values were the types of amino acids alanine, aspartic acid, glycine, glutamic acid, leucine and serine with respective values of 1639.40-1940.56ppm, 1982.17-2104.82ppm, 2199.16-2276.03ppm, 1872.09-2215.11ppm, 1020.70-1099.97ppm, 2052.42-2620.07ppm. These results indicate that the *U. reticulata* seaweed salt produced has a long shelf life and contains amino acid compounds that contribute to the salty and umami flavor.

INTRODUCTION

Seaweed is one of the potential marine organisms to be developed in the fields of pharmaceutical, cosmetic, food, and nutraceutical (Cotas *et al.*, 2024). Seaweed contains macronutrients, such as proteins (essential/non-essential amino acids), fat (fatty acids), carbohydrates, and fiber (Nite *et al.*, 2022; Meiyasa *et al.*, 2023; Tarigan *et al.*, 2023; Yiwa & Meiyasa, 2023; Meiyasa *et al.*, 2024). In addition, seaweed also contains

micronutrients, such as vitamins (A, B1, B2, B3, B5, B6, B9, B12, C, D, and E), polyphenols, sterols, pigments (chlorophyll, fukoxanthin, astaxanthin, and phycobilin), and minerals (iodine, selenium, iron, and zinc) (Badmus *et al.*, 2024; Bhat *et al.*, 2024; Bhuyan *et al.*, 2024; Matos *et al.*, 2024; Xie *et al.*, 2024). Furthermore, seaweed is rich in bioactive compounds such as alkaloids, flavonoids, saponins, tannins, phenols, steroids, sitosterol, and stigmasterol (Tarigan *et al.*, 2023; Bouzenad *et al.*, 2024; Jang *et al.*, 2024; Kim *et al.*, 2024; Meiyasa *et al.*, 2024; Sadeghi *et al.*, 2024). These bioactive compounds have antioxidant, antimicrobial, anti-inflammatory, antitumor, anticancer, antiviral, and antidiabetic effects (Meiyasa *et al.*, 2024). *Ulva reticulata* is a green seaweed that can be developed in the nutraceutical field.

U. reticulata is quite abundant in Indonesian waters, such as in the waters of Maluku, Bali, Java, and East Nusa Tenggara, as well as in the waters of East Sumba (Lokollo, 2019; Shobir, 2019; Meiyasa *et al.*, 2020; Kolo *et al.*, 2021; Meiyasa *et al.*, 2024). However, to date, *U. reticulata* has not been optimally utilized. Based on previous research, *U. reticulata* from Sumba Waters has been reported to be safe and non-toxic using brine shrimp lethality test (BSLT). *U. reticulata* is reported to have 15.24% moisture, 23.10% ash, 0.33% fat, 10.68% protein, and 33.82% carbohydrates (Tarigan *et al.*, 2023). It has also been reported that *U. reticulata* has complete amino acids as essential amino acid (phenylalanine, isoleucine, valine, arginine, lysine, leucine, threonine, and histidine) and non-essential amino acids (serine, glutamic acid, alanine, glycine, aspartic acid, tyrosine, and proline) (Meiyasa *et al.*, 2023). Aspartate acid (0.52%) and glutamate acid (0.63%) had higher values than the other amino acids in *U. reticulata* (Meiyasa *et al.*, 2023). This indicates that *U. reticulata* can be developed as a raw material for manufacturing healthy salt. The healthy salt category has NaCl <60% with a Na/K ratio <1.0 (Seulalae *et al.*, 2023).

Several studies have reported that seaweed can also be used as a preparation for salt-making (Notowidjojo *et al.*, 2021; Seulalae *et al.*, 2023). Types of seaweed that have been used as salt preparations were *Ulva lactuca* (Kurniawan *et al.*, 2019; Nurjanah *et al.*, 2024), *Sargassum* sp. (Alfath 2020; Manteu *et al.*, 2021), *Caulerpa lentilifera* (Nomleni *et al.*, 2022), *Actinotrichia fragilis* (Nurjanah *et al.*, 2023), and *Chaetomorpha* sp. (Nurjanah *et al.*, 2024). However, to date, the development of *U. reticulata* seaweed as an alternative ingredient in the manufacture of healthy salt has not been reported. Therefore, this study aimed to determine the best preparation of *U. reticulata* powder as a salt based on chemical composition, water activity, and amino acid profile.

MATERIALS AND METHODS

1. Preparation of *U. reticulata*

U. reticulata was obtained from the coastal waters of Maudolung, East Sumba. The seaweed was washed with seawater, placed in a plastic bag, and transported to the

laboratory. Seaweed was dried using sunlight for 2-3 days. The dried seaweed was then prepared in the form of simplisia and was used to prepare the seaweed salt.

2. *Ulva reticulata* seaweed salt extract

The salt making process refers to Nurjanah *et al.* (2018). Samples of *U. reticulata* were weighed as much as 50g and were added with distilled water as much as 500mL (1:10). *U. reticulata* was extracted using a water bath at 60°C for 30 min and was then filtered using a 500 micron size filter cloth. This extraction process was carried out three times. The extract results were treated with the addition of 0, 2, or 4% maltodextrin. In the next stage, the extract was poured into a Petri dish and oven at 80°C for 22h. Next, the resulting salt was blended to produce an even salt particle size, followed by testing of its chemical composition, amino acid profile, and water activity of *U. reticulata* seaweed salt.

3. The parameters test

3.1 Proximate analysis of *U. reticulata* seaweed salt

The proximate analysis performed on *U. reticulata* included measuring moisture, ash, protein, fat, and carbohydrate content (carbohydrates based on differences) as per the outlines of AOAC (2005).

3.2 Amino acid profile of *U. reticulata* seaweed salt

Amino acid profile analysis was performed using Ultra-Performance Liquid Chromatography (UPLC) 18-5-17/MU/SMM-SIG (Waters, 2012).

3.3 Water activity of *U. reticulata* seaweed salt

Analysis of water activity of *U. reticulata* seaweed salt samples was carried out using an aw meter. The procedure was to put the sample into the container on the aw meter, and then let it stand for 15min. The aw value of the sample was observed using the tools.

4. Statistical analysis

The experimental design was a completely randomized design (CRD) for proximate data, water activity, and amino acid profiles with the addition of 0, 2, and 4% maltodextrin, and repeated four times. The data obtained were analyzed using ANOVA with SPSS (Statistical Process for Social Science). The test results were significantly different ($P < 0.05$), and the Duncan test was performed. Data were analyzed using SPSS 22.0 software.

RESULTS

1. Proximate and water activity of *U. reticulata* seaweed salt

U. reticulata seaweed salt was tested for its chemical composition, including moisture, ash, fat, protein, carbohydrate, and water activities. Water is an important

parameter for ensuring that the product is safe and has a long shelf life (**Hedegaard & Skibsted, 2024; Huang *et al.*, 2024**). The results showed that the *U. reticulata* seaweed salt produced had an average moisture content of 4.60 - 6.83%. Treatment P0 (without the addition of maltodextrin) had a water content of 6.83%, treatment P1 (the addition of 2%) of 6.33%, and treatment P2 (the addition of 4%) of 4.60% (Table 1). Furthermore, water activity (A_w) is a major factor inhibiting microbial growth. In some cases, A_w is the main parameter responsible for food stability, modulation of microbial responses, and determination of the types of microorganisms found in foodstuffs (**Tapia *et al.*, 2020**). A good A_w to maintain the shelf life of food by inhibiting microbial activity and enzymatic processes is <0.7 (**Allai *et al.*, 2023; Lima *et al.*, 2023**). This study showed that A_w in *U. reticulata* seaweed salt has a value <7 , which is an average of 0.33 - 0.39 (Table 1).

Table 1. Chemical composition and water activity of *U. reticulata* seaweed salt with different maltodextrin additions

Parameter	Treatment		
	P0	P1	P2
Moisture (%)	6.83 ^c ±0.04	6.33 ^b ±0.03	4.60 ^a ±0.01
Ash (%)	62.17 ^c ±0.24	41.16 ^b ±0.22	29.39 ^a ±0.73
Fat (%)	0.96 ^a ±0.02	1.06 ^b ±0.06	1.45 ^c ±0.04
Protein (%)	3.87 ^c ±0.06	2.79 ^b ±0.08	2.02 ^a ±0.05
Carbohydrate (%)	26.17 ^a ±0.35	48.66 ^b ±0.32	62.54 ^c ±0.27
Water activity	0.39 ^c ±0.32	0.36 ^b ±0.20	0.33 ^a ±0.01

Note: P0=No addition of maltodextrin; P1=Addition of 2% maltodextrin; Addition of 4% maltodextrin.

2. Amino acid profile

Amino acids (AA) are biomolecules that serve as building blocks of proteins and are precursors of important biological substances, such as neurotransmitters and nucleotides nukleotida (**Khan *et al.*, 2024**). The amino acids produced in this study included isoleucine (473.59-543.41ppm), leucine (1,020.70-1,099.97ppm), lysine (336.07-548.95ppm), methionine (3.92-7.46ppm), phenylalanine (642.44-709.78ppm), histidine (374.96-1,789.28ppm), and threonine (792.98-1,006.83ppm), valine (782.44-818.07ppm), arginine (788.61-1052.94ppm), alanine (1,639.40-1,940.56ppm), aspartic acid (1,982.17-2,104.82ppm), glycine (2,199-2,276.03ppm), proline (740.95-967.89ppm), serine (2,052.42-2,620.07ppm), tyrosine (<608.01 ppm), glutamate acid (1,872.09-2,215.11ppm) (Table 2).

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Table 2. Amino acid profile of *U. reticulata* seaweed salt with different maltodextrin additions

Amino acid profile (ppm)	Treatment		
	P0	P1	P2
Essential amino acids			
L-Isoleucine	473.59 ^a ±0.00	536.39 ^b ±1.21	543.41 ^c ±1.49
L-Leucine	1099.97 ^c ±2.96	1053.59 ^b ±2.29	1020.70 ^a ±1.70
L-Lysine	548.95 ^c ±0.00	535.20 ^b ±5.08	336.07 ^a ±0.00
L-Methionine	7.46 ^c ±0.00	5.12 ^b ±0.02	3.92 ^a ±0.00
L-Phenylalanine	709.78 ^c ±5.57	676.39 ^b ±5.96	642.44 ^a ±1.94
L-Histidine	374.96 ^a ±0.45	732.15 ^b ±2.16	1789.28 ^c ±0.45
L-Threonine	792.98 ^a ±0.00	867.74 ^b ±5.01	1006.83 ^c ±7.38
L-Tryptophan	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
L-Valine	818.07 ^c ±0.00	813.51 ^b ±4.02	782.44 ^a ±6.22
L-Arginine	1052.94 ^c ±3.42	967.13 ^b ±2.90	788.61 ^a ±2.03
Total essential amino acids	5878.7^a	6187.22^b	6913.7^c
Non essential amino acids			
L-Alanine	1940.56 ^c ±5.80	1717.34 ^b ±4.38	1639.40 ^a ±4.22
L-Aspartic acid	1982.17 ^a ±3.67	2082.83 ^b ±5.14	2104.82 ^c ±2.09
L-cysteine	0.00 ±0.10	0.00 ±0.00	0.00 ±0.00
L-Glicine	2276.03 ^b ±1.82	2199.16 ^a ±0.48	2199.16 ^a ±1.59
L-Proline	967.89 ^c ±0.53	771.55 ^b ±3.88	740.95 ^a ±6.50
L-Serin	2052.42 ^a ±5.12	2487.70 ^b ±0.38	2620.07 ^c ±6.94
L-Tyrosine	<608.01	<608.01	<608.01
L-Glutamic acid	2215.11 ^c ±1.83	2111.67 ^b ±2.96	1872.09 ^a ±0.56
Total non essential amino acids	12042.19^c	11978.26^b	11784.50^a
Total amino acids	17920.89^a	18165.48^b	18698.20^c
% Essential amino acids	32.80^a	34.06^b	36.98^c
% Non-essential amino acids	67.20^c	65.94^b	63.02^a

Note: PO=No addition of maltodextrin; P1=Addition of 2% maltodextrin; Addition of 4% maltodextrin

DISCUSSION

The results showed that the *U. reticulata* seaweed salt produced had a low moisture content value. The decrease in moisture content was influenced using high temperatures (80°C for 22h) to produce seaweed salt. In addition, the decrease in moisture content is also influenced by the addition of maltodextrin, where maltodextrin has hygroscopic properties that can absorb water, causing the moisture content to decrease (**Lozano-**

Aguirre *et al.*, 2023; Ma *et al.*, 2023; Maharani *et al.*, 2023). The low moisture content is in line with the water activity (A_w) of the *U. reticulata* seaweed salt produced, where A_w of seaweed salt for treatments P0, P1, and P2 had values of 0.39, 0.36, and 0.33, respectively. The resulting *U. reticulata* seaweed salt can be concluded to have a long shelf life. The ash content of the results of this study was also studied. The results showed that the ash content of *U. reticulata* seaweed salt produced in this study averaged 29.39 - 62.17%. The ash content of *U. reticulata* seaweed salt was high because *U. reticulata* seaweed was recorded with a high ash content of 36.76% (**Meiyasa *et al.*, 2023**). Furthermore, **Nurjanah *et al.* (2023)** reported that *Ulva lactuca* has an ash content of 41.05%. This contributes to the ash content of *U. reticulata* salt, where the results of the study showed that the addition of maltodextrin can reduce the ash content. Similar results were also reported by **Erbay and Koca (2015)** postulating that white cheese powder with maltodextrin added had a lower value of ash content than those without maltodextrin added. **Caliskan and Dirim (2016)** also reported that the addition of maltodextrin in the manufacture of sumac powder affects the decrease in the ash content.

This is because the addition of maltodextrin will increase evaporation, resulting in a decrease in the ash content (**Agustina *et al.*, 2019**). Fat content in *U. reticulata* seaweed salt produced in this study averaged 0.96 - 1.45%. The fat content in this study was very low. This is because seaweeds have low fat content. **Meiyasa *et al.* (2023)** reported that *U. reticulata* derived from the waters of Moudolung, Sumba – Indonesia, has a fat content of 2.28% (**Meiyasa *et al.*, 2023**). Similarly, **Ferdinand *et al.* (2024)** reported that the fat content of *U. reticulata* from Hambuang waters in Sumba, Indonesia was 3.04%. The production of seaweed salt requires a temperature of 80°C for 22h, causing the fat content to decrease. However, the addition of maltodextrin increased the fat content (Table 1). This is inversely proportional to the resulting protein levels, where the addition of maltodextrin causes a decrease in the protein levels of *U. reticulata* seaweed salt. As seen in Table (1), P0 (without the addition of maltodextrin), P1 (the addition of maltodextrin 2%), and P2 (the addition of maltodextrin 4%) each had protein contents of 3.87, 2.79, and 2.02%, respectively. The decrease in protein content of *U. reticulata* seaweed salt correlates with the nitrogen chain in the bond between maltodextrin and seaweed salt, where protein levels decrease with the addition of maltodextrin. Carbohydrates were also investigated in this study. The average carbohydrate content in this study was 26.17 - 61.16%. Based on Table (1), treatment P0 (without the addition of maltodextrin), P1 (the addition of maltodextrin 2%), and P2 (the addition of maltodextrin 4%) have a carbohydrate value of 26.17, 48.66, and 62.54%, respectively. The addition of maltodextrin increased the carbohydrate content. This is because maltodextrin is part of carbohydrates, so it contributes to increasing carbohydrates value.

Water activity is positively correlated with moisture content, where low water content and activity are also low. This is in line with the report by **Mayasari *et al.* (2018)** that instant seasoning of san-sakng leaves (*Albertisia papuana* Becc.) exhibited a low A_w

value of 0.51 (Mayasari & Ulfa, 2018). Similarly, Wang and Zhou (2013) reported that soy sauce powder, which is claimed to be a seasoning, has a low Aw value (0.53). The decrease in the Aw value in the treatment with the addition of maltodextrin was due to the hygroscopic properties of maltodextrin, which can absorb water. A similar thing was also reported by Meiyasa *et al.* (2024) that *U. reticulata* flavoring powder with the addition of maltodextrin was able to reduce the Aw value. A low value of water activity is considered stable against browning reactions, fat oxidation, microbial growth, and hydrolytic and enzymatic reactions (Caliskan & Dirim, 2016). Thus, *U. reticulata* seaweed salt has a long shelf-life.

According to Jönsson *et al.* (2023), amino acids are derivatives of proteins that contribute to sweet (serine, glycine, alanine, proline), bittersweet (lysine, threonine, valine), bitter (arginine, histidine, isoleucine, leucine, methionine, tyrosine, phenylalanine), umami (aspartic acid and glutamic acid), and sulfur (cysteine) flavors. Of the amino acids produced, the highest values were alanine, glycine, serine (switch), and glutamic acid (umami). Amino acids are contained in *U. reticulata* seaweed salt because *U. reticulata* contains high enough protein which are 10.68 - 12.93% (Tarigan *et al.*, 2023; Ferdinand *et al.*, 2024). This protein content contributes to the amino acids in seaweed salt. In addition, Meiyasa *et al.* (2023) also reported that the total amino acids produced in *U. reticulata* is 45000ppm. High levels of aspartic acid and glutamic acid are responsible for the umami flavor (Cotas *et al.*, 2024; Meiyasa *et al.*, 2024; Wendin *et al.*, 2024). In addition, Jönsson *et al.* (2023) added that aspartic acid and glutamic acid can also increase the saltiness of food. The high level of aspartic acid and glutamic acid in *U. reticulata* seaweed salt is because seaweed is rich in amino acids, especially aspartic and glutamic acids (Meiyasa *et al.*, 2023a). Similarly, Son and Lee (2024) reported that aspartic acid and glutamic acid have higher values than other amino acids in seaweed species such as *Capsosiphon fulvescens*, *Hizikia fusiforme*, *Porphyra yezoensis*, *Saccharina japonica*, and *Undaria pinnatifida*. The same was also reported by Figueroa *et al.* (2022) that aspartic acid and glutamic acid have high values compared to other amino acids in *U. lactuca* seaweed (Figueroa *et al.*, 2022). The presence of aspartic acid and glutamic acid in seaweed is used to enhance the flavor of certain dishes, thus creating healthier and more flavourful food (Son & Lee, 2024). It is also one of the alternatives to minimize monosodium glutamate (MSG) consumption (Figueroa *et al.*, 2022; Son & Lee, 2024).

CONCLUSION

Ulva reticulata salt contains varying levels of water, ash, fat, protein, and carbohydrates. In addition, the low water activity indicates that *U. reticulata* salt has a long shelf-life. Furthermore, the amino acids produced in *U. reticulata* salt are quite complete, especially aspartic acid and glutamic acid, which have higher values than other amino acids. Thus, *U. reticulata* salt contributes to umami and salty taste and can be used as an alternative ingredient to replace monosodium glutamate.

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