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Comparative Analysis of the Amino Acid Composition and Phylogenetic Diversity of Five Seaweed Species

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Seaweeds represent a widely harnessed marine resource that are valued for their abundant supply of essential nutrients, particularly proteins and amino acids. In Korea, where over 500 species of seaweed thrive and more than 50 are utilized for culinary purposes, seaweed has become a staple in regular diets. In this study, we focused on five of the most commonly consumed seaweed species in Korea: *Capsosiphon fulvescens*, *Hizikia fusiforme*, *Porphyra yezoensis*, *Saccharina japonica*, and *Undaria pinnatifida*. We closely examined the amino acid compositions of these five species. High-performance liquid chromatography showed that aspartic acid, glutamic acid, alanine, and leucine were the most abundant amino acids in the seaweeds. Principal component analysis revealed that the five seaweed species could be classified into three clusters according to their amino acid composition, partially corroborating findings from the phylogenetic analysis. Among various amino acids, glutamic acid, aspartic acid, and alanine were the primary amino acids driving differentiation. Notably, *U. pinnatifida* and *C. fulvescens*, which demonstrated close phylogenetic proximity, exhibited remarkably similar amino acid profiles. Conversely, although *P. yezoensis* and *S. japonica* shared a phylogenetic relationship, they displayed distinctly different amino acid compositions. *H. fusiforme* emerged as a distinct group in both analyses.

Key words : Amino acid, phylogenetic classification, principal component analysis, seaweed

Introduction

Marine ecosystems contain a wealth of resources and a diverse array of species, rendering them invaluable for various applications. Seaweeds, in particular, stand out due to their rich reservoir of essential nutrients, which contributes to their global utilization. With a significant presence in the Pacific Ocean, they have been a dietary staple in China, Japan, and Korea since ancient times [17]. Korea, which is geographically surrounded by sea on three sides, hosts a staggering 500 seaweed species, and over 50 are utilized in the local cuisine [5]. According to the Fisheries Production Trend Survey, as of 2022 [6], Korean seaweed production amounted to 1.74 million metric tons, exhibiting an average annual growth rate of 3.1% since 2016. The major types, consisting of *Undaria pinnatifida* (34.1%), *Porphyra yezoensis* (32.3%), *Saccharina japonica* (31.7%), *Hizikia fusiforme* (0.8%), and *Capsosiphon fulvescens* (0.2%) collectively account for 99.1 % of the total seaweed production.

The diverse nutritional functions of seaweeds present immense potential for enhancing dietary and functional food options, aligning with an evolving emphasis on health consciousness. In recent years, the research on seaweed-derived nutrition has significantly grown. In this emerging field, researchers have explored the varied nutritional content of seaweed, which includes proteins, carbohydrates, fiber, vitamins, and minerals [8, 12]. Concurrently, investigators have focused on identifying functional compounds such as phycocyanins, fucoidans, and carotenoids, which have potential as antioxidants, anti-inflammatory agents, and even anticancer agents [4, 10]. Additionally, researchers have explored the prebiotic properties, antimicrobial activities, and potential anti-obesity and anti-diabetic effects of seaweed [11, 19].

Studying the amino acid composition of seaweed is important for several reasons. First, it provides crucial insights

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into the nutritional value of seaweed, which has attracted increasing attention as a potential sustainable food source due to its abundant protein content (up to 47% of protein on a dry weight basis) [16]. Understanding the specific amino acids present and their respective quantities allows for a comprehensive assessment of the protein's quality and its potential to meet dietary requirements [2, 13]. Furthermore, from a food science perspective, amino acids, which impart distinctive tastes to food, such as umami and sweetness, play a pivotal role in determining the taste of food. Consequently, the release of amino acids during food processing has a critical influence on the overall flavor profile of products. Leveraging this knowledge, we can make informed decisions in the production of various foods and fermented products, capitalizing on the significant impact that amino acids have on flavor development. Moreover, examining the amino acid profile of seaweed is instrumental in elucidating its physiological and biochemical properties. This knowledge is pivotal for various applications, ranging from food processing and formulation to the development of functional food products and supplements [13].

Many researchers have analyzed the amino acid composition of seaweeds [1, 13, 14, 18]; however, a systematic investigation comparing the profiles of these amino acids is lacking. In this study, we conducted amino acid analysis using high-performance liquid chromatography (HPLC) and employed chemometric tools to identify similarities and commonalities among the amino acid compositions of the five most widely consumed seaweed species in Korea. These findings were then compared with a dendrogram constructed based on genetic data, specifically data from cytoplasmic ribosomes, which are fundamental components of eukaryotic cells. This comparative approach allowed us to gain insights into the biological relationships and relatedness among these species.

Material and Methods

Sample collection

Capsosiphon fulvescens, *Hizikia fusiforme Porphyra yezoensis*, *Saccharina japonica*, and *Undaria pinnatifida* were procured from the Bu-Jeon Market located in Busan, Korea in 2022. *Porphyra yezoensis* was acquired in the dry form, while the other seaweeds were obtained in their original form. Each type of seaweed was sourced from three different vendors and processed separately. Subsequently, the seaweed samples were immersed in tap water for one hour and rinsed five times to eliminate excess salt. The samples were then gently pressed to expel any remaining water. Afterward, a freeze dryer (Hanil, Gimpo, Korea) was used to desiccate the samples, which were subsequently pulverized using a mixer (Hanil, Seoul, Korea). The resulting ground samples were passed through a standard 60-mesh sieve (Chunggyesanggongsa, Seoul, Korea) prior to their use in the experimental procedures.

Standards and chemicals

Hydrochloric acid (HCl) and sodium phosphate dibasic were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol and acetonitrile were obtained from Fisher Scientific (Fair Lawn, NJ, USA). The amino acid standard solution (Agilent Technologies, Santa Clara, CA, USA) comprised a total of 17 amino acids. Specifically, L-aspartic acid (Asp), glycine (Gly), L-alanine (Ala), L-valine (Val), L-leucine (Leu), L-isoleucine (Ile), L-serine (Ser), L-threonine (Thr), L-tyrosine (Tyr), L-proline (Pro), L-arginine (Arg), L-histidine (His), L-glutamic acid (Glu), L-cystine (Cys), L-phenylalanine (Phe), L-lysine (Lys), and L-methionine (Met) were dissolved in 0.1 M HCl and further diluted if necessary.

Sample preparation

Each sample, weighing 100 mg, was combined with 5 ml of 6 N HCl in a sealed test tube, and subsequently subjected to hydrolysis within an oven set to 110°C for a duration of 22 hr [3]. HCl was removed using nitrogen gas via a sample concentrator (Hangzhou Miu Instruments Co. Ltd, Hangzhou, China). The resultant solution was then diluted with 30 ml of ultrapure water and further diluted 3- or 5-fold. Samples were filtered through a 0.45 μm PTFE membrane filter (Whatman, Maidstone, Kent, UK).

Derivatization

The derivatization process was executed using an Agilent HPLC autosampler program as proposed in the manufacturer's protocol [7]. The derivatization reagents, borate buffers, o-phthaldialdehyde (OPA), and 9-fluorenylmethoxycarbonyl chloride (FMOC) were ready-made solutions supplied by Agilent Technologies (Santa Clara, CA, USA).

High-performance liquid chromatography (HPLC) analysis

An Agilent 1220 Infinity LC system (Agilent Technologies, Santa Clara, CA, USA) was used in this study. The instru-

ment was equipped with a gradient pump, integrated degasser, autosampler, column oven, and variable wavelength detector (VWD). The HPLC analysis involved an Agilent Poroshell HPH C18 column (4.6×150 mm, 4 μm). The mobile phase consisted of two solutions, A and B. Mobile phase A was prepared by combining 10 mM sodium phosphate dibasic and 10 mM sodium tetraborate decahydrate, and adjusted to pH 8.2 with HCl. Mobile phase B consisted of a mixture of acetonitrile (45%), methanol (45%), and water (10%). Amino acids were separated using linear gradient elution conditions with the time and percentage of mobile phase B set as follows: 2% B at 0 min, 2% B at 0.50 min, 57% B at 20 min, 100% at 20.10 min, 2% B 3.60 min, and 2% B at 25 min. The flow rate was maintained at 1.5 ml/min. Analysis was performed at ultraviolet wavelengths of 338 nm and 262 nm for primary and secondary amino acids, respectively.

Similarity classification

Five species of marine macroalgae commonly produced in Korea were selected for genetic analysis: *Capsosiphon fulvescens*, *Hizikia fusiforme*, *Porphyra yezoensis*, *Saccharina japonica*, and *Undaria pinnatifida*. Their 18S rRNA gene sequences retrieved from the US National Bioinformatics Center (https://www.ncbi.nlm.nih.gov/). 18S rRNA was used for phylogenetic classification as a cytoplasmic ribosomal component among the basic components of eukaryotes (http://www.ncbi.nlm.nih.gov). 18S rRNA, a conserved component of eukaryotic ribosomes, serves as a valuable marker for similarity-based classification due to its ubiquitous presence and slow evolutionary rate. The accession numbers of the 18S rRNA gene, widely utilized in phylogenetic studies as a fundamental eukaryotic component, were obtained (Table 1). ClustalW was employed to gather essential information about the five seaweed species for subsequent similarity tree construction (http://www.ddbj.nig.ac.jp/). Subsequently, a comparative system for the five seaweed species was established using MEGA11 software [15] to determine the level of similarity among 18S rRNA sequences. A dendrogram was built using the Maximum Likelihood approach and the Tamura-Nei model. 500 bootstrap analyses were performed to create a consensus tree, where branches represent groups supported by less than 50% of the replicates. The topology with the highest log likelihood was chosen and the frequency was labeled above the branches.

Table 1. Five seaweed species that were included in the phylogenetic analysis

Seaweed species	GenBank accession number
	18S rRNA gene
Capsosiphon fulvescens	AF499664
Hizikia fusiforme	AB011428
Porphyra yezoensis	D79976
Saccharina japonica	EU293553
Undarid pinnatifida	JQ936016.1

Statistical analysis

All analyses were performed in triplicate, and the results are presented as the mean values with corresponding standard deviations (n=3). Principal component analysis (PCA) was carried out using R software package (www.r-project.org; version 3.2.2).

Results and Discussion

Amino acid compositions of five seaweed species

To analyze the 17 amino acids constituting the proteins of five seaweed species, we employed the protocol provided by the HPLC manufacturer. The method linearity was greater than 0.95 for all amino acids, which indicated reliable amino acid quantification. The limit of quantification (LOQ) was established to be 0.2 mg/g dry weight using a signal-to-noise ratio of ten. The results of this analysis are presented in Fig. 1. Table 2 presents the collective amino acid contents across the five seaweed species.

The protein content of seaweed varies depending on the species, and generally exhibits low levels in brown algae (4–24% of dry weight) and higher levels in red (8–47% of dry weight) and green algae (9–33% of dry weight); these protein levels are comparable to those of other protein sources such as soybean [13]. Our analysis confirmed a similar trend in the results. Among the brown algae (Phaeophyceae), *Hizikia fusiforme*, *Saccharina japonica*, and *Undaria pinnatifida* displayed low amino acid contents of 106.10 mg/g dry weight, 128.43 mg/g dry weight, and 213.25 mg/g dry weight, respectively. In contrast, the green algae (Chlorophyta) *Capsosiphon fulvescens* and red algae (Rhodophyta) *Porphyra yezoensis* exhibited higher values of 311.95 mg/g dry weight and 321.06 mg/g dry weight, respectively, exceeding the protein content of brown algae and aligning with previous studies.

Protein quality can be assessed based on amino acid composition, and seaweeds contain all the amino acids essential for human nutrition [16]. Table 2 presents the essential amino acid profiles of five different seaweed species. Among these, leucine, valine, threonine, isoleucine, lysine, and the aromatic

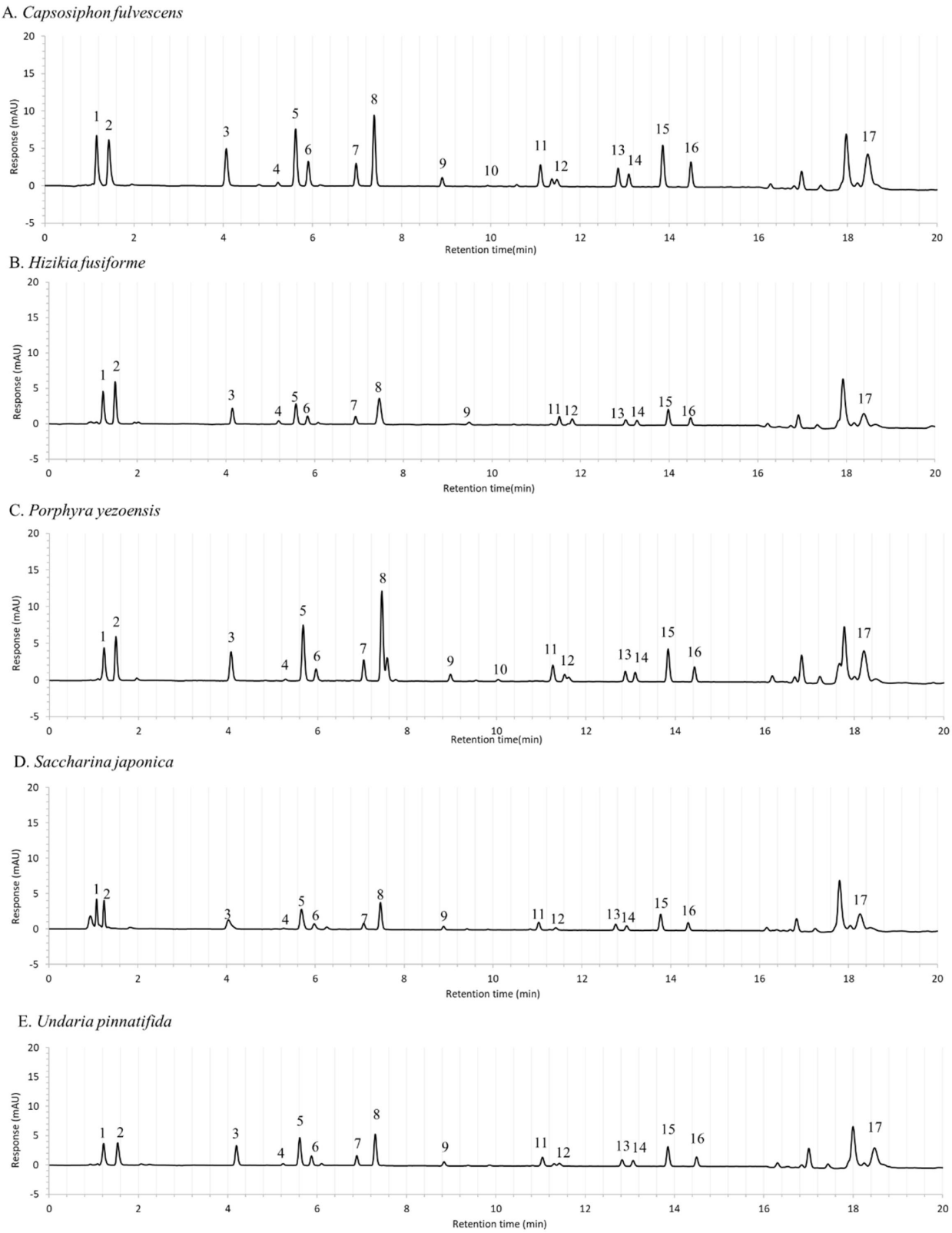

Fig. 1. HPLC chromatograms of (A) *Capsosiphon fulvescens*, (B) *Hizikia fusiforme*, (C) *Porphyra yezoensis*, (D) *Saccharina japonica*, and (E) *Undaria pinnatifida*. Peaks: 1. Aspartic acid, 2. Glutamic acid, 3. Serine, 4. Histidine, 5. Glycine, 6. Threonine, 7. Arginine, 8. Alanine, 9. Tyrosine, 10. Cystine, 11. Valine, 12. Methionine, 13. Phenylalanine, 14. Isoleucine, 15. Leucine, 16. Lysine, 17. Proline.

Table 2. Amino acid composition of *Capsosiphon fulvescens*, *Hizikia fusiforme*, *Porphyra yezoensis*, *Saccharina japonica*, and *Undaria pinnatifida*

Amino acids	Amino acid contents (mg/g of dry weight)				
	Capsosiphon fulvescens	*Hizikia fusiforme*	*Porphyra yezoensis*	*Saccharina japonica*	*Undaria pinnatifida*
Aspartic acid	44.12±6.81	20.00±2.27	40.42±7.19	19.28±5.57	31.70±1.85
Glutamic acid	45.06±5.01	24.61±1.52	45.25±7.64	18.88±5.43	29.88±2.79
Serine	18.22±1.77	4.85±0.69	17.21±0.55	7.50±2.28	12.59±0.31
*Histidine	4.89±0.59	3.04±1.20	4.15±1.32	2.68±0.63	3.88±0.25
Glycine	16.09±1.41	4.44±0.51	19.54±2.20	6.90±2.45	12.51±0.20
*Threonine	17.77±2.06	5.11±0.95	16.54±1.79	6.83±2.18	11.18±0.99
Arginine	20.61±1.63	4.93±0.50	22.68±0.40	7.33±2.62	13.78±0.76
Alanine	25.47±2.11	9.39±0.55	40.27±1.37	9.97±3.08	17.01±0.20
Tyrosine	8.59±1.04	2.03±0.26	7.54±1.34	4.00±1.60	5.40±1.28
Cystine	1.32±0.61	N.D.[a]	1.46±1.57	N.D.	N.D.
*Valine	13.73±1.59	3.56±0.31	14.92±1.30	5.35±1.50	9.35±0.44
*Methionine	4.86±0.59	1.14±0.37	6.50±2.58	2.19±1.26	3.29±0.57
*Phenylalanine	17.83±2.41	3.30±0.30	12.25±1.15	6.44±2.29	9.86±0.36
*Isoleucine	9.80±1.18	2.31±0.37	9.40±1.13	3.91±1.58	6.80±0.50
*Leucine	24.48±2.38	6.53±0.47	23.94±0.36	10.18±3.59	17.67±0.07
*Lysine	19.09±1.13	5.10±1.15	18.96±1.52	7.80±2.59	14.52±1.73
Proline	20.02±3.09	5.74±0.73	20.05±1.35	9.17±4.47	13.73±1.67
Total	311.95±29.34	106.10±9.01	321.06±23.72	128.43±42.11	213.25±5.57

The experiment was conducted in triplicate (n=3). The results are given as the means ± standard deviations.
[a]N.D. means not detected.
*Asterisks indicate essential amino acids.

amino acid phenylalanine were the most prevalent essential amino acids in seaweeds. Notably, leucine accounted for the highest proportion among the five studied seaweed species.

It is worth noting that, depending on the species of seaweed, there may be some variations; however, an overall similarity in the primary amino acid content was observed. Among the five types of seaweed analyzed, glutamic acid and aspartic acid were found to predominate, followed by leucine and alanine. These results align with those reported in previous studies [1, 2]. Particularly, in *Hizikia fusiforme*, the relative amount of glutamic acid and aspartic acid was the highest, at 42%. Dawczynski *et al.* reported an amount of 30%, which is lower than our observation. The discrepancy in amino acid composition is believed to be influenced by factors such as season, maturity, and environmental conditions, affecting the overall composition of amino acids [14, 20]. In *Saccharina japonica*, the relative amount of glutamic acid and aspartic acid was 30%. Aspartic and glutamic acids exhibit intriguing properties in flavor development, with glutamic acid being a key component in eliciting 'umami' [9]. Umami from seaweeds can be utilized to enhance the flavor of certain dishes, thereby creating healthier and more flavorful meals.

Principal component analysis (PCA)

To compare the characteristics of the five seaweed samples, we conducted principal component analysis (PCA) based on their amino acid composition. PCA was applied using the percentage of amino acid content. PC1 and PC2 accounted for 73.7% and 16.9% of the variability in the dataset, respectively. The results revealed three distinct groups: Group I (*Porphyra yezoensis*), group II (*Undaria pinnatifida, Saccharina japonica, Capsosiphon fulvescens*), and group III (*Hizikia fusiforme*), as depicted in Fig. 2. PC1 effectively distinguished Group III (*Hizikia fusiforme*) from the other seaweeds. On the other hand, PC2 set Group I (*Porphyra yezoensis*) apart from the other seaweeds. PCA biplot highlighted that glutamic acid was the amino acid with the most significant variance along the PC1 axis, followed by aspartic acid and alanine. Glutamic acid (Glu) and aspartic acid (Asp) emerged as the major distinguishing amino acids for *Hizikia fusiforme*, constituting 23% and 19% of its amino acid content, respectively. Conversely, alanine (Ala) played a pivotal role in distinguishing *Porphyra yezoensis*, accounting for

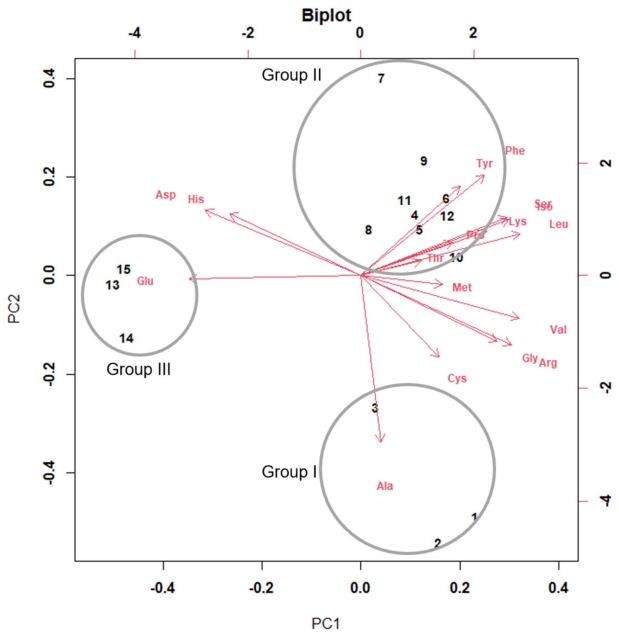

Fig. 2. PCA biplot obtained from the amino acid composition of five seaweed species samples. 1-3: *Porphyra yezoensis*, 4-6: *Undaria pinnatifida*, 7-9: *Saccharina japonica*, 10-12: *Capsosiphon fulvescens*, 13-15: *Hizikia fusiforme*.

12% of its amino acid content.

Dendrogram analysis

A similarity tree is a graphical model representing relationships among organisms. Species that share the most recent common ancestor are positioned closely, while those without a common ancestor are located farther apart. This serves as a tool to visualize similarities, common ancestry, and evolutionary history among species. The five species of seaweed were analyzed for the presence of mitochondrial genes of the 18S ribosomal RNA available in GenBank. A similarity tree was constructed using the accession numbers of the five seaweed species within the MEGA11 program. Initially, they were classified into three distinct clusters (Fig. 3). *Porphyra yezoensis* and *Capsosiphon fulvescens* were found to be closely related from a phylogenetic perspective. Similarly, *Undaria pinnatifida* and *Saccharina japonica* showed close phylogenetic proximity. In contrast, *Hizikia fusiforme* did not cluster with the other seaweeds.

The study reveals remarkable concordance between amino acid composition and 18S rRNA-based phylogeny for five seaweed species, with fine-scale resolution exceeding taxonomic classification. Notably, conspecifics *Undaria pinnatifida* and *Capsosiphon fulvescens* exhibit virtually identical

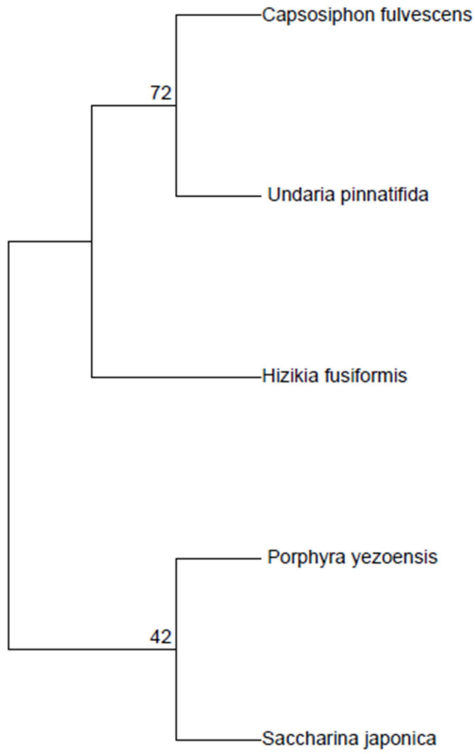

Fig. 3. A dendrogram of five seaweed species constructed in the MEGA11 program.

amino acid profiles, confirming their close evolutionary relationship. However, *Porphyra yezoensis* and *Saccharina japonica*, despite phylogenetic proximity, showcase divergent amino acid signatures, highlighting the potential for independent biochemical adaptations within closely related lineages. Interestingly, *Hizikia fusiforme* consistently forms a unique group, suggesting its distinct evolutionary trajectory and metabolic composition.

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 해조류 5종의 아미노산 조성 및 계통 다양성 비교 분석

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해조류는 풍부한 필수 영양소 공급으로 인해 귀중한 해양 자원으로 여겨지며 특히 단백질과 아미노산이 풍부하게 함유되어 있다. 한국에서는 500종 이상의 해조류가 서식하며 그 중 50종 이상이 식품용으로 이용되어 일상 식단의 중심 역할을 한다. 본 연구는 한국에서 가장 흔히 섭취되는 해조류 5종(매생이, 톳, 김, 다시마, 미역; *Capsosiphon fulvescens, Hizikia fusiforme, Porphyra yezoensis, Saccharina japonica, Undaria pinnatifida*)의 단백질 구성 아미노산을 분석하였다. 고성능 액체 크로마토그래피 분석 결과, 아스파르트산, 글루탐산, 알라닌, 류신이 가장 풍부한 아미노산 성분임을 알 수 있었다. 주성분 분석에서는 이 다섯 종류의 해조류가 아미노산 구성에 따라 세 개의 군집으로 분류될 수 있었고, 이는 부분적으로 계통 분류 결과와 일치하였다. 다양한 아미노산 중에서도 글루탐산, 아스파르트산, 알라닌이 구분을 주도하는 주요 아미노산이였다. 특히, 가까운 계통적 근접성을 보이는 *Undaria pinnatifida*와 *Capsosiphon fulvescens*는 뚜렷하게 유사한 아미노산 프로필을 나타내었다. 그에 비해 *Porphyra yezoensis*와 *Saccharina japonica*는 계통적 관계를 공유하더라도 다른 아미노산 구성을 보였다. *Hizikia fusiforme*는 두 분석 모두에서 독특한 군집으로 나타났다.