



Influence of harvest time and cultivation region on primary and secondary metabolites of Korean laver (*Porphyra* spp.)

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ABSTRACT

Laver (*Porphyra* spp.) is an economically important red seaweed primarily consumed in East Asia, yet detailed information on its nutritional composition remains limited. This study reports a first pseudo-targeted, in-depth seasonal and regional metabolite profiling of Korean laver harvested between December and March from five major cultivation regions during the 2021–2023 seasons. Using GC-MS/MS and LC-MS/MS, a total of 109 primary and nutrient metabolites were identified. Multivariate analysis (VIP > 1.0) revealed 43 primary metabolites that varied with harvest time and cultivation region in this dataset. Sweet-taste amino acids (alanine, glycine, and serine) and umami-taste amino acids (aspartic acid and glutamic acid) were more abundant in samples harvested in December and January. Additionally, seven carotenoids (fucoxanthin, lutein, zeaxanthin, β -apo-8'-carotenal, β -cryptoxanthin, α -carotene and β -carotene) and seven free phenolic acids (dihydroxyphenylacetic acid, 3,4-dihydroxybenzoic acid, hydroxybenzoic acid hexoside, 4-hydroxybenzoic acid, gallic acid gallate, dimethoxy hydroxycinnamic acid, and salicylic acid) were identified, several of which showed higher levels in early-winter samples. Taken together, these results suggest quality relevant markers for distinguishing harvest times and cultivation regions, thereby providing a scientific basis for product differentiation and the implementation of targeted quality control in laver production.

1. Introduction

Laver (*Porphyra* spp.) is a red seaweed traditionally consumed in East Asian countries such as Korea, Japan, and China. Its global popularity has grown recently due to increasing recognition of its characteristic flavor and health benefits (Cho & Rhee, 2019). In Korea, laver is primarily cultivated from December to March, particularly in the southwestern coastal region, and is mainly exported to the United States, China, and Japan (Kang et al., 2018; Kim et al., 2021). By 2024, Korea's laver exports reached USD 997 million (Kati, 2025), highlighting its pivotal role as one of the world's leading producers of laver.

A comparative study across of 11 seaweed species revealing that laver had the highest protein level at 45.6 g/100 g dry weight (Yoon

et al., 2022). Korean laver is typically a hybrid of *P. tenera* and *P. yezoensis* (Sanjeeva et al., 2018). *P. yezoensis* contains 38.63 % protein (dry weight) and is particularly rich in amino acids such as alanine and glutamic acid (Baek et al., 2019; Lee et al., 2012). Protein quality depends on amino acid composition, which is known to vary with seasonal and environmental conditions (Tanna et al., 2019), and free amino acids are essential taste-related compounds in aquatic products (Cao et al., 2021).

In addition to amino acids, Korean laver contains various metabolite classes, including fatty acids, organic acids, free sugars, and phenolic acids, which are common plant secondary metabolites with diverse bioactivities (Baek et al., 2019; Kim et al., 2014; Lee et al., 2012; Park et al., 2014; Mudondo et al., 2025). While total phenolic content (TPC)

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has been reported using spectrophotometric methods, TPC provides only an overall estimate and does not resolve the diversity of individual phenolic compounds (Baek et al., 2019; Lee et al., 2012). In addition, carotenoid reports in Korean laver have mainly focused on lutein and β -carotene (Baek et al., 2019; Park et al., 2014).

Most previous studies on Korean laver have focused on dried samples, which are typically produced by combining fresh laver harvested at different times and from various regions. The drying and sheet-forming processes involve mixing and physical processing steps that can obscure or alter harvest-specific biochemical signatures, limiting the extent to which dried-laver datasets capture true temporal and regional variation in the raw material. Understanding such variation is essential because environmental factors—including temperature, light, and salinity—fluctuate throughout the cultivation season and strongly influence seaweed metabolism (Kim et al., 2021). Indeed, early-harvested laver has been reported to contain higher levels of protein and bioactive peptides than later harvests (Park et al., 2001; Yang et al., 2022a), underscoring the importance of harvest-specific biochemical characterization. However, previous metabolomic studies have often targeted narrow metabolite classes, providing only a partial view of biochemical diversity. Addressing these gaps requires comprehensive, region- and month-resolved metabolomic profiling of fresh laver.

Metabolomics enables comprehensive characterization of seaweed biochemistry using modern mass spectrometry platforms (GC-MS, LC-MS) (Park et al., 2023) and offers valuable insights into the underlying biochemical pathways and interactions (Kim et al., 2025). However, metabolomic studies of red seaweeds such as laver remain limited compared with the emphasis on brown seaweeds.

Despite growing interest in laver as a functional food, integrated metabolomic data on fresh laver that jointly resolve harvest time and cultivation region remain limited. The focus on dried products or narrow metabolite classes makes it difficult to understand how seasonal and regional factors shape the primary and secondary metabolite landscape of the raw material that ultimately determines finished product quality.

The present study hypothesized that metabolome changes in fresh laver vary with region and harvest time, with the potential to identify markers of quality. To test this hypothesis, metabolomic profiling was conducted using gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Samples were collected from five regions during December–March of the 2021–2022 and 2022–2023 seasons. Multivariate modeling and pathway analysis were used to identify discriminating metabolites and to assess the extent to which metabolomic patterns corresponded to concurrent environmental conditions (sea surface temperature (SST), salinity, solar irradiance, and dissolved inorganic nitrogen (DIN)). These findings provide a scientific basis for quality differentiation and support the development of value-added laver products.

2. Materials and methods

2.1. Chemicals and reagents

Adonitol ($\geq 99\%$, A5502), fucoxanthin ($\geq 95\%$, 16337), α -carotene ($\geq 97\%$, 50887), β -apo-8'-carotenal ($\geq 96\%$, 10810), β -carotene ($\geq 95\%$, C4582), β -cryptoxanthin ($\geq 97\%$, C6368), 3,4-dihydroxybenzoic acid ($\geq 97\%$, 37580), 3,4-dihydroxyphenylacetic acid ($\geq 97\%$, 11569), 4-hydroxybenzoic acid ($\geq 99\%$, 240141), salicylic acid ($\geq 99\%$, 247588), sinapic acid ($\geq 98\%$, D7927), gallic acid ($\geq 99\%$, G7384), methoxyamine hydrochloride (MeOX, 226904), pyridine (reagent grade, $\geq 99\%$, 690570) and formic acid (reagent grade, $\geq 95\%$, F0507) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Lutein ($\geq 92\%$, 0306S) and zeaxanthin ($\geq 95\%$, 0307S) were purchased from Extrasynthese (Genay Cedex, France). *N*-Methyl-*N*-(trimethylsilyl)tri-fluoroacetamide with 1% trimethylchlorosilane (MSTFA with 1% TMCS, TS-48915) and HPLC grade hexane (H302), and methyl *t*-butyl

ether (MTBE) (E127) were purchased from Thermo Fisher Scientific (Asheville, NC, USA). LC-MS grade acetonitrile (ACN) (9017–88) and methanol (MeOH) (AH230) were purchased from J.T. Baker (Radnor, PA, USA) and Honeywell Burdick & Jackson (Morristown, NJ, USA), respectively.

2.2. Fresh laver samples

Fresh laver samples were collected from five major cultivation regions in South Korea: Jangheung (JH), Wido (WD), Busan (BS), Seochon (SC), and Goheung (GH). The geographic locations of these regions are indicated on the map in Fig. 1. The samples from JH and WD were collected monthly from December 2021 to March 2022, while those from BS, SC, and GH were collected monthly from December 2022 to March 2023. Each sample was labeled using a two-part code denoting region and harvest month (e.g., BS12 indicates Busan harvested in December, and GH1 indicates Goheung harvested in January). The numerical part of the code represents the harvest month: December (12), January (1), February (2), and March (3). At each harvest time, approximately 3 kg of fresh laver was harvested, immediately frozen, lyophilized, and stored at $-80\text{ }^{\circ}\text{C}$ until analysis. For each region and harvest month, a 3-kg pooled fresh-laver sample was collected and thoroughly homogenized. The homogenized material was then divided into portions designated for different analyses (primary and nutrient metabolites, crude protein, carotenoids, and phenolic acids). Each analysis was conducted using chemical replicates: five chemical replicates were prepared for primary and nutrient metabolite measurements, and three chemical replicates were prepared for crude protein, carotenoid, and phenolic acid analyses. All samples were freeze-dried prior to extraction, and metabolite concentrations were expressed on a dry-weight basis.

Environmental data (SST, salinity, solar irradiance, and DIN) were obtained from national monitoring programs (National Institute of Fisheries Science, 2023; Korea Hydrographic and Oceanographic Agency., 2023) and are presented in Fig. S1.

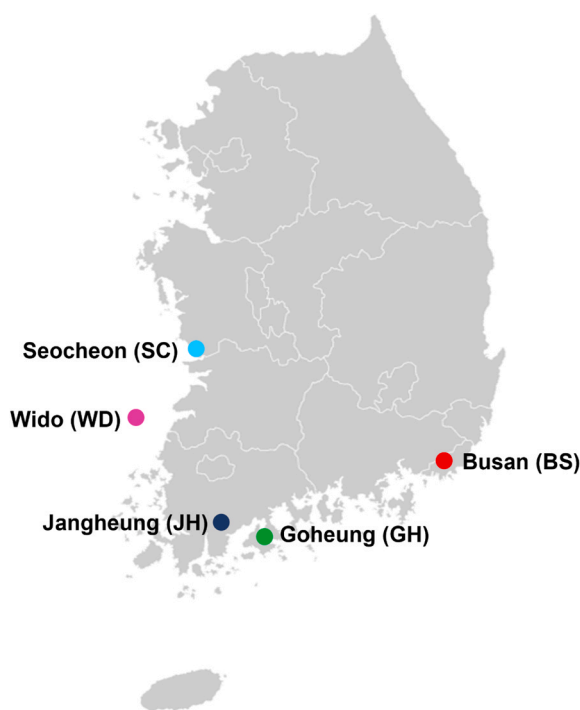


Fig. 1. Sampling locations of laver collected from five coastal regions in Korea.

2.3. GC-MS/MS analysis of primary and nutrient metabolites

Sample preparation was performed according to a previous method with minor modification (Mack et al., 2017). Lyophilized laver powder (50 mg) was placed into a 2-mL tube containing five beads (3 mm diameter) and grinding was performed for 5 min at a vibration frequency of 30 Hz using a Mixer Mill MM400 (Retsch, Haan, Germany). The milled laver was mixed with 750 μ L of methanol and 20 μ L of 1000 ppm internal standard solution (adonitol), and vortexed for 30 s. The mixture was centrifuged at 848 $\times$ g for 10 min and the supernatant was collected. The process was repeated and the combined supernatants

$$\text{Crude protein (\%)} = \frac{(\text{titrated HCl volume (mL)} - \text{blank volume (mL)}) \times \text{HCl molarity} \times 14.01}{\text{sample weight (mg)}} \times F \times 100 \quad (2)$$

were centrifuged at 16,100 $\times$ g for 5 min. A 30- μ L aliquot of the supernatant was concentrated under vacuum (SPD2010, Thermo Fisher Scientific, Asheville, NC, USA). For derivatization, the dried residue was treated with 35 μ L of MeOX in pyridine (25 mg/mL) at 37 $^{\circ}$ C for 90 min (methoximation), followed by silylation with 80 μ L of MSTFA with 1 % TMCS at 37 $^{\circ}$ C for 30 min.

The primary and nutrient metabolites were analyzed via gas chromatography with a triple quadrupole tandem mass spectrometer (GC-MS/MS) (GCMS-TQ8050, Shimadzu, Kyoto, Japan) equipped with a BPX-5 column (30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness, TRAJAN Scientific and Medical, Victoria, Australia). Helium was used as the carrier gas at a flow rate of 1 mL/min. A 1.0 μ L aliquot of the sample was injected in a split mode with a split ratio of 50:1. The oven temperature was initially set to 60 $^{\circ}$ C for 2 min, then increased at 10 $^{\circ}$ C/min to 320 $^{\circ}$ C, and held at 320 $^{\circ}$ C for 15 min. The electron ionization energy was 70 eV. The ion source and transfer line temperatures were 230 and 280 $^{\circ}$ C, respectively.

The metabolites were identified and quantified in multiple reaction monitoring (MRM) mode with the metabolites database comprising 332 metabolites from the Shimadzu Smart Metabolites Database and 62 metabolites from an in-house library constructed by our group. Data processing was conducted using GCMS Solution software (version 4.53, Shimadzu). Relative concentrations of the identified metabolites were calculated according to Eq. (1), as previously described (Lee et al., 2014). The peak area of each metabolite was normalized to that of the internal standard (i.e., adonitol), and the relative concentration was derived by multiplying the internal standard amount (20 μ g) divided by sample weight (0.05 g).

$$\text{Relative conc. } \left(\frac{\mu\text{g}}{\text{g}}\right) = \frac{\text{peak area of metabolite}}{\text{peak area of internal standard (I.S.)}} \left[\left(\frac{20 \mu\text{g I.S.}}{0.05 \text{ g sample}} \right) \right] \quad (1)$$

2.4. Crude protein content analysis

The crude protein content was determined according to the Korean Food Code (Ministry of Food and Drug Safety (Ministry of Food and Drug Safety MFDS of Korea. Food. Code., 2025). Laver (1000 mg) was placed into a digestion tube along with two digestion catalyst tablets and 12 mL of concentrated sulfuric acid (H₂SO₄). The sample was digested at 420 $^{\circ}$ C for 45–60 min until the solution became clear light blue or yellow.

After cooling to room temperature, 80 mL of distilled water was added to the digested solution. A receiving solution containing 25 mL of mixed indicator solution was placed into a conical flask and positioned on the distillation unit. Then, 50 mL of 40 % sodium hydroxide (NaOH), corresponding to four times the amount of sulfuric acid used, was added to the digestion tube. The sample was distilled for 3–4 min and the distillate was titrated with a hydrochloric acid (HCl) solution until the end point (light pink color) was reached. The crude protein content was calculated according to Eq. (2), where F is the nitrogen-to-protein conversion factor.

2.5. Carotenoid analysis via HPLC

Sample preparation for carotenoid analysis was performed following a previous method with some modifications (Koizumi et al., 2018; Othman et al., 2018). The lyophilized laver (500 mg) was rehydrated with 5 mL of distilled water and 10 mL of MeOH was added. The methanolic extract was left for 24 h at room temperature in darkness, after which it was centrifuged for 5 min at 10,000 $\times$ g and the supernatant was collected. The extraction process was repeated and the combined supernatants were concentrated under vacuum. For further purification, 2.5 mL of distilled water and 2.5 mL of hexane were added; the hexane layer was collected and re-extracted until colorless (up to six times). The combined hexane layers containing carotenoids were dried under a gentle stream of nitrogen gas, re-dissolved in 1 mL of MTBE and MeOH (1:1, v/v), and filtered through a 0.2- μ m nylon syringe filter.

Carotenoids were analyzed via high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection, using an Agilent 1260 Infinity II HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a variable wavelength detector (VWD). The detection wavelength was set to 450 nm for fucoxanthin, lutein, zeaxanthin, β -cryptoxanthin, α -carotene, and β -carotene and to 470 nm for β -apo-8'-carotenol. A YMC C30 carotenoid column (4.6 $\times$ 250 mm, 5 μ m, YMC Co., LTD., Kyoto, Japan) was used at 25 $^{\circ}$ C. The mobile phase comprised MeOH (A) and MTBE (B), and gradient elution was performed as follows; 5–70 % B, 0–25 min; 70–5 % B, 25–30 min; 5–5 % B, 30–35 min. The flow rate was 1 mL/min and the injection volume was 10 μ L.

Quantification of fucoxanthin, lutein, zeaxanthin, β -apo-carotenol,

β -cryptoxanthin, α -carotene, and β -carotene was achieved using authentic standards (Fig. S2). Standard curves were constructed at different concentrations (0.5–100 ppm) with good linearities (coefficient of determination $\geq$ 0.99) (Table S1). The carotenoid content of each sample was calculated using the standard curve, and the calculated value was divided by the sample weight (500 mg).

2.6. Free phenolic acid analysis via UHPLC-QqQ-MS/MS

Extraction of the phenolic acid was conducted following a previous

method with some modifications (Farvin & Jacobsen, 2013). The lyophilized laver (500 mg) was mixed with 5 mL 80 % MeOH and extracted overnight at room temperature, followed by centrifugation for 10 min ($10,621 \times g$ at 5°C) and collection of the supernatant. The extraction process was conducted in triplicate, and the combined supernatants were concentrated under vacuum. The dried residue was re-dissolved in 1 mL of 0.1 % formic acid in water and filtered through a $0.2\text{-}\mu\text{m}$ PVDF filter.

The phenolic content was analyzed using a 1290 ultra-high-pressure liquid chromatograph coupled to triple quadrupole tandem mass spectrometry (UHPLC-QqQ-MS/MS, Agilent Technologies, Santa Clara, CA, USA) equipped with a Poroshell C18 column ($2.1\text{ mm} \times 100, 2.7\text{ }\mu\text{m}$, Agilent Technologies) maintained at room temperature. The mobile phase consisted of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B), and gradient elution was performed as follows; 5–10 % B, 0–5 min; 10–12 % B, 5–8 min; 12–15 % B, 8–10 min; 15–15 % B, 10–15 min; 15–55 % B, 15–18 min; 55–90 % B, 18–20 min. The flow rate was 0.4 mL/min, and the injection volume was 5 μL . Electrospray ionization (ESI) in the negative mode was employed. The drying gas temperature and flow rate were 225°C and 8.0 L/min, respectively. The sheath gas temperature and flow rate were 300°C and 10 L/min, respectively. The nebulizer gas pressure, capillary voltage, and dwell time were 45 psi, 3.5 kV, and 20 ms, respectively. Across the 10–1000 ppb calibration range, linearity was high ($r^2 \geq 0.99$; Table S2). The MRM mode was used for quantification, using authentic standards of 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, and salicylic acid. Dimethoxy hydroxycinnamic acid, dihydroxyphenylacetic acid, hydroxybenzoic acid hexoside, and gallic acid gallate were relatively quantified using the standards of sinapic acid, 3,4-dihydroxyphenylacetic acid, 4-hydroxybenzoic acid, and gallic acid, respectively. The MRM transitions used for phenolic acid quantification are listed in Table S3. A representative chromatogram of phenolic acids in fresh laver is shown in Fig. S3.

2.7. Statistical analysis and metabolic pathway analysis

Statistical analysis was performed using IBM SPSS statistics software (v.25.0, SPSS, Inc., Chicago, IL, USA) and MetaboAnalyst 6.0 (<https://www.metaboanalyst.ca>). For multivariate data analysis, the data were log-transformed and auto-scaled, placing all metabolites on a correlation-based scale. The significant differences in secondary metabolite contents among the samples were analyzed using the analysis of variance (ANOVA). The post-hoc test was conducted by Tukey's tests ($p < 0.05$). Principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were used to analyze the grouping trends. The robustness of the PLS-DA model was validated using 5-fold cross-validation and permutation testing ($n = 2000$ permutations). Variable importance of projection (VIP) values (from the PLS-DA) greater than 1 and p -value < 0.05 were considered differential metabolites that contributed to grouping. Differential metabolite analysis was performed using volcano plots with a fold change threshold of 2.0 and a p -value cutoff of 0.05. Raw p -values were used without applying any multiple-testing correction. The comparison was based on the crude protein content, with high-protein samples ($\geq 30\%$) used as the reference group. Correlation analysis was conducted to investigate the relationships among individual amino acids, total free amino acids, and crude protein content in fresh laver samples. Pearson correlation coefficients (r) were calculated and heatmaps were generated. The color scale was fixed between -1 and $+1$, and hierarchical clustering was not applied. Metabolic pathway analysis plots were generated using MetaboAnalyst 6.0, and pathways were identified based on the *Cyanidioschyzon merolae* (red alga) KEGG library.

3. Results and discussion

3.1. Primary and nutrient metabolites in fresh laver samples

GC–MS/MS analysis of fresh laver identified a total of 104 primary metabolites (27 amino acids and derivatives, 10 nitrogen-containing compounds, 24 organic acids and derivatives, 17 fatty acids and derivatives, and 26 sugars and derivatives) and 5 nutrient metabolites (vitamins) (Table S4). These compounds encompass functions related to energy metabolism, structural maintenance, and antioxidant defense. Notably, 37 metabolites—including citramalic acid, glycolic acid, norvaline, decanoic acid, elaidic acid, and niacinamide—were detected in laver for the first time, expanding the known primary and nutrient metabolite repertoire of this red seaweed.

The absence of these metabolites in prior studies was likely due to methodological limitations. Earlier work predominantly analyzed dried laver (Baek et al., 2019; Choe et al., 2013), which undergoes repeated washing and high-temperature processing, leading to the loss or degradation of water-soluble and thermolabile metabolites. In addition, most previous studies relied on targeted analytical platforms that quantified only a limited, predefined set of compounds, restricting metabolite detectability. In contrast, the pseudo-targeted GC–MS/MS approach used here, combined with fresh lyophilized samples, afforded broader metabolite coverage and improved sensitivity, enabling the detection of these additional compounds.

Several of the newly identified metabolites have biological roles relevant to laver physiology. Citramalic acid has been associated with salinity-stress responses in plants (Wan et al., 2022), while glycolic acid participates in oxidative-stress mitigation pathways (Ma et al., 2017a). Norvaline, a non-proteinogenic amino acid, has been linked to nitrogen metabolism and stress adaptation in photosynthetic organisms (Li et al., 2025). Newly detected fatty acids such as decanoic acid and elaidic acid are also implicated in cellular responses to environmental and oxidative stress (Huang et al., 2014; Ma et al., 2017b). Niacinamide, a vitamin B3 derivative, contributes to redox regulation as a precursor of $\text{NAD}^+/\text{NADP}^+$ -related metabolic pathways (Arora & Philippidis, 2023). Collectively, the presence of these compounds suggests potential roles in metabolic adjustments associated with laver's adaptation to fluctuating environmental conditions.

3.1.1. Multivariate statistical analysis of primary metabolites

Principal component analysis (PCA) was first performed to explore overall metabolic variation (Fig. S4A). The first two principal components (PC1 and PC2) explained 45.4 % of the total variance, indicating that substantial variation remained unaccounted for. Therefore, the scores of the first four components (PC1–PC4; cumulative 67.3 % variance) were used as input for a PERMANOVA (Fig. S4B), which confirmed a significant regional effect on the overall metabolic profile ($p < 0.05$).

To further investigate class-related metabolic differences and to identify metabolites contributing to these patterns, a PLS-DA model was applied to the GC-MS/MS dataset (Fig. 2A). Model performance was evaluated using 5-fold cross-validation, which yielded a maximum Q^2 of 0.728 at five components (Fig. 2B). A 2,000-iteration permutation test further supported model robustness ($p = 0.0145$; Fig. 2C).

The PLS-DA score plot indicated a tendency for samples to cluster into three groups (BS; GH and JH; SC and WD), broadly corresponding to geographical proximity. This grouping pattern appeared to reflect regional differences in marine environmental conditions. As shown in Fig. S1A–B, SST and salinity differentiated WD and SC, GH and JH, and BS, with BS exhibiting distinctly higher SST and salinity relative to the other regions.

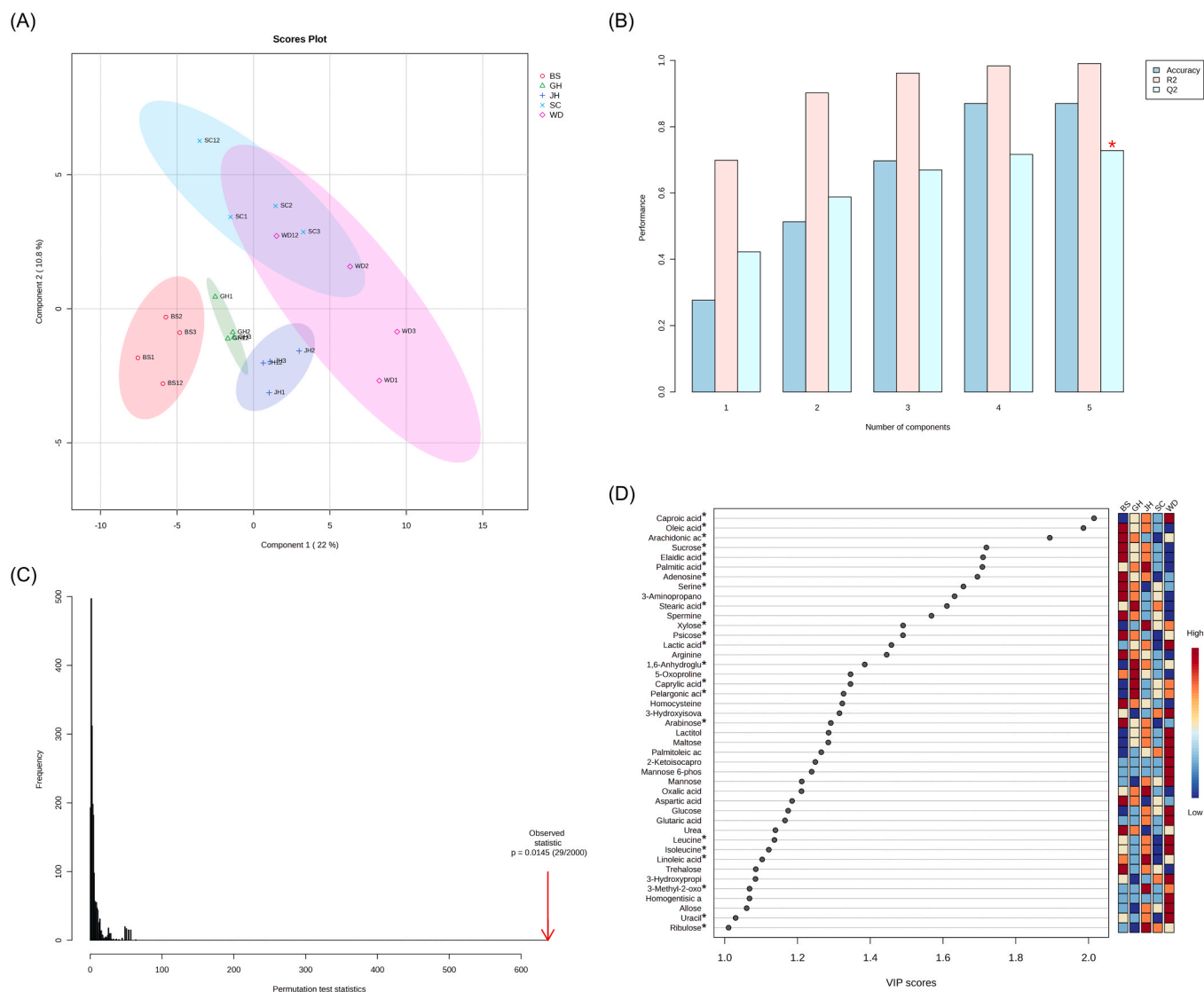


Fig. 2. Multivariate statistical analysis results of GC-MS/MS-based metabolite profiles in fresh laver samples varying with harvest time and regions: (A) partial least squares-discriminant analysis (PLS-DA) score plot of fresh laver. The horizontal axis represents the first principal component, and the vertical axis represents the second principal component. (B) Cross-validation of the PLS-DA model, showing Q^2 values for different numbers of components. (C) Permutation test of the PLS-DA model ($n = 2000$), confirming that the observed separation is not due to chance ($p = 0.0145$). (D) Variable importance in projection (VIP) scores of the top 43 metabolites ($VIP > 1.0$), indicating the most influential features in group separation; metabolites showing statistical significance ($p < 0.05$) are marked with an asterisk (*). Each sample is color-coded by region: red for Busan (BS), green for Goheung (GH), blue for Jangheung (JH), light blue for Seochon (SC), and purple for Wido (WD).

To identify the most influential features contributing to group discrimination, variable importance in projection (VIP) scores were calculated. A total of 43 metabolites had $VIP > 1.0$ (Fig. 2D, Table S5), indicating their potential as discriminatory biomarkers. As suggested by Kim et al. (2015), $VIP > 1.0$ indicates a strong contribution to class separation by representing the X block relative to the Y block. Among the 43 metabolites with $VIP > 1.0$ (Table S5), 22 showed significant group differences ($p < 0.05$; denoted by * in Fig. 2D). These included caproic acid, oleic acid, arachidonic acid, sucrose, elaidic acid, palmitic acid, adenosine, serine, stearic acid, and xylose, among others. These metabolites spanned multiple chemical classes, including fatty acids, sugars, and amino acid derivatives. Notably, oleic acid, palmitic acid, and arachidonic acid are major fatty acids involved in membrane structure and flavor formation (Santos et al., 2019). Adenosine participates in nucleotide metabolism, whereas sucrose is associated with cellular energy status and carbon flux.

3.1.2. Hierarchical clustering and heatmap analysis of primary metabolites

As shown in Fig. 3A, the heatmap visualizes the overall abundance patterns of the primary metabolites. To delineate class-level structure, heatmaps of primary metabolite subclasses are shown in Fig. 3B–F, and Fig. 3G presents the vitamins. For the month-by-region comparisons, metabolite features (rows) were hierarchically clustered while maintaining a fixed sample order across columns. Metabolites exhibiting differential patterns are marked with black dots, indicating unadjusted p -values < 0.05 . The red, white, and blue gradients indicate relatively high, average, and low metabolite levels, respectively. All samples were harvested monthly from December to March; JH and WD samples were harvested from December 2021 to March 2022, while BS, GH, and SC samples were harvested during 2022–2023. Consistent with previous reports that seaweed metabolite composition is highly sensitive to seasonal variation, particularly in antioxidant- and energy-related compounds (Marinho et al., 2019), the observed seasonal shifts highlight the importance of month-by-region comparisons.

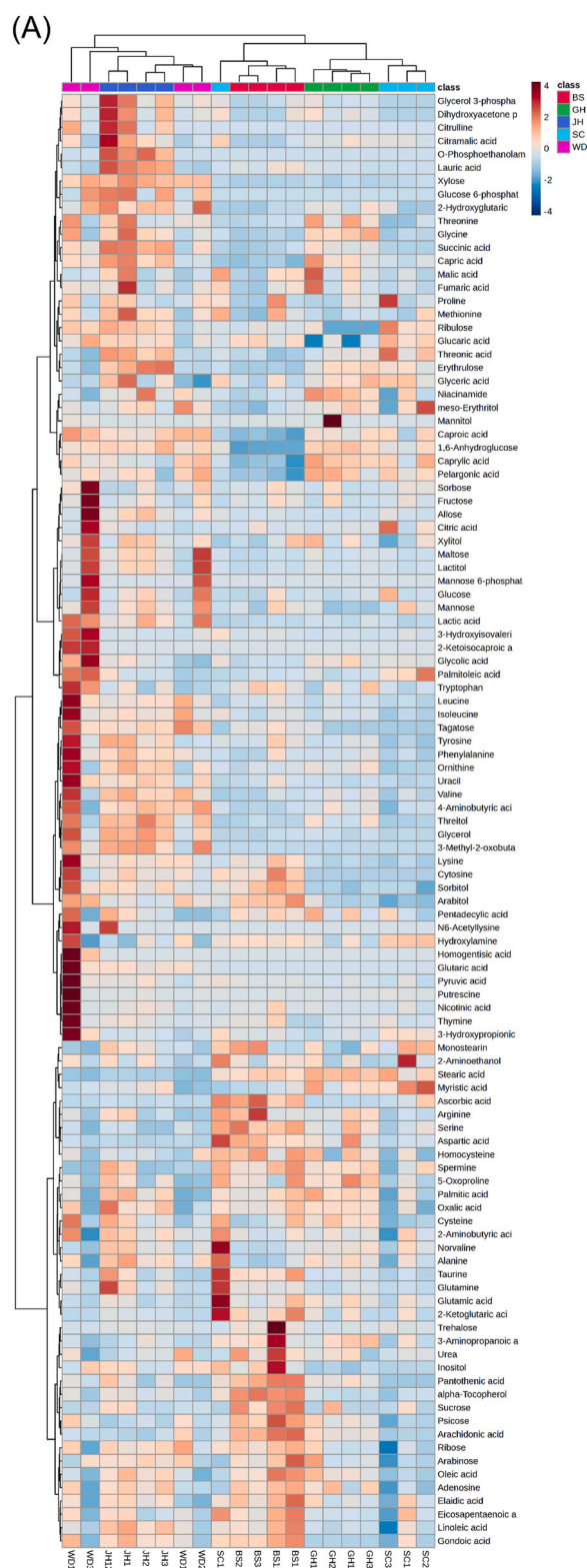


Fig. 3. Heatmap based on GC-MS/MS-based metabolite profiles in fresh laver samples varying with harvest time and region: (A) total metabolites, (B) amino acids and derivatives, (C) nitrogen-containing compounds, (D) organic acids and derivatives, (E) fatty acids and derivatives, (F) sugars and derivatives, and (G) vitamins. Red and blue colors presented in the heatmap indicate values above and below the mean centered and scaled expression values, respectively. White indicates values close to the mean. Each sample is color-coded by region: red for Busan (BS), green for Goheung (GH), blue for Jangheung (JH), light blue for Seochon (SC), and purple for Wido (WD). Black dots next to metabolite names indicate features that meet the unadjusted significance threshold ($p < 0.05$).

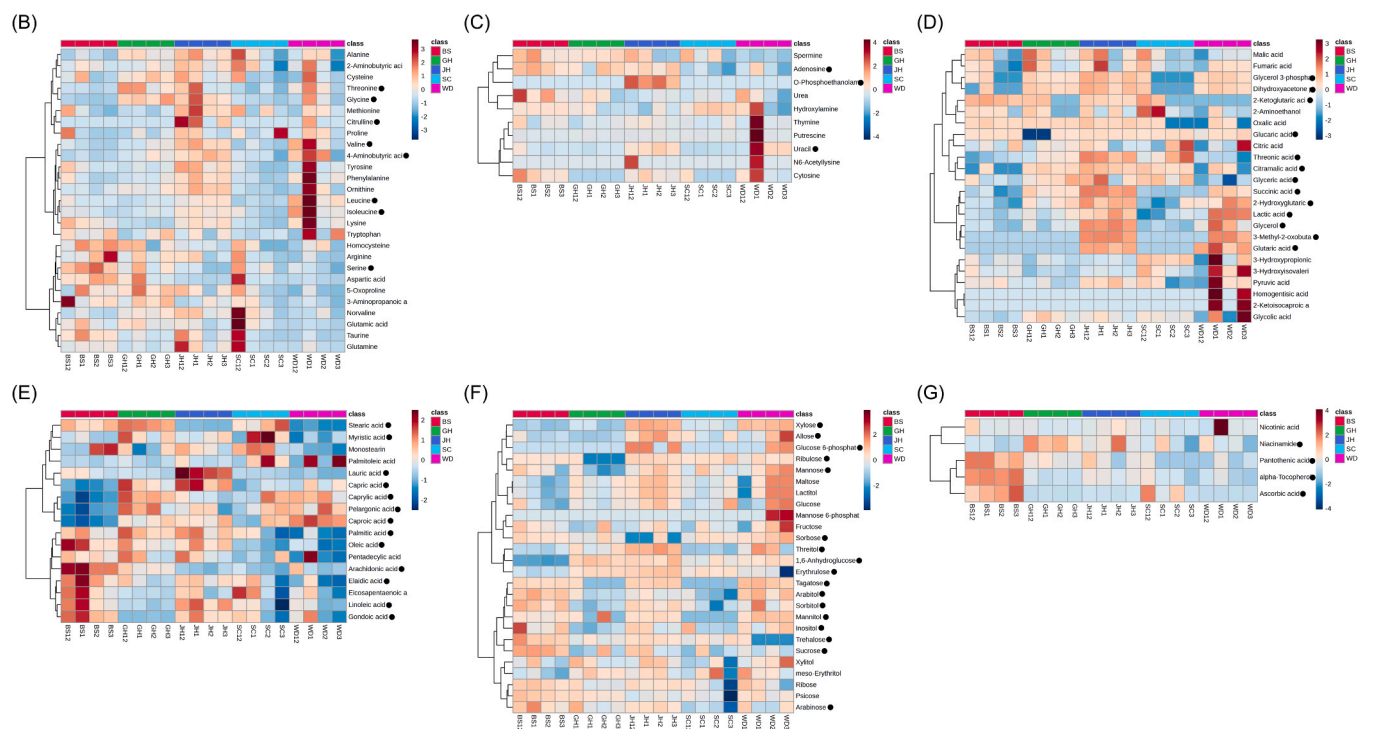


Fig. 3. (continued).

3.1.3. Amino acids and their derivatives in fresh laver samples

A broad range of amino acids and their derivatives were identified in the collected laver samples, including alanine, valine, leucine, glycine, glutamic acid, and aspartic acid. Notably, 4-aminobutyric acid (GABA), a non-proteinogenic amino acid with known bio-functional properties, was also detected. GABA functions as an inhibitory neurotransmitter in the mammalian central nervous system and has been associated with various health benefits, including antidiabetic, diuretic, and sedative effects (Poojary et al., 2017). Although GABA has been reported in several green and brown seaweeds, its presence in red seaweeds such as laver remains largely understudied (Park et al., 2023).

The heatmap of amino acids and their derivatives (Fig. 3B) revealed clear variation of the GABA content by harvest time and cultivation region. The JH samples generally exhibited the highest levels, with early harvests (December and January) exhibiting elevated concentrations across regions. The WD1 samples contained high concentrations of branched-chain amino acids (BCAAs)—valine, leucine, and isoleucine—which are associated with muscle metabolism and fatigue reduction. Amino acids related to neurotransmitter and mood regulation, including tyrosine, tryptophan, and phenylalanine, were also abundant in WD1. These amino acids were largely grouped within a single cluster in the heatmap (Fig. 3B), showing elevated levels particularly in the early-harvested samples from JH and WD. Notably, the BCAAs exhibited statistically significant differences among subclasses ($p < 0.05$).

Previous studies have shown that low-light conditions promote amino acid accumulation in seaweeds (Zhou et al., 2025). Consistent with this, the higher levels of alanine, serine, isoleucine, lysine, and threonine observed during the early-harvest period likely reflect the lower solar irradiance at that time. In SC, solar irradiance increased markedly from January to February, whereas proline levels peaked in March (SC3). This pattern is consistent with the role of proline as a stress-responsive amino acid involved in osmotic adjustment and the mitigation of reactive oxygen species under high-light conditions (Zhou et al., 2025). Taste-related amino acids such as alanine, glycine, and serine (sweet-taste related amino acids), as well as aspartic acid and glutamic acid (umami-taste related amino acids), were also identified.

These compounds are known to influence the sensory profile of laver (Cao et al., 2021; Zhao et al., 2016; Yuan et al., 2024). Taurine, a sulfonic acid compound commonly found in seafood, was also identified. Taurine not only contributes to umami enhancement by modulating sensory perception but also exhibits antioxidant and cytoprotective properties (Wang et al., 2020; Terriente-Palacios & Castellari, 2022).

To further examine the variation in these taste- and function-related compounds, their relative abundances were compared across regions and harvest times (Fig. 4A-C). In all three compound groups, the SC12 samples exhibited the highest levels and were set as the reference (100 %). Early-harvest samples (December and January) generally showed higher abundances of these compounds. In particular, GH1 and JH1 exhibited elevated levels of sweet-taste-related amino acids, whereas BS1 and GH1 showed higher levels of umami-taste-related amino acids relative to the other samples. However, because no sensory validation or dose-over-threshold evaluation was performed, these findings should be interpreted as indicating biochemical potential for taste-related differences rather than direct evidence of sensory perception. Taurine levels were particularly high in the SC12, BS1, and JH12 samples, and remained relatively high across BS samples.

Among the five regions, the SC samples contained the highest levels of taurine and other taste-active amino acids, particularly in SC12. Similarly, the BS samples were characterized by high levels of taurine and umami-taste amino acids. The JH samples had high levels of sweet-taste types. Meanwhile, the WD samples showed relatively high levels of BCAAs and mood-related amino acids.

Overall, these findings highlight the influence of both regional factors and harvest time on the metabolite composition of laver. Samples harvested from SC and BS in early winter (December and January) exhibited higher levels of umami-taste related amino acids and functional compounds such as taurine, suggesting that early winter harvesting may enhance the sensory and health-promoting qualities of laver. These regions and harvest time dependent variations in amino acid composition provide biochemical and physiological insights that could support targeted blending strategies and improve the flavor and functional quality of processed laver products. Furthermore, the results suggest that, in addition to BCAAs, taste-related amino acids may serve

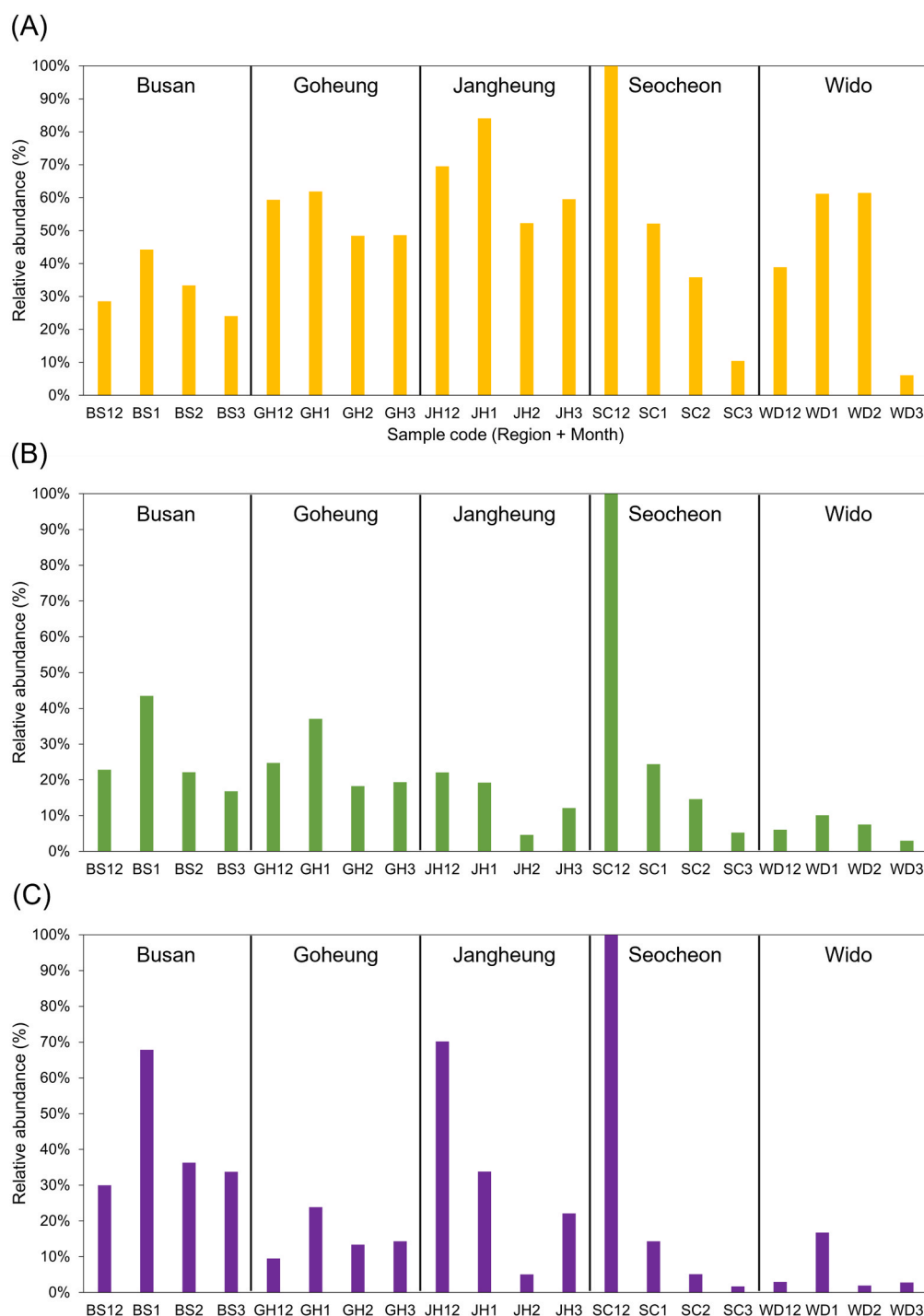


Fig. 4. Relative abundance (%) of representative amino acids in laver samples varying with harvest time and regions: (A) sweet-taste amino acids (alanine, glycine, serine), (B) umami-taste amino acids (aspartic acid, glutamic acid), and (C) taurine. The sample with the highest concentration in each group was set as 100 %, and the others were expressed as relative abundance(%) to it. Each sample was labeled using a two-part code based on region and harvest month (e.g., BS12 indicates laver samples harvested from Busan in December).

as potential quality indicators reflecting regional and seasonal characteristics of laver.

3.1.4. Nitrogen-containing compounds in fresh laver samples

Marine macroalgae exhibit variable nitrogen contents across taxa, with certain groups of red algae demonstrating exceptionally elevated levels of protein-derived nitrogen, sometimes exceeding 75 % of the total organic nitrogen found in mature seaweeds (Krishna Pillai, 1957). Beyond their industrial utility, nitrogen-containing compounds (NCCs)

within seaweed are increasingly recognized for their multifaceted biological functions and nitrogen composition. Fig. 3C presents the heatmap of 10 identified NCCs in laver samples, including nucleobases (uracil, thymine, cytosine), polyamines (spermine, putrescine), and other minor metabolites such as urea, O-phosphoethanolamine, hydroxylamine, and N6-acetyllysine. The heatmap clustering revealed a tendency for nucleobases to cluster together, indicating that they share similar accumulation patterns across samples. These metabolites varied significantly by harvest time and cultivation region. Notably, sample

WD1 exhibited relatively elevated concentrations of putrescine, N6-acetyllysine, and hydroxylamine, suggesting a distinct nitrogen metabolic profile. Nucleobases were more abundant in WD samples, particularly in WD1, while *O*-phosphoethanolamine, a precursor to phospholipids, was found in abundance in JH samples, especially JH12. Such findings imply the accumulation of specific metabolites associated with stress or nitrogen intermediates in laver, in response to variations in the cultivation environment and timing of harvest. According to the DIN levels at each site and harvest time (Fig. S1D), the DIN concentration in WD during December was markedly higher than in the other regions and months. This elevated DIN level in December may have contributed to the increased levels of NCCs observed in the WD1 samples. Variations in nitrogen levels throughout the seasons might be influenced by human activities like farming runoff and the release of treated wastewater, which often become more pronounced in winter due to higher rainfall and melting snow. Such heightened inputs of anthropogenic and environmental nitrogen may partially justify the elevated levels of NCCs detected in laver samples harvested during the winter season (December and January).

3.1.5. Organic acids and their derivatives in fresh laver samples

A total of 24 organic acids and their derivatives were identified, including malic acid, lactic acid, succinic acid, and citric acid, which are commonly found in seaweeds (Gravot et al., 2010; Hamid et al., 2019; Tanna et al., 2018). The heatmap in Fig. 3D shows that organic acid profiles varied by harvest time and cultivation region, reflecting distinct site-specific patterns. Succinic acid, a key umami-taste related compound in kelp (Milinovic et al., 2021), was consistently abundant in samples collected in December and January. The JH samples showed a higher total organic acid content, with JH12 exhibiting elevated levels of threonic acid, citramalic acid, and glyceric acid. Compared with WD12, WD1 showed higher glycolic acid and glycerol levels. Additionally, lactic acid was more abundant in the WD samples, while the JH samples exhibited a higher succinic acid content. These differences suggest that regional variations in organic acids may influence the flavor profile of laver.

3.1.6. Fatty acids and their derivatives in fresh laver samples

A total of 17 fatty acids and their derivatives were identified, encompassing both saturated and unsaturated types, such as eicosa-pentaenoic acid (EPA), linoleic acid, oleic acid, palmitic acid, and stearic acid. According to the heatmap in Fig. 3E, the palmitic acid and EPA levels were higher in samples harvested in December and January, indicating a seasonal influence on the lipid composition of laver. Seaweeds modulate the degree of fatty acid saturation to maintain membrane stability under fluctuating temperature and light conditions, reflecting adaptive changes in membrane fluidity (Vinuganesh et al., 2022; Liang et al., 2022). In particular, the relative proportions of polyunsaturated fatty acids (PUFAs) tend to increase under low-temperature and low-light conditions, enhancing membrane fluidity and supporting efficient light utilization in photosynthetic tissues (Liang et al., 2022).

According to the regional environmental data (Fig. S1A, C), SST was lowest in January and February, whereas solar irradiance was lowest in December and January. Consistent with this, PUFA levels (Fig. 3E) were highest in December and January, suggesting that low SST and irradiance induced a physiological adjustment in membrane lipid composition. Among these factors, solar irradiance likely exerted a more pronounced influence than temperature.

Palmitic acid, a predominant fatty acid in red seaweeds such as *Gracilaria* (Norziah & Ching, 2000), was particularly abundant in the GH and JH samples. The BS and SC samples exhibited high concentrations of EPA, a representative PUFA found in red seaweeds (Holadt & Kraan, 2011). Due to its high degree of unsaturation, EPA is more prone to oxidation, which may lead to the degradation of bioactive lipids and the formation of off-flavors (Choe & Oh, 2013). Oleic acid, an omega-9 fatty

acid known for its LDL cholesterol-lowering and anti-inflammatory properties, was most abundant in BS, followed by JH and GH.

In addition, GH, JH, and BS samples showed higher levels of pentadecylic acid, palmitic acid, and oleic acid compared with SC and WD. Previous studies have reported that salt stress can increase palmitic acid levels (Atikij et al., 2019), and the similar regional patterns observed here (Fig. S1B) suggest that pentadecylic acid and oleic acid may also participate in adaptive responses to salinity stress.

Overall, these results indicate that laver modulates its fatty acid metabolism to maintain membrane structural stability under environmental stresses such as low temperature, low light, and high salinity. These adaptive lipid profiles provide scientific insights that could support the optimization of harvest timing and cultivation-site selection to improve the nutritional and functional quality of laver products.

3.1.7. Sugars and their derivatives in fresh laver samples

A total of 26 sugars and their derivatives were identified, including sucrose, glucose, fructose, xylose, and glucose-6-phosphate. Several of these compounds have been consistently reported in seaweed metabolomics studies (Hamid et al., 2019). According to the heatmap presented in Fig. 3F, the sugar profiles differed among regions, with the JH samples generally exhibiting a higher sugar content. The BS samples maintained relatively elevated sucrose levels across harvest periods, whereas the WD samples showed a gradual increase in xylitol, maltose, glucose, and fructose in later harvests, with WD3 exhibiting the highest intensities.

The accumulation of free sugars in late harvest may reflect metabolic adjustments associated with storage compound buildup, partial hydrolysis of structural polysaccharides, and stress responses during tissue maturation. Furthermore, the rising seawater temperatures in February and March may further enhance the activity of carbohydrate-metabolizing enzymes, promoting sugar accumulation (Kumar et al., 2021). While an increased sugar content may enhance sweetness, it is often accompanied by undesirable changes such as softening and discoloration.

These findings highlight the complex interplay between harvest timing and regional conditions in determining sugar composition in laver, underscoring the need to optimize harvest schedules to balance sweetness and overall product quality.

3.1.8. KEGG pathway highlights in fresh laver

Metabolic pathway changes in fresh laver were evaluated by region and harvest month. For each region (with harvest month treated as a factor), metabolites showing significant differences were identified first ($p < 0.05$). The selected metabolite sets were subsequently subjected to pathway analysis. Top three pathways per region were selected primarily by significance ($-\log_{10}(p) > 3$); pathway impact > 0.4 was used as a prioritization heuristic rather than a strict cutoff (thus arginine biosynthesis is included in some regions despite lower impact) (Fig. 5). Across regions, alanine, aspartate and glutamate metabolism, glycine, serine and threonine (GST) metabolism, and arginine biosynthesis are consistently highlighted. To visualize concentration dynamics within these pathways (Fig. 6), JH and GH in Jeollanam-do were chosen as representative sites, given their high production (Korea Maritime Institute, 2025) and the difference in sampling years, enabling an assessment of interannual variation within the province. For each selected pathway, line plots of metabolite levels for JH and GH are shown together with solar irradiance, SST, and DIN to explore potential environmental associations (Fig. S1A, C, and D).

Studies on GST metabolism have largely focused on terrestrial plants, with comparatively limited work in macroalgae (Goh et al., 2025). In plants, threonine contributes to chemical defense against abiotic stress and plays key roles in growth and development, cell division, and phytohormone regulation (Muthuramalingam et al., 2018). In our dataset, threonine decreased sharply from January to February in both JH and GH. This pattern is plausibly associated with reduced availability

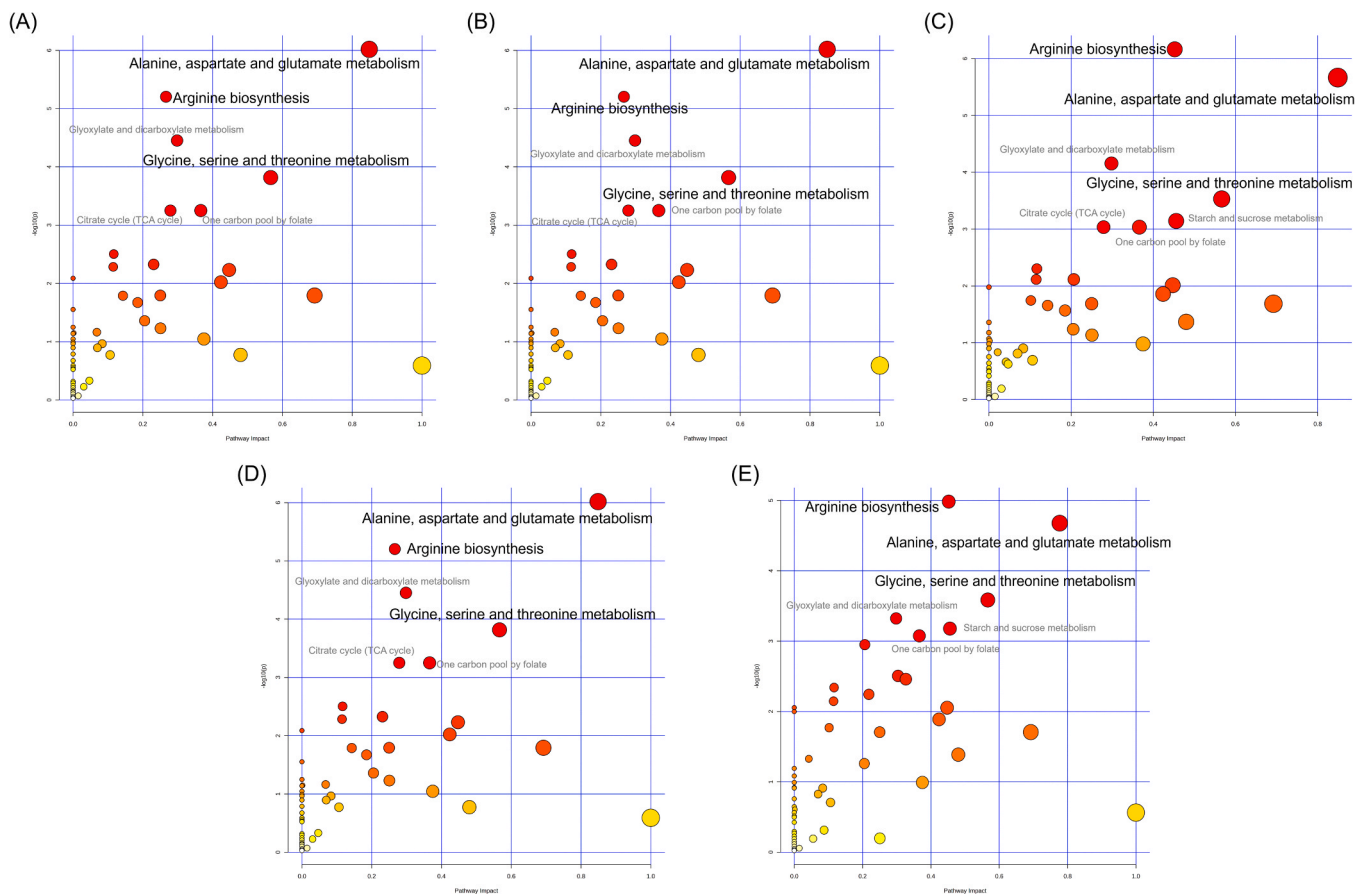


Fig. 5. Regional pathway enrichment bubble plots generated using metabolites with $p < 0.05$ for each region. The x-axis represents topology-based pathway impact, and the y-axis shows $-\log_{10}(p)$ values from the overrepresentation analysis. Bubble size reflects pathway impact, and bubble color indicates statistical significance, with redder bubbles representing higher significance. The top pathway and three closely related pathways are labeled in black for each regional panel (A: BS; B: GH; C: JH; D: SC; E: WD).

of assimilable nitrogen in February, when DIN declined (Fig. S1D), and with a transition to late season growth that can diminish free amino acid pools. Serine is a precursor to glycine, and glycine often accumulates under stress (Igamberdiev, Kleczkowski, 2018). Coinciding with the sharp December-to-January decrease in SST (Fig. S1A), region-specific differences were observed: glycine rose in JH, while serine rose in GH. This pattern is consistent with a temperature stress response at the serine to glycine step, such that JH may have channeled serine toward glycine while GH had not yet undergone the same conversion. These differences between nearby regions likely reflect interannual and microenvironmental variation.

Elevated temperature has been reported to upregulate the alanine, aspartate, and glutamate pathway in many seaweeds, supporting nitrogen metabolism, osmotic stability, and energy balance (Jahan et al., 2024). The elevated levels of alanine and glutamate (Fig. 6) observed in December are compatible with the relatively higher SST and DIN at that time (Fig. S1A, D). Glutamate is implicated in reallocating nitrogen to maintain nitrogen homeostasis (Liao et al., 2025). During the transition from high DIN in December to lower DIN in February (Fig. S1D), a pattern of decreasing glutamate together with increasing GABA (Fig. 6) can reflect temporary nitrogen storage via the GABA shunt. In March, higher solar irradiance may increase photosynthetic demand; in this context, a shift toward decreasing GABA and increasing glutamate and glutamine suggests a reallocation of nitrogen to support photosynthesis. The rise in glutamate and glutamine aligns with reports that these amino acids are linked to strengthened metabolic pathways that support photosynthetic capacity (Liao et al., 2025).

Arginine serves as a nitrogen reservoir and exhibits antioxidant

properties through its capacity to generate nitric oxide (NO), thereby protecting cells from oxidative stress (Farjallah et al., 2024). Under high DIN in December, nitrogen accumulation may have been followed by a decline in arginine when DIN decreased in February, whereas increases in SST and solar irradiance in March could have promoted a rebound in arginine as part of oxidative stress defense.

In summary, early-winter DIN likely enlarges the free amino acid pool and emphasizes the alanine, aspartate and glutamate and the GST pathways, whereas rising irradiance with declining SST may promote Ser-Gly cycling and the GABA shunt. Future studies should undertake targeted enzyme or transcript analyses to substantiate these causal links.

3.1.9. Nutrient metabolites in fresh laver samples

A total of 5 vitamins—pantothenic acid (vitamin B5), nicotinic acid (vitamin B3), niacinamide (vitamin B3), ascorbic acid (vitamin C), and α -tocopherol (vitamin E)—were identified in fresh laver samples. According to the heatmap presented in Fig. 3G, the metabolites exhibited distinct region-specific patterns, with the BS samples exhibiting the highest overall vitamin content. Regional differences exerted a stronger influence on vitamin levels than harvest time, underscoring the importance of geographical factors in determining the vitamin profile of fresh laver. In particular, α -tocopherol, a fat-soluble antioxidant, was especially abundant in BS samples. Previous findings identified α -tocopherol, as the dominant lipid-soluble vitamin in seaweeds (Panayotova et al., 2013). The water-soluble niacinamide and pantothenic acid were especially enriched in the GH and BS samples, respectively. These site-specific distributions suggest that regional differences in environmental conditions and metabolic activity influence vitamin

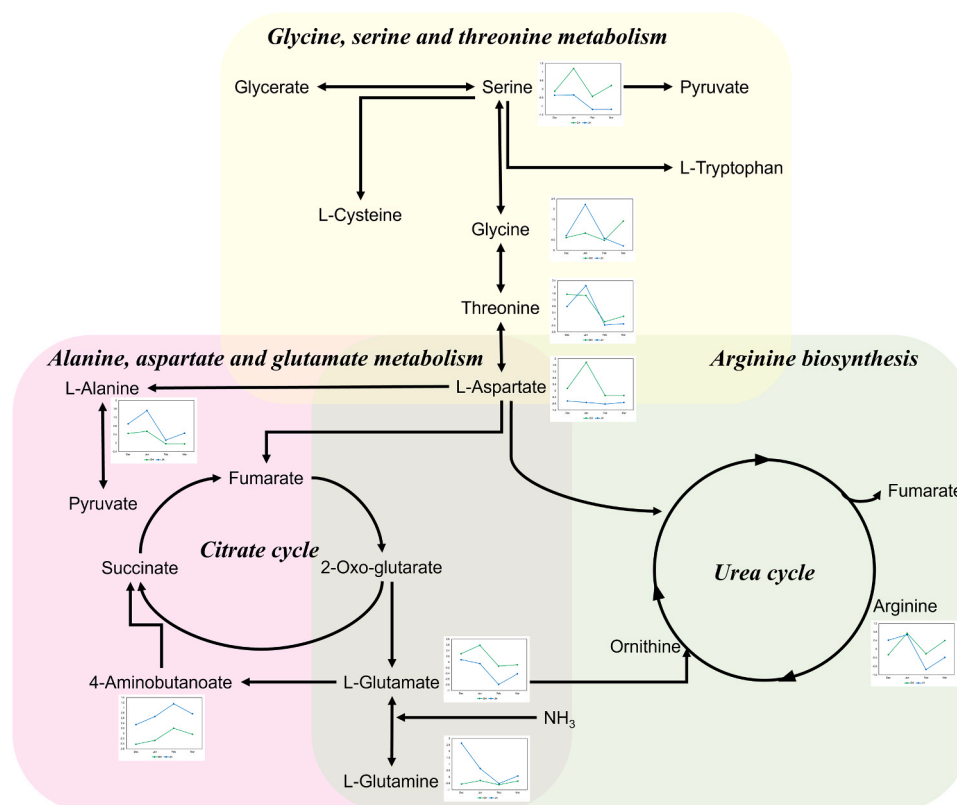


Fig. 6. Metabolic context of three enriched pathways with regional monthly line plots of relative metabolite abundance. The schematic highlights key reactions within glycine, serine and threonine metabolism; alanine, aspartate and glutamate metabolism; and arginine biosynthesis. Mini line plots adjacent to each metabolite node display monthly relative abundance patterns for JH (blue) and GH (green), and shaded backgrounds delineate the three metabolic pathways.

accumulation in laver. Given the known roles of pantothenic acid and α -tocopherol in antioxidant defense and coenzyme function (Kucharská, 2008), these patterns may also contribute to functional quality.

3.1.10. Summary of primary and nutrient metabolites in fresh laver

From a multivariate perspective, amino acids were generally higher in December and January, and selected PUFAs (e.g., EPA, linoleic acid) showed similar increases during this period. Regionally, amino acids were more abundant in JH, whereas fatty acids were relatively enriched in BS and JH. These abundance patterns, summarized in the clustered heatmaps, were broadly consistent with the concurrent environmental conditions (SST, salinity, solar irradiance, and DIN; Fig. S1). An exploratory PCA is provided in Fig. S4A; although the separation was limited, the overall tendencies were qualitatively consistent with the PLS-DA results (Section 3.1.1).

Environmental factors such as DIN, dissolved inorganic phosphorus (DIP), and light availability are key drivers of nutrient metabolism in seaweeds (Liang et al., 2022). These parameters regulate the biosynthesis of amino acids, fatty acids, and galactolipids, thereby shaping the overall metabolite profile. In this study, the amino acid concentrations were generally higher in December and January, possibly due to increased DIN availability during early winter. PUFAs such as EPA and linoleic acid also accumulated during this period, potentially as a response to light limitation or cold-induced stress (Guschina & Harwood, 2009). Regionally, the JH samples showed higher amino acid levels, while the BS and JH samples were enriched in fatty acids, reflecting site-specific environmental inputs.

Such findings highlight the significant influence of both temporal and spatial variables on the nutritional profile of laver. From an industrial perspective, selecting or blending fresh laver based on harvest time and cultivation region could enhance the flavor and functional properties of final products. For example, combining early harvested laver from

SC and BS (rich in umami-related amino acids and EPA) with JH laver containing high levels of sweet-tasting amino acids, could enhance both palatability and nutritional value. Such targeted blending approaches could afford premium laver products tailored to specific consumer demands.

3.2. Relationship between crude protein content and amino acids in fresh laver samples

In Korea, the crude protein content (CPC) is a key quality indicator for dried laver. According to the Korean Industrial Standards (KS Korean industrial standards., 2004), dried laver is classified as top grade if its CPC value is ≥ 35 %, high grade for $\text{CPC} \geq 30$ %, and normal grade for $\text{CPC} < 30$ %. To investigate the relationship between CPC and amino acid composition in fresh laver, we examined metabolic features associated with protein accumulation at the raw material stage and identified potential quality markers.

The CPC levels of the JH and WD laver samples were measured, to assess their relationship with amino acid composition in the fresh state. Based on the measured CPC, the tested samples were grouped into high-protein (≥ 30 %) and low-protein (< 30 %) categories. The high-protein group included JH12 (36.22 %), JH1 (33.39 %), and JH3 (33.46 %), while the low-protein group consisted of JH2 (29.29 %), WD12 (27.73 %), WD1 (26.39 %), WD2 (26.96 %), and WD3 (12.36 %).

To investigate the association between CPC and amino acid composition in fresh laver, volcano plot and correlation analyses were performed (Fig. 7A, B). Volcano plot analysis was performed to identify amino acids that showed significant differences between high-protein (≥ 30 %) and low-protein (< 30 %) laver samples (Fig. 7A). Seven amino acids, including glutamic acid, 5-oxoproline, glutamine, taurine, citrulline, arginine, and norvaline, were significantly enriched in the high-protein group, with fold changes ≥ 2 ($\log_2\text{FC} \geq 1$) and p -values

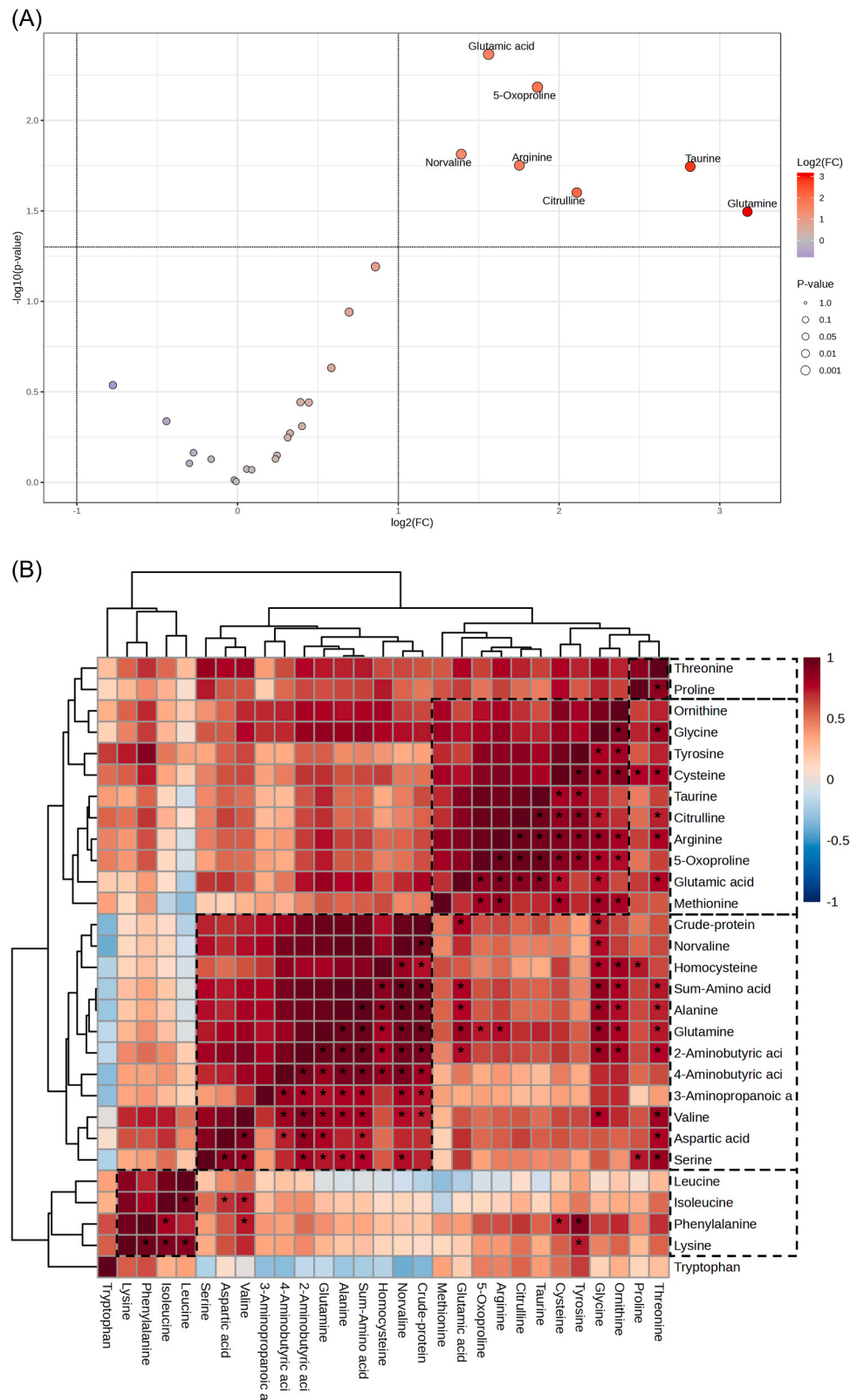


Fig. 7. Multivariate analysis results of the relationship between crude protein content and amino acid content in laver samples: (A) volcano plot showing differential amino acid between high-protein ($\geq 30\%$) and low-protein ($< 30\%$) laver groups. Metabolites with $|\log_2 \text{fold change}| \geq 1$ and $p < 0.05$ are highlighted, (B) Pearson's correlation heatmap showing the relationships between individual amino acids, total free amino acids, and crude protein content without applying a correlation cut-off. The color scale was fixed between 1- and + 1 for all panels. Correlation coefficients with $|r| > 0.7$ and statistically significant correlations ($p < 0.05$) are marked with an asterisk (*).

< 0.05. Among them, glutamic acid exhibited the greatest difference, suggesting that it may play a key role as a quality-related metabolite in high-protein laver. In the low-protein group, no amino acid was significantly enriched.

To investigate the relationship between CPC and amino acid composition in fresh laver, we examined metabolic features associated with protein accumulation at the raw material stage and identified potential quality markers. Another module consisted largely of amino acids considered conditionally essential in humans under specific physiological stress (e.g., arginine, cysteine, glycine, and tyrosine), which displayed tight inter-correlations ($p < 0.05$). This pattern likely reflects coordinated metabolism of nitrogen- and sulfur-containing amino acids, supporting cellular stress adaptation and redox homeostasis. According to Wang et al. (2023), amino acids including arginine, cysteine, glycine, and tyrosine are conditionally essential in humans under specific physiological stress. The high inter-correlation among these amino acids in laver therefore suggests nutritional and economic value, insofar as they may complement essential amino acids in plant-based protein sources.

3.3. Evaluation of carotenoid and phenolic acid method performance

The limit of detection (LOD) and limit of quantification (LOQ) were determined from the signal-to-noise (S/N) ratios of chromatographic peaks at the lowest calibration level. LOD and LOQ were defined as $S/N \geq 3$ and $S/N \geq 10$, respectively. The analytical method used in this study exhibited reliable performance and high sensitivity (LOD, LOQ, analysis time, etc.) compared with previously reported methods.

For both carotenoids and phenolic acids, the LOD and LOQ values were comparable to or lower than those reported in earlier studies (Mok et al., 2016; Xu et al., 2023; Van Meulebroek et al., 2014; Akcay et al., 2024; Song et al., 2019; Gültekin-Özgüven et al., 2015). For example, Xu et al. (2023) reported LOD and LOQ values of 0.020 and 0.067 $\mu\text{g/g}$ for lutein, respectively, while Song et al. (2019) reported values of 10 and 33 ng/g for 3,4-dihydroxybenzoic acid. Detailed values are provided in Table S6.

The analysis time for carotenoids was 35 min per injection, which falls within the 30–40 min range reported previously (Heffernan et al., 2016; Susanto et al., 2019; Othman et al., 2018; Marinho et al., 2019). Phenolic acid analysis was completed within 20 min, consistent with earlier reports (Kumar et al., 2020; Souza et al., 2011).

3.4. Carotenoids as secondary metabolites in fresh laver

Seven carotenoids (i.e, fucoxanthin, lutein, zeaxanthin, β -apo-8'-carotenal, β -cryptoxanthin, α -carotene, and β -carotene) were identified and quantified in fresh laver samples (Table 1). Carotenoids are natural antioxidants that have been associated with protective effects against various chronic diseases, including cardiovascular and neurodegenerative disorders, macular degeneration, obesity, and cancer (Perera & Al-Zahrani, 2022). While β -carotene and lutein have previously been reported in laver (Oh et al., 2013), ucoxanthin, β -apo-8'-carotenal, and α -carotene are, to our knowledge, newly confirmed in this species.

Total carotenoid contents varied significantly by harvest time and cultivation region (Table 1). The highest levels were observed in SC12 (932.3 $\mu\text{g/g}$), GH12 (925.4 $\mu\text{g/g}$) and SC1 (922.8 $\mu\text{g/g}$), whereas while lower levels were observed in BS3 (560.9 $\mu\text{g/g}$), JH2 (462.2 $\mu\text{g/g}$), and WD3 (344.3 $\mu\text{g/g}$). Overall, December and January harvests exhibited greater totals than later months, consistent with lower solar irradiance in early winter (Fig. S1B) and reports that total carotenoids can be higher under low-light than high-light conditions (Zepeda et al., 2025). In contrast, β -carotene occasionally peaked under higher irradiance in February and March (Fig. S1A), suggesting photoprotective acclimation (e.g., enhanced β -branch flux) under elevated light (Xie et al., 2020).

Among the individual compounds, lutein was the most abundant carotenoid among the major ones, ranging from 132.8 $\mu\text{g/g}$ (SC3) to 778.2 $\mu\text{g/g}$ (SC12). This contrasts with previous studies that identified β -carotene as the dominant carotenoid in laver (Oh et al., 2013), but aligns with more recent reports on red seaweeds (Park et al., 2023). α -Carotene, a provitamin A carotenoid convertible to vitamin A and an antioxidant (Aryee et al., 2018), was detected in all samples, with notably high levels in JH1. Fucoxanthin, a carotenoid commonly found in brown seaweeds, was detected at levels ranging from 2.7 to 84.2 $\mu\text{g/g}$. Beyond its well-documented antioxidant, anti-inflammatory, anti-obesity, and anti-diabetic activities (Yang et al., 2022b), its consistent presence across regions suggests that laver may serve as an additional dietary source of this compound. Zeaxanthin, a xanthophyll carotenoid known to protect against macular degeneration (Ganesan et al., 2019), exhibited higher concentrations in early-harvested samples. Among the minor carotenoids, β -apo-8'-carotenal—an oxidative cleavage product in the carotenoid pathway (Zebua et al., 2023; Shi et al., 2021)—was detected at low but consistent levels across all regions.

Table 1
Carotenoid composition of fresh laver.

Samples	Carotenoid contents ($\mu\text{g/g}$, dry weight)							
	Fucoxanthin	Lutein	Zeaxanthin	β -Apo-8'-carotenal	β -Cryptoxanthin	α -Carotene	β -Carotene	Total carotenoids
BS12	6.9 $\pm$ 0.4 j	697.2 $\pm$ 7.1 bc	80.6 $\pm$ 1.0 de	6.9 $\pm$ 0.1 g	4.6 $\pm$ 0.1 c	29.9 $\pm$ 0.2 g	25.1 $\pm$ 0.5 hij	851.2 $\pm$ 7.5 bc
BS1	13.4 $\pm$ 0.6 hi	655.0 $\pm$ 25.9 c	85.9 $\pm$ 0.5 bcd	8.4 $\pm$ 0.3 bcd	5.0 $\pm$ 0.0 ab	23.6 $\pm$ 0.3 hi	23.1 $\pm$ 0.5 ij	814.5 $\pm$ 26.1 cd
BS2	7.7 $\pm$ 0.5 j	555.8 $\pm$ 3.4 d	61.8 $\pm$ 0.3 g	7.7 $\pm$ 0.2 def	4.7 $\pm$ 0.1 bc	20.3 $\pm$ 0.3 ij	21.6 $\pm$ 0.5 jk	679.6 $\pm$ 3.6 e
BS3	8.1 $\pm$ 0.3 j	451.4 $\pm$ 3.2 ef	59.7 $\pm$ 1.3 g	5.7 $\pm$ 0.1 h	3.0 $\pm$ 0.1 g	14.8 $\pm$ 0.2 kl	18.3 $\pm$ 0.1 k	560.9 $\pm$ 4.1 g
GH12	18.2 $\pm$ 1.3 g	728.9 $\pm$ 17.6 ab	85.5 $\pm$ 0.8 cd	9.8 $\pm$ 0.3 a	4.8 $\pm$ 0.2 abc	42.2 $\pm$ 1.0 f	36.0 $\pm$ 1.2 fg	925.4 $\pm$ 17.7 a
GH1	14.7 $\pm$ 0.4 h	714.9 $\pm$ 20.0 b	78.7 $\pm$ 0.6 e	8.2 $\pm$ 0.3 cd	4.0 $\pm$ 0.1 d	29.2 $\pm$ 0.1 g	36.7 $\pm$ 0.5 fg	886.4 $\pm$ 20.3 ab
GH2	4.3 $\pm$ 0.2 k	475.3 $\pm$ 22.7 e	57.8 $\pm$ 5.0 g	8.7 $\pm$ 0.1 bc	5.0 $\pm$ 0.1 a	29.4 $\pm$ 0.2 g	38.7 $\pm$ 0.4 efg	619.2 $\pm$ 25.5 f
GH3	24.1 $\pm$ 0.3 f	533.1 $\pm$ 18.5 d	70.5 $\pm$ 2.4 f	7.3 $\pm$ 0.1 efg	3.9 $\pm$ 0.1 d	25.8 $\pm$ 0.7 h	35.3 $\pm$ 1.1 g	700.1 $\pm$ 20.7 e
JH12	11.8 $\pm$ 0.1 i	542.7 $\pm$ 34.6 d	111.6 $\pm$ 3.5 a	1.5 $\pm$ 0.0 jk	3.5 $\pm$ 0.2 ef	58.4 $\pm$ 0.3 e	61.4 $\pm$ 0.3 c	790.9 $\pm$ 38.2 d
JH1	6.8 $\pm$ 0.1 j	557.4 $\pm$ 12.0 d	88.2 $\pm$ 3.9 bc	1.9 $\pm$ 0.0 j	2.5 $\pm$ 0.1 h	67.7 $\pm$ 1.3 d	97.8 $\pm$ 0.7 a	822.3 $\pm$ 12.4 cd
JH2	30.4 $\pm$ 0.8 e	235.8 $\pm$ 3.3 gh	34.0 $\pm$ 0.8 ij	1.0 $\pm$ 0.0 k	2.9 $\pm$ 0.1 g	76.2 $\pm$ 0.8 bc	82.0 $\pm$ 0.9 b	462.2 $\pm$ 2.4 ij
JH3	33.1 $\pm$ 0.6 d	276.7 $\pm$ 19.6 g	32.7 $\pm$ 1.2 j	1.6 $\pm$ 0.1 jk	3.4 $\pm$ 0.1 f	89.9 $\pm$ 2.3 a	101.3 $\pm$ 4.1 a	538.6 $\pm$ 16.7 gh
SC12	2.7 $\pm$ 0.2 k	778.2 $\pm$ 4.9 a	91.5 $\pm$ 1.5 b	8.0 $\pm$ 0.1 cde	4.8 $\pm$ 0.0 abc	17.8 $\pm$ 0.3 jk	29.4 $\pm$ 0.4 h	932.3 $\pm$ 3.7 a
SC1	70.9 $\pm$ 0.9 b	679.1 $\pm$ 44.9 bc	89.8 $\pm$ 0.5 bc	9.0 $\pm$ 0.9 b	3.7 $\pm$ 0.1 de	30.4 $\pm$ 0.1 g	39.8 $\pm$ 0.2 ef	922.8 $\pm$ 44.0 a
SC2	84.2 $\pm$ 0.1 a	188.2 $\pm$ 3.5 hi	26.1 $\pm$ 0.4 k	7.1 $\pm$ 0.1 fg	2.8 $\pm$ 0.2 gh	22.1 $\pm$ 0.4 i	41.5 $\pm$ 0.4 e	372.2 $\pm$ 3.5 kl
SC3	N.D.	132.8 $\pm$ 2.2 i	37.9 $\pm$ 1.0 ij	2.0 $\pm$ 0.0 j	0.7 $\pm$ 0.0 k	7.5 $\pm$ 0.1 m	26.4 $\pm$ 0.2 hi	207.3 $\pm$ 3.3 m
WD12	N.D.	281.6 $\pm$ 8.1 g	39.4 $\pm$ 1.2 i	3.0 $\pm$ 0.0 i	1.7 $\pm$ 0.1 i	79.3 $\pm$ 3.4 b	17.5 $\pm$ 0.2 k	422.5 $\pm$ 10.2 jk
WD1	13.1 $\pm$ 0.5 hi	402.6 $\pm$ 17.8 f	50.1 $\pm$ 1.0 h	1.9 $\pm$ 0.0 j	2.8 $\pm$ 0.0 g	70.9 $\pm$ 1.0 d	46.3 $\pm$ 2.5 d	587.8 $\pm$ 20.0 fg
WD2	54.0 $\pm$ 2.4 c	242.4 $\pm$ 4.8 gh	25.3 $\pm$ 0.5 k	1.0 $\pm$ 0.0 k	1.6 $\pm$ 0.1 ij	74.7 $\pm$ 0.5 c	99.2 $\pm$ 2.8 a	498.0 $\pm$ 7.0 hi
WD3	24.8 $\pm$ 0.9 f	209.7 $\pm$ 5.9 h	45.6 $\pm$ 1.5 h	N.D.	1.3 $\pm$ 0.0 j	12.8 $\pm$ 0.2 l	50.2 $\pm$ 1.6 d	344.3 $\pm$ 6.6 l

N.D.: not detected
Mean values followed by different letters indicate significant sample differences at $P < 0.05$.
BS, GH, JH, SC, and WD indicate Busan, Goheung, Jangheung, Seocheon, and Wido.

Table 2
Phenolic acid composition of fresh laver.

Samples	Phenolic acid content (ng/g, dry weight)							
	4-Hydroxybenzoic acid*	Dimethoxy hydroxycinnamic acid	3,4-Dihydroxy benzoic acid*	Salicylic acid*	Dihydroxy phenylacetic acid	Hydroxybenzoic acid hexoside	Gallic acid gallate	Total free phenolic compounds
BS12	20.3 ± 1.4 de	436.4 ± 32.3 f	13.1 ± 1.3 f	23.4 ± 0.3 abc	716.0 ± 46.8 cd	8.9 ± 0.2 j	10.7 ± 0.4 jk	1228.8 ± 79.5 de
BS1	13.8 ± 1.0 hij	841.3 ± 74.1 cd	12.6 ± 1.0 f	18.0 ± 1.6 fghi	137.2 ± 8.9 gh	N.D.	10.0 ± 0.2 k	1033.0 ± 75.1 ef
BS2	19.1 ± 0.7 e	128.4 ± 12.0 ij	11.4 ± 0.8 f	18.5 ± 1.3 efghi	211.4 ± 12.4 efgh	8.7 ± 0.0 j	11.6 ± 0.6 ijk	409.2 ± 7.6 hijk
BS3	31.0 ± 1.1 a	170.0 ± 7.5 hi	13.3 ± 1.3 f	19.4 ± 1.0 cdefghi	240.7 ± 22.7 efgh	N.D.	16.9 ± 0.5 fg	491.3 ± 17.8 hij
GH12	16.4 ± 0.1 fg	1202.5 ± 111.7 b	6.1 ± 0.3 g	13.0 ± 1.0 j	120.5 ± 1.3 gh	11.9 ± 0.1 ef	N.D.	1370.5 ± 111.9 cd
GH1	27.0 ± 0.9 b	445.2 ± 24.9 f	6.6 ± 0.2 g	16.8 ± 0.3 hij	146.9 ± 1.4 gh	12.1 ± 0.0 de	14.3 ± 0.1 ghi	668.8 ± 24.8 gh
GH2	20.2 ± 0.3 de	429.7 ± 24.3 f	18.4 ± 0.2 cd	15.2 ± 0.5 ij	177.5 ± 2.3 fgh	11.7 ± 0.2 ef	20.1 ± 0.4 de	692.9 ± 26.2 gh
GH3	21.7 ± 0.5 cd	337.0 ± 6.2 fg	16.9 ± 0.0 cd	17.6 ± 0.1 ghi	198.5 ± 2.8 efgh	17.1 ± 0.1 a	18.2 ± 0.4 ef	627.0 ± 7.4 ghi
JH12	19.2 ± 0.2 e	373.0 ± 28.2 fg	7.6 ± 0.6 g	22.4 ± 1.7 abcde	728.4 ± 4.7 c	13.7 ± 1.0 c	20.0 ± 0.5 de	1184.2 ± 23.6 de
JH1	23.2 ± 0.9c	274.9 ± 11.6 gh	N.D.	20.9 ± 1.7 cdefgh	487.5 ± 16.1 cde	15.6 ± 0.2 b	24.0 ± 2.0 bc	846.1 ± 21.4 fg
JH2	15.7 ± 0.6 gh	1087.4 ± 70.2 b	13.7 ± 1.1 ef	16.7 ± 0.8 hij	438.9 ± 33.1 def	17.8 ± 1.2 a	16.3 ± 1.1 fgh	1606.4 ± 105.3 c
JH3	14.6 ± 0.7 ghi	761.8 ± 23.2 d	6.9 ± 0.5 g	18.9 ± 1.5 defghi	406.1 ± 15.1 efg	15.3 ± 0.5 b	25.6 ± 1.3 b	1249.2 ± 36.0 de
SC12	27.1 ± 1.0 b	584.8 ± 39.6 e	16.2 ± 0.2 de	17.6 ± 0.8 fghi	322.9 ± 13.0 efgh	10.8 ± 0.2 efgh	32.6 ± 1.5 a	1012.0 ± 40.6 ef
SC1	15.1 ± 0.4 gh	83.2 ± 4.9 ij	27.8 ± 1.0 b	22.0 ± 2.1 bcdef	170.4 ± 3.0 fgh	10.5 ± 0.2 fghi	13.7 ± 0.4 hi	342.8 ± 2.9 ijk
SC2	12.7 ± 0.5 ij	N.D.	19.2 ± 1.5 c	23.2 ± 1.3 abcd	65.8 ± 4.1 h	9.5 ± 0.1 hij	22.1 ± 1.7 cd	152.6 ± 6.0 k
SC3	18.7 ± 0.7 ef	82.0 ± 5.6 ij	6.6 ± 0.4 g	21.3 ± 1.3 bcdefg	159.6 ± 7.2 fgh	9.3 ± 0.1 ij	12.9 ± 0.9 ij	310.4 ± 7.8 jk
WD12	14.3 ± 1.2 ghij	41.5 ± 2.1 ij	13.4 ± 1.2 ef	26.6 ± 2.3 a	357.2 ± 30.3 efg	11.2 ± 0.6 efg	9.2 ± 0.0 k	473.3 ± 30.0 hij
WD1	16.4 ± 0.7 fg	942.6 ± 33.4 c	19.0 ± 1.5 c	25.7 ± 2.5 ab	6818.2 ± 407.6 a	11.9 ± 0.3 ef	17.7 ± 0.6 ef	7851.5 ± 384.6 a
WD2	14.2 ± 0.4 ghij	602.6 ± 57.8 e	35.3 ± 1.4 a	21.6 ± 1.8 bcdefg	396.4 ± 16.9 efg	13.4 ± 0.8 cd	11.8 ± 0.2 ijk	1095.3 ± 68.6 def
WD3	12.0 ± 0.4 j	1921.4 ± 44.0 a	5.5 ± 0.3 g	23.4 ± 1.2 abc	1406.2 ± 51.0 b	9.9 ± 0.6 ghij	9.7 ± 0.5 k	3388.1 ± 95.6 b

N.D.: not detected

Mean values followed by different letters indicate significant sample differences at $P < 0.05$.

BS, GH, JH, SC, and WD indicate Busan, Goheung, Jangheung, Seocheon, and Wido.

*Identified with authentic standard.

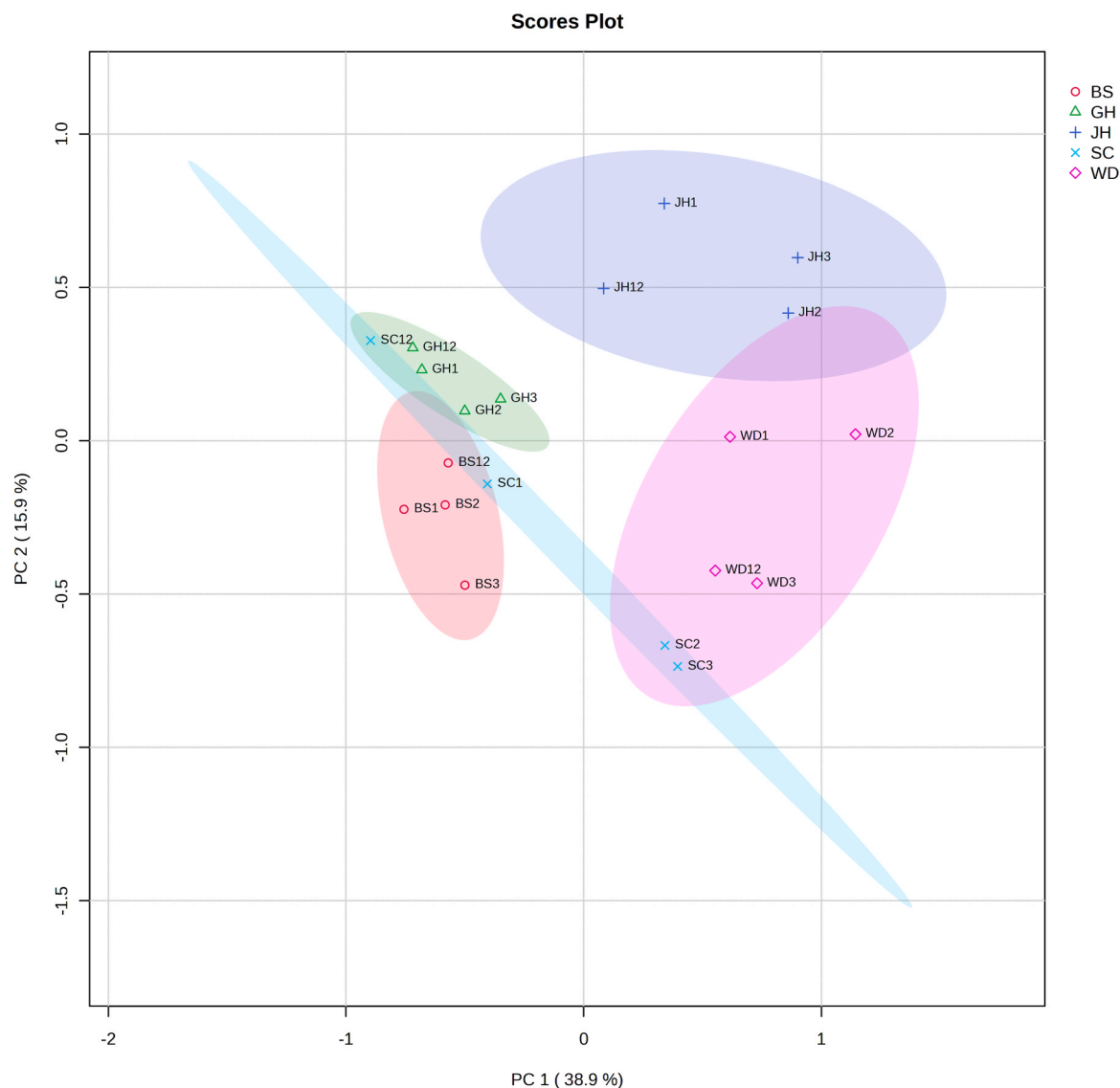


Fig. 8. PCA score plot based on the profiles of secondary bioactive metabolites (i.e., carotenoids, phenolic compounds) in laver samples varying with harvest time and region. The horizontal axis represents the first principal component, and the vertical axis represents the second principal component. Each sample is color-coded by regions: red for Busan (BS), green for Goheung (GH), blue for Jangheung (JH), light blue for Seocheon (SC), and purple for Wido (WD).

Overall, these results indicate that a compact marker panel may be useful for raw-material grading: a xanthophyll index (lutein and zeaxanthin) for identifying carotenoid-rich early-winter lots, and a provitamin-A index (α -carotene and β -carotene) for evaluating nutritional value. The newly confirmed carotenoids (fucoxanthin and β -apo-8'-carotenal) expand the pool of potential markers. Because post-harvest processing can lead to carotenoid loss, sourcing carotenoid-rich fresh laver and verifying compound retention after processing will be essential for linking raw-material markers to finished-product standards.

3.5. Free phenolic acids as secondary metabolites in fresh laver

Seven phenolic acids (4-hydroxybenzoic acid, dimethoxy hydroxycinnamic acid, 3,4-dihydroxybenzoic acid, salicylic acid, dihydroxyphenylacetic acid, hydroxybenzoic acid hexoside, and gallic acid gallate) were identified and quantified in fresh laver samples via LC-MS/MS (Table 2). Of these, five compounds have been previously reported in seaweeds, particularly in brown species with a high phenolic content (Generalić Mekinić et al., 2019; Park et al., 2023; Subbiah et al., 2023).

The total content of free phenolic acids ranged from 152.5 ng/g

(SC2) to 7851.5 ng/g (WD1). Samples collected in December generally exhibited higher concentrations, although exceptions such as JH2 (February) and WD1 (January) showed region-specific maxima. These findings suggest that phenolic acid accumulation in laver is modulated by seasonal and local environmental factors.

Compared to previous studies on Korean laver, which mainly reported total phenolic content (TPC) in dried samples (Baek et al., 2019; Lee et al., 2012), this study offers the first comprehensive profile of individual free phenolic acids in fresh laver. The total concentrations ranged from 0.15 to 7.85 ppm in fresh samples, while previous studies on TPC in dried laver reported a range from 2.45 to 3.51 ppm depending on region (Lee et al., 2012).

Among the identified compounds, dihydroxyphenylacetic acid and dimethoxycinnamic acid were predominant, reaching up to 6818.2 ng/g in WD1 and 1921.4 ng/g in WD3, respectively. Both compounds are known for their antioxidant and anti-inflammatory properties (Cotas et al., 2020). In contrast, salicylic acid and gallic acid gallate were consistently detected but showed no significant variation across samples.

The high phenolic content observed in WD3 may be linked to stress-

Table 3

Ranges of total carotenoids and free phenolic compounds in laver samples: (A) by harvest time, (B) by harvest region.

(A)	
Harvest time	Range
Total carotenoids (μg/g, dry weight)	
December	422.5–932.3
January	587.8–922.8
February	372.2–679.6
March	207.3–700.1
Total free phenolic acids (ng/g, dry weight)	
December	473.3–1370.5
January	342.8–7851.5
February	152.6–1606.4
March	310.4–3388.1
(B)	
Harvest region	Range
Total carotenoids (μg/g, dry weight)	
Busan	560.9–851.2
Goheung	619.2–925.4
Jangheung	462.2–822.3
Seocheon	207.3–932.3
Wido	344.3–587.8
Total free phenolic acids (ng/g, dry weight)	
Busan	409.2–1228.8
Goheung	627.0–1370.5
Jangheung	846.1–1606.4
Seocheon	152.6–1012.0
Wido	473.3–3388.1

related metabolic responses, as seaweeds often upregulate phenolic biosynthesis in response to environmental stimuli such as UV radiation, temperature fluctuation, and microbial stress (Aina et al., 2022). Overall, these findings reveal distinct phenolic profiles in fresh Korean laver and highlight their potential contribution in developing health-promoting food products.

3.6. Multivariate clustering of secondary metabolites in fresh laver samples

To examine the overall variation in secondary metabolites, PCA was performed using combined data for carotenoids and free phenolic acids (Fig. 8). The first two principal components (PC1 and PC2) accounted for 38.9 % and 15.9 % of the total variance, respectively. The PCA score plot revealed clear separation of samples by harvest time and cultivation region. Notably, the JH and WD samples clustered distinctly from those from BS, GH, and SC, indicating region-specific differences in secondary metabolite composition. In the loading plot, the GH samples were closely associated with lutein and zeaxanthin, while the JH samples aligned with β-carotene and α-carotene. Both the JH and WD samples showed proximity to phenolic acids, suggesting a higher contribution of phenolic metabolites in these regions.

The total carotenoid and free phenolic acid contents were summarized by harvest time and region (Table 3). Samples harvested in December and January exhibited higher levels of both compound groups compared to those collected in February and March. Regionally, SC and GH showed the highest total carotenoid contents, whereas WD and JH exhibited elevated phenolic acid levels.

These results highlight the combined influence of seasonal and regional factors on the distribution of bioactivity-associated secondary metabolites in laver and provide integrative context for the compound-specific findings described in Sections 3.3 and 3.4.

4. Conclusion

Using integrated GC-MS/MS and LC-MS/MS analyses, we characterized the metabolite composition of fresh laver across regions and

harvest times. A total of 104 primary metabolites and five vitamins were identified, including 37 metabolites newly reported in laver. Distinct seasonal and regional metabolite differences were observed. Amino acids and selected polyunsaturated fatty acids increased during December–January. Carotenoids peaked in early winter, with lutein predominating, and three carotenoids—α-carotene, β-apo-8'-carotenal, and fucoxanthin—were newly confirmed in laver. Vitamins and EPA were enriched in BS and SC, while phenolic acids were most abundant in WD and JH.

These findings demonstrate that metabolite composition varies substantially by region and harvest time, underscoring the importance of monitoring the chemistry of fresh laver when selecting raw materials for dried laver and other value-added products. A xanthophyll index (lutein and zeaxanthin), a provitamin-A index (α-carotene and β-carotene), an amino-acid panel (glutamic acid, glutamine, alanine, and valine), and EPA are proposed as candidate indicators for quality evaluation. This study establishes a foundational database linking environmental conditions with metabolite variation and provides a scientific basis for quality control. Future work should determine the carryover rates of key indicators during drying and roasting and quantify their relationships with sensory attributes.

CRedit authorship contribution statement

Seul-Ki Park: Writing – review & editing, Resources, Conceptualization. **Jeong-Ho Lim:** Writing – review & editing, Resources, Conceptualization. **Haeun Lee:** Writing – review & editing, Visualization. **Jihyun Lee:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization. **Kee-Jai Park:** Writing – review & editing, Resources, Conceptualization. **Hyang Sook Chun:** Writing – review & editing, Data curation. **Hahyeong Yu:** Writing – original draft, Visualization, Validation, Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2025.108849.

Data availability

Data will be made available on request.

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