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# Microbial Dynamics and Metabolite Profiles in Different Types of Salted Seafood (*Jeotgal*) During Fermentation

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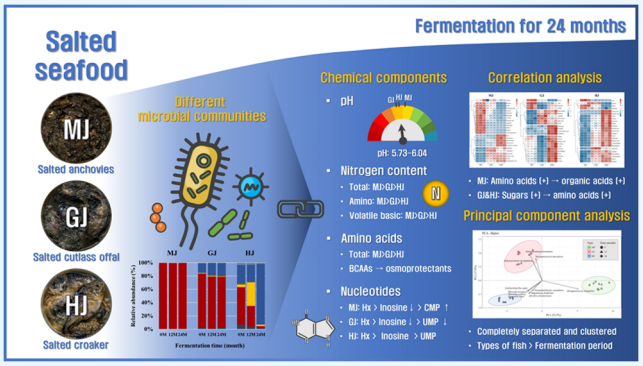
**ABSTRACT:** Salted and fermented seafood (*jeotgal*) is known for its long shelf life and unique flavor. Despite the existence of various types of salted seafood, the factors influencing this quality have yet to be identified. These factors are essential for improving the quality of salted seafood, optimizing the fermentation process, and advancing the industrialization of fermented foods. Therefore, in this study, we explored microbial dynamics and changes in quality characteristics in three salted seafood items − salted anchovies (MJ), salted cutlass offal (GJ), and salted croakers (HJ), over a 24-month fermentation period. Distinct microbial community profiles, dominated by *Tetragenococcus halophilus*, *Halanaerobium fermentans*, and *Chromohalobacter canadensis* in MJ, GJ, and HJ, respectively, affect the metabolic pathways and the corresponding flavor profiles. The pH of all samples ranged from 5.7−6.0. The titratable acidity was highest in MJ at 1.4% and lowest in HJ at approximately 0.7%. Salinity was below 25% in all samples but slightly lower in MJ. Significant differences were observed in the amino acid, nucleotide, and overall metabolite profiles. MJ exhibited the highest amino acid and nitrogen-related factor levels, such as glutamic acid and hypoxanthine, enhancing flavor complexity. Correlation analysis revealed significant associations among the types, metabolites, and microbial communities. Microbial survival mechanisms in high-salt environments result in the production of unique metabolites, including umami and aroma components as well as precursors of biogenic amines, which can affect the overall quality of the final product. These differences were primarily influenced by the fish type rather than the fermentation time. Our findings provide foundational insights for enhancing fermentation strategies, improving product consistency, and advancing the industrial application of microbial management in seafood fermentation. This study not only fills a significant gap in the current understanding of fermented seafood but also outlines practical approaches for industry applications for the optimization of product quality.




## 1. INTRODUCTION

Salted and fermented seafood, valued for its long shelf life and unique flavor profile, has been a major food source in various cultures for centuries.[1,2] Salted seafood typically has a 20−25% (w/v) salt content and an umami flavor and is thus often added to other foods or eaten as a side dish or source.[3,4] When added to vegetables or dishes containing them, it can provide essential nutrients, as it contains large amounts of proteins and amino acids.[5] In Korea, the term "*jeotgal*" is used to collectively refer to salted and fermented seafood. Among the various types of *jeotgal*, *myeolchijeot* (salted anchovies; *Engraulis japonicus*), *galchisokjeot* (salted cutlass offal; *Trichiurus lepturus*), and *hwangseogeojeot* (salted croaker; *Collichthys lucidus*) are frequently consumed.[6]

During the fermentation process of *jeotgal*, the proteins from raw fish and shellfish are converted through enzymatic hydrolysis into various nitrogen compounds such as amino acids, peptides, amines, and ammonia.[7,8] The decomposition of different nitrogen compounds can result in various flavors; for instance, it may yield compounds with unpleasant flavors, such as ammonia and trimethylamine, or those with pleasant flavors, such as amino acids.[8] In addition, *jeotgal* is decomposed by various microorganisms to produce diverse metabolites, including organic acids, alcohols, and esters.[9] Therefore, the complex interactions between enzymes and microorganisms play a key role in determining the flavor and quality of *jeotgal*.

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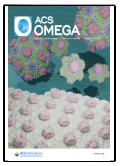

Among previous studies, some have confirmed the correlation between microbial communities and metabolites but were limited to specific fish and shellfish.[10,11] In a changing climate environment, research on various species could provide essential data that suggest potential replacement options. Furthermore, our research delves into the unique microbial profiles and metabolite compositions of each fish type, which sheds light on how these factors influence flavor and quality characteristics. By explicitly linking our findings to individual fish types, we offer valuable insights into the variability and complexity of salted seafood fermentation, filling a significant gap in the literature.

On the other hand, while the focus of previous similar studies was on describing microorganisms or quality characteristics, correlation analysis was not conducted, nor was microbial metabolism specifically described.[12−14] Indeed, while existing studies recognize the significance of microbiology and quality traits, they frequently lack in-depth exploration and direct connections to individual fish types. Our study addresses this gap by conducting a correlation analysis of microbial profiles, metabolites, and quality characteristics across different salted seafood, providing a more nuanced understanding of their interaction.

In the salted and fermented seafood industry, investigating and understanding the interactions among quality characteristics, metabolites, and microorganisms are becoming increasingly important to meet consumer preferences. These factors are significant, as they account for the elements that enhance the quality of salted fish and have the potential to advance the fermented food industry through optimized fermentation processes. Therefore, this study aimed to investigate the quality variations among different types of *jeotgal* (*myeolchijeot*, MJ; *galchisokjeot*, GJ; and *hwangseogeojeot*, HJ) and identify the differences in their microbial and metabolite profiles. Additionally, we examined the correlation between metabolites and microorganisms, which fluctuate during the fermentation period. This is the only study to date that has directly caught and investigated the effects of salting three types of fish, anchovies, cutlassfish, and croaker, confirmed the fermentation patterns for 2 years and described the correlations. This correlation analysis not only addresses the limitations of the existing literature but also aids in optimizing salted seafood production technology. By enhancing our understanding of the relationships between microbial communities, metabolites, and quality traits, the quality and flavor of products can be improved, ultimately ensuring and improving consumer satisfaction.

## 2. MATERIALS AND METHODS

**2.1. Experimental Materials.** Different types of salted seafood were obtained from Shinan Saeujeot (Shinan-gun, Republic of Korea). Each fish was salted with 20−25% salt immediately after being caught, stored at refrigerated temperatures, transported to the fermentation room within 2 h, and then fermented at 10 °C for 24 months. All experimental analyses were performed using first-grade analytical reagents obtained from Daejung (Gyeonggi-do, Republic of Korea). Chromatographic grade water and acetonitrile (Merck, Rahway, NJ) were used for high-performance liquid chromatography (HPLC, Ultimate 3000, Thermo Dionex). The standard chemicals for the analysis of amino acids and nucleotides (including hypoxanthine and inosine) were obtained from Agilent Technologies (Palo Alto, CA) and Sigma-Aldrich (St. Louis, MO), respectively.

**2.2. Microbial Community Analysis.** Total DNA was extracted using the DNA Isolation Kit (DNeasy 96 PowerSoil Pro Kit, QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA concentration and purity were measured using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The sequencing libraries were prepared according to the Illumina 16S Metagenomic Sequencing Library protocols to amplify the V3 and V4 regions. The input genomic DNA 5 ng was PCR amplified with 5× reaction buffer, 1 mM deoxynucleotide triphosphate (dNTP) mix, 500 nM each of universal forward and reverse PCR primers, and Herculase II fusion DNA polymerase. The first PCR cycle conditions included a 3 min activation at 95 °C, followed by 25 cycles of 30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C, with a final extension at 72 °C for 5 min. The universal primer pair with Illumina adapter overhang sequences used for the first amplification was as follows: 16S Amplicon PCR Forward Primer (5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3′) and 16S Amplicon PCR Reverse Primer (5′-TCT CGT GGG CTC GGA GAT GTG TAT AAG AGA CAG GAC TAC HVG GGT ATC TAA TCC-3′). The first PCR product was purified using AMPure beads (Agencourt Bioscience, Beverly, MA). For final library construction, 10 μL of the first PCR product was PCR amplified with NexteraXT Indexed Primer under the same conditions, except for the number of cycles, which was set at 10 for this process. The final PCR product was purified with AMPure beads, quantified using quantitative PCR (qPCR) according to the KAPA Library Quantification kits for Illumina Sequencing platforms, and qualified using a D1000 ScreenTape TapeStation system (Agilent Technologies, Waldbronn, Germany).

The total DNA extracted from the samples was subjected to PCR using the 16S V4 primer. Subsequently, sequencing was performed on the Mi-SeqTM platform (Illumina, San Diego, CA) by Macrogen (Macrogen Inc., Seoul, Korea). The CD-HIT-OTU analysis program was used to determine the species-level operational taxonomic units (OTUs) to cluster sequences with a similarity of 97% after eliminating sequencing errors and ambiguous and chimeric sequences. The representative OTU sequence was used to perform UCLUST (v.1.2.22) in the reference database (SILVA DB) and generate taxonomic assignments based on homology. Microbial communities were analyzed using classifiers from the Ribosomal Database Project in QIIME (v.1.9.0).

**2.3. Analyses of pH, Acidity, and Salinity.** Each sample was ground in a blender, and the extracted juice was used to measure the pH, acidity, and salinity. The pH and acidity values were measured using a TitroLine 5000 (SI Analytics GmbH, Mainz, Germany) at 24−26 °C, and salinity was measured using a salt meter (PAL-SALT, ATAGO, Tokyo, Japan). The samples were diluted 10- or 100-fold; all measurements were performed in triplicate. The instruments were rinsed with distilled water before each measurement.

**2.4. Analyses of Nitrogen-Related Factors.** *2.4.1. Total Nitrogen (TN).* The total nitrogen (TN) content of the samples was determined by using the persulfate digestion method (HACH Method 10072). The test sample was prepared by adding 0.5 mL of the sample and Total Nitrogen Persulfate Reagent Powder Pillow (26718-46, HACH, IA) to a


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Table 1. High-Pressure Liquid Chromatography (HPLC) Conditions for Free Amino Acid and Nucleotide Analysis

| parameters | conditions | |
|---|---|---|
| | free amino acid | nucleotides |
| HPLC system | Ultimate 3000 | |
| column | Inno C18 column (4.6 × 150 mm$^2$, 5 $\mu$m) | Inno C18 column (4.6 × 250 mm$^2$, 5 $\mu$m) |
| mobile phase | A: 40 mM sodium phosphate | A: 0.05 M potassium phosphate |
| | B: water/acetonitrile/methanol (10:45:45 v/v%) | B: potassium phosphate/methanol (90:10 v/v%) |
| flow rate | 1.5 mL/min | 0.7 mL/min |
| oven temperature | 40 °C | |

Total Nitrogen Hydroxide Digestion Reagent Vial (27140-45, HACH, IA). The solution was then subjected to heat treatment at 105 °C for 30 min. Following this, Total Nitrogen Reagent A Powder Pillow (26719-46, HACH, IA) was added, followed by Total Nitrogen Reagent B Powder Pillow (26720-46, HACH, IA), and allowed to react. Subsequently, 2 mL of this solution was transferred to a Total Nitrogen Reagent C Vial (26721-45, HACH, IA) and mixed thoroughly, and TN concentration was measured using a colorimeter (T-6800, Sinsche Technology Co., Ltd., Shenzhen, China).

*2.4.2. Amino Nitrogen (AN).* The amino nitrogen (AN) content of the samples was measured using the formol method.[15] A total of 2 g of sample was suspended in distilled water to obtain a final volume of 100 mL, and then, the sample was sonicated for 30 min. Following this, 20 mL of the sample extract was added to 20 mL of a neutral formalin solution, mixed, and titrated to a pH of 8.3 with 0.1 N NaOH. The AN content was calculated using eq 1:

$$\text{AN content (mg/100 g)} = \frac{(A - B) \times 1.4 \times 0.1\,\text{N NaOH factor} \times D}{\text{sample weight (g)}} \times 1000 \quad (1)$$

where $A$ is the titrated volume of 0.1 N NaOH consumed by the sample (mL), $B$ is the titrated volume of 0.1 N NaOH consumed by the blank reagent (mL), 1.4 is the AN content corresponding to milliliters of 0.1 N NaOH, and $D$ is the dilution.

*2.4.3. Volatile Basic Nitrogen (VBN).* Volatile basic nitrogen (VBN) was determined using Conway's microdiffusion method, with minor modifications.[16] The sample (10 g) was minced with 10 mL of distilled water. The minced sample was then blended with 20 mL of 10% trichloroacetic acid (TCA) and extracted for 30 min. Following extraction, the filtrate was filtered using filter paper (Whatman International Ltd., U.K.) and adjusted to a final volume of 50 mL by using 5% TCA. Subsequently, 1 mL of the filtrate was placed in the outer chamber of the Conway unit. Additionally, 1 mL of 0.01 N $H_3BO_3$ and 2−3 drops of Conway reagent (0.066% methyl red: 0.066% bromocresol green, 1:1) were added to the inner chamber. The Conway unit was promptly sealed after 1 mL of saturated $K_2CO_3$ was added to the outer chamber. The sealed Conway apparatus was gently shaken and incubated at 37 °C for 90 min. Finally, the sample was titrated by adding 0.02 N of $H_2SO_4$ to the inner chamber. The VBN value was calculated using eq 2:

$$\text{VBN content (mg/100 g)} = \frac{(A - B) \times 0.28 \times 0.02\,\text{N}\ H_2SO_4\ \text{factor} \times D}{\text{sample weight (g)}} \times 100 \quad (2)$$

where $A$ is the titrated volume of 0.02 N $H_2SO_4$ consumed by the sample (mL), $B$ is the titrated volume of 0.02 N $H_2SO_4$ solution by the blank (mL), 0.28 is the VBN content corresponding to a volume of 0.02 N $H_2SO_4$ in milliliters, and D is the dilution.

**2.5. Analyses of Free Amino Acids and Nucleotides.** For free amino acid and nucleotide analyses, the samples were homogenized and diluted, and the filtered solutions were used as samples for further analysis. Free amino acids and nucleotides were analyzed using an HPLC system (Ultimate 3000, Thermo Dionex) equipped with an Inno C18 column (4.6 × 150 mm$^2$, 5 $\mu$m particle size, Youngjin Biochro, Korea) for free amino acid analysis and an Inno C18 column (4.6 × 250 mm$^2$, 5 $\mu$m particle size, Youngjin Biochro, Korea) for nucleotide analysis. The operational conditions for the HPLC analysis of free amino acids and nucleotides are described in Table 1.

**2.6. Metabolome Analysis.** After freeze-drying, 100 $\mu$L of O-methoxyamine hydrochloride in a pyridine solution (20 mg/mL) was added to each sample. All samples were incubated at 30 °C for 90 min under dark conditions. Silylation was performed by adding 50 $\mu$L of *N*-methyl-N-trimethylsilyl-trifluoroacetamide containing 1% trimethylchlorosilane. Each sample was vortexed for 30 s and incubated at 37 °C for 30 min. Ten microliters of ribitol (0.5 mg/L) was added as the internal standard (IS). The samples were centrifuged at 13,000 rpm for 10 min, and the supernatant was collected. The derivatized samples were analyzed using a gas chromatography−mass spectrometer (GC/MS, QP2020, Shimadzu, Kyoto, Japan). An Rtx-5MS with a fused silica capillary column (30 m × 0.25 mm ID, J&W Scientific, CA) was used for the separation of metabolites. The operational conditions for the GC/MS analysis of the metabolome are described in Table 2.

Table 2. Gas Chromatography-Mass Spectrometer (GS/MS) Conditions for Metabolome Analysis

| parameters | conditions |
|---|---|
| GC-MS system | QP2020 |
| separation column | Rtx-5MS (30 m × 0.25 mm ID, 0.25 $\mu$m) |
| injection temperature | 230 °C |
| carrier gas flow | 1 mL/min (He) |
| oven temperature | 80 °C (2 min) → 330 °C (15 °C/min) (6 min) → 250 °C (10 min) |



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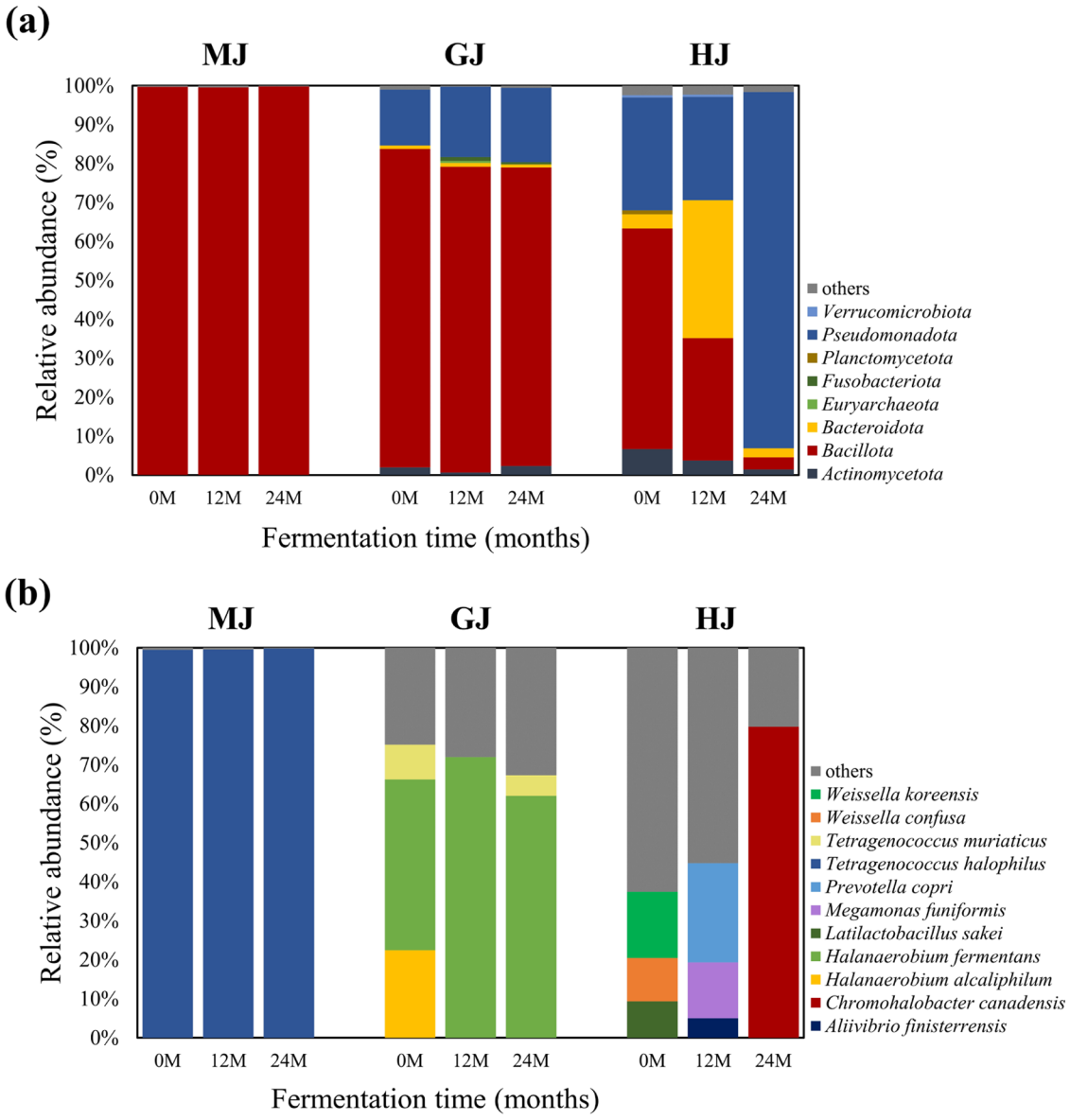


**Figure 1.** Bacterial community composition at the phylum and species levels in different types of salted seafood determined using the SILVA rRNA database. "Others" show a percentage of reads <0.5 and <5% of the total reads in all salted seafood samples in analyses at the phylum (a) and species levels (b), respectively. MJ, salted anchovies; GJ, salted cutlass offal; HJ, salted croakers.

Ionization was achieved using a 70 eV electron beam. The helium gas flow rate through the column was 1 mL/min. Twenty scans per second were recorded over the mass range of 85−500 $m/z$. Chromatograms and mass spectra were acquired using a Shimadzu GC solution (Shimadzu, Kyoto, Japan). Metabolites were identified by comparing their mass spectra using the AIoutput software, NIST 14.0 library, and human metabolome database (HMDB, http://www.hmdb.ca).

**2.7. Statistical Analysis.** All physicochemical data were measured in triplicate, and values, excluding those from microbial analysis, were expressed as the mean ± standard deviation. Statistical analyses were performed using GraphPad Prism 9.0 (GraphPad Software, Inc., San Diego, CA). R 4.3.2 (R Development Core Team 2022) was used to perform a one-way ANOVA test to determine statistical significance, and Duncan's multiple range test was performed for posthoc testing to test for significant intersample differences ($P < 0.05$).[17] Additionally, Pearson's correlation coefficient analysis was used to examine the correlation between salted shrimp varieties and metabolites. Principal component analysis (PCA) was performed using R version 4.3.2 to analyze the changes in metabolites during fermentation.

## 3. RESULTS AND DISCUSSION

**3.1. Microbial Community.** Figure 1 shows the differences in the microbial communities of different samples at different fermentation times at the phylum and species levels. At the phylum level, MJ and GJ exhibited a significantly high *Bacillota* (*Firmicutes*) ratio, whereas HJ showed a high *Bacillota* ratio only at the beginning of the fermentation process. As fermentation progressed, the three phyla (*Pseudomonadota*, *Bacteroidota*, and *Bacillota*) competed, and *Pseudomonadota* gradually became more dominant during the late fermentation period. Considering that *Bacillota* is generally dominant in salted seafood, the significant difference in the microbial community of HJ was also confirmed at the phylum level.[18]


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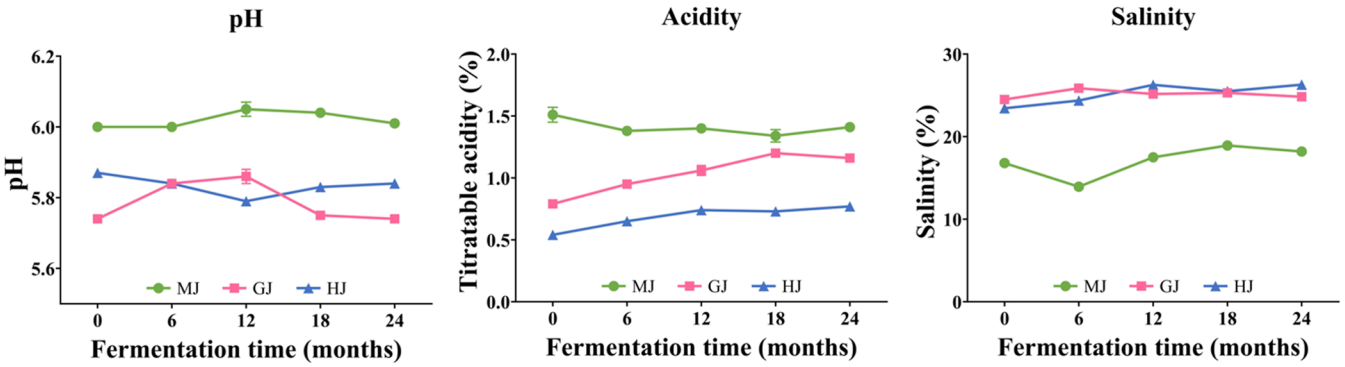


**Figure 2.** Changes in general characteristics during fermentation. MJ, salted anchovies; GJ, salted cutlass offal; HJ, salted croakers.

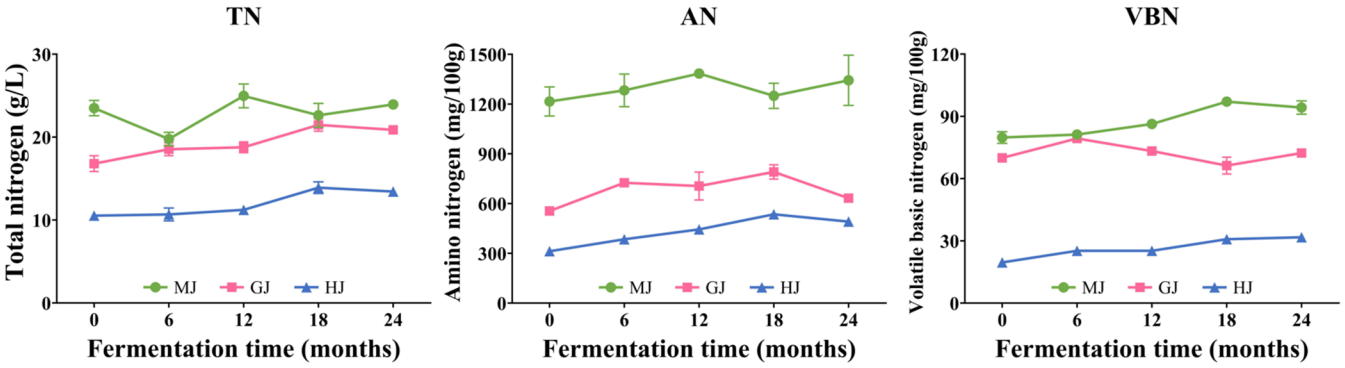


**Figure 3.** Changes in nitrogen-related factors during fermentation. MJ, salted anchovies; GJ, salted cutlass offal; HJ, salted croakers; TN, total nitrogen; AN, amino nitrogen; VBN, volatile basic nitrogen.

The microbial communities significantly differed at the species level depending on the fish species. The results confirmed that *Tetragenococcus halophilus* was the dominant species in MJ from the beginning to the end of fermentation. Many previous studies have confirmed that *Tetragenococcus* is predominant in salted anchovies, consistent with our findings.[12,18,19] *T. halophilus* is a Gram-positive halophilic lactic acid bacterium that produces organic acids at concentrations of 5−20% (w/v) NaCl.[20] It can improve the flavor profile of high-salt fermented foods by producing flavor-related compounds such as glutamic acid, alanine, benzeneacetaldehyde, and 2-methyl-propanal.[21]

In the case of GJ, between the two *Halanaerobium* species, namely, *H. fermentans* and *H. alcaliphilum*, *H. fermentans* was the dominant species throughout fermentation. This species is a Gram-negative halophilic and anaerobic bacterium often found as a dominant species in various salted seafood.[10,14] Although it was the dominant species in both MJ and GJ since the beginning of the fermentation process, we believe that it competed with numerous other bacteria in HJ from the commencement of fermentation. Approximately 216 species were detected at the beginning of the fermentation process, with *Chromohalobacter canadensis* ultimately becoming dominant over the two-year period of fermentation. The genus *Chromohalobacter* comprises halophilic Gram-negative bacteria commonly isolated from saline environments, such as salt pans, saline soils, and the Dead Sea.[22] It is plausible that *C. canadensis* in HJ originated from seawater or the surrounding saline environment where croaker, the raw material used for HJ, was caught.

**3.2. Changes in Quality Characteristics during Fermentation.** The pH and titratable acidity of fermented foods fluctuate depending on the concentration of organic acids, such as lactic and citric acid, produced by microorganisms throughout the fermentation process, serving as key indicators of their quality characteristics.[11] Figure 2 shows the changes in pH, acidity, and salinity during fermentation. The pH was similar throughout the fermentation period at 6.02 ± 0.02, 5.79 ± 0.06, and 5.83 ± 0.03 in MJ, GJ, and HJ, respectively. This finding was consistent with previous research showing that the pH of salted seafood was in the 5.5−6.5 range.[23] Moreover, as reported in previous studies, the pH change during salted anchovy sauce fermentation was insignificant.[11,24]

Titratable acidity tended to increase over 12 months in GJ and HJ but not in MJ. Generally, the acidity of salted seafood tends to increase during the fermentation process and stabilize or decrease slightly afterward.[25,26] Salinity was marginally lower in MJ but remained at a similar level for each sample throughout the fermentation. Salted seafood usually has a salinity of about 20−25% (w/v), and the Korean Industry Standard recommends that the salinity of salted shrimp be lower than 25% (w/v).[27] All samples in this study adhered to this recommendation; however, the salinity of MJ was marginally lower.

**3.3. Changes in Nitrogen-Related Factors during Fermentation.** The concentrations of nitrogen-related factors in each sample during the fermentation period are listed in Figure 3. Total nitrogen (TN) content is an important factor in determining the quality of salted seafood. A higher TN content correlates with increased levels of various amino acids, which contribute to a richer flavor.[11] By adding anchovy sauce preparations with different TN content to kimchi, Choi et al.[28]


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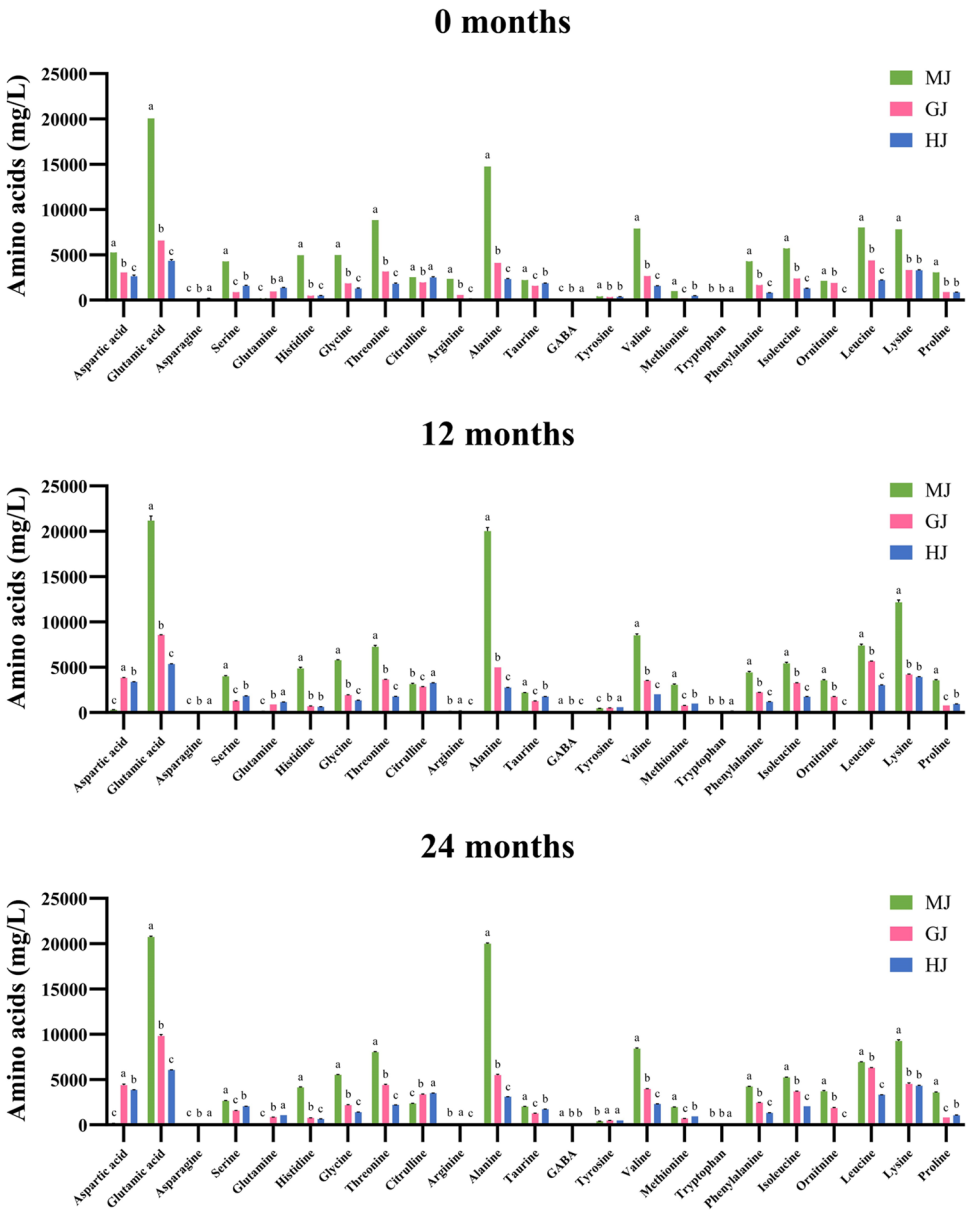


**Figure 4.** Changes in the contents of amino acids among different types of salted seafood during fermentation. Mean values with different letters (a−c) are significantly different ($P < 0.05$). MJ, salted anchovies; GJ, salted cutlass offal; HJ, salted croakers.

confirmed that the concentration of glutamic acid, which affected the umami taste, increased.

From highest to lowest, the TN content was in the order of MJ > GJ > HJ (19.2−26.2, 15.9−22.0, and 9.8−14.4 g/L, respectively). The amino nitrogen (AN) content was also the highest in MJ, but the difference in AN between MJ and GJ was greater than the difference in TN, confirming that the proportion of AN in TN was lower in GJ than in MJ. Considering that AN reflects the accumulation of nitrogen in the form of free amino acids, which occurs as a result of the hydrolysis of fish proteins, this implies that a higher level of protein degradation occurred in MJ than in GJ.[29] The metabolic activity of *T. halophilus* in anchovies may have contributed to faster protein degradation than in the other samples. Lee et al.[18] also confirmed that *Tetragenococcus* has a strong correlation with both TN and AN. Furthermore, although not observed in MJ, the amino nitrogen (AN) levels of GJ and HJ exhibited a steady increase, followed by a slight decrease in the late stages of fermentation. Consistent with our findings, previous reports have indicated a gradual rise followed by a decline in AN content or a reduction in the rate of increase during the fermentation period of salted shrimp and salted anchovies.[23,25]

Volatile basic nitrogen (VBN) serves as a freshness indicator for fish and shellfish. It encompasses low-level compounds, such as ammonia, basic amines, and trimethylamine (TMA). As the freshness diminishes, the VBN content rises. Therefore, VBN is utilized to assess both fish meat freshness and the fermentation level of fermented seafood.[30] Shim et al.[31] reported that the VBN levels of commercially distributed salted fish and salted shellfish were in the ranges of 21.94−598.99 mg/100 g and 8.53−120.93 mg/100 g, respectively. In the present study, the VBN levels of all samples that had undergone a 24-month fermentation period ranged from 30.8 to 98.0 mg/100 g, indicating a relatively low range. The VBN level tended to increase but was consistently the lowest in


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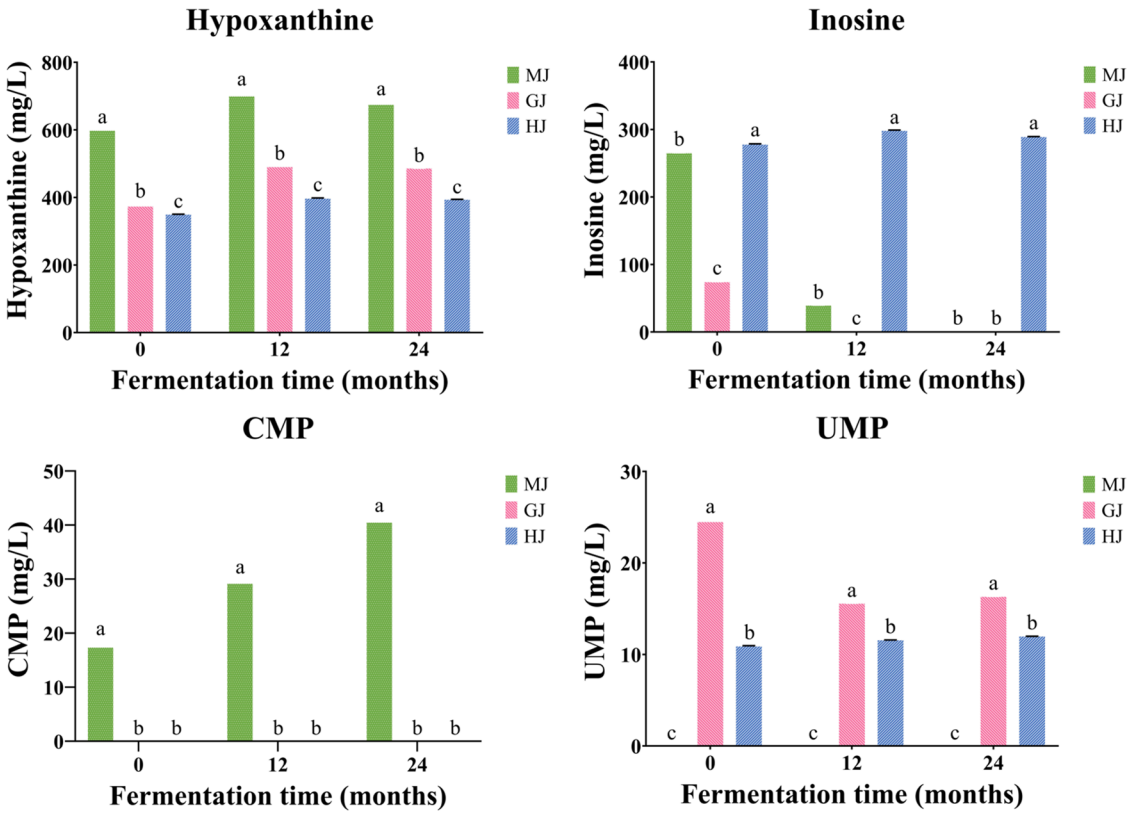


**Figure 5.** Changes in the contents of nucleotides among different types of salted seafood during fermentation. Mean values with different letters (a−c) are significantly different ($P < 0.05$). MJ, salted anchovies; GJ, salted cutlass offal; HJ, salted croakers; CMP, cytidine monophosphate; UMP, uridine monophosphate.

HJ. A previous study showed that higher fermentation temperatures significantly elevated the VBN levels.[11] Considering that all samples were fermented at the same temperature, the rate of VBN production could vary depending on the type of fish being salted.

**3.4. Changes in Free Amino Acid Content during Fermentation.** Amino acids are crucial in the flavor profile and provide essential nutrients in fermented salted seafood. For instance, amino acids such as glutamic acid, glycine, and alanine contribute to a unique umami taste, whereas others such as arginine, leucine, and isoleucine are associated with a bitter flavor.[32] Therefore, the analysis of various amino acid compositions in different types of salted seafood is important for identifying differences in the taste and quality characteristics.

The observed changes in the 23 major amino acids in different salted seafood during fermentation are listed in Figure 4. MJ had the highest amino acid content, followed by GJ and HJ. Notably, the amino acids analyzed, namely, glutamic acid, threonine, alanine, valine, leucine, lysine, and isoleucine, exhibited significantly high levels in all samples. Glutamic acid, which accounted for the highest proportion of amino acids, is responsible for imparting an umami flavor, while threonine and lysine contribute to sweetness.[32] Additionally, leucine and isoleucine contribute to a bitter taste.[6] Therefore, these amino acids might be the key elements contributing to the unique flavor profile of salted seafood. From a functional perspective, leucine and isoleucine are classified as branched-chain amino acids (BCAAs). They serve as fundamental components for building muscle tissue and enhancing protein synthesis in both animals and humans.[33]

The histidine content was significantly higher in MJ. Histamine is produced in fermented fish products through enzymatic histidine decarboxylation by certain microorganisms[34] and is a type of biogenic amine that can cause clinical diseases if the intake exceeds 100 mg/100 g.[35] Therefore, MJ, which had a relatively high content of histidine − a precursor to histamine, necessitates further research for its improvement.

Additionally, the aspartic acid content, which was detected in MJ at month 0, rapidly decreased as fermentation progressed. Gencbay and Turhan[36] reported that aspartic acid was the major constituent in anchovy (*Engraulis encrasicholus*). Furthermore, *T. halophilus*, the dominant species in MJ, has been reported to increase alanine, glycine, and proline pools and reduce aspartic acid and BCAA under salt stress.[37] Consistent with this, we confirmed that *T. halophilus* showed the same tendency, even if fermentation continued for a long period in a high-salt environment. This is related to the fact that various bacteria produce high levels of glutamate, glycine, and proline under osmotic stress, and in the presence of 2-oxoglutarate, BCAAs are converted to osmoprotectants such as glutamate and proline by BCAA aminotransferase (BCAT).[37−39] This tendency was observed consistently across different samples.

Our findings revealed a rapid consumption of arginine during fermentation, contrasting with the results of our previous study[40] where arginine was detected at high levels in salted shrimp, likely due to the elevated arginine content in shrimp shells. Thus, we confirmed that amino acid composition can differ depending on the raw materials used in salted seafood production. Additionally, while arginine levels decreased, citrulline levels increased across all samples. This


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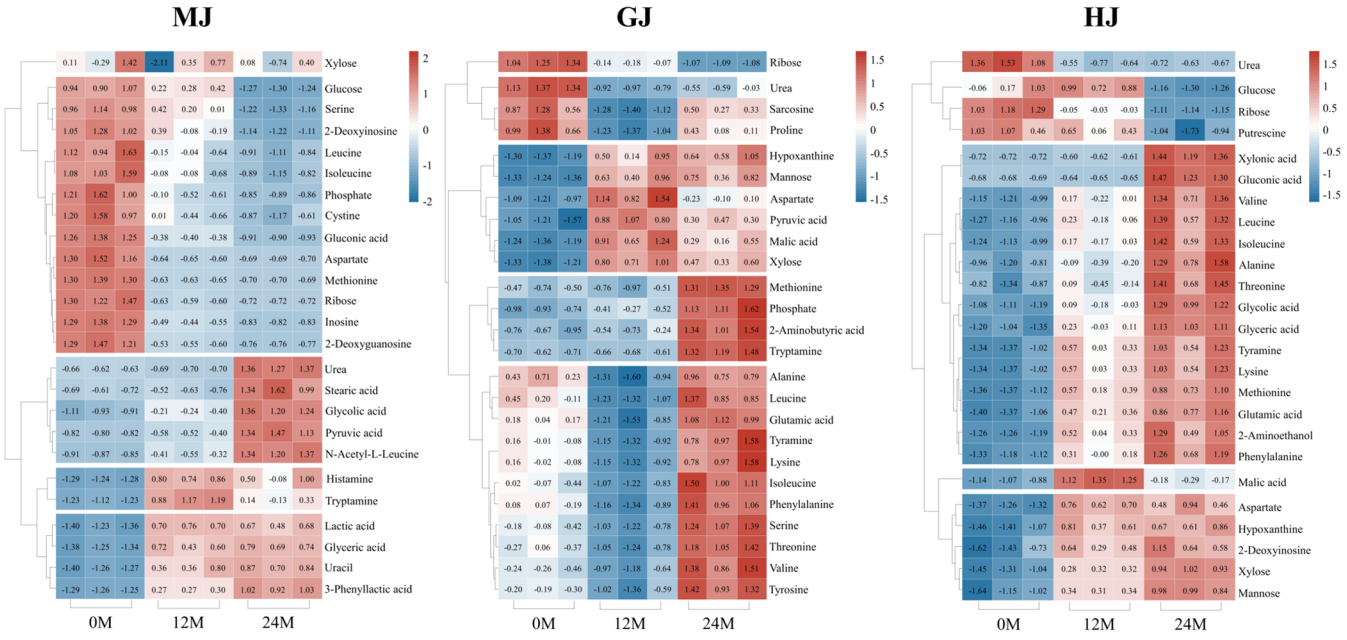


**Figure 6.** Heat maps showing the correlations among the top 25 metabolites in different types of salted seafood during fermentation. Blue and red indicate negative and positive correlations, respectively. The numbers represent the correlation coefficients. MJ, salted anchovies; GJ, salted cutlass offal; HJ, salted croakers.

could be attributed to the conversion of arginine to citrulline via the arginine deiminase (ADI) pathway, which is accelerated under salt stress.[41] Citrulline, acting as a compatible solute, helps microorganisms adapt to osmotic stress, and it appears that the increased citrulline levels were related to the adaptation of different microorganisms to high-salt conditions.[41,42] Therefore, the accumulation of specific amino acids during salted seafood fermentation might be related to the survival strategies of microorganisms aimed at mitigating osmotic pressure, which could affect the unique flavor profile and functional characteristics of salted seafood.

**3.5. Changes in Nucleotide Contents during Fermentation.** Nucleic acids, including DNA and RNA, are abundant in fish tissues. During decomposition, microorganisms release nucleases that break down nucleic acids into nucleotide monomers. Nucleic acid-related substances, including adenosine triphosphate (ATP), adenosine diphosphate (ADP), hypoxanthine (Hx), and inosine, contribute to the umami taste.[43] Autodigestive enzymes in fish and shellfish muscle break down ATP and nucleic acid-related substances.[44] Hx, inosine, cytidine monophosphate (CMP), and uridine monophosphate (UMP) were detected in the present study, and the changes in their content during the fermentation period are shown in Figure 5. Among them, Hx had the highest content in all samples and increased as fermentation progressed. On the other hand, the inosine content decreased with the progress of fermentation in MJ and GJ but was maintained at a high level in HJ. Meanwhile, CMP was detected only in MJ, and its amount gradually increased, whereas UMP was detected only in GJ and HJ.

We inferred that this is due to differences in the degradation mechanism depending on the type of salted seafood. During the ATP degradation process, adenosine is deaminated to form inosine as an intermediate, which is subsequently converted to Hx by another deaminase enzyme.[44,45] In MJ and GJ, the conversion from inosine to Hx appears to have occurred rapidly, with inosine being gradually and completely consumed.

Cytidine 5′-monophosphate (5′-CMP) and uridine 5′-monophosphate (5′-UMP) are pyrimidine nucleotides and the key intermediates for the production of several nucleotide derivatives.[46] Evrin et al.[47] reported that the regulatory mechanism of UMP kinase differs between Gram-positive and Gram-negative bacteria. Therefore, GJ and HJ showed a tendency different than that of MJ, which was dominated by Gram-positive bacteria. In addition, this may be due to differences in preferred metabolic pathways and mechanism regulation.

**3.6. Correlation Analysis between Types of Salted Seafood and Metabolites or Microbial Community.** Figure 6 shows a heat map of the correlation between the metabolites produced in each salted seafood type, according to the fermentation period. The top 25 metabolites were identified and clustered based on the similarity of each sample and metabolome.

Since the beginning of the fermentation process, MJ showed a positive correlation with amino acids, such as serine, leucine, and isoleucine, and a strong positive correlation with inosine, 2-deoxyguanosine, and ribose. In contrast, its correlation with organic acids, such as stearic, glycolic, pyruvic, lactic, and glyceric acids, increased toward the later stages of fermentation. *Tetragenococcus*, which dominates the microbial community in MJ, is involved in the production of lactic acid, ethanol, and acetic acid.[48] Therefore, the production of various organic acids or esters among the metabolites is closely related to the presence of *Tetragenococcus*. GJ showed a strong positive correlation with ribose in the early stage of fermentation and gradually showed a strong correlation with bases such as hypoxanthine and phosphate and amino acids such as leucine, lysine, and thiamine as fermentation progressed. Similar to GJ, HJ exhibited a strong correlation with sugars, such as glucose and ribose, in the early stage of fermentation. However, its


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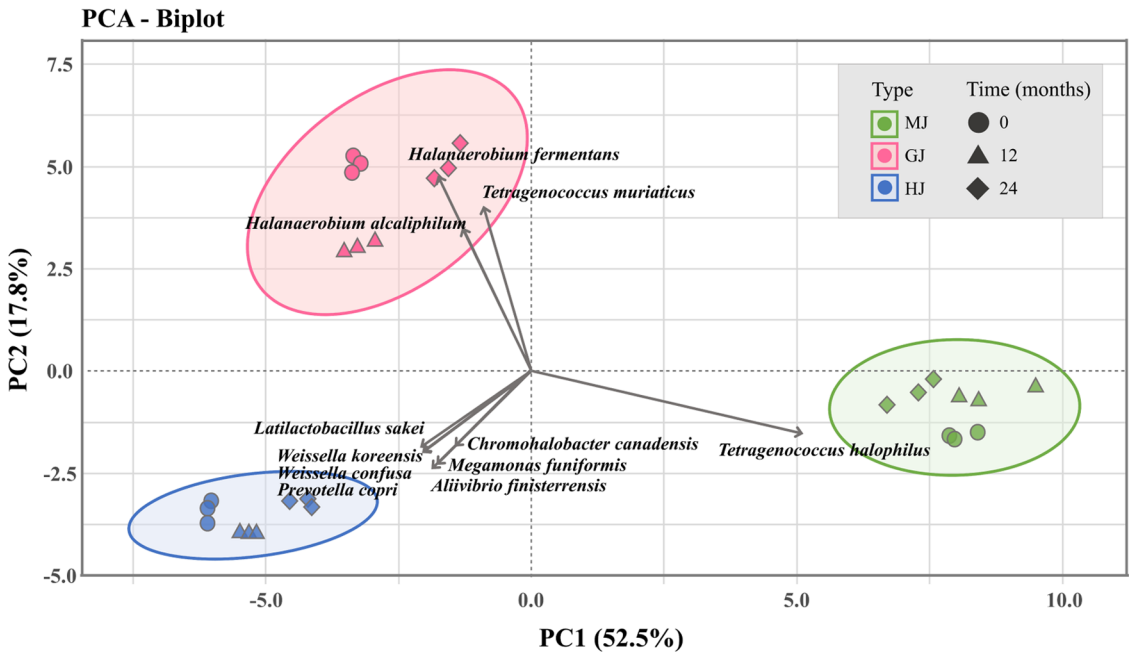


**Figure 7.** Biplot of the principal component analysis (PCA) of metabolite compounds detected in different types of salted seafood. MJ, salted anchovies; GJ, salted cutlass offal; HJ, salted croakers.

correlation with amino acids increased as the fermentation progressed.

In all three groups, sugars, such as glucose and ribose, showed a positive correlation in the early stages and a negative correlation in the later stages, indicating that these sugars were used for microbial fermentation. In contrast, in the case of urea, the correlation decreased as fermentation progressed in GJ and HJ, but the opposite trend was found in MJ. This is assumed to be due to the role of the urea cycle in processing nitrogenous wastes generated in MJ, which had a comparatively higher protein content.

Figure 7 shows a biplot of the principal component analysis (PCA) of metabolite compounds detected in different types of salted seafood. Principal components (PCs) 1 and 2 explained 52.2 and 17.8% of the variation, respectively, which together explain 70.3% of the total fluctuation. Each type of salted seafood was completely separated and clustered. In addition, PC1 confirmed that MJ was completely separated and showed relatively different aspects from the metabolites of GJ and HJ. In addition, the microbial species affecting the salted seafood differed significantly depending on the salted seafood type rather than the fermentation period. In conclusion, we confirmed that the quality characteristics of different kinds of salted seafood vary significantly under the influence of each microbial succession.

## 4. CONCLUSIONS

In this study, we identified differences in the microbial communities and metabolites of three types of salted seafood during fermentation and analyzed their correlations. The observed variations in microbial community composition, quality characteristics, amino acids, nucleotides, and metabolite profiles highlight the importance of understanding species-specific adaptations and fermentation mechanisms. Our results showed that *T. halophilus*, *H. fermentans*, and *C. canadensis*, which were the final dominant species in MJ, GJ, and HJ, respectively, produced different compounds through different metabolic processes. For example, the high levels of glutamic acid and hypoxanthine in MJ, as a result of the metabolic activity of *T. halophilus*, contribute to its rich umami flavor. The production of different metabolites might act as a mechanism for protecting the microbes from the high-salt environment or could represent a selection of metabolic pathways that confer survival advantages. These findings underscore the importance of tailored fermentation strategies for each type of jeotgal, emphasizing the need for species-specific approaches to optimize the product quality.

The insights from our study will lead to enhancement of the quality and taste of salted seafood by forming the selection of specific starter cultures and optimizing fermentation conditions for different types of fish. Understanding these species-specific metabolic processes allows for adjustments to promote the growth of beneficial microbes while suppressing the growth of undesirable microbes. Furthermore, by adjustment of factors such as pH, salinity, and fermentation time, it is possible to modulate the production of key flavor compounds, thereby improving the overall sensory quality of the final product. In conclusion, the findings of this study will not only contribute to the scientific understanding of microbial dynamics during the salted fish fermentation process but will also provide practical strategies for improving product quality and consumer satisfaction in the fermented food industry. Future research can build on the findings of this study by exploring its role in additional microbial species and other types of fermented foods, potentially leading to further innovations in food production techniques.

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### Notes

The authors declare no competing financial interest.

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