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Investigation of Metabolite Differences in Salted Shrimp Varieties during Fermentation

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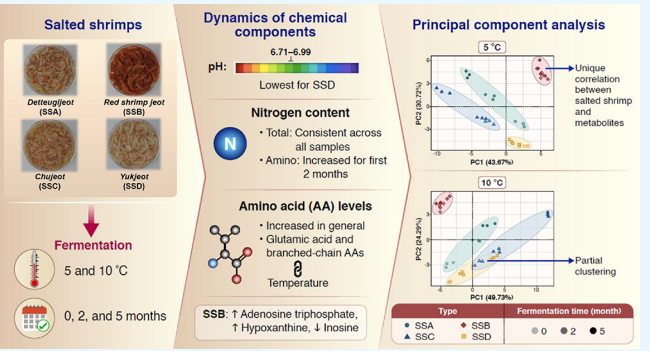
ABSTRACT: Fermentation of salted shrimp involves the interaction of multiple factors. However, studies of the effects of shrimp variety and fermentation temperature on metabolites generated during fermentation are limited. Therefore, we investigated the effects of the shrimp variety, fermentation temperature, and fermentation period on the composition of fermented salted shrimp. Four different varieties of salted shrimp, namely, *Detteugijeot* (SSA), *Red shrimp jeot* (SSB), *Chujeot* (SSC), and *Yukjeot* (SSD), were prepared and stored at 5 and 10 °C for 5 months. The pH values ranged from 6.71 to 6.99, with SSD showing the lowest pH at both temperatures. Although total nitrogen content remained relatively constant, amino nitrogen exhibited an upward trend after 2 months and was particularly increased at 10 °C. This increase was attributed to variations in microorganisms and enzymes in the salted shrimp. Except for proline, citrulline, and ornithine, amino acid levels increased during fermentation with the highest amounts detected in SSA. Additionally, the levels of glutamic acid and branched-chain amino acids were found to be sensitive to fermentation temperature. Amino acid levels were apparently affected by species-specific metabolic pathways of the microorganisms present in each salted shrimp. Compared to the other varieties, SSB had significantly higher contents of adenosine triphosphate and hypoxanthine. A high hypoxanthine content could contribute to increased bitterness and an umami taste profile. Furthermore, the correlation between salted shrimp and metabolites was unique in SSB, whereas partial clustering was observed between the SSA and SSC.



1. INTRODUCTION

Fermentation has long been used as a preservation method for various food products, including seafood, allowing for the development of unique flavors, enhanced nutritional profiles, and extended shelf life.[1−3] *Jeotgal*, the fermented seafood of Korea, is usually made by fermenting seafood, such as fish, roe, intestine, and shellfish.[4] *Jeotgal* (salted seafood) includes *saeujeot* (salted shrimp), *myeolchijeot* (salted anchovies), *myeongnanjeot* (salted pollack roe), and *jogaejeot* (salted clam meat). Nitrogen compounds change most during the *Jeotgal* fermentation process. Proteins from fish and shellfish are converted into various nitrogen compounds, such as peptides, amino acids, amines, and ammonia, through enzymatic hydrolysis.[5] The resulting trimethylamine and ammonia cause unpleasant odor and taste, whereas amino acids are often associated with a pleasant flavor.[6]

Among the multitude of *Jeotgal* varieties, the salted shrimp called *saeujeot* is one of the most consumed in Korea.[7] It is added to other foods, such as kimchi, stew, and steamed egg, or used as a sauce in place of salt owing to its 20−25% salt content and a unique umami flavor.[8,9] Depending on the season in which the shrimp was caught, *saeujeot* is given different names—*Detteugijeot* (spring), *Red shrimp jeot* (summer), *Chujeot* (autumn), and *Yukjeot* (June in the lunar calendar).[10] *Pandalopsis japonica*, *Leptochela gracilis*, *Acetes japonicus*, and *A. chinensis* are commonly species used as raw material for *saeujeot*.[11]

The fermentation of salted shrimp involves the interaction of multiple factors, including salt concentration, temperature, microbial communities, and protein composition.[12−14] The interaction among these factors significantly changes the chemical properties and metabolite composition of salted shrimp.[12,15] As the salted shrimp fermentation temperature increases, the content of volatile basic nitrogen and histamine increases.[13] Moreover, the metabolite and flavor profiles of raw and fermented shrimp have been reported to be different.[16] Therefore, understanding these changes is crucial for ensuring

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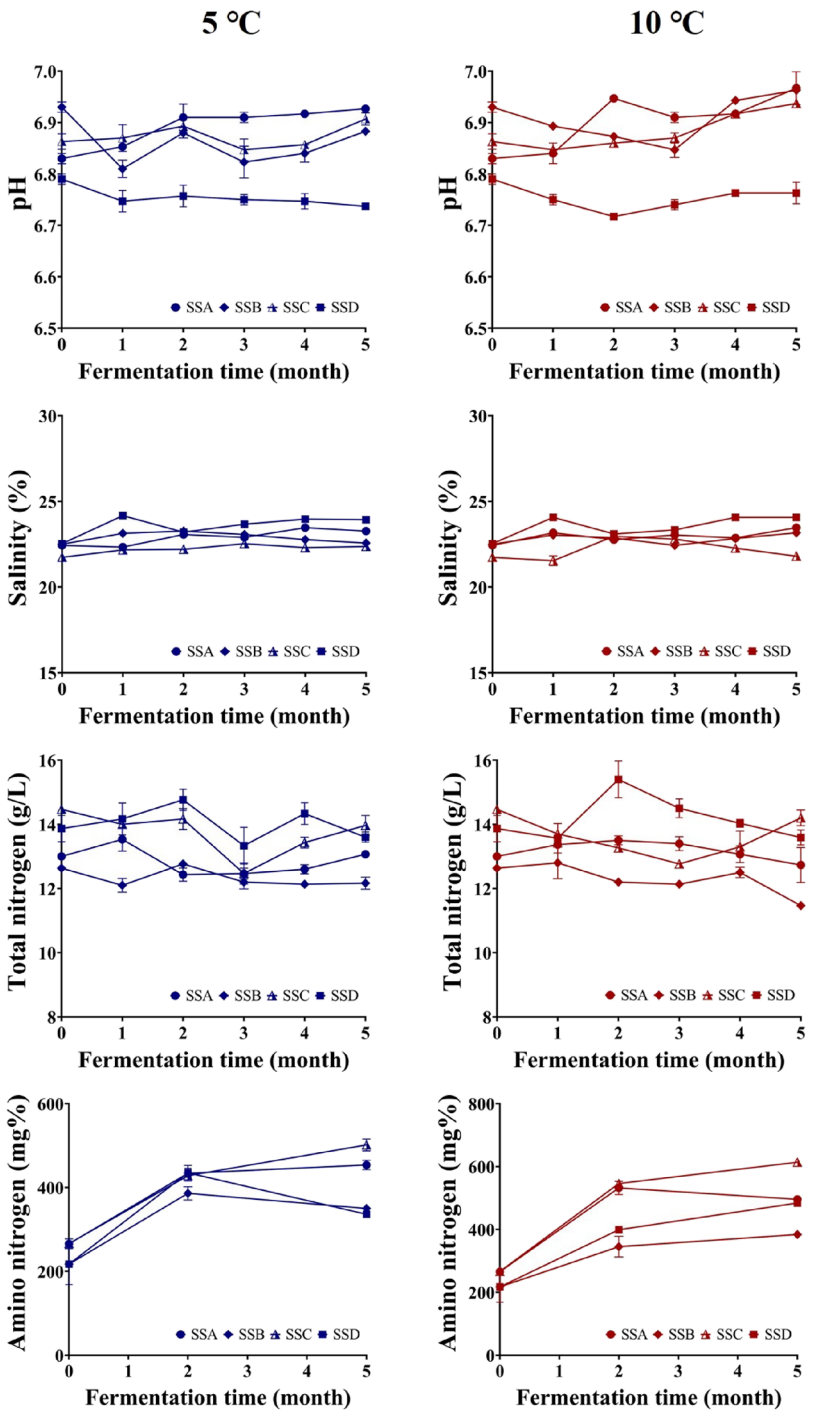

Figure 1. Changes in the pH, salinity, total nitrogen, and amino nitrogen levels of different salted shrimp varieties during fermentation.

the safety of the final product and optimizing its sensory attributes.

The effect of temperature on microbial succession and metabolite changes during *saeujeot* fermentation was comprehensively examined in a previous study;[17] however, information on differences among different varieties remains insufficient. The chemical composition of 11 types of salted shrimp paste (*Kapi*) was also investigated, but the changes during fermentation or changes in metabolites were not studied.[14] The optimal temperature for fermentation of salted shrimp was determined by studying the correlation between the microbial community in the shrimp and metabolites and by investigating the changes in microbial succession and metabolites with changes in salt concentration or fermentation period.[12,15,17] However, studies on changes in the chemical components and metabolites generated during the fermentation of different shrimp varieties and by the conductivity of fermentation at different temperatures are insufficient.

The main objective of this study was to analyze the changes in chemical components and metabolites generated during the fermentation of different shrimp varieties and to determine the effect of different fermentation temperatures. Moreover, we investigated whether the correlation between varieties and metabolites and differences in chemical composition could

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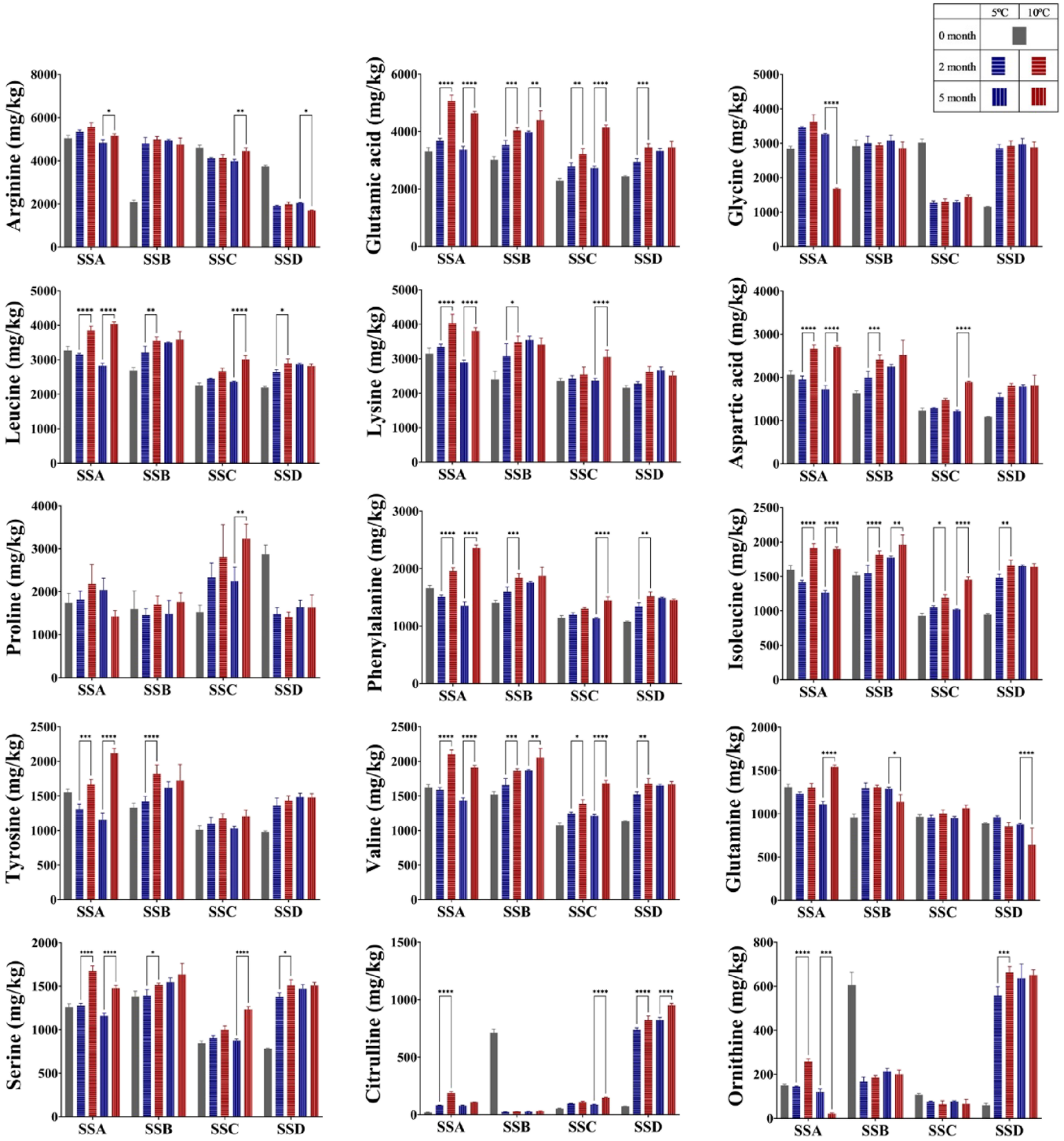

Figure 2. Changes in the content of major amino acids among different salted shrimp varieties during fermentation. The blue and red lines represent 5 and 10 °C, respectively, and the horizontal and vertical lines represent months 2 and 5 of fermentation, respectively. The asterisks indicate the significance level between the two temperatures at the same fermentation time (****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$).

account for the differences in the fermentation of salted shrimp varieties caught in different seasons (*Detteugijeot*, SSA; *Red shrimp jeot*, SSB; *Chujeot*, SSC; and *Yukjeot*, SSD).

2. RESULTS AND DISCUSSION

2.1. Changes in General Quality Characteristics during Fermentation. General changes in the quality of the salted shrimp samples are listed in Figure 1. The initial pH of salted shrimp samples ranged from 6.78 to 6.94; after 5 months of fermentation, the pH was similar, being 6.73−6.93 and 6.74−6.99 at 5 and 10 °C, respectively. Generally, the pH of salted seafood is 5.5−6.5; however, crustaceans, such as shrimp, have a high pH because of the influence of amines or dissociation of calcium ions in their shells.[18−20] Except for SSD, the pH tended to increase slightly, albeit insignificantly, during the fermentation period. In a previous study, the pH of anchovy sauce fermented at 10 °C did not change significantly compared with that of anchovy sauce fermented at 30 °C.[21] As

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both fermentation temperatures used in this study were low, distinguishing the temperature-induced changes in pH was challenging. Furthermore, SSD had the lowest pH throughout the fermentation, confirming a pH difference depending on the shrimp variety. Oh et al. also showed that the pH of salted shrimp varied depending on the shrimp variety, and as in our study, the pH of *Yukjeot* tended to be slightly lower than that of the other varieties.[18] In addition, they mentioned that various characteristics, such as the taste and color, of salted shrimp differed depending on the raw shrimp variety. The difference in pH that depends on the shrimp variety is believed to be caused by the seawater temperature, pH, or salinity; however, additional research is needed to verify this. In general, the pH of fermented foods varies depending on the content of organic acids, such as lactic acid and citric acid, secreted by microorganisms during fermentation.[21] Therefore, the relatively minor pH variations could be attributed to the slower microbial metabolism associated with acid production at lower temperatures.

On the contrary, during the entire fermentation period, the salinity of salted shrimp was maintained at 22.94 ± 0.65% (w/v), conforming to the Korean Industry Standard, which recommends salinity lower than 25% (w/v).[22] Total nitrogen (TN) content is an important index for evaluating the quality of fermented foods, such as salted seafood.[21] The higher the TN level, the higher the amino acid content in salted seafood, and the decomposition of shrimp proteins into amino acids imparts a unique umami taste to the salted shrimp.[23,24] According to Korean Industrial Standards, TN is recommended to be at least 0.8 g per 100 g and amino nitrogen (AN) is recommended to be at least 350 mg per 100 g.[22] The TN of the samples was 13.25 ± 0.89 g/L, which satisfied the specification.

The AN of the samples was initially 231.79 ± 33.45 mg/100 g, which did not meet the standard; however, from the second month of fermentation, all samples contained more than 350 mg of AN per 100 g, which is in accordance with the Korean Industrial Standard. In addition, AN tended to increase more significantly at higher fermentation temperatures, and in particular, the AN content of SSA and SSC was high, with maximum contents of 553.00 and 623.00 mg/100 g, respectively. These results were similar to the those of Park et al. in that the AN content increased at higher temperatures during the fermentation of salted anchovies.[21] The increase in AN was thought to be related to the increase in the content of low molecular weight peptides through the decomposition of proteins into amino acids by enzymes and microorganisms in salted shrimp.[7,25] Therefore, the higher temperature may have increased the activity of enzymes and microorganisms, which may have affected protein degradation and increased AN.

2.2. Changes in Free Amino Acid Content during Fermentation. In fermented salted seafood, amino acids, such as glutamic acid, glycine, and alanine, are known to provide a unique umami taste, whereas amino acids, such as arginine, leucine, and isoleucine, impart a bitter taste.[7,24] Endogenous enzymes originating from the muscles and digestive tract of fish promote fermentation and produce abundant amino acids.[26,27] The changes in 15 major amino acids of salted shrimp during fermentation are shown in Figure 2 and Tables S1 and S2. The total amount of 23 amino acids measured in the salted shrimp samples before fermentation was 40.20 ± 0.67, 33.75 ± 0.87, 31.39 ± 0.82, and 29.32 ± 0.31 g/kg in SSA, SSB, SSC, and SSD, respectively. Among the amino acids, arginine, glutamic acid, glycine, leucine, lysine, and alanine were dominant, and their sum accounted for 44−52% of the total amino acid content. This finding was similar to that of a previous study wherein glutamic acid, asparagine, glycine, and leucine in shrimp samples were shown to constitute more than 40% of the total amino acids.[28]

Our results showed that SSA was relatively sensitive to fermentation temperature; thus, the amino acid content tended to increase as the temperature increased. The content of glutamic acid, leucine, lysine, aspartic acid, phenylalanine, isoleucine, tyrosine, valine, and serine increased significantly, whereas that of glycine decreased significantly during fermentation. The increase in the content of various essential amino acids in SSA was consistent with the findings of a previous study that *jeotgal* had abundant essential amino acids, such as lysine and threonine.[24] This result was slightly different from that of a previous study in which the amino acid content increased regardless of the fermentation temperature.[17] Unlike SSA, SSD showed a small difference in the amino acid content depending on the temperature, indicating that the temperature sensitivity could also depend on the shrimp variety.

As fermentation progressed, the content of most amino acids in SSB tended to increase, and some amino acids showed a remarkable difference in the increase/decrease trend. In the beginning, the arginine level in SSB was lower than that in the other samples but increased rapidly to a level similar to that in the other samples, whereas the content of citrulline and ornithine decreased more rapidly than that of other amino acids. Shrimp shells are rich in amino acids, such as arginine; SSB, which has a thick shell compared with other shrimp varieties, exhibited a significant increase in arginine content as the decomposition of the shell occurred during the fermentation process.[29]

In SSC, the glycine content decreased rapidly and the content of aspartic acid, phenylalanine, isoleucine, tyrosine, serine, and ornithine was relatively low, whereas the content of proline increased rapidly and was eventually the highest. In some invertebrates, cold stress causes proline accumulation.[30−32] In the present study, among the four varieties, SSC shrimp were captured in the coldest season and could have contained a large amount of proline-generating enzymes to prepare for cold stress; however, this notion needs to be validated in future research. Unlike in SSA, in SSC, temperature-dependent difference in amino acid content was noted from month 5, and not from month 2.

In SSD, the contents of arginine and proline decreased rapidly and those of glycine, serine, citrulline, and ornithine increased significantly during fermentation. As the fermentation progressed, the contents of citrulline and ornithine became remarkably high. In the urea cycle, a large amount of arginine present in salted shrimp is converted to ornithine by arginase, and ornithine is again converted to citrulline.[33] Therefore, SSD is thought to contain a large amount of arginase, which converts arginine to ornithine.

Overall, the content of all amino acids, except for proline, citrulline, and ornithine, was high in SSA. On the contrary, SSB initially had a low arginine content and a very specific high content of citrulline and ornithine; however, the content of the latter two rapidly decreased as fermentation proceeded. Initially, the content of citrulline and ornithine was very low in both SSC and SSD, but it subsequently increased only in SSD. Considering that citrulline is metabolized to arginine by arginine deiminases, the activity of arginine deiminase is

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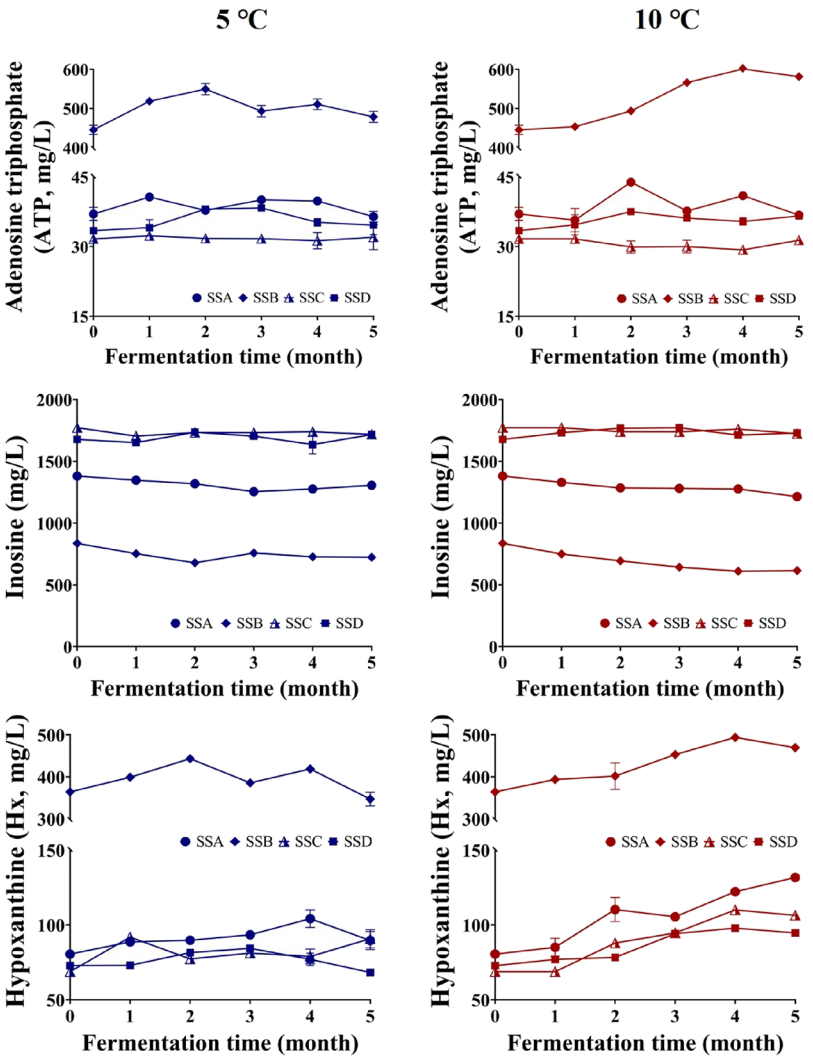

Figure 3. Changes in the content of nucleotides in different salted shrimp varieties during fermentation.

considered to be limiting in SSD during fermentation. The glycine content decreased sharply in SSC but increased rapidly in SSD, and the proline content was specifically high in SSD at first and then decreased. Therefore, the content of certain amino acids showed significant variations between different shrimp varieties; specifically, the content of arginine, glycine, proline, serine, citrulline, and ornithine in the early stage and of arginine, glycine, proline, citrulline, and ornithine in the later stage of fermentation varied. Moreover, amino acids, such as glutamic acid, leucine, phenylalanine, isoleucine, valine, and serine, were sensitive to changes in temperature.

One notable factor contributing to the diverse amino acid profiles is the presence of distinct microbial communities in different shrimp varieties. Salted shrimp is known to contain numerous halophilic bacteria, such as *Halomonas*, *Salinicoccus*, *Salinimicrobium*, *Salinivibrio*, and *Staphylococcus*.[7] These microbial communities exhibit diverse enzymatic activities, leading to variations in amino acid transformation. For example, *Staphylococcus* species have been identified as contributors to the production of various volatile compounds, lactate, glutamine, and branched-chain amino acids (BCAAs), such as valine, leucine, and isoleucine. This ability is linked to the presence of specific genes in *Staphylococcus* encoding aldehyde dehydrogenases (NAD^+) or associated with the BCAA metabolic pathway.[34] Consequently, the changes in amino acid composition are believed to stem from species-specific microbial metabolic pathways.

2.3. Changes in Nucleotide Content during Fermentation. Adenosine triphosphate (ATP) and nucleic acid-related substances are degraded by autodigestive enzymes in fish and shellfish muscle.[35] In a previous study, as shrimp fermentation progressed, the content of nucleic acid-related substances, such as 5′-nucleotides (adenosine monophosphate, AMP; inosine monophosphate, IMP; and uridine monophosphate, UMP) in shrimp sauce increased.[36] In addition, nucleic acid-related substances, such as ATP, adenosine diphosphate (ADP), hypoxanthine (Hx), and inosine, are responsible for the umami taste.[37] Numerous studies have reported that the content of nucleic acid-related substances increases during fermentation, which positively affects umami and savory taste.[38,39] In addition, in a previous study, we confirmed that Hx was strongly positively correlated with umami and the sensory evaluation was different depending on the variety of salted shrimp added as an ingredient of kimchi.[40] Therefore, the problem of selection of the shrimp variety is directly related to the sensory characteristics of the food.

The changes in the nucleotide content of salted shrimp during fermentation are listed in Figure 3. The most noticeable

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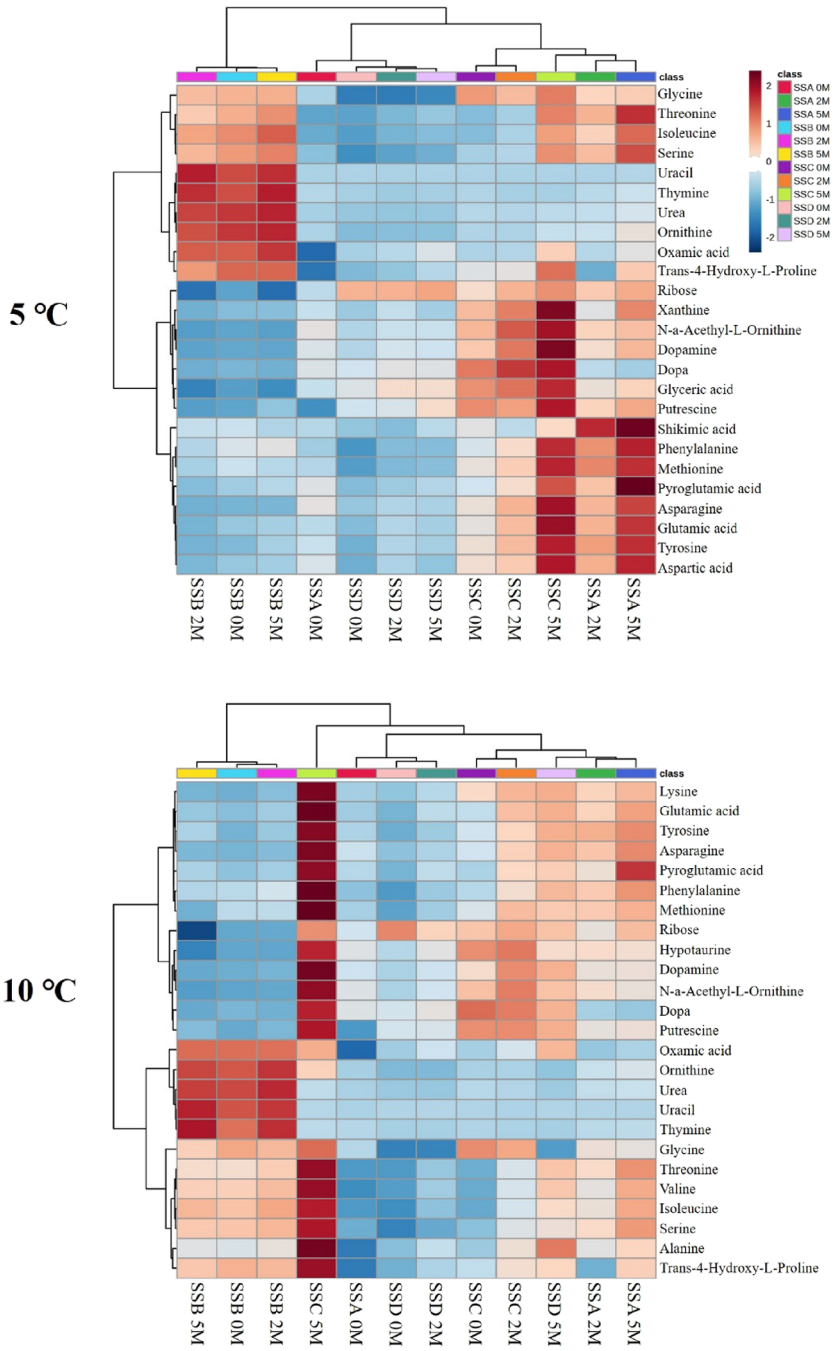

Figure 4. Correlation analysis and heatmap clustering of the top 25 metabolites according to Pearson's correlation analysis. Each row represents one metabolite, and each column represents a sample.

difference was in the content of ATP and Hx in SSB, which was more than 10 and approximately five times higher than in the other varieties, respectively, whereas its inosine content was the lowest. The levels of ATP and inosine remained similar at both temperatures, but the Hx level tended to increase slightly faster at higher temperatures. In addition, the inosine content was similarly the highest in SSC and SSD, followed by that in SSA, and it was the lowest in SSB.

In the ATP degradation process, adenosine is deaminated to form inosine as an intermediate, which is further converted to Hx by another deaminase enzyme.[35,41] Considering the pathway of ATP → ADP → AMP → inosine → Hx, it can be expected that SSB with a high initial ATP content would eventually yield a significant amount of hypoxanthine. However, several factors should be considered to explain the low inosine content in SSB. One possible reason is the presence of specific enzymatic activities and regulatory mechanisms within SSB. Enzymes involved in the degradation pathway, such as inosine phosphorylase or adenosine deaminase, could play a role in the rapid breakdown of inosine, limiting its accumulation.[42] Additionally, certain microorganisms in SSB might have the ability to metabolize or convert inosine, leading to its depletion in shrimp. Sun et al. reported that *Escherichia coli* survived in an acidic environment by converting adenine into inosine or NH_3.[43] Similarly, microorganisms may have developed strategies to counteract

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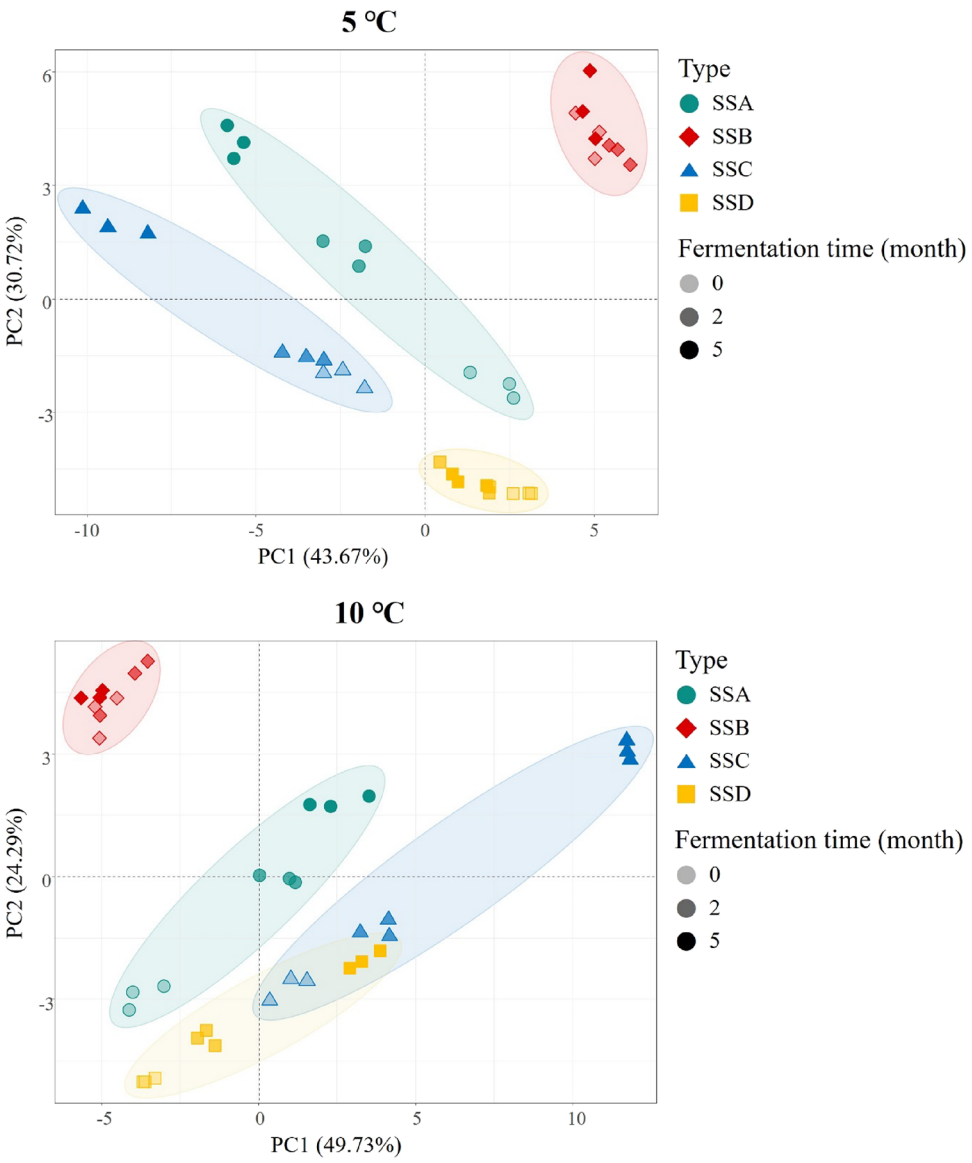

Figure 5. Principal component analysis (PCA) score plot of the metabolites in different salted shrimp varieties during fermentation at different temperatures. The symbols with different colors and shapes represent salted shrimp samples, and the symbols with different transparencies denote different months of fermentation.

the acidic substances produced during fermentation. These adapted microorganisms might be distributed within the salted shrimp, contributing to the depletion of the inosine levels through their metabolic activities.

2.4. Correlation between Shrimp Varieties and Metabolites at Different Temperatures. Figure 4 shows the correlation between the shrimp varieties and metabolites at each temperature. The top 25 metabolites were marked and clustered based on the similarity of each sample and metabolome.

In SSA, the content of aromatic amino acids, such as phenylalanine and tyrosine, as well as of sensory-related substances, such as shikimic, glutamic, and pyroglutamic acids, increased during fermentation. Aromatic amino acids are found in many fermented foods, and they provide a unique flavor or odor to fermented salted shrimp.[36,44]

In the correlation analysis of shrimp varieties and metabolites, SSB was the most prominently distinguished. Compared with that in other varieties, it showed a strong positive correlation with uracil, thymine, urea, ornithine, and oxamic acid and a strong negative correlation with ribose, glyceric acid, *N*-a-acetyl-L-ornithine, and dopamine. The skin of *Pandalopsis japonica*, the raw material of SSB, contains various bioactive substances, such as carotene protein, pigment, chitin, and chitosan.[45−47] It has also been reported to have antioxidant and inhibitory activities against xanthine oxidase.[48] Therefore, the difference in SSB and its metabolites and other shrimp varieties and their metabolites, which was thought to be related to the unique structure of the SSB chitin surface and various bioactive materials, prevented microbial invasion and enzyme action.

Similar to SSA, SSC became more positively correlated with fermentation-related metabolites as fermentation progressed. In the fifth month of fermentation, SSC showed a very strong positive correlation with amino acids at both 5 and 10 °C. The sharp increase in this correlation was related to the rapid increase in the content of amino acids such as arginine, glutamic acid, leucine, lysine, aspartic acid, and proline. It is

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Table 1. Appearance and Color of Different Salted Shrimp Varieties[a]

Label	SSA (*Detteugijeot*)	SSB (*Red shrimp jeot*)	SSC (*Chujeot*)	SSD (*Yukjeot*)
Appearance				
L*	50.18±0.43^{A}	41.88±1.02^{B}	51.54±1.10^{A}	62.28±0.38^{A}
a*	4.96±0.20^{B}	11.35±0.35^{A}	4.38±0.15^{B}	3.23±0.23^{B}
b*	7.13±0.20	13.61±0.19	6.41±0.31	7.69±0.12

[a]All values are the mean ± SD. Mean values in the same row with different letters (A-B) are significantly different ($p < 0.05$).

possible that microorganism enzyme action and metabolic activity increased during fermentation; however, this requires additional research.

At the beginning of fermentation, SSD showed a negative correlation with most metabolites, except ribose; at 5 months of fermentation, it began to show a similar correlation as did SSA at 10 °C. This tendency could be because SSD inherently had the lowest total amount of amino acids. Additionally, SSD differed from SSA in that it was positively correlated with the content of dihydroxyphenylalanine (DOPA) and oxamic acid. Overall, the results confirmed that the shrimp variety and temperature interacted in a complex manner to control the fermentation rate and change the composition of metabolites and even bioactive substances.

Changes in total metabolite content in the salted shrimp samples during 5 months of fermentation were expressed as score plots using principal component analysis (PCA, Figure 5). At 5 °C, principal components (PCs) 1 and 2 explained 43.67 and 30.72% of the variation, respectively, which could explain 74.39% of the total fluctuation at 5 °C. At 10 °C, PCs 1 and 2 explained 49.73 and 24.29% of the variation, respectively, which could explain 74.02% of the total fluctuation. At 5 °C, as fermentation progressed, PC1 moved in the negative direction and PC2 in the positive direction and each shrimp variety was distinctly classified. Conversely, at 10 °C, with the progress of fermentation, PC1 and PC2 tended to shift in the positive direction. Notably, SSA and SSC exhibited a similar trend and SSA in early fermentation and SSD in late fermentation overlapped at 10 °C. These clustering patterns were also observed in the results depicted in Figure 4. Furthermore, the fermentation patterns exhibited overall similarities between SSA and SSC, whereas SSB and SSD were separated, particularly at 5 °C. This indicated a unique metabolite characteristic specific to each shrimp variety. In our previous study, changes in the profiles of amino acids, as a type of metabolite, showed similarities between kimchi (Korean fermented vegetable product) with SSA and kimchi with SSC.[40] On the contrary, kimchi made with SSB and kimchi made with SSD were clearly distinguished. This showed that the metabolite composition of the raw material continues to affect even the final food products.

3. CONCLUSIONS

In this study, the effects of the shrimp variety and temperature on the fermentation of salted shrimp were investigated. The pH was the lowest in SSD throughout the fermentation period, and all samples showed an increasing tendency for the AN content to be above 350 mg per 100 g at 2 months of fermentation. As the fermentation proceeded, the change in amino acid content differed among the shrimp varieties, with the effect of temperature being the strongest in SSA and the weakest in SSD. Among the nucleotides, the content of ATP and Hx was uniquely high in SSB whereas the inosine content was the lowest. This difference might be related to specific enzymatic activities or certain microorganisms in the SSB. Additionally, differences in the metabolome changes in salted shrimp were observed among the varieties during the fermentation period. The correlation analysis between salted shrimp varieties and metabolites showed that the difference between the SSB and other samples was large, which could be related to its specific structural characteristics. Although SSA and SSC had different fermentation rates, they were clustered in a similar manner because of the similar compositional tendencies of their metabolites. The results obtained in this study provide a basis for a comprehensive understanding of salted shrimp fermentation and changes in its components. Our findings contribute novel information about the fermentation process and should enable the development of improved production techniques, quality control measures, and potential applications in the food industry.

4. METHODS

4.1. Experimental Materials. Four different varieties of salted shrimp were obtained from Shinan saeujeot (Shinan-gun, Republic of Korea) and fermented for 5 months at 5 or 10 °C. Information on the color and appearance of the samples is provided in Table 1. The sizes of SSA, SSB, SSC, and SSD were 20−45, 23−37, 16−30, and 20−42 mm, respectively. All of the experimental analyses were performed using first-grade analytical reagents obtained from Daejung (Gyeonggi-do, Republic of Korea). The water and acetonitrile used for high-pressure liquid chromatography (HPLC) were of chromatographic grade (Merck, New Jersey, USA). The standard chemicals for the analysis of amino acids and nucleotides (including hypoxanthine and inosine) were obtained from Agilent Technologies (Pal Alto, California, USA) and Sigma-Aldrich (St. Louis, Missouri, USA), respectively.

4.2. Analyses of the pH and Salinity. Each sample was ground in a blender, and the extracted juice was used to

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measure the pH 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, and all measurements were performed in triplicate, each time after being rinsed with distilled water.

4.3. Analyses of Total Nitrogen and Amino Nitrogen Content. The TN content of the samples was evaluated using the persulfate digestion method (HACH Method 10072). Total Nitrogen Persulfate Reagent Powder Pillow (26718-46, HACH, Iowa, USA) was put into a Total Nitrogen Hydroxide Digestion Reagent Vial (27140-45, HACH, Iowa, USA), and 0.5 mL of the sample was added. This solution was used as the sample. After heating at 105 °C for 30 min, Total Nitrogen Reagent A Powder Pillow (26719-46, HACH, IA, USA) was added and allowed to react, after which Total Nitrogen Reagent B Powder Pillow (26720-46, HACH, Iowa, USA) was added and allowed to react. Thereafter, 2 mL of this solution was added to a Total Nitrogen Reagent C Vial (26721-45, HACH, Iowa, USA) and mixed and the TN concentration was measured using a colorimeter (T-6800, Sinsche Technology Co., Ltd., Shenzhen, China).

The AN content of the samples was measured using the formol method.[49] Two grams of sample was suspended in distilled water to obtain a final volume of 100 mL and then sonicated for 30 min. Twenty milliliters of the sample extract was added to 20 mL of neutral formalin solution, mixed, and titrated to a pH of 8.3 with 0.1 N NaOH. The AN content was calculated using the following equation:

$$\text{AN content (ppm)} = \frac{(A - B) \times 1.4 \times 0.1\ \text{N NaOH factor} \times D}{\text{sample weight (g)}} \times 1000$$

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

4.4. Analyses of Free Amino Acids and Nucleotides. For the analyses of free amino acids and nucleotides, the salted shrimp 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, USA) equipped with an INNO C18 column (4.6 × 150 mm, 5 μm particle size, Youngjin Biochro, Korea) and an INNO C18 column (4.6 × 250 mm, 5 μm particle size, Youngjin Biochro, Korea), respectively. The HPLC operational conditions for free amino acids were as follows: mobile phase A, 40 mM sodium phosphate; mobile phase B, 10:45:45 (v/v%) water:acetonitrile:methanol. For nucleotides, the conditions were as follows: mobile phase A, 0.05 M potassium phosphate; mobile phase B, 90:10 (v/v%) potassium phosphate:methanol. The flow rates were 1.5 and 0.7 mL/min of free amino acids and nucleotides, respectively. The oven temperature was set to 40 °C.

4.5. Metabolome Analysis. After freeze-drying, 100 μL 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 in the dark. Silylation was performed by adding 50 μL of *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide containing 1% trimethylchlorosilane. After vortexing each sample for 30 s, it was incubated at 37 °C for 30 min and 10 μL of ribitol (0.5 mg/L) was added as the internal standard (IS). After centrifuging the samples at 13,000 rpm for 10 min, the supernatants were subjected to a GC−MS analysis. The derivatized samples were analyzed using GC−MS (QP2020, Shimadzu, Kyoto, Japan). An Rtx-5MS with a fused silica capillary column (30 m × 0.25 mm ID, J&W Scientific, California) was used for the separation of metabolites. The front inlet temperature was 230 °C. The column temperature was held at 80 °C for 2 min isothermally and then raised at 15 °C/min to 330 °C and held there for 6 min isothermally. The transfer line and ion source temperatures were 250 and 200 °C, respectively. Ionization was achieved with a 70 eV electron beam. The flow rate of helium gas 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).

4.6. Statistical Analysis. Statistical analyses were performed using GraphPad Prism 9.0 (GraphPad Software, Inc., San Diego, California, USA). The R statistical system 4.1.3 (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 post hoc testing to test for significant intersample differences (p Duncan's.[50] Pearson's correlation coefficient analysis was performed using MetaboAnalyst 5.0 (www.metaboanalyst.ca) to examine the correlation between the salted shrimp varieties and metabolites. The PCA of changes in metabolites during fermentation was performed using R 4.1.3.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c06046.

Changes in the free amino acid contents of salted shrimp during fermentation (5 °C) and changes in the free amino acid contents of salted shrimp during fermentation (10 °C) (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

ADP, adenosine diphosphate; AMP, adenosine monophosphate; AN, amino nitrogen; ATP, adenosine triphosphate; BCAAs, branched-chain amino acids; DOPA, dihydroxyphenylalanine; Hx, hypoxanthine; IMP, inosine monophosphate; PCA, principal component analysis; TN, total nitrogen; UMP, uridine monophosphate

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