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UHPLC-MS/MS metabolomics analysis of sea cucumber (*Apostichopus japonicus*) processed using different methods

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ABSTRACT

The effects of different processing methods on the nutritional components of sea cucumber (*Apostichopus japonicus*) are of concern to consumers who select sea cucumber products. This study employed liquid chromatography tandem mass spectrometry to examine the metabolites in fresh, unsoaked salted, soaked salted, and instant sea cucumber body wall samples sourced from Dalian, China. Metabolites were evaluated utilizing partial least squares discriminant analysis (PLS-DA) and subsequently subjected to KEGG metabolic pathway analysis for further investigation. PLS-DA effectively discriminated the body wall metabolites of sea cucumbers obtained via various processing techniques. The differential metabolites identified predominantly encompassed amino acids, lipids, and carbohydrates. Subsequent KEGG metabolic pathway analysis demonstrated a significant association between lipid, carbohydrate, and amino acid metabolism and the specific processing methods employed. The assessment of nutritional differences corresponding to the various *A. japonicus* processing methods was conducted. The findings of this study can assist in the choice of sea cucumber products and the selection of suitable processing methods.

1. Introduction

Apostichopus japonicus, also called *Apostichopus* or sea cucumber, is in the phylum Echinodermata and class Holothuroidea. In China, this marine organism has a rich culinary history spanning more than a millennium and is esteemed as a highly nutritious and premium seafood choice [1]. *A. japonicus*, being the foremost commercially sought-after species, attained an annual production of almost 200,000 tons in 2020 [2], boasting a market value of approximately 9.4 billion USD. Nutritionally, *A. japonicus* has high nutritional value and is rich in proteins [3], polysaccharides fatty acids, and other important chemicals such as pigments and trace elements [4], which makes it a tonic food material [5]. Many bioactive substances have recently been discovered in sea cucumber, including compounds with anticoagulant effects [6], antitumor effects [7], and anti-aging effects [8]. However, after being caught, the

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fresh sea cucumber readily undergoes enzymatic autolysis [9]. Therefore, more than 80 % of fresh sea cucumber are currently processed to give dehydrated products chosen for their convenience in storage [9]. The primary edible portion of the sea cucumber is its body wall. Sea cucumber body wall that is processed by different procedures has different nutrient compositions.

Metabolomics possesses fundamental features such as high throughput and high sensitivity, making it an essential component of biology. Traditionally, the scope of nutritional metabolism mainly encompasses blood biochemical compounds and hormone levels to evaluate the nutritional status of one or more biological indicators. Nevertheless, the factors that influence the body are intricate and dynamic. Throughout the transmission chain of genetic lineage, the body strives to maintain system stability and dynamic equilibrium with the external environment by effectively regulating its complex metabolic network. Consequently, finding a novel scientific approach becomes exceptionally crucial. In this context, employing the metabolic group in the realm of animal nutrition holds significant importance [10]. Sensitive and reproducible metabolomics methods using liquid chromatography (LC) mass spectrometry (MS) are widely used to detect various low-molecular-weight compounds [11]and also suited to identifying large hydrophobic compounds produced through secondary metabolic pathways [12]. A metabolomic method for simultaneously and rapidly identifying cattle, chicken, deer, pig, and sheep meat from seven peptides specific to meat products has been successfully established [13]. A metabolomic approach was developed for assessing the quality of cured ham, focusing on both typical dryness and deteriorated dryness, along with metabolites and texture characteristics, as outlined in a prior study [14]. This investigation revealed that amino acid derivatives and oligopeptides play a pivotal role in discriminating between normally dried and spoiled ham, The primary metabolic pathways associated with ham spoilage have been recognized as purine metabolism, pyrimidine metabolism, and protein degradation. Analyzing lipid, amino acid, and protein metabolism in *A. japonicus* suggests that the lipid, amino acid, and protein contents can be used to identify the geographical source of *A*. japonicus [15].

Changes in the nutritional content of sea cucumbers caused by processing are a concern for consumers when selecting sea cucumber products. Earlier investigations [16–18] have evaluated the nutritional composition of both dehydrated and fresh sea cucumbers. However, no metabolomics study has been conducted to simultaneously assess the different nutrient contents of fresh sea cucumber, unsoaked salted sea cucumber, soaked salted sea cucumber, and instant sea cucumber. The nutrient composition of sea cucumbers is clearly apparent vary by species, geographical source [15] and are influenced by processing procedures [18].

In this study, Ultrahigh-performance (UHP) LC-MS metabolomics was employed to identify the metabolites generated during different processing methods in Dalian, evaluate the effects of these changes caused by processing, and determine the nutritional values of sea cucumber products processed using various methods. The analysis revealed significant differences in metabolites such as amino acids, lipids, carbohydrates, and more, caused by different processing methods. Furthermore, quantitative analysis of fatty acids and amino acids was conducted to support these findings. The results of this study will facilitate the selection of appropriate sea cucumber processing methods and products for consumers.

2. Materials and methods

2.1. Ethics statement

Since *Apostichopus japonicus* are invertebrates, ethical review and approval were waived for this study. The content of this article does not involve human or animal research in the institutional review board statement.

2.2. Sample collection

Sea cucumber specimens were sourced from Dalian Xinyulong Marine Biological Seed Technology Co., Ltd. (Dalian, China) and conformed to the national standards, specifically SC/T 3308–2014 for instant sea cucumbers [19] and SC/T 3215–2014 for salted sea cucumbers [20]. The sample details are presented in Table 1, involving the extraction of half of the body wall from unsoaked salted sea cucumbers, which were subsequently soaked to obtain soaked salted sea cucumbers. Upon their arrival at the laboratory, all samples underwent immediate cleaning, dissection, and the collected body walls were stored in a laboratory freezer at −80 °C. The production process is briefly outlined in Table 2. Unique abbreviations were assigned to each type of sea cucumber sample: US (unsoaked salted sea cucumbers), SS (soaked salted sea cucumbers), F (fresh sea cucumbers), and I (instant sea cucumbers).

2.3. Metabolite extraction

The ground tissues (100 mg) were homogenized in well-chilled 80 % methanol using liquid nitrogen. After a 5-min incubation on ice, the samples were centrifuged at 15,000 g and 4 °C for 20 min. The supernatant was diluted by adding LC-MS grade water, resulting in a final composition of 53 % methanol. The samples were then transferred and subjected to another 20-min centrifugation at 15,000 g

Table 1
Sample information of *Apostichopus japonicus*.

Sample Name	Weight (g)	Body Length (cm)
F	144.57 ± 32.95	16.22 ± 2.14
US	21.78 ± 2.69	7.93 ± 0.25
I	73.015 ± 8.1	11.92 ± 0.56

Table 2
Different processing methods for sea cucumber products.

Different processing methods	Processing methods
unsoaked salted sea cucumbers (US)	fresh sea cucumber → remove internal organs, wash → cook → salt → refrigerated
soaked salted sea cucumbers (SS)	unsoaked salted sea cucumbers → wash→ adopt an intermittent simmering process → cooled → wash → add pure water for three days
instant sea cucumbers (I)	fresh sea cucumber → remove internal organs → cleaning → pre -cooked → high temperature pre -cook → soaked→ packing → injection of pure water → seal → sterilization → refrigerated preservation

and 4 °C in a fresh Eppendorf tube. The final supernatant was analyzed by injecting it into the LC-MS/MS system [21].

2.4. UHPLC-MS/MS spectrometry analysis

At Novogene Co., Ltd. In Beijing, advanced UHPLC-MS/MS techniques were employed using a Vanquish UHPLC system (ThermoFisher, Germany) coupled with an Orbitrap Q ExactiveTM HF mass spectrometer (Thermo Fisher, Germany). Analysis was conducted on a Hypesil Gold column (100 × 2.1 mm, 1.9 μm) with a 17-min linear gradient at a flow rate of 0.2 mL/min. In positive polarity, eluents consisted of 0.1 % Formic Acid in water and Methanol, and in negative polarity, 5 mM ammonium acetate (pH 9.0) and Methanol was used : 2 % B (1.5 min), a transition to 85 % B (3 min), 100 % B (10 min), a decrement to 2 % B (10.1 min), and stabilization at 2 % B (12 min). The mass spectrometer was set to operate in dual polarity mode, with the following parameters: a 3.5 kV spray voltage, 320 °C capillary temperature, 35 psi sheath gas, using 10 L/min of auxiliary gas, an *S*-lens RF level set at 60, and an auxiliary heater temperature of 350 °C.

2.5. Metabolite identification and data processing

The initial data underwent processing, including alignment, peak selection, and metabolite quantification, using Compound Discoverer 3.1 (CD3.1, ThermoFisher). Key parameters included a 0.2-min retention time tolerance, 5 ppm actual mass tolerance, 30 % signal intensity tolerance, a signal/noise ratio of 3, and a minimum intensity threshold, among other settings. Following this, peak matching against databases such as mzCloud (https://www.mzcloud.org/), mzVault, and MassList was conducted to achieve precise qualitative and relative quantitative outcomes. Statistical analyses were performed using R (version R-3.4.3), Python (version 2.7.6), and CentOS (release 6.6). When the data deviated from a normal distribution, efforts were undertaken to normalize them through an area-based normalization approach.

2.6. Data analysis

Metabolite profiling involved a comprehensive search in the KEGG database (https://www.genome.jp/kegg/pathway.html), HMDB database (https://hmdb.ca/metabolites), and LIPID Maps database (http://www.lipidmaps.org). Subsequently, data analysis was carried out through Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA), facilitated by metaX [22], in conjunction with simca14.1. Statistical significance was assessed using univariate t-tests to calculate p-values. Metabolites meeting strict criteria (VIP >1, $p < 0.05$, and fold change ≥2 or ≤0.5) were categorized as differential metabolites. The functionality of the identified metabolites and their roles within various metabolic pathways were explored by cross-referencing the KEGG database. Additionally, enrichment analysis of metabolic pathways was conducted for the differential metabolites. Enrichment was determined when the x/n > y/N ratio criterion was met, and A metabolic pathway was deemed statistically significant with a *p*-value less than 0.05.

2.7. Determination of fatty acid content in sea cucumber body wall samples by GC-MS

Refer to the " National standards for food safety - Determination of fatty acids in food (GB 5009.168–2016)" [23], information on reagents, equipment, and standards is provided in the supplementary file (Table S1、Table S2).

2.8. Determination of hydrolyzed amino acid content in sea cucumber body wall samples using amino acid automatic analyzer

Refer to the " National standards for food safety - Determination of amino acids in food (GB 5009.124–2016)" [24], Equipment and reagents are shown in the supplementary file (Table S3-Table S7).

3. Results and discussion

3.1. UHPLC-MS/MS metabolomics analysis of the sea cucumber samples

The sea cucumber samples were analyzed using both positive and negative ionization modes. 487 metabolites were identified in the

Dalian samples for both positive and negative ionization modes. The stability of the instrument was indicated by stable spectrum baselines. Fig. 1A and B demonstrate a robust correlation within the Quality Control (QC) samples, with R2 values nearly reaching 1, thereby signifying a stable detection process and exceptional data quality. Principal component analysis (PCA) was performed on the sea cucumber sample data, and the resulting score plots are presented in Fig. 2A. However, unsupervised PCA models have limitations [25] and are not effective in differentiating between metabolites. To address this, we utilized partial least-squares discriminant analysis (PLS-DA), this allows for the prediction of sample categories and provides stronger classification abilities compared to PCA. The PLS-DA score plot for the sea cucumber samples from Dalian demonstrated clustering according to the processing method, as depicted in Fig. 2B. The permutation test results presented in Fig. 2C reveal that the R2 and Q2 values for the sea cucumber samples in positive ionization mode were 0.568 and −0.768, respectively. These values indicate a good level of repeatability and predictability in the model. Based on these findings, a Venn diagram was generated for display (Fig. 3).

3.1.1. PLS-DA analysis of the sea cucumber samples

PLS-DA was used with pairwise contrasts among the processing techniques to identify differential metabolites, then metabolite expression was analyzed to establish its correlation with the processing technique, enabling the modeling and prediction of samples (Fig. 4). A PLS-DA model was developed to effectively predict the results of sample processing in paired sea cucumber techniques by leveraging the differences observed in the metabolites produced during the process. For the US/F pair (Fig. 4A), the R2 and Q2 were each 0.99, This suggests the reliability of the PLS-DA model. To avoid overfitting, 200 permutation tests were performed on the PLS-DA model parameters (R2 and Q2) for the comparison group. The results indicate that Q2 was <0, indicating that the results were not overfitted and that analysis was accurate (Table 3). 19 significantly different metabolites were identified between US and F (Table 4) with VIP >1 and *P*-value <0.05, palmitic acid, docosapentaenoic acid, adenosine and cGMP appear to be up-regulated, cytidine-5′-monophosphate, UDP-N-acetylglucosamine inositol, L-glutamic acid, α,α-trehalose, D-gluconic acid, D-alanine, homovanillic acid, L-thyroxine, L-asparagine, prostaglandin F2α, D-glucosamine 6-phosphate, porphobilinogen and inosine appear to be up-regulated. Differential metabolites for the remaining pairs can be found in the supplementary file (Table S8 through Table S11).

3.2. Differential metabolite analysis

Hierarchical clustering analysis (HCA) was conducted on the full set of differentially identified metabolites from the comparison groups, and these metabolites' relative quantitative measurements were subjected to normalization and clustering (see Fig. 5A and B). The analysis revealed that the majority of the differential metabolites were lipids. To further illustrate this finding, a classification annotation map based on LIPID maps (Fig. 6A and B) was created for visual representation.

3.2.1. Comparative analysis of metabolites in processed sea cucumber and fresh sea cucumber

The main differences between the metabolites in fresh and processed *A. japonicus* from Dalian are lipid metabolites and amino acid metabolites.

Fatty acids can be used as nutritional biomarkers. The utilization of fatty acid profiling in the study of marine foods has seen a growing trend [26]. In the US samples, the concentrations of C20:5n3 and palmitic acid (C16:0) were significantly elevated ($P < 0.05$) compared to the F samples, this might have been caused by sea cucumber undergoing treatment with hot water in addition to salting [27]. This was also found when the data from instant sea cucumber and soaked salted sea cucumber, which also underwent this treatment step, were compared to fresh sea cucumber data, respectively, we found that, fatty acids such as C20: 4, C16: 0, and C20: 5n3 (DPA) all exhibited a significant ($P < 0.05$) up-regulation trend. This is consistent with previous studies that higher C22:6n3 concentrations have been found in salt drying salmon eggs than fresh salmon eggs [28]. This difference might have been caused by the salting and drying procedures used [29], or microbial activity during the salting and drying processes [30]. Compared to fresh sea

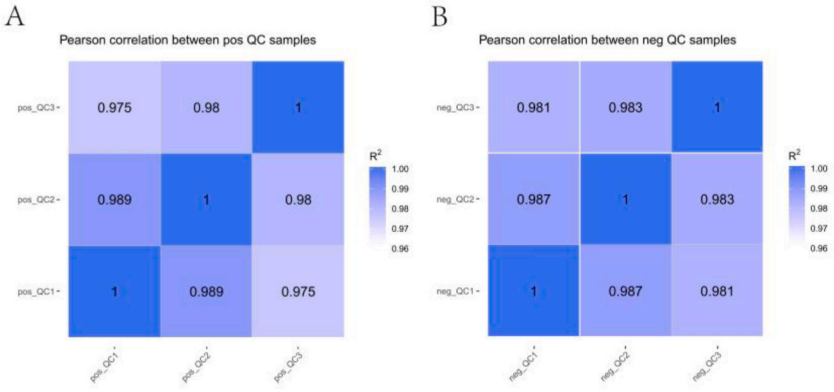

Fig. 1. QC sample correlation analysis chart, A : Positive ion mode, B : negative ion mode.
Note: To determine the Pearson correlation coefficient among QC samples using the relative quantification values of metabolites. A higher correlation among QC samples (with an R2 value closer to 1) suggests greater overall analysis process stability.

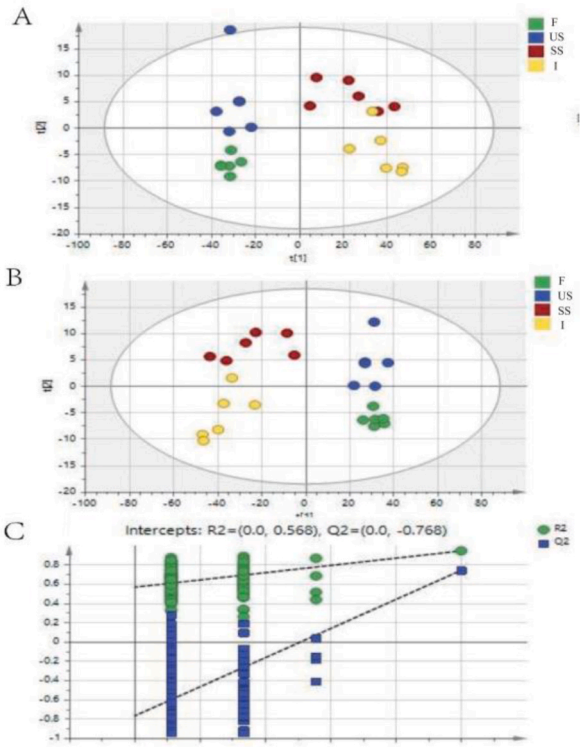

Fig. 2. Multivariate statistic analysis. (A) : The PCA plot of sea cucumber samples in Dalian; (B) : The PLS-DA model of sea cucumber samples in Dalian; (C) : The permutation test for PLS-DA model of serum samples.

cucumbers, there was a significant up-regulation ($P < 0.05$) in the levels of the polyunsaturated fatty acid, arachidonic acid, in both salted after processing and instant sea cucumbers. Similar results were found in a previous study that compared fresh sea cucumber with salted sea cucumber [31]. All of them underwent the process of boiling in salted water, which might have been caused by microorganism activity in a saline environment, leading to the production of large quantities of esterases. Glycerides and phospholipids are continually degraded by enzymes to produce fatty acids, resulting in the continuous accumulation of free fatty acids. Zhao et al. [32] found that the addition of table salt promoted fat degradation and fatty acid production, resulting in increased fatty acid contents in the analyzed samples. The fatty acid content of brined tilapia was higher (6.82 mg/g) compared to fresh tilapia (4.22 mg/g), with a significantly higher unsaturated fatty acid content. Based on these findings, we propose a preliminary speculation that the saline processing environment contributes to the up-regulation of fatty acids in saline sea cucumber. In addition to the observed significant differences, the metabolism of histidine was significantly decreased ($P < 0.05$) in the processed samples compared to the fresh samples. Furthermore, L-glutamine acid exhibited lower metabolic intensity in the SS and I samples compared to the F samples. It is hypothesized that rapid dehydration and high-temperature simmering during the salting process could potentially lead to the hydrolysis and reduction of amino acids, soluble nutrients, and nucleotides [33]. Xiang et al. [34] found that hot water treatment caused salted sea cucumber to lose large quantities of nutrients (particularly polysaccharides, proteins, and amino acids) and that most of the losses were caused by dissolution during the first hot water treatment. Producing unsoaked salted sea cucumber requires cooking at a high temperature and salting, thus, we compared fresh sea cucumber with unsoaked salted sea cucumber. Less amino and nucleotide metabolism was found in the US samples than the F samples, and this manifested as down-regulated UDP-N-acetylglucosamine in the US sample, this finding is consistent with the anticipated decline in nutrient concentrations, particularly polysaccharides, in the body wall of sea cucumbers when subjected to cooking in water. The water-soluble amino acids would have been degraded because of the hot processing conditions [35]. For example, when comparing the I and SS samples with the F samples, the main metabolite differences were related to niacin and nicotinamide metabolism, as well as glutamate metabolism. Amino acids serve diverse physiological functions and additionally contribute significantly to the flavor of food. Salting resulted in increased concentrations of amino acids, such as phenylalanine and glutamic acid, which are associated with taste. However, the subsequent processes of soaking in water and high-temperature simmering decreased the levels of these amino acids. Nevertheless, simmering and soaking are essential steps in the processing of numerous sea cucumber products. This could be one of the reasons why several sea cucumber products do not have an optimal taste. Higher metabolic intensity of adenosine was observed in the unsoaked salted (US) samples compared to the fresh (F) samples. Previous studies have shown that nucleotide catabolism increases with rising temperatures [36]. Adenosine triphosphate (ATP) can be broken down through two main pathways: (1) ATP → ADP → AMP → IMP → HxR → Hx, and (2) ATP → ADP → AMP → AdR → HxR → Hx [37]. Hattula and Kiesvaara [36] noted the decrease in IMP concentration and the increase in adenosine

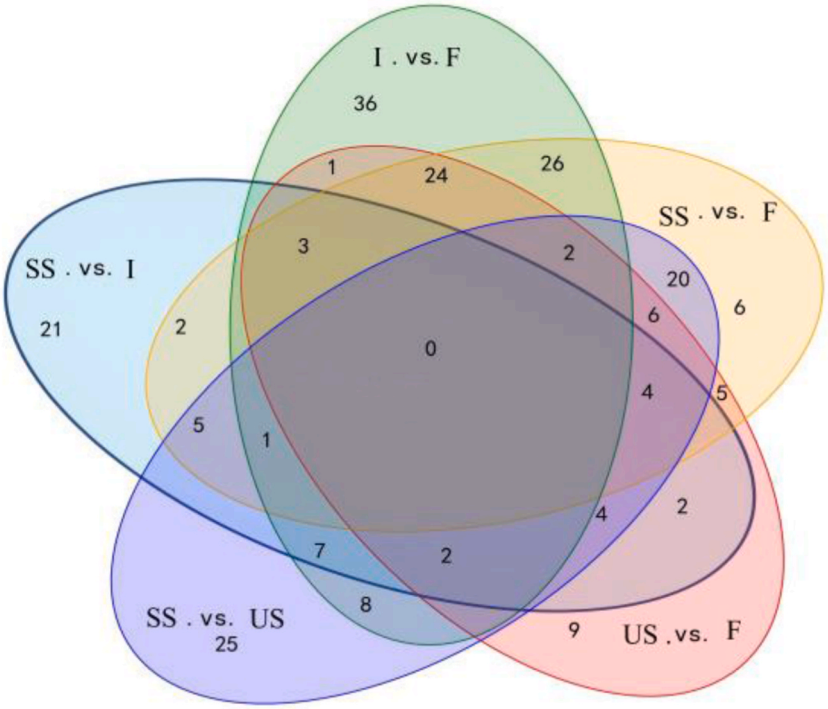

Fig. 3. Venn diagram of the total sea cucumber samples in Dalian area.
Note: By using a Venn diagram that incorporates multiple comparative groups of differentially expressed metabolites, we can visually compare the shared (overlap) and unique (unique) differentially expressed metabolites between different groups, showcasing the relationships among multiple sets of differentially expressed metabolites.

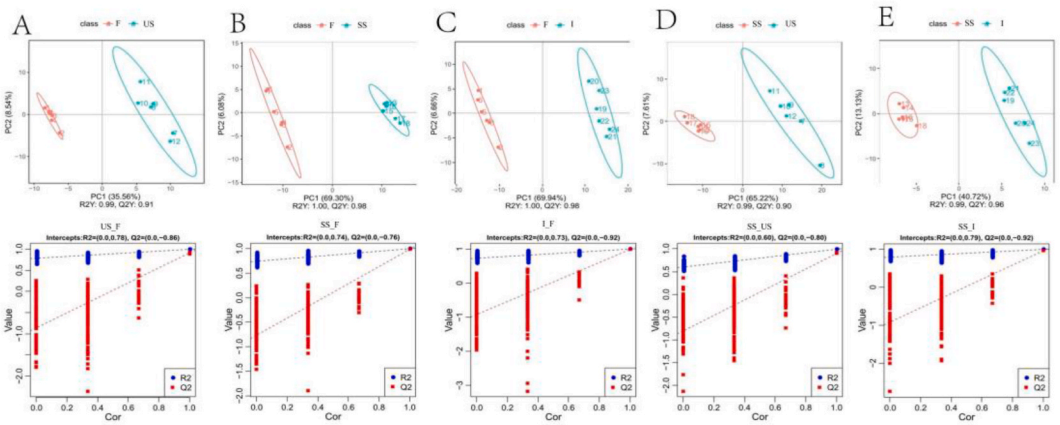

Fig. 4. The PLS-DA score chart and permutation test for each comparison group, A: US/F (unsoaked salted sea cucumber/fresh sea cucumber); B: SS/F (soaked salted sea cucumber/fresh sea cucumber); C: I/F (instant sea cucumber/fresh sea cucumber); D: SS/US (soaked salted sea cucumber/unsoaked salted sea cucumber); E: SS/I (soaked salted sea cucumber/instant sea cucumber).
Note: The x-axis displays sample scores on the first principal component, and the y-axis shows scores on the second principal component. R2Y reflects the model's explanatory capacity, while Q2Y assesses the PLS-DA model's predictive performance.

concentration during the heating of rainbow trout to 65 °C. Chen et al. [38] also reported an increase in adenosine concentration during heating, likely due to changes in enzymes (such as ATPase, adenosine diphosphate enzymes, AMP dehydrogenases, and nucleosidases) that affect ATP degradation. These findings suggest that the up-regulation of adenosine was associated with the high-temperature simmering process. This was further confirmed by the up-regulation of adenosine in the soaked salted (SS) samples compared to the unsoaked salted (US) samples. Another important compound, cyclic adenosine monophosphate (cAMP), which is derived from ATP, plays a crucial role in regulating intracellular metabolism and biological functions, acting as a second messenger in

Table 3
Parameters for each comparison group in the model.

Comparison group	R2	Q2	InterceptR2	InterceptQ2
US/F	0.99	0.91	0.78	−0.86
SS/F	1.00	0.98	0.74	−0.76
I/F	1.00	0.98	0.73	−0.92
SS/US	0.99	0.90	0.60	−0.80
SS/I	0.99	0.96	0.79	−0.92

Table 4
Different metabolites between US versus F groups.

Mode	Name	RT [min]	*m/z*	FC	*P*-value	ROC	VIP
	Adenosine	2.85	268.1039	9.468926	0.004851	0.944444	1.097873
	Palmitic Acid	6.757	274.274	3.092237	0.006905	0.972222	1.233917
ESI+	cGMP	2.565	346.0545	16.20371	0.008775	1	1.787821
	Docosapentaenoic acid	7.682	331.2631	3.288558	0.043003	0.888889	1.507125
	Cytidine-5′-monophosphate	1.47	322.0444	0.024618	3.00E-05	1	1.803047
	UDP-N-acetylglucosamine	1.536	606.0747	0.068902	8.41E-05	1	1.501466
	D-Ala-D-Ala	1.435	159.0775	0.082867	0.000295	1	1.846275
	Ergothioneine	1.458	228.0814	0.044959	0.000474	1	1.878617
	α,α-Trehalose	1.462	341.1088	0.098061	0.000523	0.972222	1.173961
ESI-	D-Gluconic acid	1.405	195.051	0.225122	0.0006	1	1.234376
	Inositol	1.349	225.0616	0.206395	0.001129	0.972222	1.07311
	L-Asparagine	1.314	131.0461	0.173133	0.001445	0.972222	1.380577
	Inosine	3.325	267.0736	0.041102	0.004089	0.972222	1.61118
	D-Glucosamine 6-phosphate	1.372	258.0382	0.267076	0.004785	0.944444	1.235241
	Prostaglandin F2alpha	6.998	353.2333	0.216601	0.009543	0.916667	1.623878
	L-Glutamic acid	1.386	146.0457	0.060956	0.013019	0.833333	1.559237
	Porphobilinogen	4.958	225.088	0.454129	0.01814	0.861111	1.271635
	L-Thyroxine	6.437	775.6799	0.276556	0.027897	0.944444	1.237124
	Homovanillic acid	5.241	181.0505	0.349433	0.04003	0.833333	1.273843

*RT：Retention time.
FC：Fold change.
VIP：Variable Importance in the Projection.
P-value：Tatistical significance level.

organisms [39]. The up-regulation of cAMP during purine metabolism indicates that *A. japonicus* expends energy in a saline environment. The primary differential metabolic pathways observed when comparing fresh *A. japonicus* with *A. japonicus* treated using different processing techniques were related to the biosynthesis of various fatty acids and the metabolism of different amino acids. These metabolic changes were largely attributed to the saline environment, high-temperature simmering, and soaking in hot water.

3.2.2. Comparative analysis of metabolites in soaked salted sea cucumber compared to unsoaked salted sea cucumber

The fatty acid, amino acid, nucleic acid and nucleic acid derivative, and some other compound concentrations were different in the soaked salted sea cucumber compared to the unsoaked salted sea cucumber. In the comparisons between I (Instant sea cucumber) and F (Fresh sea cucumber), SS (Soaked salted sea cucumber) and F, as well as SS and US (Unsoaked salted sea cucumber), a decrease in the metabolic intensity of carbohydrates was observed. During processing, polysaccharides are lost due to their high solubility in water [35]. Therefore, polysaccharides may be lost during high-temperature simmering, as well as subsequent hydration and soaking processes [33]. A notable difference was observed in the tyrosine metabolism between the SS/US sample pairs, with lower concentrations of 3,5-diiodotyrosine in the SS samples compared to the US samples. It is worth mentioning that 3,5-diiodotyrosine exhibits a superior spotting effect compared to potassium iodide [40]. The loss of 3,5-diiodotyrosine could have occurred during the high-temperature simmering and subsequent hydration steps. Arachidonic acid and adrenal acid were found to have significantly higher concentrations in the SS samples compared to the US samples. However, DHA (docosahexaenoic acid) exhibited lower concentrations in the SS samples compared to the US samples. In a previous study [27], it was observed that treatment with hot water increased the concentrations of amino acids and fatty acids but decreased the concentrations of EPA (eicosapentaenoic acid) and DHA in the sea cucumber body wall. EPA and DHA are crucial nutrients for humanhealth [41], as they play important roles in neuroimmunomodulation, neuroprotection, prevention of cardiovascular disease, lowering cholesterol levels, and decreasing blood viscosity. Therefore, it is essential to minimize the negative effects of salting and soaking on the concentrations of these compounds (EPA, DHA, polysaccharides, and 3,5-diiodotyrosine) in sea cucumber.

3.2.3. Analyzing metabolites in soaked salted sea cucumber versus instant sea cucumber

Soaked salted sea cucumber and instant sea cucumber are two products that can be consumed directly. Significantly higher concentrations of melatonin were found in the SS samples compared to the I sample. Melatonin is crucial for various physiological

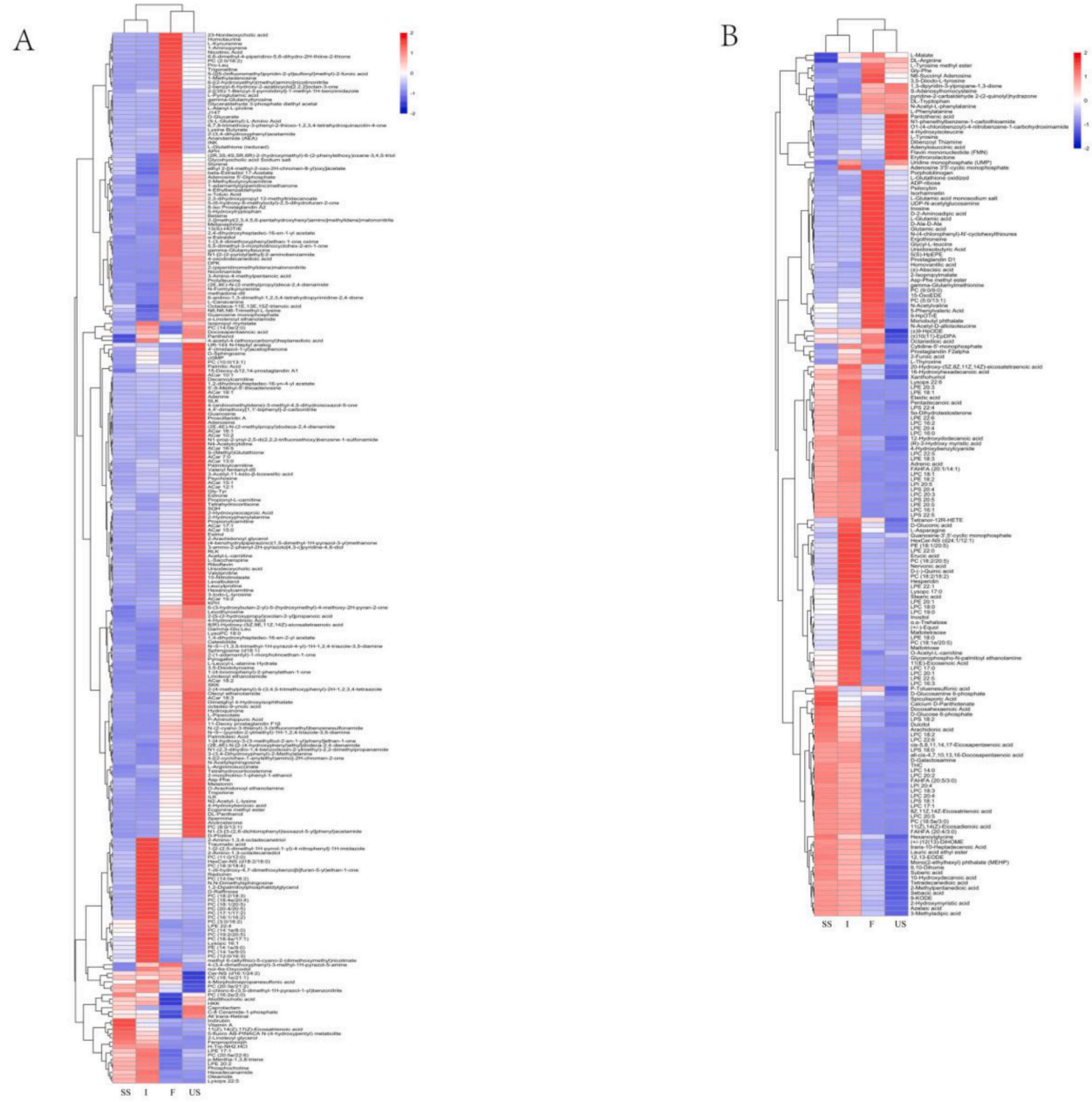

Fig. 5. Hierarchical cluster analysis heat plot, A : Positive ion mode, B : negative ion mode.
Note: Red and blue represent the expression trends of differential metabolites. Blue represents the down-regulated expression of the substance, while red represents the up-regulated expression of the substance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

functions such as controlling circadian rhythms, regulating body mass index, influencing reproduction, managing immune responses, and impacting neurological activity, cardioprotection, and exhibiting antitumor, antioxidant, hypolipidemic, anticancer, and anti-diabetic effects [42]. Therefore, soaked salted sea cucumber in Dalian may be more suitable for individuals interested in experiencing these effects compared to instant sea cucumber in Dalian. The processing methods employed also resulted in a decrease in the concentrations of various water-soluble compounds, such as D-proline and L-ornithine. As shown in Table S11, the levels of several amino acids exhibited a significant reduction ($P < 0.05$) in the instant sea cucumber samples when compared to the soaked and salted sea cucumber samples. This is likely because the presence of salt in the soaking process would increase the concentrations of free amino acids [43]. Consequently, the soaked salted sea cucumber body wall would retain higher concentrations of free amino acids compared to unsalted instant sea cucumbers. Using the findings of this study as a reference, consumers can select sea cucumber products that are suitable for their needs.

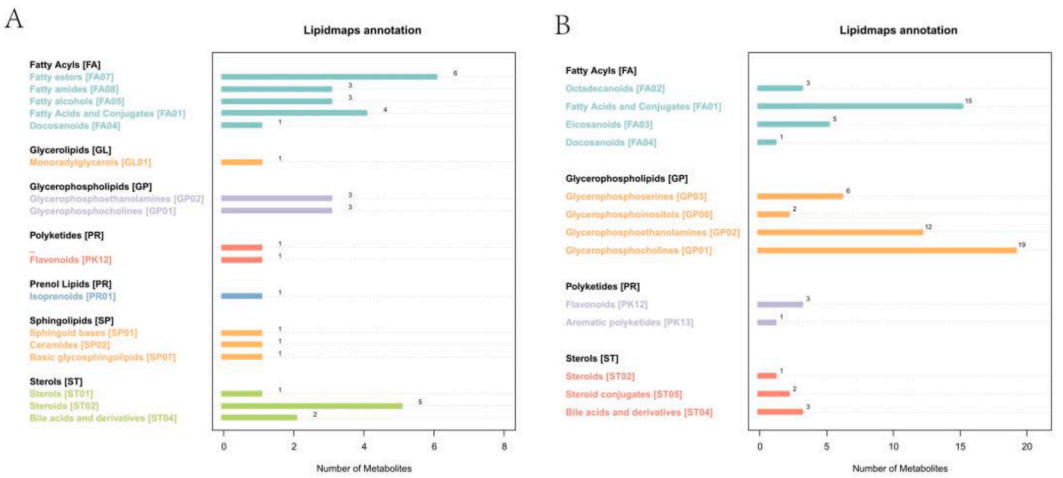

Fig. 6. LIPID MAPS classification annotation map, A : Positive ion mode, B : negative ion mode.
Note: The x-axis shows metabolite counts, and the y-axis displays LIPID MAPS annotated lipid classes.

3.2.4. Analysis of polysaccharide metabolism

Sea cucumber polysaccharide is a nutrient substance found in sea cucumbers and has been attributed to various biological activities, such as antioxidant, hypolipidemic, and anti-tumor effects [44]. Chondroitin sulfate, on the other hand, is a type of sulfated polysaccharide characterized by fucose branched chain substitution. It is primarily composed of D-acetylgalactosamine, D-glucuronic acid, and other substances [45].

In our study conducted on the sea cucumber population in the Dalian area, we conducted a comparative analysis of UDP-N-acetylglucosamine metabolic levels in fresh sea cucumbers as opposed to instant, soaked salted, and unsoaked salted sea cucumbers. The findings revealed a notable reduction in the metabolic levels of UDP-N-acetylglucosamine in the processed sea cucumber samples ($P < 0.05$).Furthermore, we observed a downregulation of D-glucuronic acid and D-glucosamine 6-phosphate in non-soaked salted sea cucumber when compared to fresh sea cucumber as a reference, while soaked salted and instant sea cucumber both showed a decrease in D-glucuronic acid salts. These differences in sugar metabolites suggest that different processing methods can lead to variations in the polysaccharide composition of sea cucumber products, potentially diminishing their potential health benefits. These differences in sugar metabolites indicate that processing methods will cause differences in polysaccharides in sea cucumber products and reduce its potential health benefits.

Table 5
Information on the fatty acid of the sea cucumber *Apostichopus japonicus*.

Fatty acids (mg/100g)	F (fresh sea cucumber)	US (unsoaked salted sea cucumber)	SS (soaked salted sea cucumber)	I (instant sea cucumber)
C14:0	3.9^{a}±0.0000	3.9^{b} ± 0.0000	0.9^{c}±0.0000	1.2^{d} ± 0.0000
C14:1	3.9^{a}±0.0000	1.8^{b} ± 0.0000	1.0^{c}±0.0000	0.9^{d} ± 0.0000
C15:0	0.4^{a}±0.0000	0.5^{b} ± 0.0000	_	_
C16:0	68.2^{a}±0.0004	50.3^{b} ± 0.0003	10.7^{c}±0.0002	10.7^{c}±0.0001
C16:1	80.9^{a}±0.0002	43.1^{b} ± 0.0001	9.2^{c}±0.0006	9.6^{c}±0.0001
C17:0	6.5^{a}±0.0000	6.5^{a}±0.0000	2.4^{b} ± 0.0000	2.2^{c}±0.0000
C18:0	60.6^{a}±0.0006	43.9^{b} ± 0.0004	12.6^{c}±0.0001	13.0^{c}±0.0001
C18:1n9t	4.9^{a}±0.0008	_	_	_
C18:1n9c	133.5^{a}±0.0004	29.2^{b} ± 0.0004	12.2^{c}±0.0001	14.5^{d} ± 0.0000
C18:2n6c	142.1^{a}±0.0001	26.7^{b} ± 0.0001	10.7^{c}±0.0000	16.1^{d} ± 0.0000
C20:0	10.1^{a}±0.0001	7.5^{b} ± 0.0000	2.2^{c}±0.0001	2.6^{d} ± 0.0000
C18:3n6	1.9^{a}±0.0000	_	_	_
C20:1	70.3^{a}±0.0005	15.1^{b} ± 0.0003	6.7^{c}±0.0001	8.6^{d} ± 0.0001
C21:0	111.0^{a}±0.0002	8.0^{b} ± 0.0002	2.2^{c}±0.0002	2.5^{d} ± 0.0000
C20:2	17.0^{a}±0.0002	7.3^{b} ± 0.0001	3.5^{c}±0.0001	4.0^{d} ± 0.0001
C22:0	7.6^{a}±0.0000	6.5^{b} ± 0.0001	2.2^{c}±0.0001	2.6^{d} ± 0.0001
C22:1n9	304.0^{a}±0.0002	22.8^{b} ± 0.0002	12.7^{c}±0.0002	13.2^{d} ± 0.0000
C20:4n6	39.1^{a}±0.0001	31.4^{b} ± 0.0002	10.7^{c}±0.0001	9.2^{d} ± 0.0001
C20:5n3	56.7^{a}±0.0007	33.5^{b} ± 0.0007	12.7^{c}±0.0002	10.4^{d} ± 0.0002
C24:1	33.0^{a}±0.0001	22.6^{b} ± 0.0002	14.6^{c}±0.0003	10.9^{d} ± 0.0000
C22:6n3	330.0^{a}±0.0002	16.2^{b} ± 0.0003	5.3^{c}±0.0000	5.7^{c}±0.0000

* Values marked with different superscript letters in the same row indicate significant differences ($P < 0.05$).

3.3. Fatty acid analysis

In the sea cucumber samples collected from Dalian, in total, 21 fatty acids were recognized, as detailed in Table 5. Among these, Eight fell into the category of saturated fatty acids, five into the monounsaturated fatty acids group, and eight were classified as polyunsaturated fatty acids. The concentrations of some fatty acids were lower in the salted sea cucumbers (before and after soaking in hot water) and instant sea cucumbers than in fresh sea cucumbers, and were lower in soaked than unsoaked salted sea cucumbers. The C16:1, C18:2n6c, and C20:0 concentrations were significantly lower ($P < 0.05$) in unsoaked salted sea cucumbers than fresh sea cucumbers and the C14:0, C14:1, C16:0, C16:1, C18:1n9c, C20:0, C20:1, C22:0, C20:4n6, C20:5n3, C24:1, and C22:6n3 concentrations were significantly lower ($P < 0.05$) in soaked salted sea cucumbers than fresh sea cucumbers. The C14:0, C16:0, C16:1, C17:0, C18:0, C18:1n9c, C18:2n6c, C20:0, C20:1, C20:4n6, C20:5n3, C22:0, C22:1n9, C22:6n3, and C24:1-concentrations were significantly lower ($P < 0.05$) in instant sea cucumbers than fresh sea cucumbers. The fatty acids except C16:0, C18:1n9t, and C18:3n6 were found at significantly lower concentrations ($P < 0.05$) in soaked salted sea cucumbers than unsoaked salted sea cucumbers. The fatty acid concentrations in the soaked salted sea cucumber and instant sea cucumbers were only slightly different. The C16:0, C18:0, and C22:6n3 concentrations in the SS and I There were no significant differences in the samples ($P > 0.05$).

Our results indicate that various processing techniques affect the fatty acid concentrations in sea cucumber products but the two processing methods of hot water simmering and hot water immersion have similar effects on the fatty acid concentrations in sea cucumber products. Hence, the disparity in fatty acid nutritional content between instant sea cucumbers and salted sea cucumbers after soaking is relatively minimal. Nevertheless, it is worth noting that Gao et al. [46] observed that sea cucumbers collected during winter exhibited higher levels of polyunsaturated fatty acids (PUFAs) compared to those harvested during the summer. Our sampled work was carried out in November ，so the conclusion does not represent the nutritional status of *A. japonicus* fatty acids throughout the season.

3.4. Amino acid analysis

17 amino acids were identified in Dalian Sea cucumber samples, respectively, as shown in Table 6. Sample F displayed a prominent presence of glutamic acid as the primary amino acid, with glycine following closely behind. This observation is consistent with the results reported in numerous sea cucumber species [47]. In processed sea cucumber products, the highest concentration was observed for glycine, followed by glutamic acid. As described by Bordbar et al. [48], both glycine and glutamic acid are crucial in regulating the immune system. The glutamic acid content in samples F, US, and SS measured 503.0 ± 0.009, 1543.0 ± 0.026, and 513.0 ± 0.012 mg/100 g, respectively, closely resembling the levels observed in fresh sea cucumbers and processed sea cucumber products [49]. "Besides the prevalence of glutamic acid and glycine, the amino acid composition in sea cucumbers is distinguished by a low ratio of lysine to arginine [48]. The lysine-to-arginine ratios in samples F, US, SS, and I were approximately 0.57, 0.37, 0.42, and 0.43, respectively. Our findings corroborate those of Meng et al. [49], who noted that the lysine-to-arginine ratio in processed sea cucumbers was lower than in fresh sea cucumbers. Numerous studies have provided evidence that proteins exhibiting a low ratio of lysine to arginine contribute to hypocholesterolemic effects [50].

The amino acids except cystine were found at significantly higher concentrations in the unsoaked salted sea cucumber samples than in the other three sea cucumber sample types ($P < 0.05$). The amino acid concentrations were two to four times higher in the unsoaked salted sea cucumber samples than the fresh sea cucumber samples, among them, the amino acids involved in taste that were found at the highest concentrations were Asp, Gly, Glu, Ala, and other flavor amino acids. The concentrations of some amino acids were higher in fresh sea cucumbers than salted sea cucumbers soaked in hot water, because simmering in hot water during processing decreased the concentrations of these amino acids [31]. The concentrations of the amino acids except cystine, histidine, lysine, and valine—were higher in the instant sea cucumber samples than the soaked salted sea cucumbers. The reason for this trend is determined by whether the samples underwent a salting process. The results indicate that the salting treatment increased the content of various amino acids in the body wall of *Apostichopus japonicus*, and some amino acids in the bod-y wall of the soaked salted sea cucumber were lost with the soaked process, which can be seen in the soaked salted sea cucumber versus the unsoaked salted sea cucumber. Free amino acids in the soaked salted sea cucumber body wall might therefore have been retained and been at higher concentrations than free amino acids in unsalted instant sea cucumbers.

4. Metabolic pathway analysis

After identifying metabolites in sea cucumber processing techniques through pairwise comparisons, we identified the related metabolic pathways through KEGG analysis, as depicted in Fig. 7. The data for the unsoaked salted sea cucumber, soaked salted sea cucumber and instant sea cucumber samples from Dalian were compared with the data for the fresh sea cucumber samples from Dalian (Fig. 7A, B, Fig. 7C). The significantly different metabolites were lipid metabolites, amino acid metabolites, nucleotides and analogs; they are mainly associated with lysine degradation, tryptophan metabolism, nicotinate and nicotinamide metabolism, lipolysis regulation in adipocytes, vascular smooth muscle contraction, general metabolism, and purine metabolism. The amino acid metabolites, lipid metabolites, and various organic compounds were significantly different between the unsoaked salted sea cucumbers and soaked salted sea cucumbers from Dalian (Fig. 7D). According to the KEGG metabolic pathway analysis, these compounds predominantly participate in ABC transport and the metabolism of galactose. KEGG metabolic pathway analysis indicates that these compounds were mainly involved in biosynthesis of unsaturated fatty acids, the cAMP signaling pathway, and neuroactive ligand–receptor interactions. The purine metabolite and amino acid metabolite were significantly different in the soaked salted sea cucumbers and the

Table 6
Information on the amino acid of the sea cucumber *Apostichopus japonicus*.

Amino acids (mg/100g)	F (fresh sea cucumber)	US (unsoaked salted sea cucumber)	SS (soaked salted sea cucumber)	I (instant sea cucumber)
Asp	387.0^{a}±0.005	1110^{b} ± 0.014	373.0^{a}±0.005	310.0^{c}±0.000
Thr	197.0^{a}±0.005	547.0b ± 0.009	183.0a±0.005	153c.0 ± 0.005
Ser	153.0^{a}±0.005	487.0^{b} ± 0.009	163.0^{a}±0.005	130.0^{c}±0.000
Glu	503.0^{a}±0.009	1543.0^{b} ± 0.026	513.0^{a}±0.012	413.0^{c}±0.005
Gly	513.0^{a}±0.005	1893.0^{b} ± 0.017	630.0^{c}±0.008	510.0^{a}±0.000
Ala	203.0^{a}±0.005	813.0^{b} ± 0.012	250.0^{c}±0.008	200.0^{a}±0.008
Cys	5.0^{a}±0.000	4.0^{b} ± 0.000	2.0^{c}±0.000	5.0^{d} ± 0.000
Val	160.0^{a}±0.000	440.0^{b} ± 0.008	147.0^{c}±0.005	123.0^{d} ± 0.005
Met	17.0^{a}±0.001	62.0^{b} ± 0.002	_	9.0^{c}±0.000
IIe	110.0^{a}±0.00	310.0^{b} ± 0.016	103.0^{a}±0.005	82.0^{c}±0.003
Leu	183.0^{a}±0.005	450.0^{b} ± 0.014	167.0^{a}±0.005	137.0^{c}±0.005
Tyr	82.0^{a}±0.003	187.0^{b} ± 0.005	73.0^{c}±0.002	63.0^{d} ± 0.000
Phe	107.0^{a}±0.005	277.0^{b} ± 0.005	89.0^{c}±0.003	67.0^{d} ± 0.001
Lys	123.0^{a}±0.005	287.0^{b} ± 0.005	107.0^{c}±0.005	82.0^{d} ± 0.002
His	49.0^{a}±0.001	90.0^{b} ± 0.001	34.0^{c}±0.002	32.0^{c}±0.001
Arg	217.0^{a}±0.005	777.0^{b} ± 0.025	253.0^{c}±0.005	193.0^{d} ± 0.005
Pro	233.0^{a}±0.005	927.0^{b} ± 0.009	280.0^{c}±0.000	220.0^{a}±0.008

* Values marked with different superscript letters in the same row indicate significant differences ($P < 0.05$).

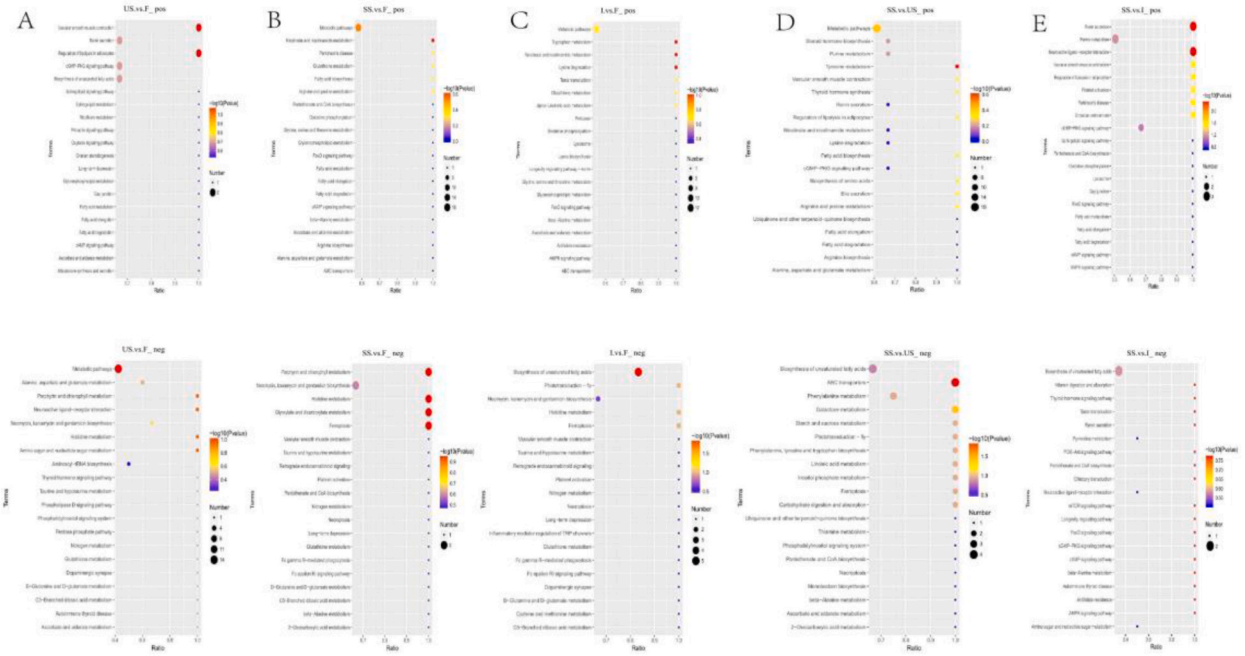

Fig. 7. The KEGG metabolic pathway analysis, A: US/F (unsoaked salted sea cucumber/fresh sea cucumber); B: SS/F (soaked salted sea cucumber/fresh sea cucumber); C: I/F (instant sea cucumber/fresh sea cucumber); D: SS/US (soaked salted sea cucumber/unsoaked salted sea cucumber); E: SS/I (soaked salted sea cucumber/instant sea cucumber).

instant sea cucumbers from Dalian (Fig. 7E). KEGG metabolic pathway analysis indicates that these Compounds primarily linked to purine, arginine, and proline metabolism.

5. Conclusions

In this study, UHPLC-MS/MS metabolomics studies were used to reveal the metabolite changes of *A. japonicus* in different processing methods. PLS-DA was used to classify the metabolites, and the differential metabolites of sea cucumbers under different processing methods were determined. It was found that the significantly different metabolites caused by different processing methods were amino acid metabolites, lipid metabolites, carbohydrate metabolites, etc.

Compared with fresh sea cucumbers, the salt processing process makes 16 kinds of amino acids (such as ASP, THR, GLY, etc.) and fatty acids C16: 1, C18: 0, C18: 2N6C, C20: 4N6 ($P < 0.05$) decline. The processing process of high-temperature simmering and soaking

in hot water causes a significant ($P < 0.05$) up-regulation of C20: 4, C16: 0, C20: 5N3 (DPA). Compared with the salted sea cucumber before and after soaked, the soaked process makes 16 kinds of amino acids ($P < 0.05$) decreased and Unsaturated fatty acids like DHA and EPA experience a decrease. Compared with the instant sea cucumbers, due to the salt processing, the content of various amino acids of soaked salted sea cucumbers is higher than that of the instant sea cucumbers but it is observed that the DHA content is lower than that of the instant sea cucumber. And the quantitative analysis of fatty acids and amino acids was further verified. This information about nutritional varies caused by different *A. japonicus* processing methods will make it easier for people to choose the suitable sea cucumber processing method as well as sea cucumber products. In the future, more attention should be paid to the processing of *A. japonicus* products and their nutritional retention, and new process methods should be optimized and explored.

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Data availability statement

No; Data will be made available on request.

CRediT authorship contribution statement

Jinyuan Zhang: Data curation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Pengfei Hao:** Data curation, Formal analysis, Investigation. **Lingshu Han:** Formal analysis, Investigation, Software. **Jiahui Xie:** Conceptualization, Funding acquisition, Investigation. **Chuang Gao:** Conceptualization, Software, Validation. **Yuanxin Li:** Formal analysis, Supervision. **Xianglei Zhang:** Data curation, Software. **Peng liu:** Validation. **Chao Guo:** Formal analysis, Methodology. **Zhenlin Hao:** Supervision. **Jun Ding:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources. **Yaqing Chang:** Conceptualization, Investigation, Resources, Supervision. **Luo Wang:** Funding acquisition, Investigation, Methodology, Supervision.

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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21854.

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