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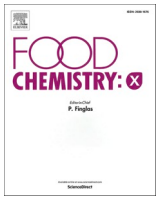

Insight into the relationship between metabolite dynamic changes and microorganisms of sea urchin (*S. intermedius*) gonads during storage

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ABSTRACT

Sea urchin gonads have high nutritional value and degenerate rapidly during storage. Previous assessment of the freshness of sea urchin gonads was based on experience without valid biochemical indicators. Thus, the current study is to find biochemical indicators representing the freshness of sea urchin gonads. Results showed that the dominant genera of sea urchin gonads were changed from *Psychromonas*, *Ralstonia*, and *Roseimarinus* to *Aliivibrio*, *Psychrilyobacter*, and *Photobacterium*. The differential metabolites of sea urchin gonads were mainly produced through amino acids metabolism. Among them, GC-TOF-MS based differential metabolites had the greatest enrichment in the valine, leucine and isoleucine biosynthesis pathway, while LC-MS based differential metabolites had the greatest enrichment in the alanine, aspartate and glutamate metabolism pathway. The growth of dominant genus (*Aliivibrio*) had a great influence on the production of differential metabolites. These results will provide valuable information for accurately judging the freshness and shelf life of sea urchin gonads.

Introduction

Sea urchin gonads are regarded as a high-nutritional-value food, and are consumed in some Southeast Asian countries, especially in the sushi market of Japan. Recently, the demand for quantity of sea urchin gonads has increased rapidly in China due to the popular intrinsic sensory features (unique sweet and umami) (Wang, Xue, Xue, Li, Lv, & Zhang, 2011). In actual scenarios (such as restaurants), the gonads consume mainly raw. However, the changes in quality of raw sea urchin gonads during storage have rarely been studied, while refrigerating of gonads occurs frequently when production is greater than consumption or transported at a long distance (Camacho et al., 2023). Previous assessments of the freshness of sea urchin gonads are based on the experience, such as granular surface without melting (Verachia, Lazzarino, Niven, & Bremer, 2013). Baião, Rocha, Lima, Marques, Valente, and Cunha (2021) also reported that appearance is the key attributes for choice of sea urchin gonads. It is of application value to screen out an effective biochemical index to accurately judge the freshness and shelf life of sea urchin gonads (Freitas, Vaz-Pires, & Câmara, 2021).

The growth of microorganisms and the metabolic processes catalyzed by endogenous enzymes have a great impact on the freshness and shelf life of aquatic products (Olafsdóttir et al., 1997; Hong, Regenstein, & Luo, 2015). Meanwhile, there may be a correlation between growth of microorganisms and metabolic processes (Zhao, Hu, & Chen, 2022). Therefore, it is beneficial to find biochemical indicators representing the freshness of sea urchin gonads by exploring the production of differential metabolites and the transfer of dominant genera during storage, as well as the correlation between them. There is limited information on the differential microorganisms and metabolites of sea urchin gonads during storage. Verachia et al. (2013) studied the adenine nucleotide profile of sea urchin gonads during storage, while Wang et al. (2011) focused on the changes in TVB-N values and K-values. These indicators are commonly used to characterize the freshness of aquatic products. Camacho et al. (2023) studied the contribution of nucleotides and free amino acids to the freshness and taste of the gonads, which provided some insight into the metabolic pathways of sea urchin gonads.

As a novel but well-established tool, high-throughput sequencing (HTS) can apply to determine the transfer of dominant genera by more precisely characterizing microbial diversity in complex environmental ecosystems (Zhang, Li, Zhang, Jiang, Chen, & Dong, 2021). Polymerase chain reaction cloning and 16S rRNA sequencing are utilized in HTS for cultivation-independent determination, which can reflect the dynamics

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in complex microbial communities (Shen et al., 2020). As a powerful approach, metabolomics can provide a holistic and comprehensive analyses of differential metabolites (Adebo, Kayitesi, Tugizimana, & Njobeh, 2019). The key metabolic pathways, metabolic networks and the function of metabolites can be explored by identifying and classifying a large number of metabolites (Jia, Fan, Shi, Zhang, Wang, & Shi, 2021). GC–MS based metabolomics is the technique of choice to identify the metabolite species of volatile, semi-volatile and low-polar compounds and corresponding pathways (Lee et al., 2016). LC-MS based metabolomics can reveal the amino acid metabolism, nucleotide metabolism, etc (Zhang, Chen, Chen, Zhong, Yun, & Chen, 2020).

Thus, in this study, the abundance and diversity of microbial communities in sea urchin gonads during storage were studied by HTS technology, while the differential metabolites and corresponding pathways were studied by GC–MS and LC-MS based metabolomics. The relationship between the changes of dominant genera and the production of differential metabolites were revealed by the construction of association network. The results will provide valuable information for accurately judging the freshness and shelf life of sea urchin gonads.

Materials and methods

Preparation of sea urchin gonads

The sea urchin gonads (*S. intermedius*) were collected from Dalian Qianri Sea Food Co., Ltd (Liaoning, China), with a weight of 58.06±9.02 g and a shell width of 6.82±0.45 cm. The gonads of female sea urchin gonads were separated immediately in the sterile production plant of company after harvesting to maintain the freshness of samples. The whole process was completed within 1 h, including washing with 3% salt solution and draining at ambient temperature of 10 ℃. And then, the gonads were put in a glass petri dish and stored at 4 ℃ for determination. Analyses were performed on days 0, 3, 5 and 7 (denoted as H0, H3, H5 and H7 groups, respectively) in triplicate.

Measurement of freshness indicators

Total volatile base nitrogen (TVB-N)

Automatic kjeldahl nitrogen determination method was used to measure the TVB-N values of samples (Li, Niu, Shao, & Wu, 2021). The sea urchin gonads (10 g) were homogenized with distilled water (75 mL) and shaken for 30 min. A Kjeldahl automated distillation unit (Kjeltec 8100, Foss, Sweden) was used to determine the homogenates. TVB-N values were expressed as mg N/100 g sample.

Total viable counts (TVC)

TVC values of sea urchin gonads were measured according to Cai, Chen, Dong, Shi, Wei, and Liu (2020). Samples (25 g) were homogenized with sterile saline solution (225 mL, 0.85 g/100 mL). And then homogeneous substance (1 mL) was pipetted into sterile saline solution (9 mL) for serially diluting. 2–3 appropriate dilution gradients were selected and incubated on the plate count agar (PCA) plates for counting. TVC values were calculated and expressed as log CFU/g.

Thiobarbituric acid reactive substances (TBARS)

TBARS values of samples were measured according to Jiang et al. (2022). The sea urchin gonads (5 g) were homogenized with trichloroacetic acid (25 mL, 7.5%, v/v) (Clapping Stomacher SH-400A, Shanghai Hegong Scientific Instrument Co., Ltd. China). Thiobarbituric acid (5 mL, 0.02 mol/L) was added into the mixture after shaking for 30 min. And then, it was reacted for 20 min in a water bath (100 ℃). The reacting mixture was centrifuged at 8000 rpm for 15 min (CR22N high-speed refrigerated centrifuge, Koki Holdings Co., Ltd, Ibaraki, Japan), and the supernatant was separated and treated with chloroform (5 mL). The absorbance of samples was read at 532 nm (Infinite 200 microplate reader, Tecan Austria GmbH, Grödig, Austria). TBARS values of samples were expressed as mg malondialdehyde (MDA)/kg.

Sensory evaluation

The sensory of sea urchin gonads was evaluated as described by Quan, Benjakul, and Hozzein (2019). Ten trained panelists (including 5 male and 5 female, aged between 23 and 30) were recruited for sensorial evaluation. Overall quality was scored based on taste, color, odor and appearance with a scale of 0–25.

Measurement of color

The color of sea urchin gonads was identified by a colorimeter (YS3060, Shenzhen 3NH Technology Co., LTD, Guangdong, China) including L* (lightness), a* (redness), and b* (yellowness). Total difference of color (ΔE*) was calculated as follows:

$\Delta E^* = ((L^*\text{-}L^*_0)^2+(b^*\text{-}b^*_0)^2+(a^*\text{-}a^*_0)^2)^{1/2}$.

Where L^*_0, b^*_0 and a^*_0 were the L*, b* and a* at day 0.

The W* (whiteness) of sea urchin gonads was calculated as follows:

$W^* = 100\text{-}((100\text{-}L^*)^2 + b^{*2} + a^{*2})^{1/2}$.

Microbial diversity analysis

Bacterial collection

The bacteria from sea urchin gonads were collected according to Chen et al. (2019). The sea urchin gonads (10 g) were homogenized with normal sterile saline (95 mL), and centrifuged at 2600× g at 4 °C for 5 min (Microfuge 22R Centrifuge, Beckman Coulter Inc., MA, USA). And then, the supernatant was separated and centrifuged again at 8800× g for 5 min. The bacteria were collected from the precipitate.

DNA extractions and PCR amplification

DNA from bacteria was extracted using the CTAB method by the Qubit dsDNA HS Assay Kit (Invitrogen, CA, USA). V3-V4 regions were amplified by PCR using the primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R(5′-GACTACHVGGGTATCTAATCC-3′). The PCR reactions were conducted as described by Chen et al. (2019).

16S rDNA sequencing and data analysis

AMPure XT beads (Beckman Coulter Genomics, MA, USA) were used to purify the PCR products. Sequencing was performed by the amplicon pool on the NovaSeq PE250 platform. Chimeric sequences were filtered using Vsearch software (v2.3.4). The feature abundance was normalized by SILVA (release 138) classifier.

GC-TOF-MS conditions

The conditions of GC-TOF-MS based metabolomics were set as described in Kind et al. (2009). Samples (50 mg) were extracted by pre-cold mixture (500 μL, methanol/chloroform = 3:1) and evaporated in a vacuum concentrator (LNG-T98, Taicang Huamei Biochemical Instrument Factory, Jiangsu, China). The dried metabolites were first mixed with methoxyamination hydrochloride (20 mg/mL) and incubate at 80 ℃ for 30 min. And then, the BSTFA regent (60 μL, 1% TMCS, v/v) was added into the mixture to incubate at 70 ℃ for 1.5 h. After adding FAMEs (5 μL, in chloroform), it was identified by gas chromatograph (Agilent 7890, CA, USA) coupled with a time-of-flight mass spectrometer (GC-TOF-MS).

DB-5MS capillary column (30 m × 250 μm × 0.25 μm, J&W Scientific, Folsom, CA, USA) was utilized in the system with a carrier gas of helium. The front inlet purge flow rate was 3 mL/min and the gas flow rate through the column was 1 mL/min. The oven temperature ramp was raised at a rate of 10 °C/min to 310 °C. The ion source, transfer line, and injection temperatures were 250, 280 and280 °C, respectively. The energy in electron impact mode was −70 eV. After a solvent delay of 6.25 min, the mass spectrometry data were acquired in full-scan mode at a

rate of 12.5 spectra per second with the *m/z* range of 50–500.

Raw data was analyzed by Chroma TOF (V 4.3x, LECO) software and the metabolites were identified by LECO-Fiehn Rtx5 database with the matching of mass spectrum and retention index.

LC-MS conditions

The conditions of LC-MS based metabolomics were set as described in Alseekh et al. (2021) with some modifications. Samples (25 mg) were extracted with the mixture solution (500 μL, methanol: acetonitrile: water = 2: 2: 1) and centrifuged at 13800× g at 4 °C for 15 min. The supernatant was collected for analysis.

LC-MS based metabolomics was performed with an UHPLC system (Vanquish, Thermo Fisher Scientific, MA, USA). The Q Exactive HFX mass spectrometer (Orbitrap MS, Thermo) was coupled with a UPLC BEH Amide column (2.1 mm × 100 mm, 1.7 μm). The mobile phase consisted of ammonia hydroxide (25 mmol/L) and ammonium acetate (25 mmol/L) in water (pH = 9.75) (A) and acetonitrile (B). The injection volume and auto-sampler temperature were 2 μL and 4 °C, respectively.

Raw data were converted to the mzXML format by ProteoWizard. The metabolites were annotated by an in-house MS2 database (BiotreeDB) with a cutoff at 0.3.

Statistical analysis

The results were reported as mean ± SD. Statistical analyses of data were evaluated in SPSS Statistics 20.0 software (IBM, Armonk, NY, USA) by one-way analysis of variance (ANOVA).

Results and discussion

Analysis of freshness indicators

TVB-N analysis

The degree of protein decomposition into basic nitrogen-containing substances (such as ammonia, amines, etc) was widely evaluated by TVB-N. These products were usually produced by activity of microorganism and endogenous enzymes (Huang et al., 2022). During storage, the TVB-N values of sea urchin gonads increased significantly from 2.79 to 27.46 mg N/100 g sample (Fig. 1A), and exceeded the detection threshold of 20 mg N/100 g sample in H7 group (Chen et al., 2019). Meanwhile, the TVB-N values increased rapidly from H5 to H7. This phenomenon at the end of storage would be more related to the growth and activity of microorganisms (Liu, Liang, Xia, Regenstein, & Zhou, 2013). Wang et al. (2011) found similar results that the TVB-N values of sea urchin gonads increased from 4.74 to 24.91 mg N/100 g sample on day 8 after storing at 5 ℃ with air packaging.

TVC analysis

The TVC values of sea urchin gonads stored on days 0, 3, 5, and 7 are assessed and shown in Fig. 1B. Initial TVC values were 1.91 log CFU/g, indicating that the initial quality of experimental samples was excellent. With the increase of storage time, TVC values of samples showed a trend of first decreasing and then increasing. It was even below the detection limit of 1.10 log CFU/g in H5 group. The decrease of bacterial counts may be due to the low microbial loads at an unfavorably low storage temperature (Zhang, Yao, Gao, Wang, & Xu, 2018). Wang et al. (2011) reported that the aerobic plate count of sea urchin gonads had no significant differences when stored between 2 and 8 days. Subsequently, the TVC values increased greatly from H5 to H7 groups and showed a similar trend to that of TVB-N values, proving that the deterioration of sea urchin gonads was increasing rapidly, and there would be a correlation between microbial growth and protein degradation.

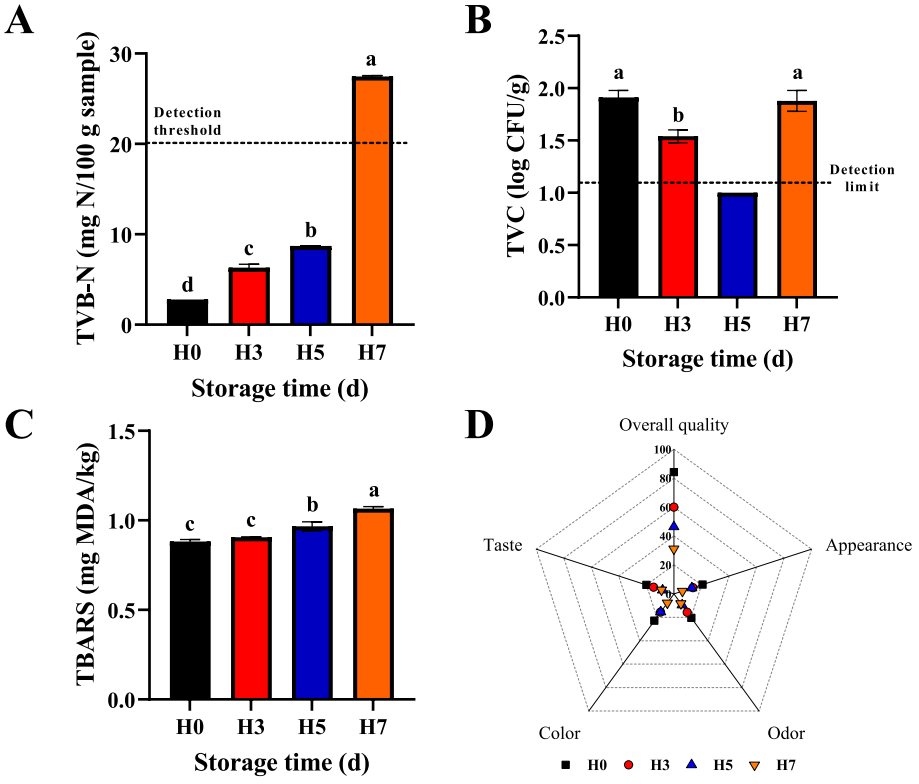

Fig. 1. Changes in TVB-N (A), TVC (B), TBARS (C) and sensory evaluation (D) of sea urchin gonads during storage. Different lowercase superscripts (a-d) in the different groups expressed significant differences ($P < 0.05$).

TBARS analysis

Changes in TBARS values of sea urchin gonads are shown in Fig. 1C. The TBARS values by detecting the content of malondialdehyde (MDA) represented the degree of secondary lipid oxidation (Jiang et al., 2022). During storage, the TBARS values increased slowly from 0.88 (H0) to 1.07 mg MDA/kg (H7) and remained below the threshold of 2 mg MDA/kg in H7 group (Santos, Matos, Casal, Delgadillo, & Saraiva, 2021). It proved that TBARS values were not the key indicator to detect freshness of sea urchin gonads. Coroneo, Corrias, Brutti, Addis, Scano, and Angioni (2022) reported that the sea urchin gonads had high protein content and low lipid content. It would be the reason why the TBARS value was not critical.

Sensory attributes

The results of sensory evaluation of sea urchin gonads (including appearance, taste, color, odor and overall quality) stored on days 0, 3, 5, and 7 are shown in Fig. 1D. It was observed that overall quality decreased obviously with the increase of storage time. There was a significant water spill on day 7, resulting in the lowest appearance and color scores in H7 group. Meanwhile, the taste and odor of sea urchin gonads had deteriorated obviously on day 5. The scores of the taste and odor decrease faster than that of appearance and color.

Changes in color

Surface color of sea urchin gonads is a critical index to consumer acceptance (Zhang, Li, Kang, & Peng, 2022). The color characteristics of sea urchin gonads stored on days 0, 3, 5, and 7 are shown in Table 1. With the increase of storage time, all the L*, a*, b*, and ΔE* values increased significantly, while W* values decreased significantly. Compared with L* and b* values, there was less difference in a* values. The increase in L* and b* values would be due to the movement of water to the surface of sea urchin gonads during storage (Osman et al., 2022). The increase of total color changes (ΔE*) indicated that the appearance quality of sea urchin gonads decreased with the storage time.

Microbiota composition

The abundance and diversity of microbial communities in the storage of sea urchin gonads were clearly analyzed by high-throughput sequencing technology. The most abundant genera (top 30) were shown in Fig. 2A with five biological parallels. In the H0 group, the relative abundance of *Psychromonas*, *Ralstonia*, and *Roseimarinus* were highest. With the increase of storage time, *Aliivibrio*, *Psychrilyobacter*, and *Photobacterium* developed into the dominant genera. *Aliivibrio* originated from the digestive system of sea urchin gonads (Jacobsen, Mikalsen, Joensen, & Eysturskard, 2019). *Photobacterium* and *Aliivibrio* were closely related, whose growing environment required a high sodium content (Broekaert, Heyndrickx, Herman, Devlieghere, & Vlaemynck, 2011). Jia et al. (2019) reported that *Photobacterium* enabled to grow and reproduce in cold temperatures as a specific spoilage organism (SSO) in seafood. The amine and sour off-odors were presented with the rapidly growth of *Photobacterium* (Macé et al., 2013). The relative abundance of *Psychrilyobacter* increased until H5 group and then decreased in H7 group. The decrease of *Psychrilyobacter* may be affected by the low lipid content in sea urchin gonads. Zhang et al. (2021) reported that *Psychrilyobacter* was associated with lipid degradation.

Changes in the relative abundance of top 30 genera were clearly observed by the heatmap (Fig. 2B). The relative abundance increased when the color changed from blue to red. With increasing of storage time, the relative abundance of about 19 genera decreased obviously, while only about 6 genera increased. It indicated that the microbial diversity of sea urchin gonads was simplified during storage. This phenomenon was caused by the storage environment and the growth inhibition in interbacterial competition (Jiang et al., 2022).

The main differential microbial communities in sea urchin gonads were identified by LDA effect size (Fig. 2C). The nodes represented species classifications and its size corresponded to abundance. Nodes with the same color as the group represented significant differences in corresponding microorganisms compared to other groups. It was observed that the number of differential microbial species decreased with the increase of storage time.

Analysis of differential metabolites identified by GC-TOF-MS

The flavor profiles of sea urchin gonads were identified by GC-TOF-MS. A total of 51 differential volatile metabolites were detected between H0 and H7 groups and clustered in Fig. 3A. The concentration of metabolite increased when the color changed from blue to red. The differential volatile metabolites were divided into 9 different categories, including amino acids and their metabolites (18), carboxylic acids and their derivatives (5), organic acids and their derivatives (10), nucleotides and their metabolites (3), benzene and substituted derivatives (2), esters (2), ketones (1), alcohols (2), and others (8). As key components in response to environmental stress factors, organic acids also acted as intermediates in carbon metabolism (Shen et al., 2022). During the storage of sea urchin gonads, the differences in flavor compounds were mainly produced through amino acids, organic acids, and carboxylic acids metabolism. For instance, the content of oleic acid in H0 group was obviously higher than that in H7 group. It may be due to the auto-oxidation or enzyme-oxidation of unsaturated fatty acids to produce hydroperoxides (Al-Dalali, Li, & Xu, 2022). Meanwhile, the content of hypoxanthine was higher in H7 group, indicating the decrease of sea urchin gonads freshness (Camacho et al., 2023). There was almost no aldehyde in the differential metabolites, which was mainly affected by the low lipid content in sea urchin gonads (Yin, Wen, Sun, Wang, Kong, & Chen, 2021).

Correlation coefficient of differential metabolites was calculated to explore mutual regulatory relationships. As shown in Fig. 3B, red and bule represented positive and negative correlation, respectively, while significant correlation marked with an asterisk. During the storage of sea urchin gonads, valine was positively correlated with L-glutamic acid. Isoleucine was positively correlated with carbamoyl-aspartic acid and methionine, and negatively correlated with *N*-Methyl-DL-alanine. Carbobenzyloxy-L-leucine was positively correlated with 4-Acetylbutyric acid and oxamide, and negatively correlated dihydroxyacetone and 2-hydroxy-3-isopropylbutanedioic acid.

The differential metabolites were mapped and annotated by KEGG (Fig. 3C). The abscissa represented the number of annotated differential metabolites in one pathway as a percentage of the total annotated differential metabolites. The results showed that amino acid metabolism was the main source of differentiated metabolites during storage of sea urchin gonads. The metabolic pathway of sea urchin gonads was further mapped in the bubble diagram (Fig. 3D). The influence factor of the pathway was expressed by abscissa and bubble. The degree of enrichment was expressed by the ordinate and color. The pathway of valine, leucine and isoleucine biosynthesis had the greatest influence and

Table 1
Changes in color of sea urchin gonads during storage.

	L*	a*	b*	ΔE*	W*
H0	51.36 ±0.16^{c}	36.58 ±0.16^{b}	86.63 ±0.20^{d}	—	−6.79 ± 0.29^{d}
H3	60.37 ±0.83^{b}	37.50 ±0.17^{b}	98.35 ±0.53^{c}	17.24 ±0.19^{c}	−14.18 ± 0.54^{c}
H5	61.68 ±0.28^{b}	38.68 ±0.17^{a}	101.39 ±0.55^{b}	19.65 ±0.77^{b}	−16.70 ± 0.52^{b}
H7	62.99 ±0.88^{a}	39.63 ±0.86^{a}	107.16 ±0.93^{a}	23.88 ±1.14^{a}	−19.05 ± 0.68^{a}

Different lowercase superscripts (a-d) in the same column expressed significant differences ($P < 0.05$).

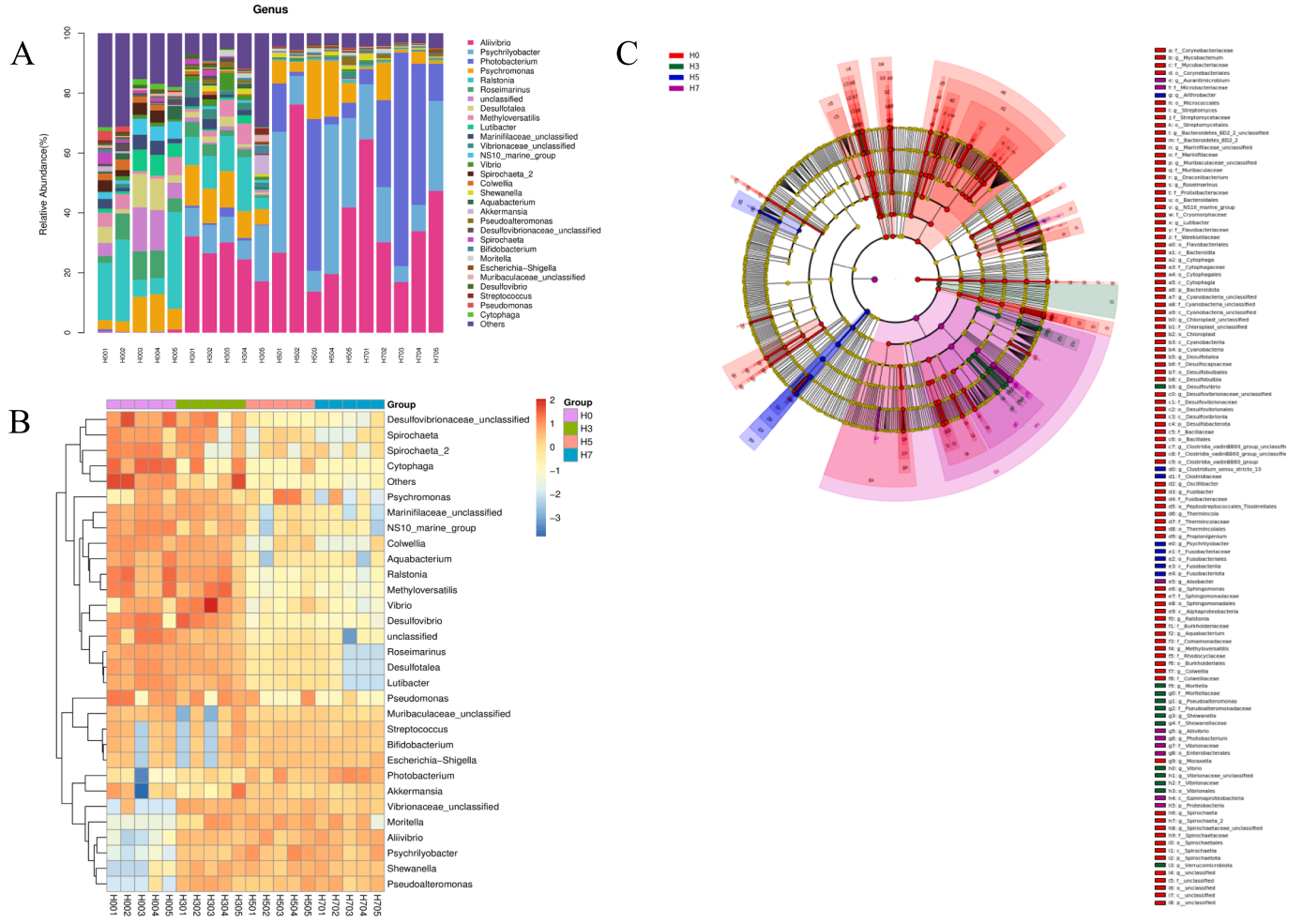

Fig. 2. Changes in stacked bar chart (A), heat-map (B), and LDA effect size (C) of sea urchin gonads during storage.

enrichment on the production of differential metabolites.

Analysis of differential metabolites identified by LC-MS

The differential metabolites in sea urchin gonads identified by LC-MS were performed by hierarchical cluster analysis and expressed as up-regulation and down-regulation (Fig. 4A and D). A total of 106 and 196 differential metabolites was selected in the negative and positive ion mode, respectively, mainly including amino acids and their metabolites, organic acids and their derivatives, nucleotides and their metabolites, and carboxylic acids and their derivatives. Meanwhile, ketones, aldehydes, and esters were identified in the positive ion mode. The differentially expressed proteinaceous amino acids of L-proline, L-serine, L-cystine, L-phenylalanine, L-glutamine, L-asparagine, L-arginine, L-histidine, L-methionine and L-lysine down-regulated, while L-norleucine, L-targinine and L-valine up-regulated during storage of sea urchin gonads. The bitterness sensor was positively correlated with L-methionine, and can be masked by L-serine and L-asparagine (Han et al., 2021).

The pathways of differential metabolites obtained from LC-MS were annotated by KEGG (Fig. 4B and E) and further mapped in Fig. 4C and F. Results showed that amino acid metabolism and nucleotide metabolism were the main source of differentiated metabolites. Additionally, some metabolites were enriched into biosynthesis of cofactors and biosynthesis of amino acids. However, the degree of differential metabolites enrichment in aminoacyl-tRNA biosynthesis and alanine, aspartate and glutamate metabolism pathway was highest, and the glycine, serine and threonine metabolism pathway had the greatest influence in the negative ion mode, while vitamin B6 metabolism pathway had the greatest influence and enrichment in the positive ion mode.

Analysis of the correlation between microorganisms and differential metabolites

The production of differential metabolites in the storage of sea urchin gonads may be associated with the changes of microbial community (Zhao et al., 2022). The top 35 genera were selected to construct a network model, and closely correlated with 62 kinds of differential metabolites (Fig. 5). The genera and differential metabolites were indicated by yellow and green squares, respectively, while the red and blue lines represented the positive and negative correlation, respectively. As shown in the correlation network, *Aliivibrio* was positively linked to D-sedoheptulose, 2-ethylacrylic, L-threonic acid, D-ribose 5-phosphate, glyceraldehyde 3-phosphate, and 3-phosphoglyceric acid. *Psychrilyobacter* was positively linked to nnal-*n*-oxide. There was no clear association between *Photobacterium* and differential metabolites. *Roseimarinus* was negatively linked to indolelactic acid, glyceraldehyde, and 3-aminoisobutanoic acid, and positively linked to oleic acid. It indicated that the growth of dominant genus (*Aliivibrio*) had a great influence on the production of differential metabolites in sea urchin gonads during storage.

Conclusions

To summarize, freshness indicators showed that protein oxidation

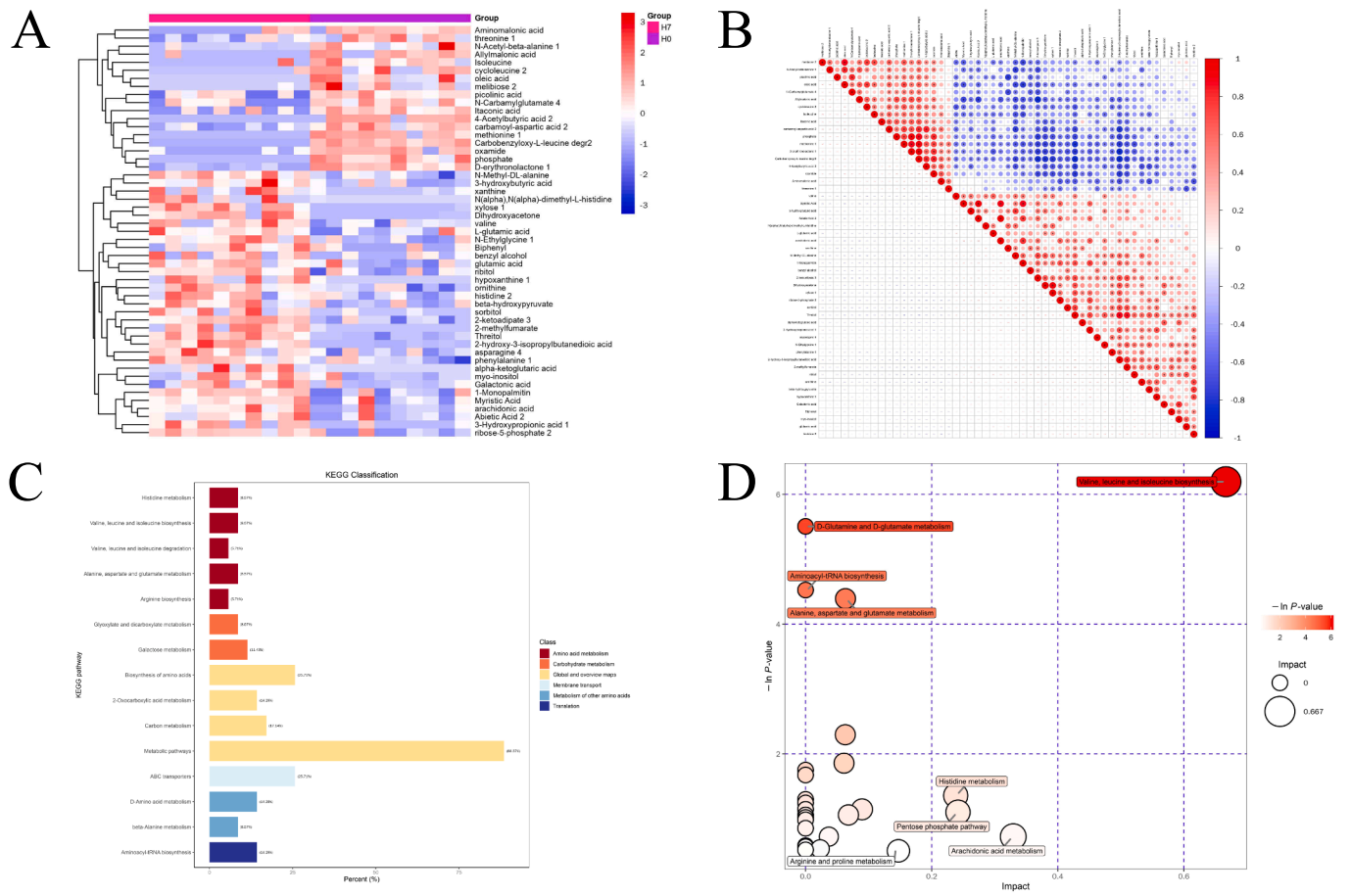

Fig. 3. Heatmap of hierarchical clustering analysis (A) and correlation analysis (B), KEGG classification (C) and Pathway analysis (D) of differential metabolites of sea urchin gonads stored on days 0 and 7 obtained from the GC-TOF-MS data.

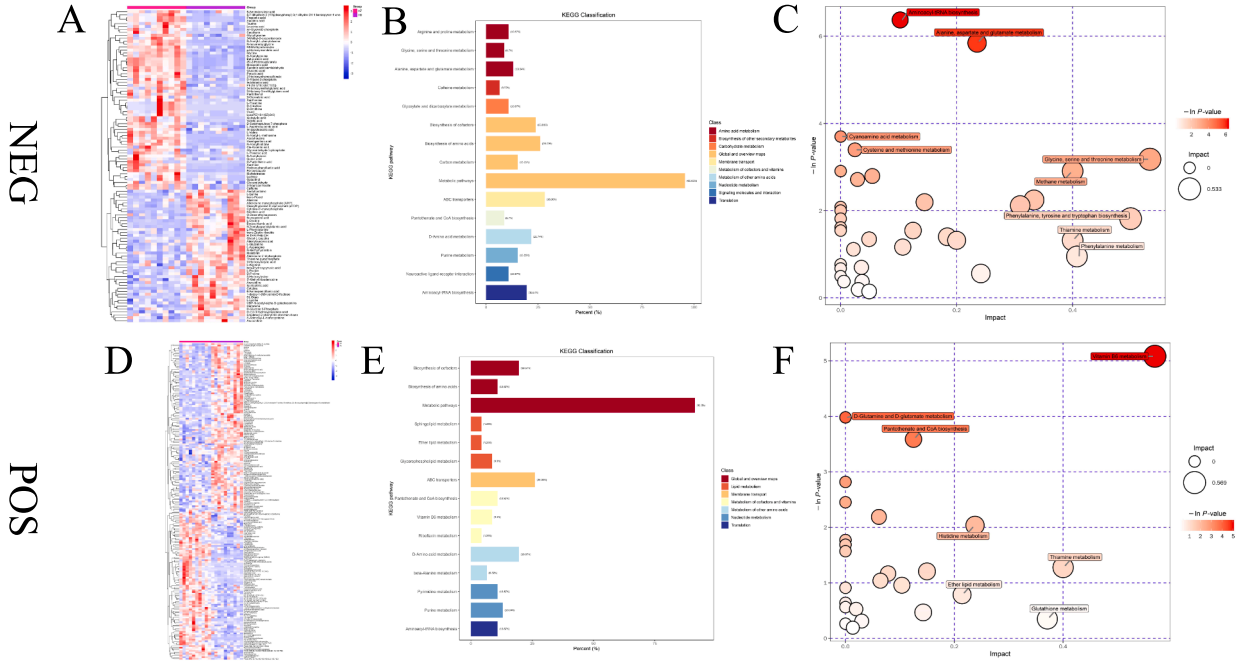

Fig. 4. Heatmap of hierarchical clustering analysis (A, D), KEGG classification (B, E) and Pathway analysis (C, F) of differential metabolites of sea urchin gonads stored on days 0 and 7 obtained from the LC-MS data (NEG: negative ion mode; POS: positive ion mode).

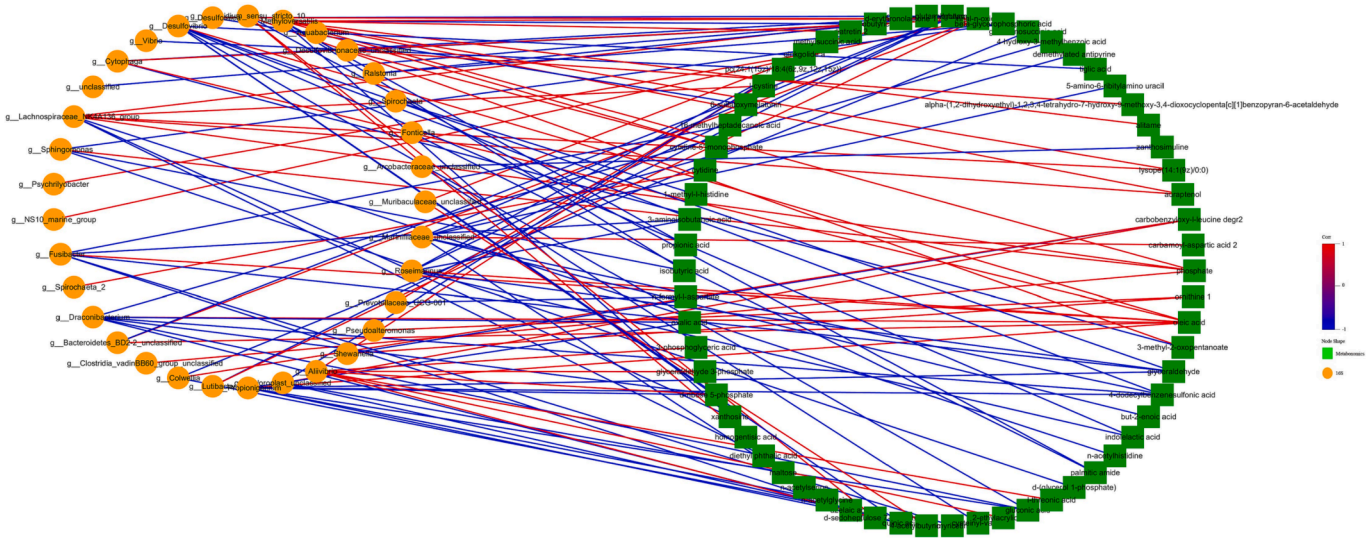

Fig. 5. Relation correlation analysis between differential metabolites and differential bacteria (genus level).

and microbial growth were the main factors for the deterioration of sea urchin gonads quality during storage. The taste and odor of sea urchin gonads had already deteriorated obviously on day 5 with a significant water spill. The dominant genera were changed from *Psychromonas*, *Ralstonia*, and *Roseimarinus* to *Aliivibrio*, *Psychrilyobacter*, and *Photobacterium*. Meanwhile, the differential metabolites of sea urchin gonads were mainly produced through amino acids metabolism, organic acids metabolism, carboxylic acids metabolism and nucleotide metabolism. Among them, the GC-TOF-MS based differential metabolites had greatest enrichment in the valine, leucine and isoleucine biosynthesis pathway, while the LC-MS based differential metabolites had the greatest enrichment in the aminoacyl-tRNA biosynthesis and alanine, aspartate and glutamate metabolism pathway. The growth of dominant genus (*Aliivibrio*) had a great influence on the production of differential metabolites in sea urchin gonads during storage. It may be feasible to accurately judge the freshness and shelf life of sea urchin gonads by measuring the number of *Aliivibrio*. These results were beneficial to reveal the relationship between microorganisms and differential metabolites production in sea urchin gonads during storage, which provided effective reference for the conservation and control of sea urchin gonads quality.

CRediT authorship contribution statement

Wen-qiang Cai: Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing, Visualization. **Cai-yan Jiang:** Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Shan Shang:** Validation, Investigation. **Shu-chen Wang:** Validation, Investigation. **Kai-yue Zhu:** Validation, Investigation. **Xiuping Dong:** Methodology, Writing – review & editing. **Da-yong Zhou:** Validation, Investigation. **Peng-fei Jiang:** Methodology, Resources, Supervision, Project administration, Funding acquisition.

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.

Data availability

Data will be made available on request.

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