



Feasibility of circular fermentation as a new strategy to accelerate fermentation and enhance flavor of Antarctic krill paste

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ABSTRACT

A recycled fermented shrimp paste made by adding old shrimp paste (Antarctic krill paste that has been fermented for 28 T in a room temperature location) and fermenting it again for 28 days. To investigate the changes in their physicochemical indices: water content, volatile salt base nitrogen, malondialdehyde content, protein content and total colony count during fermentation. The flavour substances of shrimp paste samples at the 28 T were investigated by GC-IMS, and the results showed that 31 volatile components were determined. Aldehydes isoamyl alcohol and dimethyl sulphide contributed more to the flavour of Antarctic krill paste. The microbial dynamics during fermentation were detected using high-throughput sequencing. It was found that the rate of dominant flora formation accelerated with the addition of more old shrimp paste. The higher the salinity was, the higher was the species richness. The dominant bacteria genera were more diversified.

1. Introduction

Antarctic krill has a high nutritional value, and is rich in protein various unsaturated fatty acids and 18 kinds of protein hydrolysis products of amino acids, including eight kinds of essential amino acids needed by the human body. This has good health functions for the human body. It is rich in minerals, up to 2.76%, which is higher than Japanese shrimp (1.6%), clams (2.2%), and other aquatic products. It is also rich in various active ingredients, such as enzymes, chitin and astaxanthin. All of these substances are conducive to the development of high value-added products of Antarctic krill, which greatly improves the comprehensive utilization rate of Antarctic krill resources. Wanget al. added Antarctic krill to the diet to raise leopard gill spiny perch, thereby improving antioxidant capacity, immunity and reducing lipid accumulation (Wang et al., 2024). Therefore, fermentation of shrimp paste with Antarctic krill as a raw material, such as Antarctic krill paste and Antarctic krill oil, is also a popular product in recent years (Meng et al., 2024).

Shrimp paste fermentation methods are categorised into three types: traditional fermentation, low-salt fermentation and rapid fermentation with the addition of currants. Traditional fermentation (Li et al., 2023) is characterised by a longer time consuming process, ranging from several

months or even more than a year, and some even require 2–3 years of fermentation. To inhibit the growth of spoilage microorganisms, traditional fermentation generally adopts high-salinity pickling, with a salt content of up to 30%. Although high salinity can inhibit the growth of microorganisms to a certain extent, it also inhibits the action of protease enzymes (Surya et al., 2023). Conventional rapid fermentation methods such as low-salt fermentation and fermentation with added currants can shorten the fermentation time to a certain extent. Quark fermentation is the addition of some quarks and the use of some enzymes secreted by them to break down the proteins, fats, and carbohydrates in the raw materials, and then through a series of biochemical reactions to form the fermented aquatic products unique flavour. This method is widely used, where a single addition of a fast fermenting varietal is put into shrimp paste to observe the changes in microbial dynamics or the effect on flavour. YU and others used halophilic bacteria as a fermenter to accelerate the fermentation of shrimp paste and to enhance its flavor (Yu et al., 2022).

GC-IMS is widely used in the field of food flavour analysis, which can provide essential data support for aquatic products' freshness, shelf-life, species quality, grade differentiation, processing optimisation, and origin identification, etc. (Parastar and Weller, 2024). Lu et al. (2022) investigated the effect of four heating modes: steaming, stir-frying,

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microwave and infrared on the volatile components of shrimp paste using GC-IMS technology. Chen et al. (2022) distinguished four species of Chinese freshwater fish in the raw and cooked states using an applied gas chromatography–ion mobility spectrometry (GC-IMS) and ultrafast gas chromatography–electron nose (uf-GC E-nose) coupling technique for the detection of volatile flavour compounds in freshwater fish.

High-throughput sequencing sequences hundreds of thousands to millions of small nucleic acid molecules simultaneously and randomly cuts them into fragments, adds ligations, and constructs sequencing libraries. Amplification is performed on thousands of clones. Obtain the corresponding sequence information. A large number of studies have used high-throughput sequencing to clarify the species composition of a species by conducting comprehensive and in-depth transcriptomic and genomic analyses of the species. K et al. (Kingkaew et al., 2022) isolated the genome of halotolerans SKP2-8 from a traditional shrimp paste (Ka-pi) and defined it as a salinophilic bacterium of the genus *Bacteroides albicans* using high-throughput sequencing techniques. The genome sequence data of this strain provided information for further analysis of the potential biotechnological utilization of this microorganism and guided its characterisation.

By contrast, this paper proposes a relatively simple and rapid fermentation method: adding old shrimp paste for refermentation. By adding different levels of old shrimp paste to produce a cyclic fermentation shrimp paste, the microbial dynamics of the shrimp paste made by the two fermentation methods were monitored using high-throughput sequencing technology. The microbial communities formed in shrimp paste made by ordinary fermentation and circular fermentation were observed, in which there was no obvious change in the dominant flora, e.g., the dominant flora was formed faster in the circular fermentation method than in ordinary fermentation. GC-IMS was used to analyse the volatile flavour compositions of the circulating fermented shrimp paste and to explore the volatile compounds that contributed more to its flavour. We hope to lay a theoretical foundation for a new fermented product made using the cyclic fermentation method.

2. Materials and methods

2.1. Preparation of shrimp paste samples

Antarctic krill paste with 20% and 25% salt concentrations from 28 days of fermentation was used as the old shrimp paste, which was added to Antarctic krill paste with the same salinity for another 28 days of fermentation. Antarctic krill was purchased from Dalian Ocean Foods Co. The amounts of old shrimp paste added were 5% and 10%. First, the raw material is put into the 4 °C cold storage to slow down the melting, take out and use gauze to clutch out the water, then put the raw material into the meat grinder (Foshan City Hai Xun Electrical Appliances Meat Grinder HX-J381A) and beat it into minced meat. Add salt and mix well in a beaker. The old shrimp paste and freshly made shrimp paste in the first 50 mL beaker initial mixing, then poured into the 100 mL beaker again, and finally poured into the 500 mL beaker mixing, the formation of a gradient mixing, to ensure that the old shrimp paste and shrimp paste is fully mixed state. Five cans were made for each sample to ensure uniform sampling. The beaker was sealed with two layers of gauze and placed at room temperature (24 °C–26 °C) for fermentation for 28 days, with daily stirring for 2 h to accelerate water evaporation. Samples were taken at 0, 7, 14, 21 and 28 days and were frozen at –80 °C for subsequent experiments. The group of circulating Antarctic krill paste made by adding 5% old shrimp paste at 20% salt concentration is named Ca, the group of circulating Antarctic krill paste made by adding 10% old shrimp paste at 20% salt concentration is named Cb, the group of circulating Antarctic krill paste made by adding 5% old shrimp paste at 25% salt concentration is named Cc, and the group of circulating Antarctic krill paste made by adding 10% old shrimp paste at 25% salt concentration is named Cd.

2.2. Physical and chemical indicators

2.2.1. Total moisture content

Take a clean weighing bottle and place 10 g of sea sand and a small glass rod into it, and place it in the drying box (Foshan City Hai Xun Electrical Appliances Co., Ltd. Meat Grinder HX-J381A) at 101 °C–105 °C. Weigh 5 g of shrimp paste (accurate to 0.0001 g) in a weighing bottle, stir using a small glass rod, and place it in a boiling water bath, stirring at any time until evaporation. After wiping off the water from the bottle, place it in a 101 °C–105 °C drying oven to dry for 4 h and remove the lid, and place it into a desiccator to cool down for 0.5 h after weighing. Repeat this operation until the error between two weighings does not exceed 2 mg, the constant weight. Five parallel moisture determinations were performed for each sample.

2.2.2. Total volatile base nitrogen (TVB-N)

The method of Yang (Yang et al., 2023) was adopted with slight modifications. The shrimp paste sample (10 ± 0.001 g) was added to 75 mL of distilled water and homogenised using a homogeniser (Shanghai Hegong Scientific Instrument Co., Ltd. Gastro-patterner SH-400 A). The sample was fully exposed to the solution and analysed using a fully automatic Kjeldahl nitrogen analyser after standing at room temperature for 30 min. The number of parallel samples in each group was 3.

2.2.3. Total malondialdehyde (TBARS)

The determination of TBARS was based on the method of Trejo (Trejo et al., 2021) with slight modifications. The shrimp paste sample (1.0 ± 0.1 g) was thoroughly ground and added to 5 mL of mixed solution (0.375% TBA, 15% TCA and 0.25 mol/L HCl) with rotary shaking. It was immersed in boiling water for 15 min and then cooled in tap water. The sample was centrifuged (CR22N high-speed cryo-centrifuge, Koki Holdings Co., Ltd, Ibaraki, Japan) for 10 min at 4 °C and 8000 r/min using rotor R15A. After the completion of centrifugation, 200 µL of the supernatant was taken in a 96-well plate, and the absorbance was measured at 532 nm. Three parallels were made for each set of samples. The formula for calculating the TBARS content is as follows $TBARS = A532 \times 2.77$.

2.2.4. Total protein

Protein content was determined according to the method reported by Pongsetkul (Pongsetkul et al., 2014) with slight modifications. A well-mixed sample of 2 g (to the nearest 0.001 g) was placed in a digestion tube. Add 0.4 g of copper sulphate, 6 g of potassium sulphate and 20 mL of sulphuric acid into the digestion furnace. After the temperature of the digester reached 420 °C, the sample was continuously digested for 1 h, then removed and cooled down, and 50 mL of water was added into the automatic Kjeldahl nitrogen analyser. Three parallels were made for each set of samples.

2.2.5. Total viable counts (TVC)

The total number of colonies was determined according to the method reported by Ling (Ling et al., 2022). A 1:10 mixture was made by homogenising 25 g of samples in 225 mL of sterile saline (Shanghai Hegong Scientific Instrument Co., Ltd. Gastric Clapper SH-400 A) for 1 min. Then 1 mL of threefold diluted bacterial suspension was placed into sterile petri dishes. Add 15 mL of plate counting agar medium to each Petri dish, mix well, and then, spread evenly on the plate counting agar. Place in a 30 °C incubator for 72 h and count. Three parallels were made for each set of samples.

2.3. High-throughput sequencing technology

By referring to the method of Xiong (Xiong et al., 2024). Total DNA of Antarctic krill paste was extracted by CTAB method. DNA was quantified by using Nanodrop. The quality of the DNA extraction was determined using 1.2% agarose gel electrophoresis. Microbial ribosomal

RNA sequences were used as targets to amplify the V3–V4 variable region as an amplification template using two primers 341 F (5'-CCT ACGGGGNGGCWGCAG-3') and 805 R (5'-GACTACHVGGGTATCTAA TCC-3'). Amplification products were recovered by magnetic bead purification: magnetic beads 0.8 times the volume (Vazyme VAHTSTM DNA Clean Beads) was added to 25 μ L of PCR products. Add 20 μ L of 0.8-fold magnetic bead washing solution, shake to fully suspend the supernatant, and then, place it on the magnetic rack for 5 min. Then, add 200 μ L of 80% ethanol, place it on the magnetic rack in reverse, and then, use the magnetic bead to adsorb it to the other side of the PCR tube, and aspirate the supernatant after sufficient adsorption. It was placed at room temperature and left for 5 min for the magnetic beads to crack. Finally, 25 μ L of elution buffer was added for elution. The PCR tubes were placed on an adsorption rack for 5 min to fully. The supernatants were pipetted into clean 1.5 mL centrifuge tubes for storage. The amplification products were subjected to fluorescence quantification: the fluorescence reagent was Quant-iT PicoGreen dsDNA Assay Kit and the quantification instrument was a microplate reader (BioTek, FLx800). Preparation of sequencing libraries: repair the ends of the amplification products, ligate the DNA fragments for PCR amplification, and do the final fragment selection and purification of the libraries. Finally, high-throughput sequencing was performed on the machine, and the optimal sequencing of insert fragments in the range of 200–450 bp.

2.4. GC-IMS analysis

The volatiles in shrimp paste were characterised by GC-IMS (FlavourSpec® Gas Phase Ion Mobility Spectrometry) (Dortmund, Germany). Here 2 g (± 0.01 g) of shrimp paste was placed in a 20 mL headspace vial, incubated at 60 °C for 15 min and then injected into the sample, and three parallel sets of each sample were determined. Reference was made to Lv (Lv et al., 2024), with slight modifications. Column temperature: 60 °C; carrier gas: high-purity nitrogen (purity $\geq 99.999\%$); programmed ramp-up: initial flow rate of 2.0 mL/min was maintained for 2 min, linearly increased to 10.0 mL/min within 8 min, and then linearly increased to 150.0 mL/min within 10 min and continuously maintained for 10 min. Chromatographic run time: 40 min; inlet temperature: 80 °C. Ionisation source: tritium source (3H); migration tube length: 98 mm; electric field strength: 500 V/cm; migration tube temperature: 45 °C; drift gas: high purity nitrogen (purity $\geq 99.999\%$); flow rate: 150.0 mL/min; positive ion mode.

2.5. Relative odour activity values

The ROAV of Antarctic krill paste was calculated with reference to Jiang (Jiang et al., 2022) to evaluate the contribution of different volatile flavour compounds to the overall flavour of recycled fermented shrimp paste and to define the main flavor compounds with new parameters. The flavour compound with the highest contribution to the sample was defined as ROAV_{stan} = 100, and the ROAV values of the other volatile flavour compounds were calculated as follows:

$$ROAV = \frac{C_a}{T_a} \times \frac{T_{stan}}{C_{stan}} \times 100\%$$

where,

T_{stan}: the threshold that contributes the most to the volatile flavour compounds;

T_a: the threshold corresponding to each volatile flavour compound;

C_{stan}: denotes the relative amount of the most volatile flavour compounds;

C_a: the relative amount of each volatile flavour compound.

2.6. Statistical analysis

All data were analysed using SPSS 26.0 (IBM, Inc., Armonk, NY, USA). GC-IMS data were analysed using the Laboratory Analysis Viewer, Reporter, Gallery Chart and Dynamic PCA Plug-in. The volatile flavour compounds in the samples were characterised through comparison with the instrument's built-in databases (GC \times IMS Library Search NIST database and IMS database). High-throughput sequencing was performed using QIIME2's classify-sklearn algorithm for each ASVs' feature sequence, and the pre-trained Naive Bayes classifier was used for species annotation using default parameters in QIIME2 software. The R language, ggtree, phyloseq and other R packages were used to plot the taxonomic hierarchy tree and GraPhlAn evolutionary tree diagrams, and Krona software was used for the interactive presentation of the taxonomic composition of the community to plot species composition. Correlation plots were drawn using Pearson. All experimental results were repeated three or more times with >3 parallel groups to ensure the accuracy of the experimental data.

3. Results and discussion

3.1. Results of physical and chemical indicators

3.1.1. Moisture content analysis

Changes in water content are shown in Fig. 1a: 20% salt concentration recirculating fermented shrimp paste Ca (20% salt concentration 5% old shrimp paste) decreased from 64.02% to 59.70%; Cb (20% salt concentration 10% old shrimp paste) decreased from 65.19% to 61.81%, 25% salt concentration recirculating fermented shrimp paste Cc (25% salt concentration 5% old shrimp paste) decreased from 63.94% to 59.13%, and Cd (25% salt concentration 10% old shrimp paste) decreased from 64.04% to 59.21%. At the end of fermentation, except for the Cb moisture content of 61.81%, which was higher than the shrimp paste industry standard of 60%, the moisture content of the remaining three groups of cyclic fermented shrimp paste were Ca, 59.70%; Cc, 59.13%; and Cd, 59.21% which were all in accordance with the standard. Different salinity has different effects on the water content of shrimp paste, the lower the salinity the higher the water content, this is in line with the findings of S M (Mulyani et al., 2021) et al. who fermented shrimp paste and L (Listyaningrum et al., 2022) et al. who made fish sauce.

3.1.2. TVB-N analysis

Volatile salt base nitrogen is an essential indicator for testing protein decomposition spoilage in animal food (Pellegriani et al., 2023). The green food standard limit set by China's Ministry of Agriculture is ≤ 150 mg/100 g. Cured raw animal aquatic products require volatile salt base nitrogen ≤ 25 mg/100 g. As shown in Fig. 1b, four groups of cyclic fermentation of Antarctic krill paste volatile salt base nitrogen content showed an increasing trend. The TVB-N values of the four groups of shrimp paste were 13.4, 15.7, 15.4, and 12.8 at 0 T in the early stage of fermentation, which increased to 16.4, 18.6, 16.2, and 15.4 at 14 T, respectively. Here, microbial growth and reproduction of proteins are decomposed into amino acids and should produce volatile alkaline nitrogenous substances, leading to the TVB-N content increase. At the end of fermentation, the Ca and Cb groups were higher than the Cc and Cd groups. The shrimp paste contents of the four groups at 28 T were 17.8, 21.4, 16.4 and 16.9. The TVB-N values of the Cb samples among the four samples were significantly higher than those of the other three groups, and the TVB-N values of the 10% additions were higher than those of the 5% additions at the end of the fermentation of the two salinities. The lower the salinity was, the higher the was volatile saline nitrogen content, indicating a higher level of spoilage of shrimp paste. This is in agreement with the changes in the volatile saline nitrogen content of fermented shrimp with different salinities studied by Lee (Lee et al., 2024) et al. It indicates that high salinity can inhibit the ability of

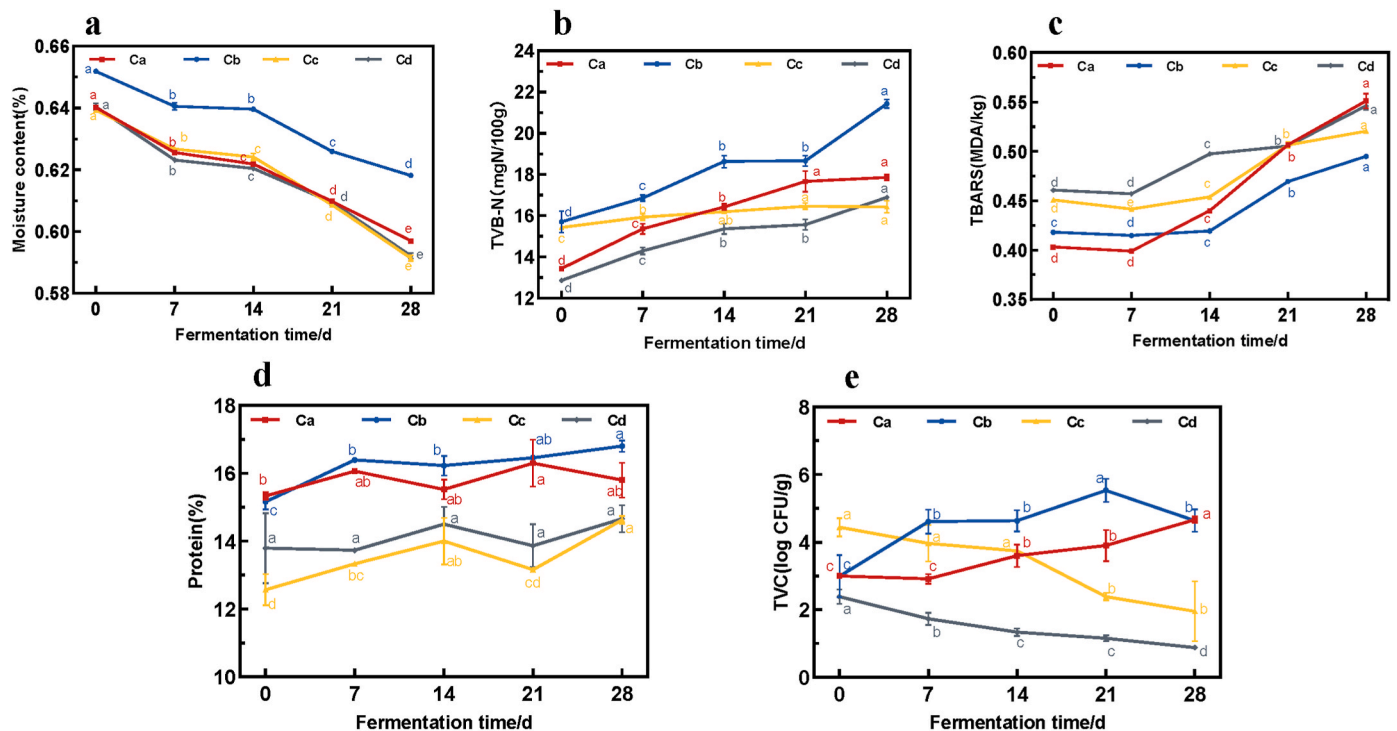


Fig. 1. Changes in moisture (a), TVB-N (b), TBARS (c), protein (d) and TVC (e) of different fermentation times of Antarctic krill sauce. Values with different letters are significantly different ($p < 0.05$).

microbial action to produce volatile nitrogen-containing substances during fermentation. The trend of TVB-N was correlated with the microbial activity during the fermentation of shrimp paste. The microorganisms multiplied rapidly during the logarithmic growth period, and the proteins in the shrimp paste were decomposed into amino acids, which were decarboxylated or deaminated to produce alkaline nitrogenous substances, such as volatile amines and ammonia, leading to a sharp increase in the TVB-N content.

3.1.3. TBARS analysis

Malondialdehyde content can reflect the degree of lipid oxidation and thus the freshness of the fermented products. Fig. 1c shows that the value of TBA increased during the fermentation process, indicating that the oxidation of shrimp paste fat was still accompanied by the fermentation process. The malondialdehyde contents of Cc and Cd were higher than those of Ca and Cb. The addition of old shrimp paste and the increase in salt content could accelerate the oxidation of fat, which led to a higher malondialdehyde content. The elevation of malondialdehyde content in the four groups of samples was more pronounced at 14–28 T, indicating that lipid oxidation in shrimp paste was more intense in the later stages of fermentation. The four groups of samples were between 0.4 and 0.56 mg/kg and the overall content changes were small, indicating that the degree of lipid oxidation is not high. All shrimp paste did not exceed the national standard GB10136-2015 'National Standard for Food Safety Animal Aquatic Products' China's marinated aquatic products (excluding herring, Spanish mackerel and salmon) ≤ 2.5 .

3.1.4. Protein analysis

The changes in the protein content of cyclically fermented Antarctic krill paste are shown in Fig. 1d. There were up and down fluctuations during the fermentation period but there was an increasing trend at the end of the fermentation period (28 days) compared with day 0. Ca shrimp paste increased from 15.3 to 15.8, Cb shrimp paste increased from 15.2 to 16.8, Cc shrimp paste increased from 12.6 to 14.6, and Cd shrimp paste increased from 13.8 to 14.7, whereas the protein content of Ca and Cb was higher than that of Cc and Cd. The change in the spatial

conformation of protein molecules at higher salt concentrations leads to weakened interactions between proteins and the phenomenon of aggregation and precipitation (Chen et al., 2024). Because of the raw material Antarctic krill has a high protein content (Li et al., 2020), all four fermented shrimp will be ≥ 10 g/100 g, in line with the Chinese aquatic industry regulations.

3.1.5. TVC analysis

Microorganisms are the main causes cause of spoilage of aquatic products. As shown in Fig. 1e, the total colony counts in Ca and Cb shrimp paste samples showed an increasing trend and those in Cc and Cd shrimp paste samples showed a decreasing trend. The total colony counts in Ca shrimp paste samples reached a maximum value in 4.7 at 28 T, and those in Cb shrimp paste reached a maximum value in 5.5 at 21 T, and then decreased to 4.6 at 28 T. The maximum values of Cc and Cd shrimp paste samples were 4.4 and 2.3 at 0 T in the early stage of fermentation, respectively, and gradually decreased to 1.9 and 0.9. The more old shrimp paste was added, the larger was the total number of colonies at 20% and 25% salinity. At 25% salinity, the more old shrimp paste was added, the smaller was the total colony size. Xie (Agustini and Amalia, 2017) et al. found that the total number of bacteria increased by decreasing the amount of salt added in shrimp paste of different salinity levels. Cc, Cd end of fermentation in line with the shrimp paste Chinese aquatic industry standard SC/T3602-2002 and shrimp paste domestic trade industry standard SB/T 10525-2009, microbial limit of ≤ 4 , Ca, Cb shrimp paste sample is slightly higher than the limits.

3.2. Analysis of high-throughput sequencing technologies

3.2.1. Species diversity analysis

The position of each ASV in the evolutionary tree, as well as the evolutionary distance between each other, was shown by plotting the evolutionary tree. As shown in Fig. 2a, the ASV feature sequences or their connected branches were coloured according to the genus classification level, and the Krona species composition map was plotted for the interactive presentation of the taxonomic composition of the

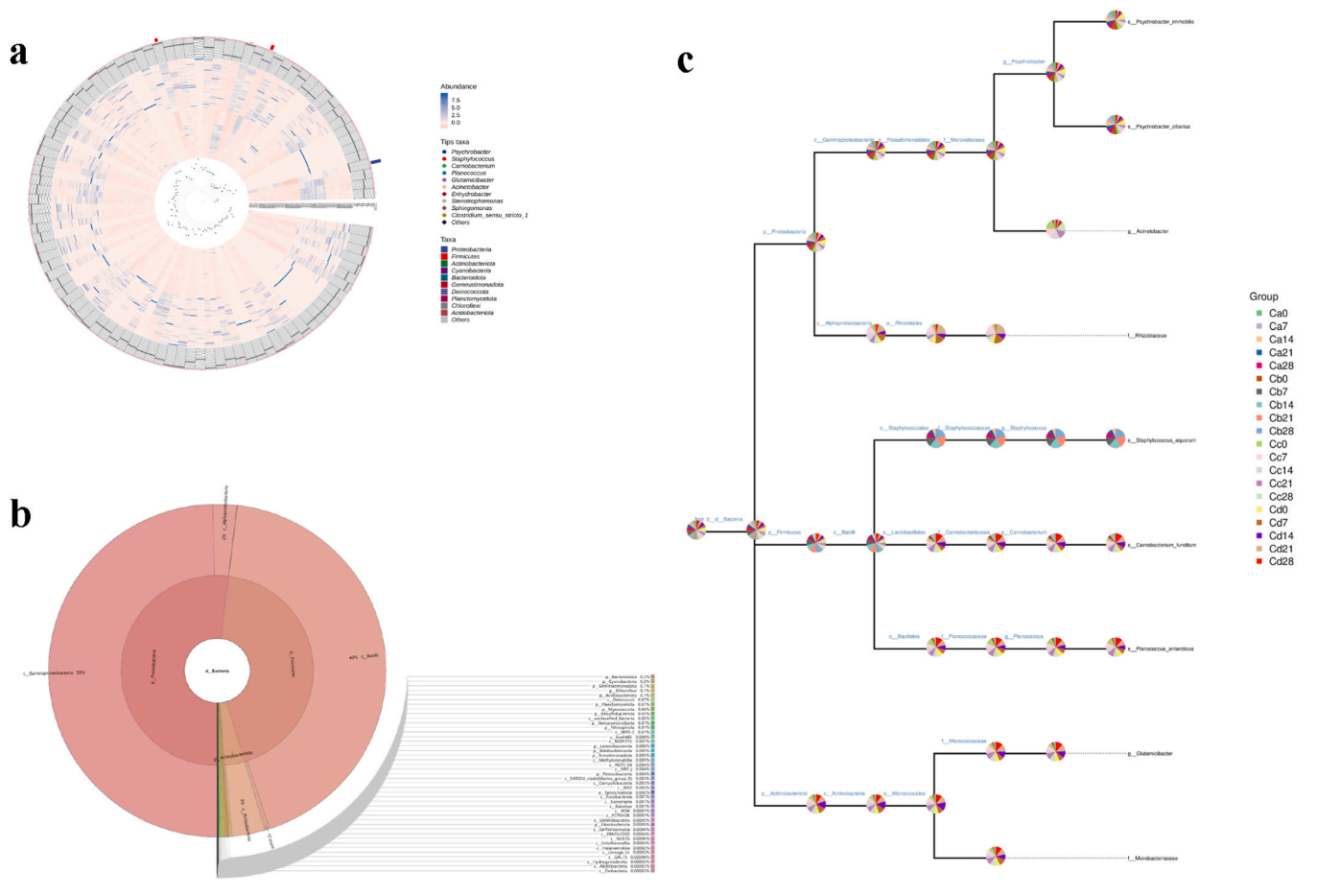


Fig. 2. Species composition of four shrimp pastes, graPhlAn phylogenetic tree diagram (a), Krona species composition map and (b) classification hierarchy tree diagram (c).

community. As shown in Fig. 2b not only allows for visual analysis of the taxonomic composition of samples, but focuses more on the interactive presentation of the data. Fig. 2c shows the species taxonomic hierarchy tree for abundance information, with pie charts at each branching node of the taxonomic hierarchy tree (threshold 0.5%). The proportion of the composition of that taxonomic unit across samples in the grouping is demonstrated. The larger the fan area was, the higher was the ratio abundance of that taxonomic unit in the corresponding sample.

3.2.2. Species composition analysis

To investigate the dynamics of the bacterial community during the fermentation process of circulating Antarctic krill paste, the same time points of 0, 7, 14, 21 and 28 days were selected as those of ordinary fermentation. It was observed whether the relative abundance levels of different bacteria in phylum and genus differed from those in normal fermentation during that period. Mean colony abundance values were plotted as a bar graph at the phylum and genus levels, with the area of each colony in the bar being the proportion of its abundance. All colony abundance values were summed to equal 1. As shown in Fig. 3a, the top 10 phyla were selected for analysis at the phylum level. Same as the first three dominant phyla of ordinary fermented old shrimp paste. These dominant phyla are common aquatic product fermentation dominant phyla. Yao et al. (2021) found that Proteobacteria and Firmicutes were the dominant phyla in the fermentation process of Beitang shrimp paste. The dominant bacterial phylum in normal fermented shrimp paste with 20% salt concentration was Proteobacteria at 0 T, with a proportion of 0.79%, and the dominant bacterial phylum was transformed into Firmicutes at the end of the fermentation at 28 T, with a proportion of

0.94%. When old shrimp paste was added to the circulating fermented shrimp paste also the number of Firmicutes was higher, which had the greatest effect on the circulating fermented shrimp paste. The dominant phylum in the Ca group of cyclic fermented shrimp paste was Proteobacteria, and there was an increase in Firmicutes at the late stage of fermentation. The proportion of Proteobacteria was 0.85% at 0 T, which decreased to 0.51% when reaching the end of fermentation at 28 T. The proportion of Firmicutes was 0.13% at 0 T, which increased to 0.49% at 28 T. By contrast, the Cb group shrimp paste was Proteobacteria at the beginning of fermentation. There was an uptick in Firmicutes in the later stages of fermentation. The proportion of Proteobacteria was 0.84% at 0 T and only 0.02% at 28 T; The proportion of Firmicutes was 0.14% at 0 T, which increased to 0.98% at 28 T. By comparing the dynamic changes of the dominant phylum during the fermentation process of Ca and Cb, it was shown that the addition of different contents of old shrimp paste had a significant effect on the dominant phylum, which resulted in the dominant phylum shifting from Proteobacteria to Firmicutes, which eventually became the absolute dominant phylum in the Cb group. The dominant phylum of normal fermentation with 25% salt concentration was always Proteobacteria. At day 0, the proportion of 0.69%, at day 28, the proportion of 0.75%. At the end of fermentation, Firmicutes also increased, from 0.14% to 0.19%. Cc, Cd two groups of shrimp paste dominant bacterial phylum in the early stage of fermentation is still for the Proteobacteria, respectively, accounted for 0.71%, 0.75%, with the gradual decline in the fermentation process, respectively, were 0.51%, 0.43%. Conversely, Firmicutes showed an increasing trend from 0.23% to 0.43% on day 0 in the Cc group and from 0.2% to 0.49% in the Cd group, surpassing the share of the Proteobacteria. Lv (Lv et al., 2020)

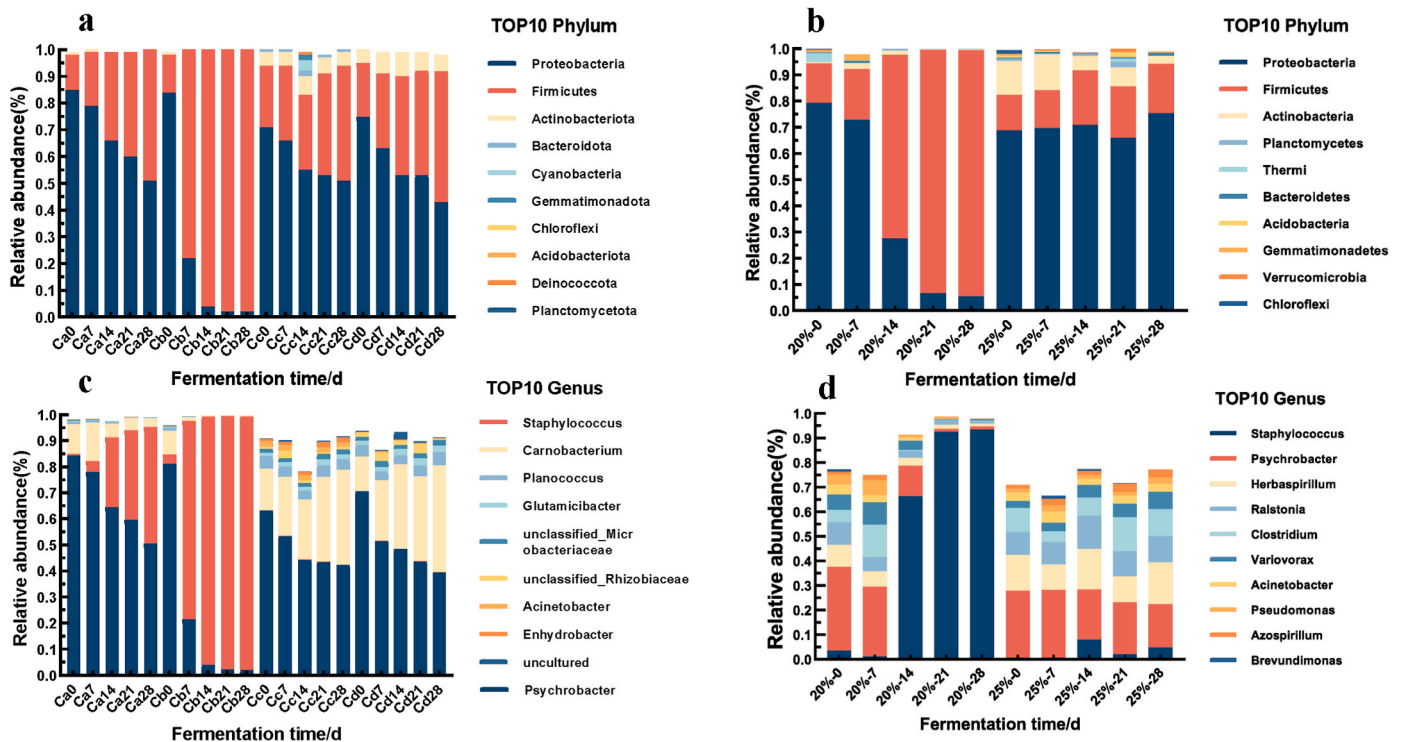


Fig. 3. Shows the Fengdu accumulation diagram of the first 10 bacteria at the phylum and genus levels: samples of cyclic fermented shrimp paste (a) and (c); ordinary fermentation samples (b) and (d).

et al. analysed the diversity of shrimp paste in Jinzhou and found that the main reasons that for Firmicutes became the dominant phylum are the raw materials and water environment. Comparison of the changes in the dominant bacterial phyla of Cc and Cd can be seen, with the increase in the amount of old shrimp paste added, the trend of the dominant bacterial phyla change is more significant.

In terms of genus level, the dominant genus on 0 T of normal fermentation at 20% salinity was Psychrobacter with 0.34% and Staphylococcus with only 0.03%. As the fermentation progressed Staphylococcus became the absolute dominant genus with a sudden increase to 0.66% on day 14, and then to 0.93% on day 28 at the end of the fermentation. The first four dominant genera in the circulating fermented shrimp paste were Psychrobacter, Staphylococcus, Carnobacterium and Planococcus. The dominant genus was Psychrobacter in the Ca group, which was highest at 0.84% on day 0 of fermentation, decreasing to 0.51% on day 28, a phenomenon that was also observed in the early stages of normal fermentation. Staphylococcus gradually increased as the fermentation progressed and became the main dominant genus at the end, from 0.007% on 0 T to a sudden increase to 0.27% on 14 T and 0.45% on 28 T. The dominant genus in the Cb group was also Psychrobacter, with a high level of 0.81, compared with Staphylococcus of 0.03%. Staphylococcus increased to 0.76% on 7 T and became the absolute dominant organism, growing to 0.97% by the end of the fermentation. Psychrobacter was the opposite of this change, becoming less and less as the fermentation progressed. The addition of old shrimp paste accelerated the formation of dominant genera by comparing Ca and Cb. Here, 25% salinity normal fermented shrimp paste was more abundant in dominant genera, which did not increase or decrease significantly during the fermentation period and did not form absolute dominant genera. The dominant genera in shrimp paste in the Cc and Cd groups were equally diverse, and the dominant genera in shrimp paste in the Cc group were Psychrobacter, Carnobacterium, and Planococcus. Psychrobacter accounted for a larger percentage, 0.63% at day 0, which decreased to 0.42% as fermentation progressed. There was an increase in Carnobacterium as fermentation progressed, from 0.16%

to 0.36%. The genera present from 0 T such as Glutamicibacter, Acinetobacter and Planococcus survived until the end of the fermentation, with Glutamicibacter and Enhydrobacter showing signs of elevation. The genera in circulating fermented shrimp paste were not quite the same as those in previous studies, which may be related to the raw materials and fermentation conditions. There were few Staphylococcus in the fermentation of group c (25% salinity) of normal fermented shrimp paste, suggesting that Staphylococcus is more likely to grow in the relatively low salinity environment in 20% versus 25% salinity. In the study by Shang (Sang et al., 2020) and others, the highest level of Staphylococcus was found in 10% salt concentration shrimp paste, which has amine-producing ability (Li et al., 2021) and brings unpleasant odour to shrimp paste. According to research, staphylococci are moderately halophilic, and the optimal salinity is generally 3%–15% (Daroonpant et al., 2019).

To further compare species composition differences between samples, a presentation of trends in the distribution of species abundance across samples was realised. Species composition analysis was performed using heat maps, where the abundance data of the top 50 genera in terms of average abundance were plotted. In Fig. 4, the red block denotes that the genus has higher abundance in that sample compared with other samples, and the blue block denotes that the genus has lower abundance in that sample compared with other samples. Staphylococcus, Pseudomonas, Psychrobacter and Psychromonadaceae in the Ca and Cb groups all have higher abundance, and the other species abundance is lower. It indicates that salt-intolerant microorganisms as dominant bacteria in low-salt environments can quickly survive to grab nutrients and dominate, leading to an increase in their abundance (Saqib et al., 2023). The Cc and Cd group shrimp paste was a little more abundant in terms of species composition heat map. Cc shrimp paste had higher abundance of Bacillus, Blastococcus, Craurococcus-Caldovatus, Deinococcus, Calothrix_PCC-6303, Hymenobacter at day 14, Chroococcidiopsis_SAG_2023, Geodermatophilus, Aliterella, and Truepera were all higher in abundance. Cd samples had higher abundance of Mycobacterium, Thermopolyspora, and Actinomadura at day 7, and

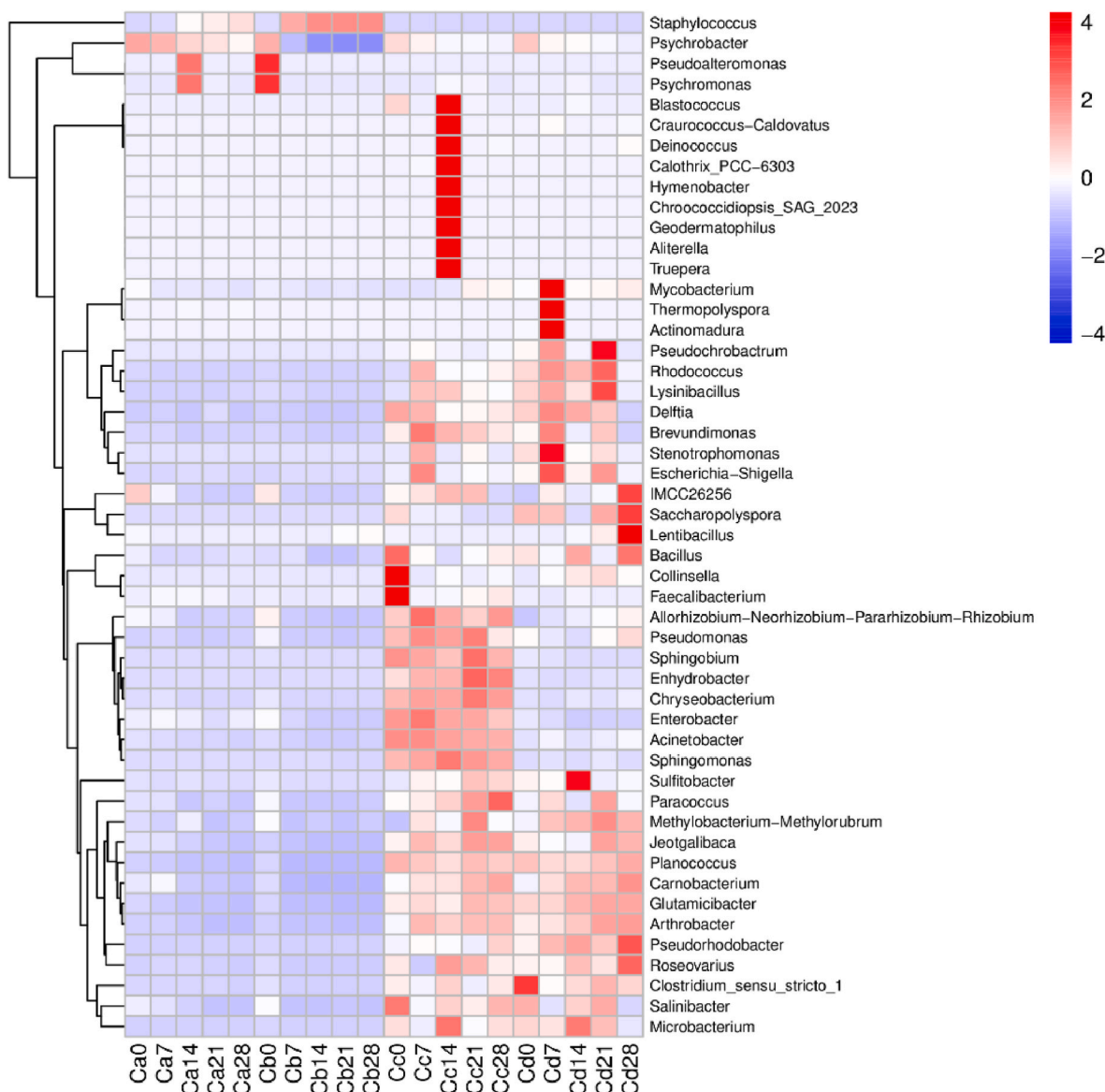


Fig. 4. Heat maps were plotted of the abundance data of the top 50 genera of different shrimp paste samples.

IMCC26256, Saccharopolyspora, and Lentibacillus at day 28. The above results indicated that fermentation at different salinities resulted in large differences in the composition and abundance of the flora in shrimp paste.

3.3. GC-IMS analysis

3.3.1. GC-IMS flavour composition spectra analysis

Fig. 5a shows the three-dimensional spectra obtained by GC-IMS for the detection of volatile flavour substances in different shrimp sauces. The three coordinate axes X-axis, Y-axis, and Z-axis denote migration time, retention time, and signal peak intensity, respectively. Each spot in the spectrogram represents a compound because some volatile substances have monomer, dimer, and multimer forms. Therefore, a compound may have one, two, or more spots. The content of a component can be determined using the shade of the colour, with darker colours indicating a higher content of that substance. The three-dimensional spectra represent, from left to right, samples of Ca28, Cb28, Cc28, and Cd28 shrimp paste.

Fig. 5b is a two-dimensional plan view. The horizontal coordinate represents the relative migration time (normalised), the red vertical line

at 1.0 is the RIP peak (reactive ion peak, normalised), and the vertical coordinate represents the retention time of the gas chromatogram (s). The points on both sides of the RIP peak represent various volatile organic compounds. The colours represent the peak intensities of the substances, from blue to red, with darker colours indicating greater peak intensities. It can be seen that there are differences in the VOCs in the four shrimp sauces.

Ca28 was selected as a reference and the spectra of the other samples were deduced from the reference to obtain the differences of the four shrimp sauces, as shown in Fig. 5c. If the contents of volatile organic compounds in the target sample and reference are the same, the background after deduction is white, red denotes that the concentration of the substance is higher than that of the target sample and blue colour represents that the concentration of the substance is lower than that of the target sample.

The volatile components in the shrimp paste samples were analysed by GC-IMS, and a total of 53 volatile components were detected, including monomers, dimers, trimers and 10 volatile components for characterisation, and 31 volatile components were determined. These included 11 aldehydes, 10 ketones, 14 alcohols, 3 esters, acetic acid, dimethyl sulphide, and 2,6-dimethylpyridine. Corresponds to Table 1

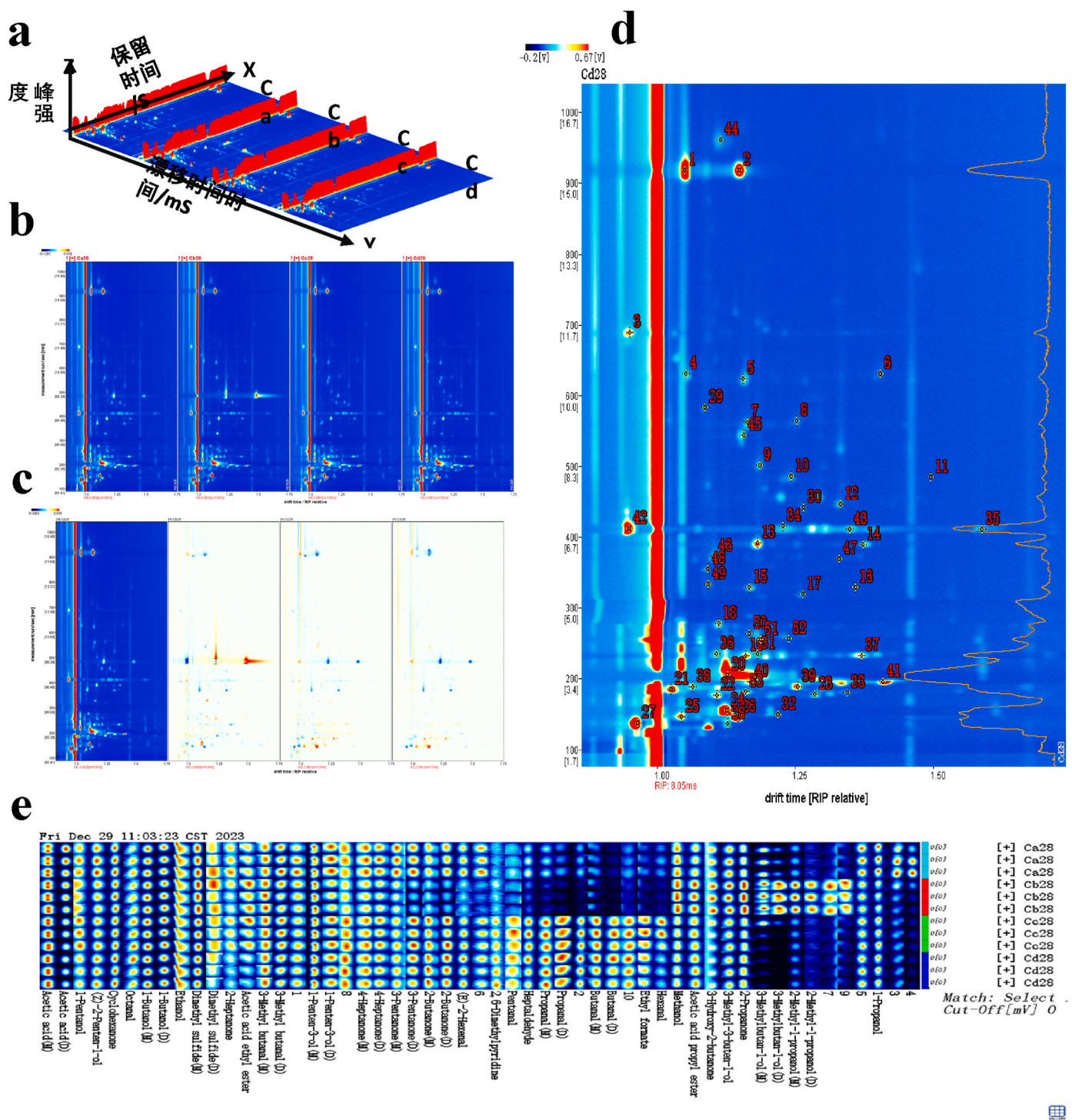


Fig. 5. Characteristics of volatile flavour compounds in different fermented Antarctic krill pastes. Three-dimensional atlas (a), two-dimensional atlas (b), comparison of differences (c), qualitative analysis of volatile compounds (d) and fingerprint (e).

and Fig. 5d.

3.3.2. Fingerprint analysis

The volatile substances in the circulating fermented shrimp paste at day 28 were further compared by generating fingerprints of all volatile substances using the aeries plot plug-in, as shown in Fig. 5e, and each sample was measured thrice in parallel, with the horizontal coordinate representing the volatile flavour substances in the samples, the vertical coordinate representing the amount of their volatile flavour substance content and the uncharacterised substances being represented by

numbers. The results of the comparative analysis of the volatile substances in the four shrimp sauces showed that the substances in the red box had the highest content in Ca28, including 1-propanol. Substances in the yellow box are most abundant in Cb28 and include isobutanol, 3-methylbutanol, acetone, 3-methyl-3-buten-1-ol, 3-hydroxy-2-butanone, propyl acetate and methanol. Substances in the green box are more abundant in Cc28 and Cd28, including hexanal, ethyl formate, butanal, propanal, heptanal, pentanal and 2,6-dimethylpyridine, with hexanal being slightly more abundant in Cc28 than in Cd28. From the fingerprints, there was a slight difference in flavour content between the Ca28

Table 1
Volatile flavour compounds and ROAV contribution values in different fermented Antarctic krill pastes.

Number	Compound	CAS	Formula	MW	Threshold (ug/ mg)	ROAV				Aroma description
						Ca	Cb	Cc	Cd	
1	Octanal	C124130	C8H16O	128.2	0.000587	23.47 ± 2.16 ^a	25.13 ± 2.78 ^a	22.78 ± 0.36 ^a	25.18 ± 1.57 ^a	fat, soap, lemon, green
2	(E)-2-Hexenal	C6728263	C6H10O	98.1	0.0885	0.23 ± 0.02 ^b	0.18 ± 0.01 ^c	0.21 ± 0.01 ^b	0.27 ± 0.01 ^a	green, leaf, apple
3	Heptaldehyde	C111717	C7H14O	114.2	0.0028	5.36 ± 0.11 ^b	3.41 ± 0.14 ^c	7.60 ± 0.10 ^a	7.47 ± 0.33 ^a	fat, citrus, rancid
4	Hexanal	C66251	C6H12O	100.2	0.005	1.39 ± 0.11 ^c	0.70 ± 0.07 ^d	2.57 ± 0.29 ^a	1.89 ± 0.19 ^b	grass, tallow, fat
5	Butanal(M)	C123728	C4H8O	72.1	0.002	10.79 ± 0.06 ^c	8.17 ± 0.43 ^d	23.74 ± 0.27 ^b	25.28 ± 0.42 ^a	pungent, green
6	Butanal(D)	C123728	C4H8O	72.1	0.002	2.12 ± 0.12 ^b	1.4 ± 0.17 ^c	10.69 ± 0.38 ^a	11.33 ± 0.57 ^a	pungent, green
7	Propanal(M)	C123386	C3H6O	58.1	0.0151	2.32 ± 0.16 ^c	1.25 ± 0.09 ^d	4.01 ± 0.05 ^b	4.48 ± 0.37 ^a	cocoa, earthy, ethery
8	Propanal(D)	C123386	C3H6O	58.1	0.0151	2.67 ± 0.09 ^c	0.91 ± 0.11 ^d	8.06 ± 0.20 ^b	9.17 ± 0.16 ^a	cocoa, earthy, ethery
9	Pentanal	C110623	C5H10O	86.1	0.012	0.55 ± 0.03 ^c	0.6 ± 0.08 ^{bc}	0.7 ± 0.06 ^b	0.92 ± 0.07 ^a	almond, malt, pungent
10	3-Methyl butanal(M)	C590863	C5H10O	86.1	0.0011	35.22 ± 2.98 ^a	38.99 ± 4.26 ^a	34.72 ± 0.69 ^a	40.53 ± 4.00 ^a	malt
11	3-Methyl butanal(D)	C590863	C5H10O	86.1	0.0011	100.00	100.00	100.00	100.00	malt
12	3-Hydroxy-2-butanone	C513860	C4H8O2	88.1	0.014	4.02 ± 0.41 ^b	4.84 ± 0.61 ^a	3.51 ± 0.22 ^b	3.89 ± 0.24 ^b	boiled potatoes
13	Cyclohexanone	C108941	C6H10O	98.1	0.28	0.14 ± 0.01 ^{ab}	0.14 ± 0.01 ^{ab}	0.12 ± 0.01 ^b	0.15 ± 0.01 ^a	earth
14	2-Propanone	C67641	C3H6O	58.1	0.832	0.38 ± 0.03 ^c	0.64 ± 0.07 ^a	0.40 ± 0.02 ^c	0.51 ± 0.02 ^b	mint, sweet
15	2-Heptanone	C110430	C7H14O	114.2	0.14	0.05 ± 0.01 ^a	0.05 ± 0.00 ^a	0.05 ± 0.00 ^a	0.05 ± 0.01 ^a	soap
16	4-Heptanone(M)	C123193	C7H14O	114.2	0.04	4.17 ± 0.21 ^b	4.20 ± 0.43 ^b	4.14 ± 0.21 ^b	4.80 ± 0.29 ^a	
17	4-Heptanone(D)	C123193	C7H14O	114.2	0.04	3.26 ± 0.42 ^a	3.02 ± 2.08 ^a	3.03 ± 0.22 ^a	3.20 ± 0.20 ^a	
18	3-Pentanone(M)	C96220	C5H10O	86.1	0.04	0.87 ± 0.12 ^a	0.75 ± 0.07 ^{ab}	0.69 ± 0.05 ^b	0.84 ± 0.07 ^a	ether
19	3-Pentanone(D)	C96220	C5H10O	86.1	0.04	1.34 ± 0.05 ^a	0.87 ± 0.04 ^b	1.42 ± 0.10 ^a	1.40 ± 0.02 ^a	ether
20	2-Butanone(M)	C78933	C4H8O	72.1	35.4002	< 0.1	< 0.1	< 0.1	< 0.1	fragrant, fruit, pleasant
21	2-Butanone(D)	C78933	C4H8O	72.1	35.4002	< 0.1	< 0.1	< 0.1	< 0.1	fragrant, fruit, pleasant
22	(Z)-2-Penten-1-ol	C1576950	C5H10O	86.1	0.72	< 0.1	< 0.1	< 0.1	< 0.1	green, plastic, rubber
23	3-Methyl-3-buten-1-ol	C763326	C5H10O	86.1	0.547125	< 0.1	< 0.1	< 0.1	< 0.1	
24	1-Pentanol	C71410	C5H12O	88.1	0.1502	< 0.1	< 0.1	< 0.1	< 0.1	turnips, vegetables
25	3-Methylbutan-1-ol (M)	C123513	C5H12O	88.1	0.004	37.82 ± 2.78 ^a	81.77 ± 9.57 ^b	19.75 ± 14.57 ^c	7.77 ± 0.24 ^c	whiskey, malt, burnt
26	3-Methylbutan-1-ol (D)	C123513	C5H12O	88.1	0.004	15.44 ± 1.23 ^b	97.65 ± 10.60 ^a	6.33 ± 6.40 ^{bc}	2.47 ± 0.56 ^c	whiskey, malt, burnt
27	1-Butanol(M)	C71363	C4H10O	74.1	0.4592	0.19 ± 0.02 ^a	0.22 ± 0.03 ^a	0.20 ± 0.01 ^a	0.22 ± 0.01 ^a	medicine, fruit
28	1-Butanol(D)	C71363	C4H10O	74.1	0.4592	< 0.1	< 0.1	< 0.1	< 0.1	medicine, fruit
29	2-Methyl-1-propanol (M)	C78831	C4H10O	74.1	6.5052	< 0.1	< 0.1	< 0.1	< 0.1	alcohol, fruity, banana
30	2-Methyl-1-propanol (D)	C78831	C4H10O	74.1	6.502	< 0.1	< 0.1	< 0.1	< 0.1	alcohol, fruity, banana
31	1-Propanol	C71238	C3H8O	60.1	8.5056	< 0.1	< 0.1	< 0.1	< 0.1	alcohol, pungent
32	Ethanol	C64175	C2H6O	46.1	950	< 0.1	< 0.1	< 0.1	< 0.1	sweet
33	Methanol	C67561	CH4O	32.0	800	< 0.1	< 0.1	< 0.1	< 0.1	
34	1-Penten-3-ol(M)	C616251	C5H10O	86.1	0.3581	0.66 ± 0.05 ^a	0.70 ± 0.07 ^a	0.64 ± 0.03 ^a	0.70 ± 0.04 ^a	wine, leather
35	1-Penten-3-ol(D)	C616251	C5H10O	86.1	0.3581	0.10 ± 0.01 ^b	0.08 ± 0.01 ^a	0.09 ± 0.01 ^b	0.09 ± 0.00 ^a	wine, leather
36	Acetic acid propyl ester	C109604	C5H10O2	102.1	2	< 0.1	< 0.1	< 0.1	< 0.1	
37	Ethyl formate	C109944	C3H6O2	74.1	8.9	< 0.1	< 0.1	< 0.1	< 0.1	sweet green floral
38	Acetic acid ethyl ester	C141786	C4H8O2	88.1	0.005	1.81 ± 0.06 ^b	2.28 ± 0.12 ^c	1.92 ± 0.30 ^b	1.50 ± 0.04 ^a	potatoes, roasted nuts
39	Acetic acid(M)	C64197	C2H4O2	60.1	99	< 0.1	< 0.1	< 0.1	< 0.1	sour; vinegar
40	Acetic acid(D)	C64197	C2H4O2	60.1	99	< 0.1	< 0.1	< 0.1	< 0.1	sour; vinegar
41	Dimethyl sulfide(M)	C75183	C2H6S	62.1	0.0012	> 100	> 100	> 100	> 100	ethereal, permeating; disagreeable;
42	Dimethyl sulfide(D)	C75183	C2H6S	62.1	0.0012	> 100	> 100	> 100	> 100	ethereal, permeating; disagreeable;
43	2,6-Dimethylpyridine	C108485	C7H9N	107.2	0.003	1.31 ± 0.26 ^c	1.49 ± 0.06 ^c	1.90 ± 0.16 ^b	2.29 ± 0.13 ^a	soap

and Cb28 shrimp paste samples, and the flavour contents in Cc28 and Cd28 shrimp paste samples were similar.

3.4. ROAV analysis

Volatile flavour substances in circulating fermented Antarctic krill paste samples were quantified by peak volume normalisation. 3-Methylbutyraldehyde, which has a low flavour threshold and high relative content, was selected as the key flavour substance. The ROAV values of other compounds were calculated, shown in Table 1. Aldehydes contributed more to the overall flavour of circulating fermented shrimp paste because of their lower threshold. They showed fruity, grassy and other odour characteristics. They are mainly produced by the oxidative degradation of unsaturated fatty acids and play an essential role in the formation of the characteristic flavour aspects of foods. The 11 aldehydes that have a high impact on flavour include the following: octanal, n-butanal and 3-methylbutanal. According to Chen et al. (2023), C6–C10 straight-chain saturated aldehydes such as octanal have a vegetative odour such as grass or mushroom at lower concentrations, and at high concentrations they produce undesirable odours such as fishy or putrid smells. The oxidation of fatty acids promotes the production of volatile substances such as aldehydes (Mengyue et al., 2021), acids, esters and ketones, including octanal. Four shrimp sauces with octanal's ROAV exceeded 20, which had a certain effect on the overall flavour of shrimp sauces, and were presumed to be the source substances of fishy smell in shrimp sauces. 3-Methylbutanal has a malty aroma and is mainly derived from the Strecker reaction of amino acids. It serves as a key flavor substance because of its low threshold value and high content, bringing malt aroma to shrimp paste. It is worth mentioning that the ROAV values of heptanal and propanal in Cb28 samples were lower than those of the other three groups. Heptanal has a fatty, citrusy and rancid odour; and propanal has a cocoa, earthy and etheric odour. These two substances bring bad odours to shrimp paste.

Ketones tend to have higher thresholds than aldehydes with the same number of carbon atoms, accompanied by floral and fruity aromas (Xiao et al., 2023), which mainly originate from the lipolysis and oxidation of alcohols. Because of their higher sensory thresholds, their contribution to the odour of shrimp paste is relatively small. It has been proved that ketones have an enhancing effect on fishy substances and their presence can enhance or change the fishy odour (Zheng et al., 2022). A total of 10 ketones were detected in circulating fermented Antarctic krill paste. Among them, compounds with ROAV greater than 0.1 included 3-hydroxy-2-butanone, cyclohexanone, acetone, 4-heptanone and 3-pentanone. Four Antarctic krill sauces with high values of 3-hydroxy-2-butanone ROAV had a boiled potato aroma, which acted as a modifier of the overall odour of the shrimp sauces. Lei et al. (Lei et al., 2023) examined the volatile flavour components of frozen pairs of fermented Mandarin fish by GC-IMS. It was found that 3-hydroxy-2-butanone was detected in the samples, which brought creamy flavour to it and bestow its special aroma.

Alcohols are mainly derived from the oxidation of fats, reduction of amino acids and metabolism of carbohydrates. Unsaturated alcohols have lower thresholds and contribute more to the flavour by having a floral, mushroomy, earthy or rancid taste. Fourteen alcohols were detected in the four shrimp sauces, but some of the compounds had ROAVs of less than 0.1 due to their higher thresholds or lower levels. These included n-butanol(D)2-methyl-1-propanol, n-propanol, methanol, ethanol, and 1-penten-3-ol. Isoamyl alcohol has higher ROAV values in Ca28 and Cb28 shrimp paste, which has a whisky, malt, burned flavour. Isoamyl alcohol is a branched-chain alcohol produced from Embden-meyerhof pathway, EMP and amino acids via the Ehrlich pathway. Decarboxylase and ethanol dehydrogenase are involved in the Ehrlich pathway (Lei et al., 2023).

Three esters and one acid (propyl acetate, ethyl formate, ethyl acetate and acetic acid) were also detected. Esters are mainly formed by the esterification of acids and alcohols (VAN et al., 2012; Grabež et al.,

2019), can also be formed by other pathways, such as protein degradation, endogenous enzyme action and fat oxidation, and present a fruity aroma and sweetness, but can only modify the flavour of shrimp paste because of its high threshold. It was shown that acetic acid production is mainly due to the metabolic activities of *Bacillus thermocellulosus* and *Lactobacillus lactis*, which has a pungent odour and acts as a modifier of the theme flavour because of its high threshold (Liu et al., 2024).

Dimethyl sulphide is a volatile sulphur-containing compound produced by microbial decomposition and metabolism, which is considered to be one of the main sources of the fishy odour of aquatic raw materials (Cecchi et al., 2018). Cheng (Cheng et al., 2023) et al. used gas chromatography-mass spectrometry (GC-MS) to characterise key volatile compounds during the cold storage of tilapia fillets and found that dimethyl sulphide had a role in their flavour presentation. With the prolongation of time, dimethyl sulphide has a diffuse onion flavour, rotten egg and other irritating odours. The dimethyl sulphide ROAV values of all four shrimp sauces were >100, bringing bad odour to the shrimp sauces. It was not found in the normal fermentation and was found in the circulating fermented paste, suggesting that the addition of old shrimp paste to promote fermentation can accelerate the fermentation rate and affect the main volatile flavours.

Nitrogen-containing heterocyclic compounds such as piperidine, pyrazine and pyridine are Maillard reaction-derived compounds (Nieva-Echevarría et al., 2018), and pyridine can also be produced by amino acid degradation. The detected 2,6-dimethylpyridine presents a soapy flavour and can have a modifying effect on shrimp paste because of its low threshold value.

3.5. Flavor and genus correlation analysis

Pearson's correlation coefficient was used to analyse the correlation between the main flavour components of circulating fermented shrimp paste at the completion of fermentation and top15 bacteria. With the numbers in Fig. 6 indicating the value of their correlation, with red representing a positive correlation and blue a negative correlation, and the darker the colour the greater the degree of their correlation. Some aldehydes, alcohols and sulphur ethers, such as trimethylbutyraldehyde, isoamyl alcohol and dimethyl sulphide, contributed more to the shrimp paste. Among them, 3-methylbutanal was positively correlated with *Planococcus*, *Carnobacterium*, unclassified *Microbacteriaceae*, and *Arthrobacter*, and negatively correlated with *Staphylococcus*. Although isoamyl alcohol was positively correlated with *staphylococci*, it was negatively correlated with *Glutamicibacter*, *Planococcus*, *Carnobacterium*, unclassified *Microbacteriaceae*, *Cryptophilus*, *Psychrobacter*, uncultured; uncultured was negatively correlated. Dimethyl sulphide (M) was positively correlated with *Staphylococcus* and negatively correlated with the same genus as isoamyl alcohol. It is evident that *Staphylococcus* has an effect on the flavor of shrimp paste. Thus, it can be seen that the main volatile compounds in Antarctic krill sauce are correlated with *Staphylococcus*. Wang et al. (2021) found that *Staphylococcus* plays a key role in the formation of the flavour of Jinhua ham. *Staphylococci* contribute to the flavour formation of shrimp paste because of their high lipase activity and protease activity (Xu et al., 2020).

4. Conclusions

Different salinities and different additions of old shrimp paste had essential effects on the physicochemical properties, flavour, and bacterial community composition and abundance of circulating fermented shrimp paste. Physical and chemical indicators, except moisture content Cb, colony total Ca, Cb slightly higher than the limit, the rest are in line with the standard. Using GC-IMS to detect volatile flavour substances at the end of the fermentation of the four shrimp sauces, 31 volatile components were determined. These contained 11 aldehydes, 10 ketones, 14 alcohols, 3 esters, acetic acid, dimethyl sulphide and 2,6-

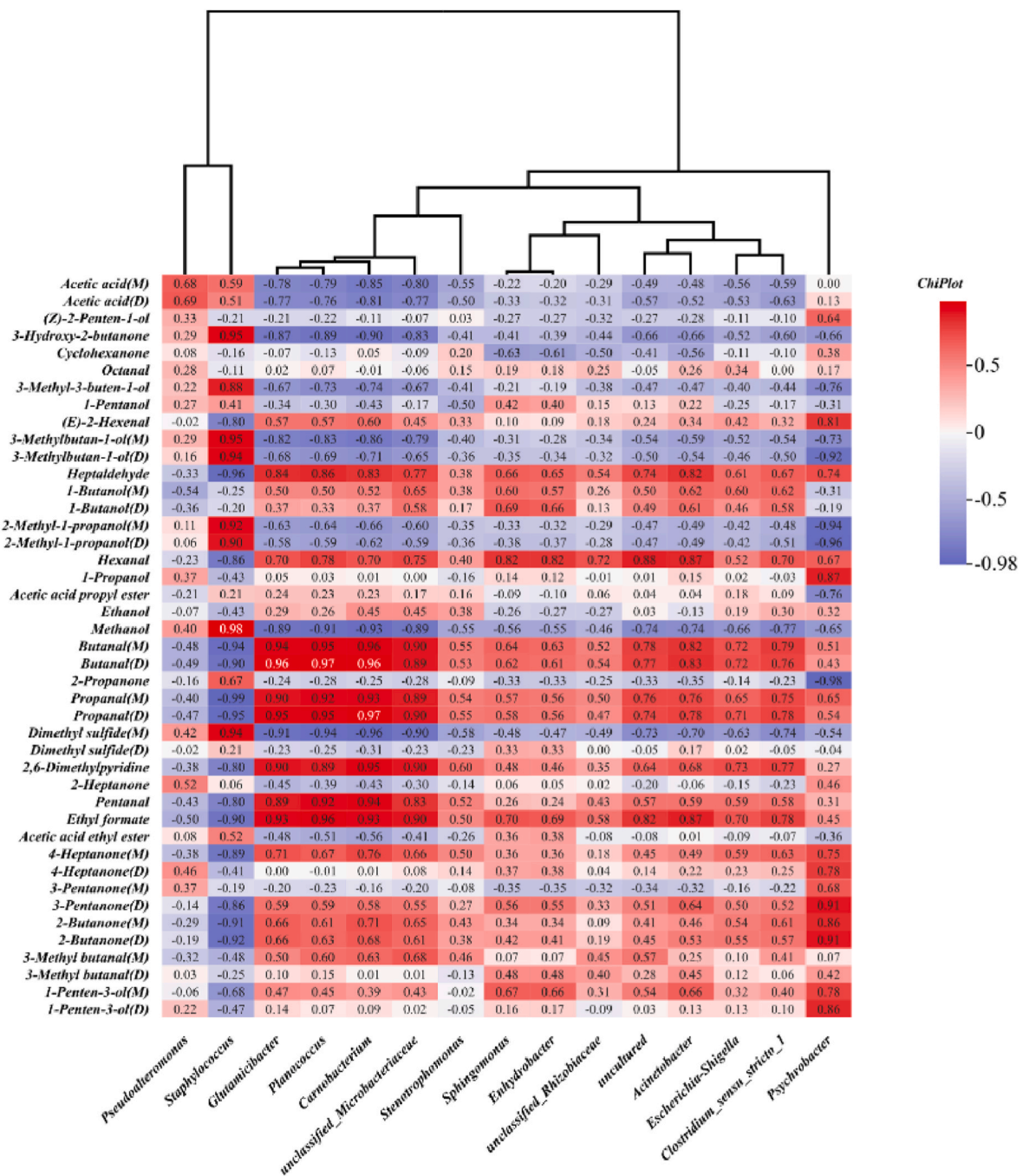


Fig. 6. Heat map of the top 15 correlations of all volatile compounds and colonies at the genus level of Antarctic krill paste samples after 28 days of cyclic fermentation.

dimethylpyridine. The substances that contributed more to the flavoUr of circulating fermented paste were octanal, 3-methylbutanal and dimethyl sulphide. Using high-throughput sequencing technology to observe the microbial community dynamics during the fermentation of shrimp paste, it was found that Staphylococcus was the dominant genus at 20% salinity, and the more the old shrimp paste was added, the faster it became the dominant genus. The abundance was very low at 25% salinity. In the heat map of the correlation between colonies and volatile compounds it can be seen that staphylococci are correlated with compounds that have a high impact on the flavour of shrimp paste. Staphylococcus plays an essential role in the cyclic fermentation of Antarctic krill sauces.

CRedit authorship contribution statement

Pengfei Jiang: Investigation, Methodology, Writing – original draft. **Yang Liu:** Investigation. **Jin-ye Yang:** Investigation. **Wen-qiang Cai:** Writing – review & editing. **Cai-yan Jiang:** Investigation. **Jia-bo Hang:** Investigation. **Xiao-qing Miao:** Writing – review & editing, Supervision. **Na Sun:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing.

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

The data that has been used is confidential.

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