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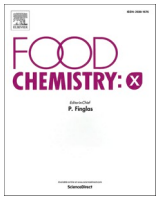

Effects of different thermal processing methods on physicochemical properties, microstructure, nutritional quality and volatile flavor compounds of silver carp bone soup

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ABSTRACT

In this study, silver carp by-products were used as raw materials to prepare silver carp bone soup under four processing conditions: CF, CY, GF, and GY. The results showed that the content of soluble protein and FAA content in fish soup increased significantly after high-pressure cooking, decreased the particle size of micro-nanoparticles of protein and fat, and rendered the system relatively more stable. It was also found that the GY samples had a significantly higher variety and abundance of flavor compounds than the other three groups of samples. The results of the correlation network model showed that m-phthalaldehyde and phenylacetaldehyde were significantly correlated with most of the FFAs, and Met, Ile and Arg were significantly positively correlated with most of the flavor compounds. In conclusion, the nutritional quality and flavor of silver carp were relatively improved after high-pressure cooking (pressure 70 kPa, cooking for 45 min).

1. Introduction

Silver carp is a freshwater fish native to the river basins of East Asia and is one of the four major freshwater fishes in China, widely distributed in all major water systems of China. Its market demand is high, trade is active, and it has an important position in world aquaculture and trade (Jawdhari et al., 2022). Silver carp is mainly used to make various types of processed products, such as fish balls, fish cakes, and surimi (Kan et al., 2024), and by-products such as heads, skins, scales, guts, and skeletons are produced during processing, which account for about 50 % of the mass of the whole fish (Tan et al., 2019). The full utilization of silver carp by-products can not only increase economic income, but also promote the development of the corresponding industries and enhance the efficiency of resource utilization. However, most of the by-products of silver carp processing are used to produce feed with low added value or directly discarded, which causes serious waste of resources and environmental pollution, and is not conducive to the sustainable development of silver carp processing industry. Therefore, the comprehensive utilization and improvement of the added value has become an important issue for silver carp processing.

Fish skeleton contains more fish meat and the bones contain collagen and various minerals (Toppe et al., 2007), which have high nutritional and developmental value. Currently, domestic and international research on the processing and utilization of fish bones focuses on value-added bioactive compounds, mineral supplements, and so on (Hemker et al., 2020), and in terms of food consumption, fish bones are often prepared into a variety of dishes such as pan-fried fish bones, fish bone soup, and so on. The Chinese Materia Medica mentions that silver carp contains proteins, fats, carbohydrates, calcium, phosphorus, iron, and some amines, and is popular among the general population (Jiang et al., 2014). Traditional fish soup is made by frying fish in oil and then adding hot water for cooking; shallots, ginger, and cooking wine are also added to improve the fishy flavor. Different thermal processing methods have different effects on the release of aroma and the dissolution of flavor-presenting substances in bone soup, and it has been found that high-pressure cooking can better preserve the original flavor of bone soup compared to atmospheric pressure cooking (Ma et al., 2023). In addition, the pre-processing method of fish stock simmering may also affect the nutritional and flavor release of fish stocks (Wu et al., 2023). However, little research has been conducted on the microstructure of

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silver carp bone soup and its nutritional flavor using different thermal processing methods.

Therefore, in this study, the flavor effects of four different thermal processing methods on silver carp soup were evaluated by electronic nose (*E*-nose), headspace-gas chromatography-ion mobility mass spectrometry (HS-GC-IMS), and headspace solid-phase microextraction-gas chromatography–mass spectrometry (HS-SPME-GC–MS), and the microstructures and free amino acids, fatty acids, etc. were analyzed. In addition, we constructed a network model of free amino acids - flavor compounds - free fatty acids to further explore the regulatory mechanism of flavor formation in silver carp soup. To provide theoretical foundations for a comprehensive understanding of the nutritional value of silver carp bone and to further promote its development and utilization.

2. Materials and methods

2.1. Preparation of silver carp bone soup

Twelve fresh silver carp (2.5 ± 0.5 kg) were purchased from a local aquatic market in Nanchang, Jiangxi Province, China, and these fish were transported to the laboratory (30 min by car) in oxygenated bags and water alive. The whole experiment was approved by the Commitment for Ethics and Welfare of Laboratory Animals of Jiangxi Normal University (Approval No. 20231018-A3), and it complies with relevant international and national laws and regulations. Silver carp were stunned by striking their head, then gutted, the middle spine was removed, cut into 5 cm × 5 cm × 1.5 cm pieces, and rinsed with running tap water.

Add 20 g of edible peanut oil, followed by 200 g of pre-treated silver carp bone pieces, 1.0 L of hot water, 18 g of ginger, and 45 g of scallion into a pot, and then the fish broth was boiled for 45 min to make the final volume of fish broth of 1.0 L. The silver carp bones, which had not been processed by frying, were directly put into an atmospheric pressure pot (YTD-6B Ceramic Soup Pot, Supor Home Appliances Manufacturing Co., Ltd., Zhejiang, China), and the resulting fish soup was defined as atmospheric pressure non-frying silver carp soup (CF). The silver carp bones were put into the hot oil and turned from time to time to ensure that they were evenly heated, and then after frying to golden brown on both sides, the fish stock was then cooked at atmospheric pressure in the above YTD-6B ceramic soup pot, and the resulting fish stock was named atmospheric frying silver carp soup (CY). The non-frying silver carp bone pieces were directly put into a pressure cooker (SY-80YC9510C Electric Pressure Cooker; Supor Home Appliances Manufacturing Co., Ltd., Zhejiang, China) for cooking, and the resulting fish broth was defined as high-pressure non-frying silver carp soup (GF). After frying the silver carp bone pieces until golden brown on both sides, followed by cooking the fish stock in the pressure cooker described above, the resulting fish stock was defined as high-pressure fried silver carp soup (GY).

2.2. Determination of color

After fish soup was cooled to room temperature, it was put into transparent glass bottles, and the color of fish soup was measured using a colorimeter (CR-400, Colorimeter Konica Minolta Investment Co., Ltd., China), and the results were expressed by L*, a* and b*. L* indicated the brightness, and the larger the value of L* indicated that the brighter the color was, a* indicated the value of red and green, and + a* indicated red, and -a* indicated green, b* indicated the value of yellow and blue, and + b* indicated yellow, and -b* indicated blue.

2.3. Determination of soluble protein

The soluble protein was determined by Bradford's method, 0.1 mL of fish bone soup was taken in a test tube, 5 mL of coomassie brilliant blue G250 (Solarbio Science & Technology Co., Ltd., Beijing, China) was added, and the mixture was homogenized and then allowed to stand at room temperature for 5 min. The absorbance was determined at 595 nm by UV spectrophotometer (Suzhou Shimadzu Instruments Co., Ltd.).

2.4. Determination of particle size and zeta potential

0.5 mL of sample was diluted to 10 mL with ultrapure water and 1.0 mL of sample solution was taken into the cuvette. Zetasizer Nano ZSP (Malvern Instruments Ltd., UK) was used to determine the particle size and zeta potential of silver carp bone soup.

2.5. Microstructure determination

The method of Wu et al. (Wu et al., 2024) was referenced with slight modifications. 0.5 mL of fish soup was taken and 0.5 mL of phosphate buffer (0.01 mol/L, pH = 7.0) was added, followed by 20.0 μL of 0.1 % Nile Red and 0.1 % Nile Blue A staining solution, which was mixed well and then allowed to stand in the dark for 30 min for the soup to be fully stained. Then 2 μL of stained fish soup was taken on a slide, covered the slide and left at room temperature for 20 min, dried and observed under a fluorescence biomicroscope (DMi8, Leica Instruments Co., Ltd., Wetzlar, Hesse, Germany).

2.6. Determination of free amino acids

Take 3 mL of fish soup samples in a centrifuge tube, centrifuge at 4 °C, 8000 *g* for 5 min, take 2.0 mL of supernatant in a centrifuge tube and add 2.0 mL of 4 % sulfosalicylic acid, mix well, and let it stand for 15 min. After centrifugation at 8000*g* for 5 min at 4 °C, the supernatant was diluted and passed through a 0.22 μm filter membrane, injected into the injection bottle, and then analyzed by an automatic amino acid analyzer (Sykam S—433D, Germany). The analytical conditions were as follows: column size of 4.6 mm × 150 mm, column temperature of 57 °C, buffer flow rate of 0.4 mL/min, reaction solution (ninhydrin reagent) flow rate of 0.35 mL/min, detection wavelength of 570 nm, injection volume of 50 μL.

2.7. Determination of free fatty acids

Take 1 mL of sample in a centrifuge tube, add 100 μL of internal standard nineteen alkanoic acid (C19:0,10 mg/mL) and 2 mL of chloroform-methanol (2:1,*V*/V), vortex to mix well and leave it to stand for 1 h. Then centrifuge the sample at 8000 *g* for 5 min and collect the lower layer of lipid solution. Finally, the organic reagent was evaporated with nitrogen to obtain the concentrated lipid. Then 500 μL of 4 % sodium hydroxide-methanol solution was added, vortexed for 30 s, 6 mL of n-hexane was added, and a water bath at 60 °C for 30 min was used, and the supernatant was taken through a 0.22 μm organic phase filter membrane, and then stored in a gas phase injection bottle for spare use. The fatty acid methyl esters were analyzed with the help of GC–MS. The specific conditions were as follows: nitrogen as carrier gas, inlet temperature of 280 °C, injection volume of 1 μL, split ratio of 1:2, flow rate of 1 mL/min; heating program: the starting temperature of 100 °C, held for 1 min; to 200 °C at a rate of 5 °C/min; and then to 230 °C at a rate of 1 °C/min and held for 10 min, the inlet and detector temperatures were set to 250 °C and 300 °C, respectively.

2.8. E-nose analysis

The flavor profile of the samples was measured using a PEN3 E-nose. A 2.0 mL sample was taken in a 50 mL centrifuge tube, sealed with plastic wrap, heated in a 50 °C water bath for 30 min, cooled to room temperature, and then inserted into an electronic nose probe for measurement. The parameters were set as follows: sample interval 1.0 s, flush time 120 s, The parameters were set as follows: sample interval 1.0 s, flush time 120 s, measurement time 140 s, chamber flow 600 mL/min,

initial injection flow 600 mL/min.

2.9. GC-IMS analysis

Volatile flavor compounds in silver carp bone soup under different thermal processing treatments were detected using a FlavourSpec® flavor analyzer. A 2.0 mL sample was taken in a 20 mL headspace bottle, sealed, and incubated at 60 °C, 500 rpm for 15 min. 500 μL of the sample was injected into the injection well using an 85 °C injection needle.

GC conditions: an Agilent-DB-WAX capillary column (30 m × 0.25

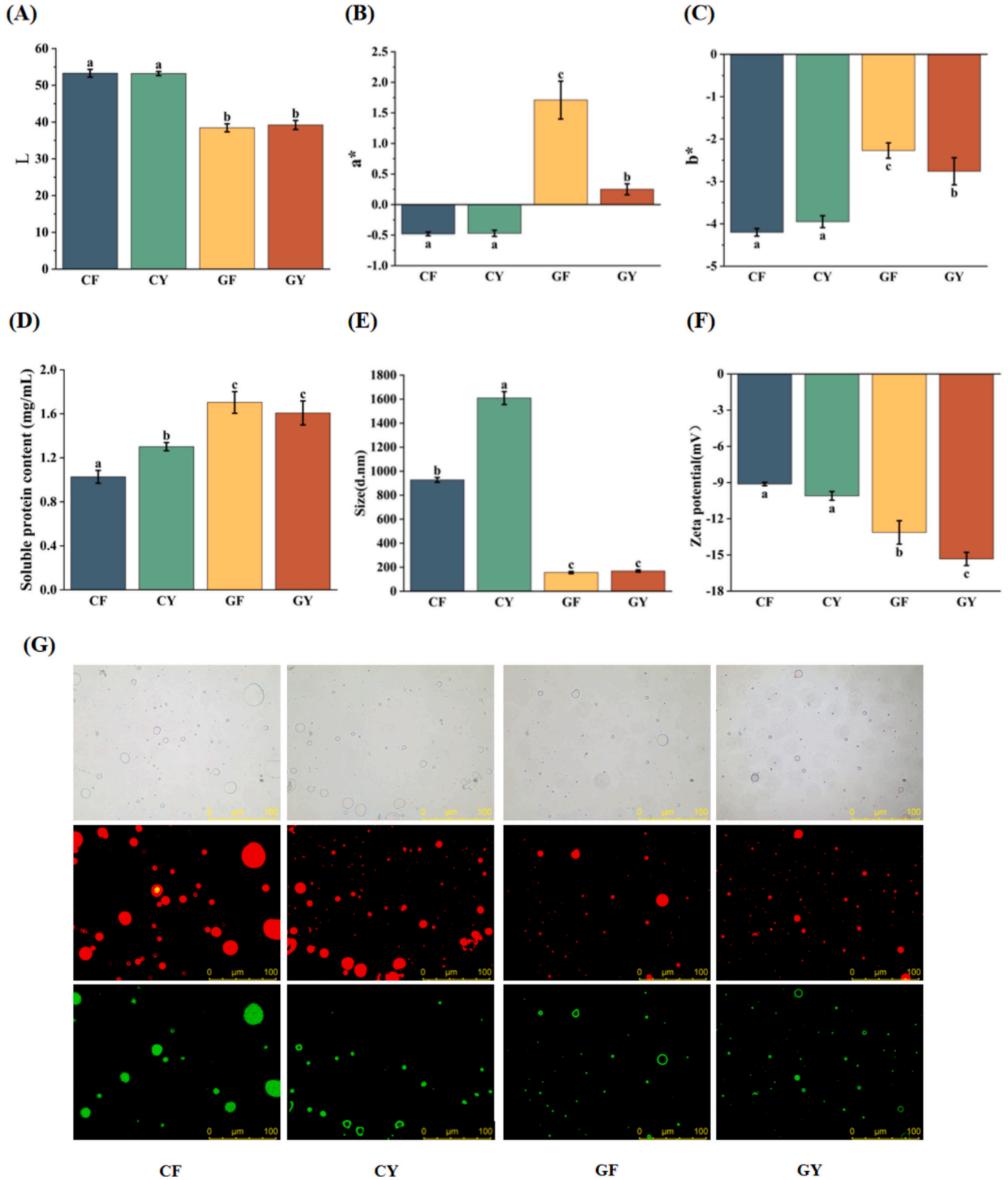

Fig. 1. Effects of different thermal processing on color (A-C), soluble protein (D), particle size (E), zeta potential (F) and microstructure (G) of silver carp soup.

mm × 0.25 μm) was used, with a column temperature of 60 °C and a high-purity nitrogen carrier gas (purity ≥99.999 %) for 30 min. The flow rate scheme was as follows: an initial flow rate of 2 mL/min was maintained for 2 min, increased linearly to 10 mL/min for 10 min, to 100 mL/min for 20 min, and to 150 mL/min for the last 10 min.

IMS conditions: the length of the drift tube was 9.8 cm, the linear voltage inside the tube was 400 V/cm, the temperature was 45 °C, the drift gas was high-purity N2 (purity ≥99.999 %), and the flow rate of the drift gas was 150 mL/min. The substances were characterized using Laboratoy Analytical Viewer (LAV), NIST database and IMS database.

2.10. HS-SPME-GC–MS analysis

2 mL of fish soup was added to a 20 mL headspace vial, 10 μL of o-dichlorobenzene (0.1306 μg/mL) internal standard was added, sealed, and equilibrated by heating in a water bath at 60 °C for 30 min, and then the solid-phase microextraction (SPME) device (57330-U Supelco) was inserted into the vial, and the headspace adsorption was continued for 30 min. The GC–MS system was equipped with a DB-WAX column (30 m × 0.25 mm × 0.25 μm). The GC conditions were as follows: inlet temperature, 250 °C; carrier gas, He; flow rate, 1.0 mL/min, splitless mode. The temperature increase program was as follows: the starting temperature was 45 °C, which was held for 5 min, increased to 240 °C at a rate of 5 °C/min, held for 10 min, and operated for 54 min. The mass spectrometry (MS) conditions: EI ionization source with an ionization voltage of 70 eV, ion source temperature of 230 °C, quadrupole temperature of 150 °C, full scanning mode, and mass scanning range of 35–400 amu. The mass spectra of the volatile components were compared to those of the NIST 17 mass spectral database and AMDIS for qualitative analysis. The aroma activity value (OAV) was calculated as follows.

$$OAV = C/T \quad (1)$$

C: absolute concentration of the olfactory substance; T: sensory threshold (thresholds from literature).

2.11. Statistical analysis

Statistical analysis was performed using SPSS 26.0 software, Origin Pro v.2021 to plot histograms, TBtools v.1.082 to generate hierarchical clustering heat maps, Person correlation coefficients were assessed using SPSS 19.0 software (IBM Corp., Armonk, USA), and Cytoscape v3.7.1 to plot correlation network models. All experiments were repeated three times.

3. Results and discussion

3.1. Color and soluble protein

As shown in Fig. 1 **(A-C)**, the effect of different thermal processing treatments on the color of silver carp bone soup, the fish soup presented a whiter color in the atmospheric pressure treatment group compared to the high pressure treatment group. This was attributed to the fact that the fish soup cooked at atmospheric pressure continuously tumbled, the fat contained inside the meat spilled out, and the lecithin and protein reacted during the cooking process to form emulsification (Wu et al., 2023). The a* and b* values of fish soup after high-pressure cooking were higher than those of the normal-pressure group, indicating that the fish soup in the high-pressure group was relatively redder and bluer.

Different thermal processing methods have important effects on the quality and nutrient composition of fish bone soup. As shown in Fig. 1D, the soluble protein content of the high pressure group was significantly higher than that of the atmospheric pressure group, which was attributed to the fact that high pressure can disrupt the spatial structure of proteins, making them more easily surrounded and solubilized by water molecules (Guyon et al., 2016), which led to an increase in the soluble protein content of fish soup. Significant differences were also found between GF and CF, CY and GY, while no significant difference was found between GF and GY, suggesting that the effect of high pressure on soluble protein content was greater than that of the oil frying treatment.

3.2. Particle size and zeta potential

During the heating and boiling process of the soup, the nutrients will self-assemble into colloidal particles with different particle sizes due to intermolecular forces. As shown in Fig. 1E, the particle sizes of fish soup boiled at high pressure and atmospheric pressure were in the range of 150–200 nm and 900–1700 nm, respectively, which suggests that the particle distribution of fish soup boiled at high pressure is more uniform and has better stability (Yao et al., 2021), and it can promote the release and decomposition of nutrients.

Zeta potential characterizes the number of charges on the surface of particles in solution, and a higher Zeta potential indicates a high electrostatic repulsion and a greater separation distance between droplets, which can reduce the generation of flocculation and aggregation (Thaiphanit et al., 2016). As can be seen from Fig. 1F, the absolute value of the potential of the fish stock after being cooked under high pressure was significantly higher than that of the atmospheric pressure cooking. In conclusion, the particle size of fish soup after high pressure cooking is smaller and the system is relatively stable.

3.3. Microstructure

As shown in Fig. 1G, protein particles (red) and fat (green) were dispersed in the fish soup, which were basically regular circles. The particle size of fish soup in the CY was obviously smaller than that in the CF, which was attributed to the fact that the fat was more likely to be broken into small particles of fat particles after frying of the fish meat, which in turn led to the reduction of the particle size of fish soup (Wu et al., 2023). It was also found that the particle size of fish soup was smaller and the particles were more evenly dispersed after high-pressure treatment, and the effect of high-pressure treatment on the particle size of fish soup was significantly greater than that of oil frying. In addition, protein particles became finer after high pressure treatment due to the pressure effect. While the fat particles were significantly reduced after high pressure and oil frying, and incomplete round fat appeared, this is because the soluble proteins in fish meat would enter the fish soup when it was cooked, and these proteins acted as emulsifiers, which were able to encapsulate the fat particles and form a stable emulsification system (Wu et al., 2024). These results are consistent with those measured for fish soup particle size.

3.4. Free fatty acids

Lipids are important precursor substances of volatile compounds in fish soup, and the oxidative degradation of fat is one of the important reactions for the formation of rich aroma in fish soup. Fish fats mainly include triglycerides and structural phospholipids, which are thermally degraded under heating conditions to generate free fatty acids, of which unsaturated fatty acids can be further oxidized to generate ketones, aldehydes and other volatile carbonyl compounds due to the presence of double bonds, resulting in the production of meaty, oily and aldehydic aromas, which ultimately have an impact on the flavor of fish soup (Qi et al., 2020). As shown in Fig. 2A, 26 fatty acids were detected in the four groups of samples, of which 14, 5 and 7 were SFA, MUFA and PUFA, respectively. Among them, only 14 fatty acids were detected in CF, which was due to the fact that the fish were not fried in oil and high pressure treated, which prevented the better solubilization of lipids from the fish tissues (Asokapandian et al., 2020). It was also found that the abundance of most fatty acids was significantly higher in CY and GF than in CF and GY.

The fatty acid composition of silver carp soup and its trend under

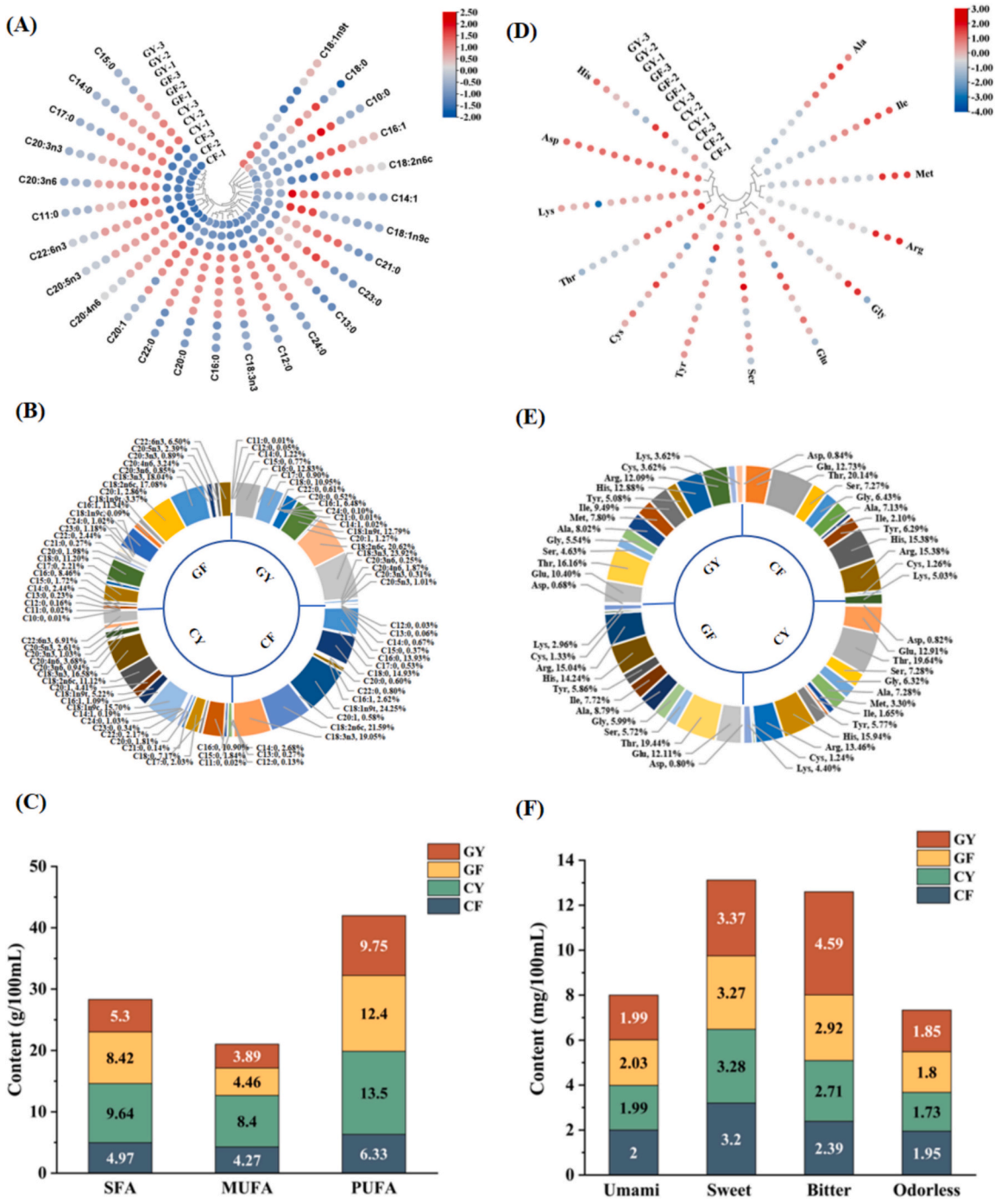

Fig. 2. Effects of different thermal processing methods on free fatty acids and free amino acids in silver carp soup. (A) Thermogram of free fatty acids, (B) graph of percentage share of each fatty acid, (C) graph of content of each type of fatty acid, (D) thermogram of free amino acids, (E) graph of percentage share of each amino acid, (F) graph of content of each type of amino acid.

different thermal processing as shown in Fig. 2B, and it was found that silver carp soup was rich in PUFA (Fig. 2C), which accounted for the highest percentage of fish soup, especially C18:3n3 and C18:2n6c. C16:0 and C18:0 were important SFAs in fish soup, with both accounting for more than 18 % of the content. Qian et al. (2019) similarly found C16:0 and C18:0 to be important fatty acids comprising SFA in tuna soup. The higher ratios of MUFA were C16:1 and C18:1n9t, in which the proportion of C16:1 in the samples of the high pressure group (GF: 11.34 %, GY: 6.49 %) was significantly higher than that in the atmospheric pressure group (CF: 2.62 %, CY: 1.09 %), which may be due to the fact that high pressure can promote the dissolution of oleic acid (C16:1). In addition, EPA and DHA were detected in the samples of CY, GF and GY groups, and the highest content was found in the CY group (EPA: 0.825 g/100 mL, DHA: 2.183 g/100 mL), followed by the GF group. EPA and DHA belong to the family of omega-3 fatty acids, which have a variety of benefits to the human body such as anti-inflammation, improvement of blood lipids, and long-term consumption also significantly improves one's memory and prevent dementia (Guo et al., 2018).

3.5. *Free amino acids*

Proteins produce small peptides under heating conditions and

further thermal degradation to produce free amino acids, which can be used directly as flavor enhancers and flavor precursors (Geng et al., 2019). Free amino acid is an important flavor presenting substance with fresh, sweet and bitter taste sensation, which can enrich the taste level of food. A total of 13 free amino acids were detected in the four groups of samples, CF, CY, GF and GY (Fig. 2C), and the total free amino acid contents were 9.53 mg/100 mL, 9.71 mg/100 mL, 10.02 mg/100 mL and 11.80 mg/100 mL, respectively (Fig. 2F). These results indicated that FAA production in silver carp soup could be significantly promoted after high-pressure and oil frying. This was attributed to the fact that high-pressure simmering allowed more efficient breakdown of proteins into amino acids in the fish, whereas oil frying damaged the surface tissues of the fish and promoted the release and breakdown of amino acids (Wu et al., 2023).

Sweet amino acids were found to be the predominant amino acids followed by bitter, fresh and odorless amino acids in the four groups of samples (Fig. 2E). Asp and Glu are typical fresh tasting amino acids found in aquatic products and synergize with NaCl to enhance the freshness and salinity of fish soup. Thr, Ser, Gly and Ala provide pleasant sweetness to the soup and the sweet amino acid content was increased in the soups that had been pan-fried and pressure-cooked, especially in the case of Ala. Coordination between Ala and Glu can increase the intensity of freshness in fish soup (Wang, Nie, et al., 2022). Arg, Met, Ile, Tyr, and His are typical bitter amino acids, and the content of bitter amino acids was increased after frying. It has been shown that subthreshold levels of bitter amino acids actually enhance umami flavor (Harmon et al., 2024). In addition, one more amino acid, Met, was detected in the oil-frying group compared to the non-oil-frying group, which may be due to the hydrolysis of the protein at high temperatures. This sulfur-containing amino acid is a flavor precursor substance that produces characteristic aroma and can generate volatile aldehydes through the Strecker reaction (Yu et al., 2018), which enriches the aroma and taste of fish soup.

3.6. GC-IMS analysis

HS-GC-IMS was used to characterize the VOCs of fish soup from different thermal processing treatments. The Fig. 3A and Fig. 3B show the 2D chromatograms and 3D chromatograms of the four groups of fish soup samples, respectively, from which it can be seen that most of the signals appeared in the retention time range of 200–600 s, with a drift time of 1.0–1.9 s. In addition, the peak types and intensities of the peaks of GY were significantly higher than those of the other three groups of samples, which indicated that the content and types of VOCs in the silver carp soup treated with high-pressure frying was higher than the other three groups of samples. A total of 39 signals were detected in the four sets of samples and 26 compounds were identified, including 12 aldehydes, 6 alcohols, 3 terpenes, 2 ketones, 2 esters and 1 acid. Certain compounds produced multiple signals, possibly due to their different concentrations and their own properties, so that these ions formed dimers and trimers when passing through the drift tank (Krisilov et al., 2017).

To further analyze the changes in volatile compounds in silver carp soup under different thermal processing treatments, the fingerprints of the volatile compounds were analyzed. As shown in Fig. 3C, the fingerprints of the four groups of samples were individually divided into three regions, and there was no significant difference in the concentrations of volatile compounds such as ethanol-M, acetic acid-M, and n-pentanal-M in region A. This indicates that these five compounds are typical flavor substances in fish soups, and among them, both n-pentanal-M and acetic acid-M have fruity flavors (Wang, Tang, et al., 2022; Wang, Wang, et al., 2022). Zone B is the newly generated or significantly increased concentration of compounds in GY, mainly aldehydes and terpenoids, which can bring sweetness and floral flavor to fish soup (Wang, Tang, et al., 2022).The reason for the significant increase in the concentration of compounds in GY may be due to the fact that high pressure and high temperature lead to the oxidation of the double bonds in unsaturated fatty acids to form peroxides, which can be further degraded into different aromatic compounds, especially aldehydes (Wang et al., 2019). In addition, high-pressure simmering increases the abundance of some flavor compounds (C-region), such as butanal, 1-propanol, and 1-pentanol, which are mainly the products of fat oxidation, of which butanal has a chocolate-like, malty flavor (Nie et al., 2022); and can enrich the flavor of fish soup.

3.7. Electronic nose analysis

An electronic nose is a device that mimics the mechanism of human olfactory perception and analysis, which identifies odor components in a sample through the sensitivity of a gas sensor array to specific gas molecules, and discriminates and analyzes the detected odors (Li et al., 2024). The results of principal component analysis (PCA) of silver carp bone soup with different thermal processing treatments are shown in Fig. 4A, which indicated that the contributions of PC1 and PC2 were 97.83 % and 2 %, respectively, and there was partial overlap between CF and CY samples and between GF and GY samples. However, there was no overlap between the atmospheric-pressure boiling samples and high-pressure boiling samples, and they could be differentiated from each other, which indicated that simmering at different pressures had a greater effect on the flavor substances of silver carp bone soup, and frying had a relatively smaller effect on the flavor substances. Large differences between the W1W, W1S, and W2W sensors (Fig. 4B), suggesting that different thermal processing methods have relatively large effects on sulfides, methylated compounds, and aromatic compounds in silver carp soup.

3.8. HS-SPME-GC–MS analysis

The volatile substances in the four groups of silver carp soups with different thermal processing methods were comprehensively analyzed by HS-SPME-GC–MS. A total of 63 volatile substances were identified, including 13 aldehydes, 7 ketones, 4 alcohols, 6 esters, 6 aromatic compounds, 8 hydrocarbons, 14 terpenes and 5 other compounds. As shown in Fig. 4C, the largest percentage of hydrocarbons (CF: 43 %, CY: 52 %) was found in the atmospheric pressure boiling samples, followed by aldehydes (CF: 35 %, CY: 22 %), whereas aldehydes (GF: 55 %, GY: 24 %) and terpenoids (GF: 32 %, GY: 54 %) accounted for the larger percentage of samples at high pressure. In addition, the species of aldehydes, ketones and esters were significantly increased in GY samples (Fig. 4D).

Aldehydes are considered to be the main flavor of aquatic products due to their low threshold and have a large contribution to flavor, and the aldehydes in fish soup are mainly derived from lipid oxidation and amino acid degradation (Nie et al., 2022). From Fig. 4E, it can be seen that the content of aldehydes in fish soup increased significantly after high-pressure treatment, especially in the samples of the GF, which is mainly due to the fact that high-pressure accelerates the process of oxidative degradation of lipids in fish soup, which leads to the production of, among others, 1-nonanal and phenylacetaldehyde, which have a pleasant sweet, citrusy, and lemony flavor. In contrast, the aldehydes content in the samples of the non-frying group (CF and GF) was significantly higher than that of the samples of the frying group (CY and GY), which was attributed to the fact that the high-temperature frying process accelerates the oxidative decomposition of lipids and introduces other complex chemical reactions, which in turn leads to the reduction of aldehydes (Wu et al., 2023). The effect of alcohols on the flavor of fish bone soup was relatively small, and volatile alcohols are mainly decomposition products of fat oxidation and derivatives of the Merad reaction (Wang et al., 2023). A total of four alcohols were detected in the four fish bone soups, such as the short branched-chain alcohol 1,4-butanediol, which had a high sensory threshold and a low contribution to the overall flavor. In contrast, (*R*)-(+)-beta-citronellol as a long straight-chain alcohol, had a lower sensory threshold and added lemongrass

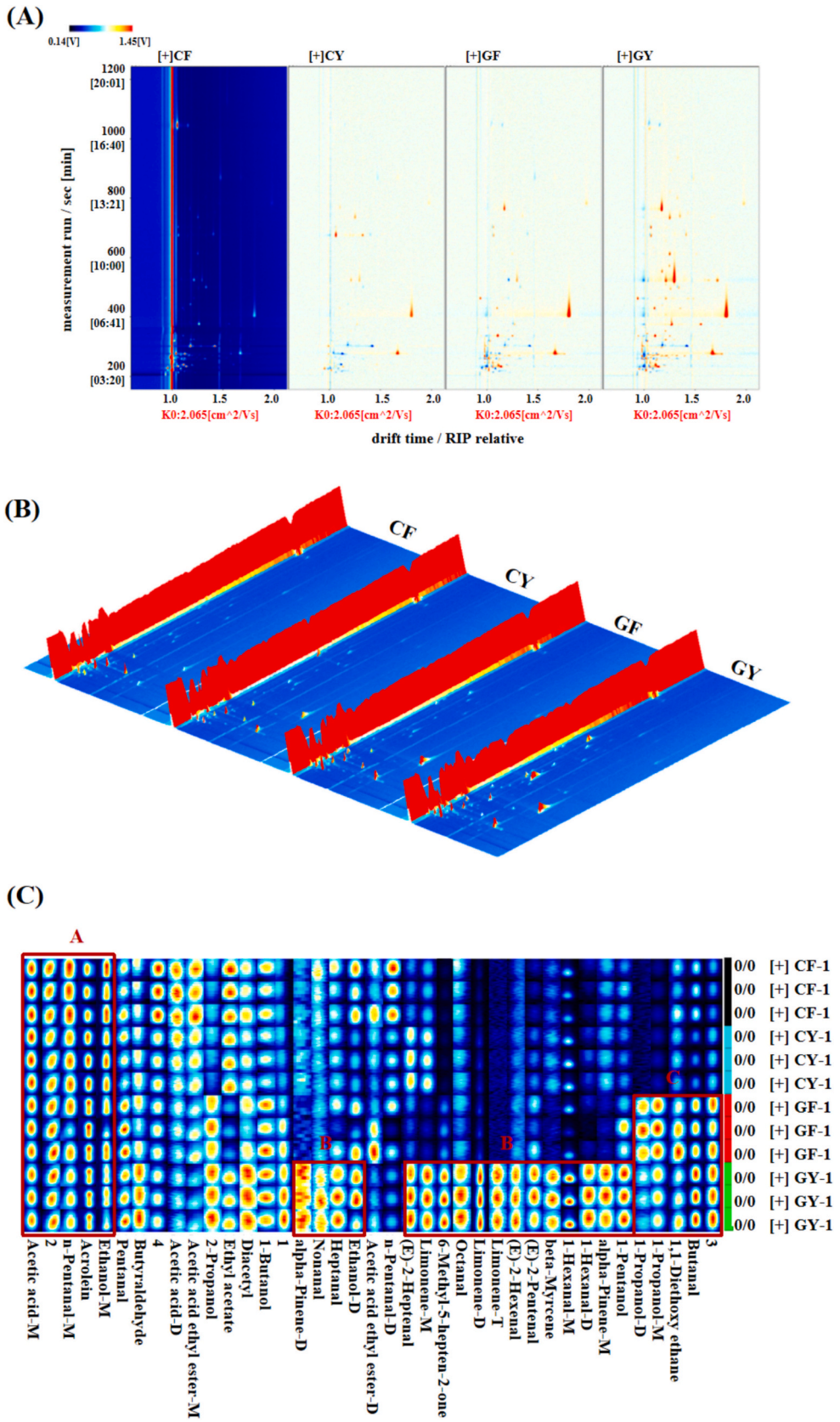

Fig. 3. GC-IMS analysis of volatile flavor compounds in silver carp soup under different thermal processing methods. (A) Comparative difference spectra of volatile components, (B) three-dimensional spectra of volatile components, (C) fingerprints of volatile flavor compounds.

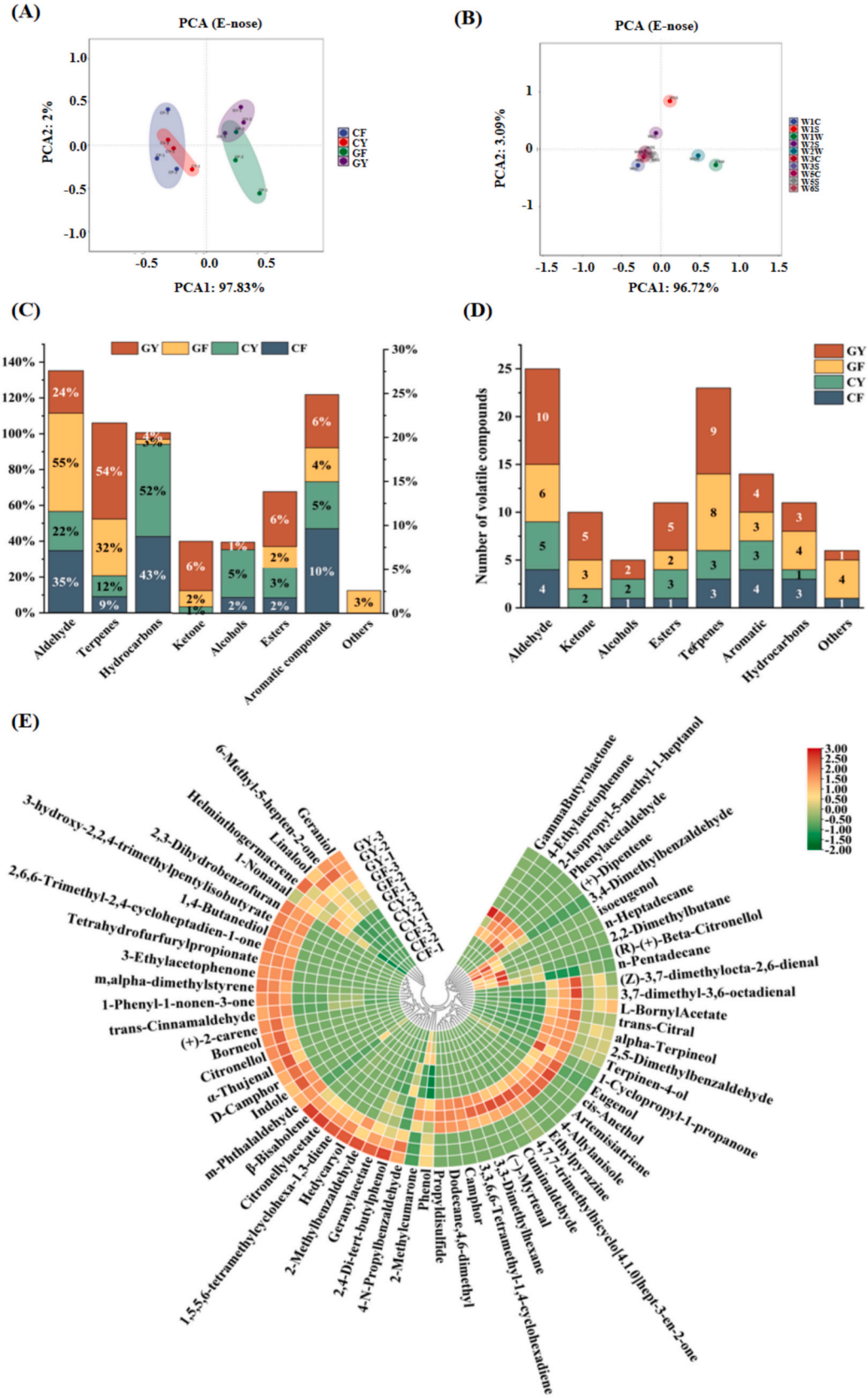

Fig. 4. *E*-nose and GC–MS analyses of volatile flavor compounds in silver carp soup under different thermal processing methods. (A) PCA of the electronic nose, (B) PCA analysis of each sensor of the electronic nose, content (C) and quantity changes (D) of volatile flavor compounds, and (E) thermogram of volatile flavor compounds.

oil, rose, and petal flavors to enrich the flavor of fish soup.

Ketones are mainly derived from oxidative degradation of unsaturated fatty acids, amino acid catabolism, and Maillard reactions, and generally have a fruity, buttery, and clean odor. 4-Ethylacetophenone belongs to the floral type of compounds and has a hawthorn odor. 2,6,6-Trimethyl-2,4-cycloheptadien-1-one is possibly from spices, has a minty odor, and was only found in the GY sample. Esters are generally produced by carboxylic acids and alcohols through esterification reactions, and the esters produced from short-chain acids have a fruity flavor, while the esters produced from long-chain acids have a slight greasy flavor. Esters such as L-bornylacetate, citronellylacetate, and geranylacetate were detected in the four fish soups, and they had fruity and sweet flavors, which improved the flavor and overall taste of the fish bone soups.

Hydrocarbons, which mainly originate from the splitting of alkoxyl groups in fatty acids, have a low impact on the flavor of fish stock due to their high odor threshold (van der Klis et al., 2011). The hydrocarbon content was significantly higher in the atmospheric pressure-treated

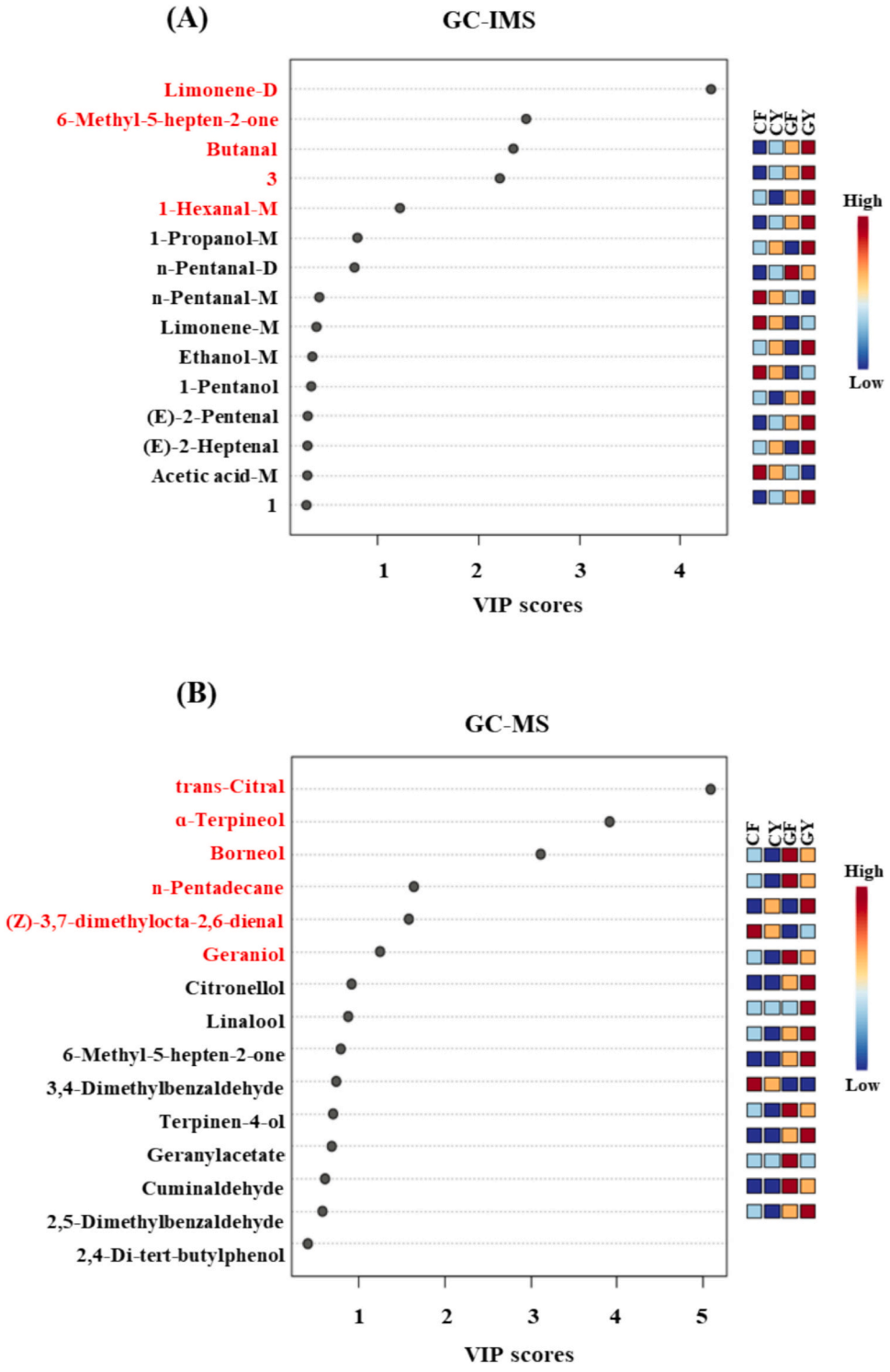

Fig. 5. The PLS-DA model was used to analyze VOCs in silver carp soup. VIP score plots for GC-IMS data (A), VIP score plots for GC–MS data (B).

group than in the high-pressure-treated group, which may be due to the fact that the atmospheric pressure heating method is more conducive to the dissolution of hydrocarbons from silver carp bones. It has been found that unsaturated hydrocarbons can be partially converted to aldehydes and ketones, which may enhance the fishy flavor of fish stock (Fu et al., 2024). Most of the aromatic compounds have aromatic odors, mainly from spices, and can enrich the flavor of fish soups. Phenol can provide a special smoky and toasty flavor to fish soups (Wang et al., 2025). Eugenol usually imparts a toasty, buttery flavor to foods, and it is only found in non-frying samples (CF and GF), probably because the high-temperature frying process volatizes eugenol and high pressure promotes the production of eugenol. The main reason for this is the high temperature and high pressure of frying. and high pressure promotes the production of eugenol. In addition, propyldisulfide and 4-allylanisole were detected in the fish bone soup of the GF, conferring an oniony, licorice-like flavor to the soup, which may be related to the seasoning of shallots in the fish bone soup. In addition to CY, small amounts of heterocyclic compounds were detected in three other silver carp bone soup samples, such as 2-methylcumarone, ethylpyrazine and 2,3-dihydrobenzofuran, which are the products of the Maillard reaction and can impart a roasted flavor to fish soup.

3.9. Characteristic volatile flavor substances identification

PLS-DA was further used to analyze the volatile compounds in the silver carp soup from different thermal processing treatments, and in general, VOCs with VIP $\geq$ 1.0 were considered as markers of product flavor. As shown in Fig. 5, a total of 10 compounds with VIP $\geq$ 1.0 were detected in GC-IMS and GC–MS, mainly including aldehydes and terpenoids. Hexanal, which has a grassy and buttery flavor, is produced by the oxidative decomposition of lipids with unsaturated fatty acyl groups, and heating accelerates its oxidation (Tsuzuki, 2019), so the content of hexanal in the samples of the GY group was significantly higher than that in the samples of the other groups. Butyraldehyde has a fruity and banana aroma, which was significantly increased in the high pressure group (GF and GY) samples. Terpenoids are mainly derived from spices, Geraniol has a rose aroma in addition to antimicrobial and antioxidant properties that inhibit the growth of certain bacteria and fungi. Limonene is a naturally occurring functional monoterpene with a citrus-orange sweet flavor (Yang et al., 2019).

The flavor of silver carp bone soup is an overall manifestation of the synergistic effect of many substances, and the volatile flavor substances of fish soups obtained from different thermal processing treatments varied considerably. As shown in **Table S1**, no characteristic components (OAV > 1) were seen in the two atmospheric pressure treatment groups. The high-pressure treatment groups GF and GY, on the other hand, contained two co-featured components (OAV > 1), linalool and geraniol, which are terpenoids with strong aroma and bioactivity, and are usually introduced by seasonings such as ginger and scallion, which added sweet floral and rosy aroma to the fish bone soup, and played an important modifying role in the overall flavor. In addition, the high-pressure non-frying group has the unique characteristic ingredient of trans-citral (OAV > 1), an aldehyde derived from the oxidation of linalool and geraniol (Sun & He, 2004), which infuses fish bone soups with a refreshing citrusy lemon flavor, helping to remove the fishy taste and enhance the soup's aroma and texture.

3.10. Amino acids, fatty acids and flavor compounds correlation analysis

The main sources of volatiles in fish stock are the thermo-oxidative degradation of lipids and proteins and the Maillard reaction that occurs between amino acids and sugars (Elmore et al., 1999). Thermally induced oxidation of fatty acids (especially unsaturated fatty acids) produces degradation products such as fatty aldehydes, ketones, and alcohols. Amino acids undergo catabolism during high pressure and heating to produce small molecule peptides and free amino acids, which are important precursors of major flavor components, including aldehydes, alcohols, ketones, and sulfur-containing compounds (Grujovic et al., 2022). In order to better analyze the effects of fatty acids and amino acids on the flavor of silver carp soup, the correlation analysis was performed between the flavor compounds (13 aldehydes, 7 ketones, and 4 alcohols) in fish soup and 26 fatty acids and 12 amino acids (Glu was not correlated because it did not change in the four samples), and those substances with $|R| > 0.7$ and $P < 0.05$ were analyzed in a further discussion,(Fig. 6).

The degree of unsaturation of fatty acids as well as the length of carbon chains are the key factors in determining the type and content of aroma compounds. From the correlation network diagram, it can be seen that m-phthalaldehyde is significantly negatively correlated with most fatty acids, such as lauric acid (C12:0), myristic acid (C14:0), C21:0, etc., while phenylacetaldehyde is significantly positively correlated with most fatty acids. Phenylacetaldehyde is an aromatic compound with a hyacinth aroma and a green leaf-like crisp fragrance. In addition, a significant negative correlation was found between nonanal and C18:1n9t. Nonanal is produced when fatty acids are attacked by free radicals due to thermal oxidation, and high temperature conditions facilitate this reaction (Elmore et al., 1999), so it is only detected in CY, GF and GY. Indeed, in addition to contributing to the aroma of fish products, aldehydes can also cause modifications to proteins, altering their nutritional and sensory properties (Guyon et al., 2016). It has been shown that thermal peroxidation of saturated fatty acids, decomposition of peroxides of unsaturated fatty acids and intramolecular electron rearrangements contribute to the production of methyl ketones and cyclic ketones (Yin et al., 2011). 1-Cyclopropyl-1-propanone was detected in fish soup and was found to be significantly positively correlated with C18:0, C21:0, and C16:1. Volatile alcohols are mainly products of oxidative breakdown of fats and 2-isopropyl-5-methyl-1-heptanol was found to be significantly correlated with C13:0, C14:0, C15:0, C20:4n6, C20:3n3, C20:5n3, and C22:6n3 fatty acids were significantly positively correlated ($r > 0.7, p < 0.05$).

Amino acids are one of the key precursors for the production of volatile odorants (Maoz et al., 2022), and some free amino acids undergo deamination and decarboxylation and decarbonylation reactions to form aldehydes, amines, and other volatile compounds capable of presenting meat flavor. In addition, the Maillard reaction between the amino group of free amino acids and the carboxyl group of sugars produces aldehydes and ketones with high contribution to flavor. α-Amino acids and natural hydroxyl compounds undergo Strecker degradation, which in turn produces a range of fatty aldehydes with flavor characteristics (Rizzi, 2008). 2-Methylbenzaldehyde is a Strecker aldehyde with a fruity flavor that can be generated from the thermal decomposition of amino acids, and correlation analysis revealed that 2-methylbenzaldehyde was significantly positively correlated with Met, Ile, and Arg, which may be due to the fact that high-pressure frying promotes the breakdown of proteins and amino acids.1-Nonanal is an aromatic aldehyde with a citrus and fatty flavor, mainly from lipid oxidative catabolism and amino acid degradation (Wang et al., 2023). A significant negative correlation ($r < -0.7, p < 0.05$) was found between the changes in the content of nonanal and Ala in the four groups of samples (Fig. 6).

Lys and Tyr can produce ketones during degradation, and ketones produced by degradation of these amino acids have a higher threshold (Lv et al., 2024). No significant correlation was found between ketones and these amino acids in any of the four groups of samples, indicating that amino acid changes in fish soup did not have a significant effect on ketones. However, most ketones such as 1-phenyl-1-nonen-3-one, 6-methyl-5-hepten-2-one, 2,6,6-trimethyl-2,4-cycloheptadien-1-one, and 3-ethylacetophenone were found to be significantly positively correlated with Met, Ile, and Arg were significantly positively correlated. Among them, 6-methyl-5-hepten-2-one is one of the three model flavor compounds released and retained by nigrosperm-like essences with fruity aroma and fresh crisp aroma (Obretenov et al., 2002). These results clarified the effects of different thermal processing methods on the

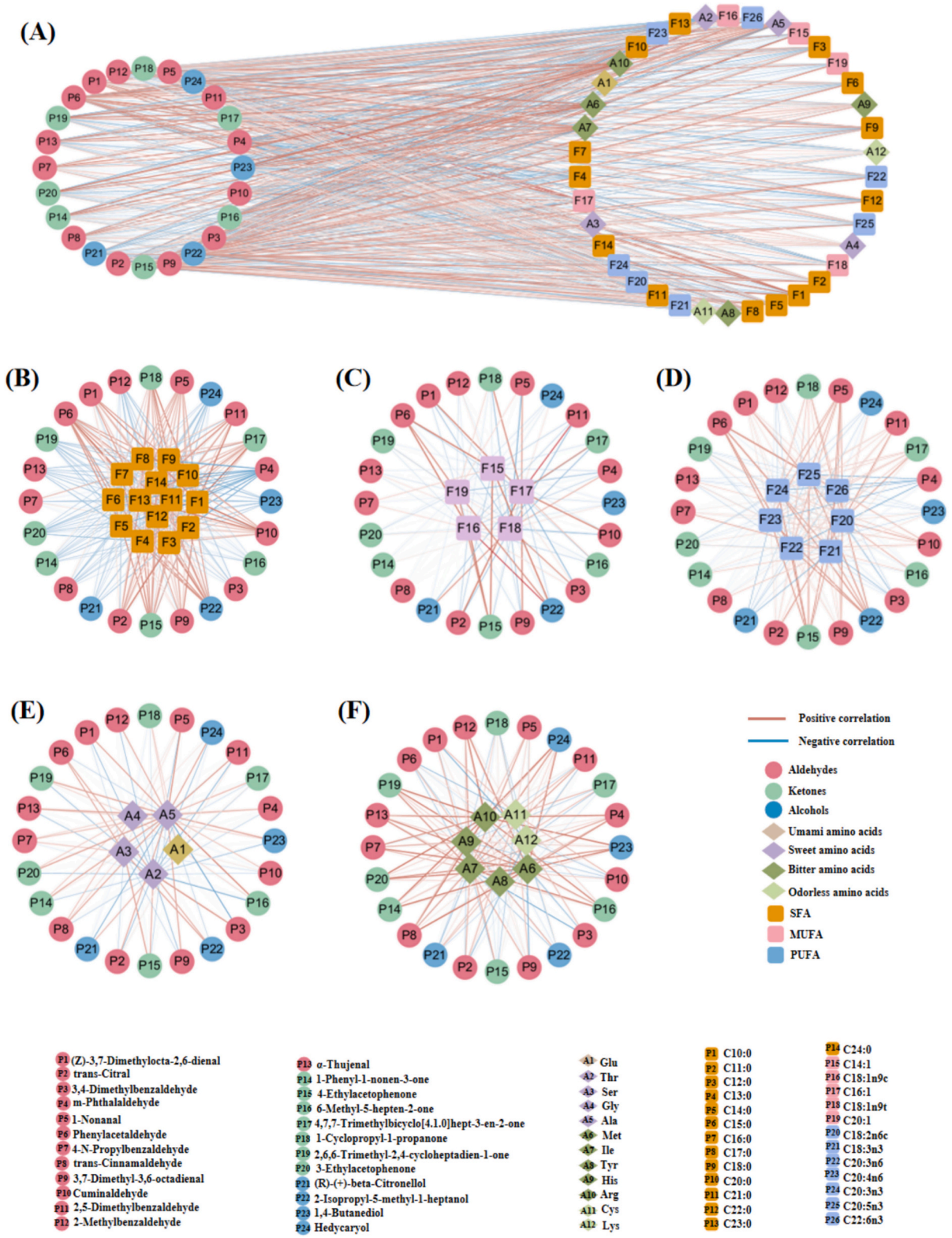

Fig. 6. Correlations between free amino acids, free fatty acids and flavor were assessed based on person correlation coefficients. (A) Network diagram of correlations between free amino acids, free fatty acids and flavor; (B) saturated fatty acids; (C) monounsaturated fatty acids; (D) polyunsaturated fatty acids; (E) umami and sweet amino acids; and (F) bitter and odorless amino acids.

nutrition, flavor and microstructure of silver carp bone soup, and provided an important theoretical basis for improving the quality and flavor of silver carp soup.

4. Conclusion

This study investigated the effects of different thermal processing methods on the nutritional quality and flavor of silver carp bone soup. Key findings emphasized the particle size of the high-pressure-treated

fish soup was smaller and the particle dispersion was more homogeneous, and the soluble protein content and free amino acid content were higher than those of the fish soup cooked at atmospheric pressure. The types and abundance of flavor compounds in the GY group were found to be significantly higher than those in the other three groups of samples. Ten key biomarkers, mainly aldehydes and terpenoids, were screened in the fish soup based on VIP values. In addition, the correlation results indicated that the flavor and nutritional value of the fish soup were improved after high-pressure treatment, and these findings not only provided a solid theoretical basis for the research and development of the soup, but also opened up new avenues for the comprehensive utilization of silver carp by-products, which enhanced the comprehensive utilization efficiency of aquatic resources and promoted the sustainable development of fishery economy. This study provides new insights to a certain extent for a deeper understanding of the influence mechanism of thermal processing methods on the flavor formation of silver carp bone soup, and lays the foundation for the subsequent optimization of the application of thermal processing technology in the food field.

CRediT authorship contribution statement

Shi Nie: Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Lu Zhang:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Yutong Xie:** Investigation, Formal analysis. **Shiru Feng:** Validation, Software. **Yaqin Yu:** Validation, Software. **Chunming Tan:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation. **Zongcai Tu:** Supervision, Project administration, Conceptualization.

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.fochx.2025.102319.

Data availability

Data will be made available on request.

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