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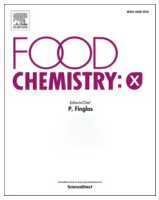

Effect of different processing steps in the production of beer fish on volatile flavor profile and their precursors determined by HS-GC-IMS, HPLC, E-nose, and E-tongue

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ABSTRACT

Beer fish is characterized by its distinctive spicy flavor and strong beer aroma. Currently, there is a lack of comprehensive research analyzing the changes in taste and volatile compounds that occur during the processing of beer fish. Thus, this study used HS-GC-IMS, electronic tongue, and electronic nose to investigate the changes in flavor components during various processing stages of beer fish. The obtained results were subsequently analyzed using multivariate statistical analysis. The results showed that the final beer fish product (SF) had the greatest amount of free amino acids (888.28 mg/100 g), with alanine, glutamic acid, and glycine contributing to the taste of SF. The inosine monophosphate (IMP) content of beer fish meat varied noticeably depending on processing stages, with deep-fried fish (FF) having the greatest IMP content (61.93 mg/100 g), followed by the final product (SF) and ultrasonic-cured fish (UF). A total of 67 volatiles were detected by GC-IMS, mainly consisting of aldehydes, ketones, and alcohols, of which aldehydes accounted for >37%, which had a great influence on the volatile flavor of beer fish. The flavor components' composition varied noticeably depending on the stage of processing. PLS-DA model screened 35 volatile flavor components (VIP > 1) as markers; the most significant differences were 1-propanethiol, isoamyl alcohol, ethanol, and eucalyptol. Ultrasonic processing, frying, and soaking sauce can significantly improve the formation of flavor compounds, resulting in a notable enhancement of the final beer fish's umami taste and overall flavor quality.

1. Introduction

Nile tilapia fish (*Oreochromis niloticus*) is the second most commercially valuable fish farmed because of its high adaptability and capacity for success in challenging environments (Zhu et al., 2021). The province of Guangxi produced 308,000 tons of tilapia fish in 2016, accounting for 17.3% of China's overall output (Ding et al., 2020). Beer fish, a local specialty in Yangshuo County, Guangxi, is widely recognized for its delicious, spicy, and aromatic flavor. It has gained significant popularity among individuals and has been officially acknowledged as a cultural heritage at the county level. In addition, the China Cuisine Association has recognized it as one of the top ten traditional meals in Guangxi, specifically within the category of "Chinese cuisine" (Wang, Wang, et al., 2021). The traditional method of preparing beer fish involves the selection of live fish sourced from the Li River. The fish are subsequently slaughtered, cleaned, and cut along the backbone using a knife. The fish is then subjected to ultrasonication with a marination solution and then fried until it achieves a desirable brown coloration. Subsequently, the fish is heated up with a combination of ingredients and seasonings, including tomatoes, peppers, and Liquan beer, until it reaches a state of optimal ripeness and flavorfulness (Pang, 2021). The flavor characteristic is important in aquatic products, serving as an important indicator for evaluating their sensory quality (Zhao et al., 2023). Aquatic products get their flavor from flavor precursor substances, which include volatile

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flavor compounds and taste-providing substances (Menis-Henrique, 2020). The taste substances primarily consist of non-volatile water-soluble compounds, including free amino acids, inosinate, peptides, and organic acids. These compounds contribute to the perception of sweetness, sourness, umami, bitterness, and saltiness in aquatic products (Dashdorj et al., 2015). The volatile flavor compounds include a range of chemical components such as aldehydes, alcohols, esters, sulfides, and other molecules. These compounds collectively contribute to the distinctive flavor of aquatic products (Zhao et al., 2023). Fresh aquatic products possess a fishy odor due to the decrease in freshness. However, as these aquatic products undergo cooking and processing, they have a distinctive and recognizable aroma (Cheng et al., 2023). Hence, understanding the changes in flavor that occur in aquatic products during the processing stages may facilitate the control of flavor formation pathways and influencing factors. This, in turn, contributes to improving processing techniques and enhancing flavor quality in aquatic products.

Currently, the majority of research related to aquatic products focuses on the effect of single processes or different heat treatment techniques on their flavor. For example, in a study conducted by Li, Sun, et al. (2022), the key flavor compounds of fried tilapia were analyzed using GC-O-MS and AEDA methods. Similarly, in their study, Wang, Wu, et al. (2021) conducted a study involving the steaming of black carp after marination. They aimed to determine the best steaming time that would enhance the flavors of black carp based on the analysis of free amino acids, flavor nucleotides, and total nitrogen. In addition, Chen, Shi, et al. (2023) investigated the volatile compounds of tilapia under different heat treatment conditions using gas chromatography and E-nose analysis. However, it is essential to note that the studies mentioned above do not provide a comprehensive and successive analysis of the flavor profiles of specific aquatic products during their processing, nor do they provide a comprehensive understanding of the changes in taste substances and volatile compounds.

This study used a combination of HPLC, GC-IMS, E-nose, and E-tongue methods, along with taste activity value, principal component analysis (PCA), cluster analysis, and partial least squares discriminant analysis (PLS-DA). The objective was to analyze the changes in taste substances and volatile compounds during the ultrasonic pickling, frying, and dipping processes of beer fish to establish a theoretical basis for understanding the flavor of beer fish and provide insights for additional studies on the flavor of aquatic products.

2. Materials and methods

2.1. Materials and reagents

Tilapia fish, ginger, green onions, red pickled peppers, shallots, sour ginger, garlic, Liquan beer, tomato paste, chili oil (food grade), etc., were purchased from Guangxi Sangu's Vegetable Network Technology Co., LTD. Xanthan gum (food grade) was obtained from Henan Zhongchen Biotechnology Co., Ltd.; methanol and acetonitrile (chromatography grade) were bought from Germany CNW company; *o*-phthalaldehyde, 3-mercaptopropionic acid, and fluorenyl methoxycarbonyl chloride (analytical pure) were purchased from Sigma Company (USA); sodium dihydrogen phosphate dihydrate, disodium hydrogen phosphate dodecahydrate, boric acid, sodium hydroxide, perchloric acid, anhydrous ethanol, potassium hydroxide, tartaric acid (analytically pure), etc. were obtained from Guangzhou Chemical Reagent Factory.

2.2. Pretreatment of raw materials

To make preparations, the tilapia fish undergoes the process of fin and scale removal. Afterward, a cut is made along the dorsal side of the fish to take out its internal organs. After being cleaned carefully, the fish is placed aside for subsequent processing.

2.3. Preparation of fish sauce

The preparation of fish sauce was conducted according to the method described by Liu et al. (2023). In brief, fill the cooking vessel with 160% water based on the fish weight, followed by 120% beer, 16% tomato paste, 12% green onion, 24% red pickled pepper, 8% shredded ginger, 12% garlic, 2% salt, 8% oyster sauce, 4% light soy sauce, 1.2% dark soy sauce, and 8% chili oil. Bring to a boil at 2200 W for 15 min, then add the 5% xanthan gum solution and mix evenly; continue to heat for 5 min, and then filter using a mesh sieve to produce the beer fish sauce.

2.4. Preparation of beer fish

The production process of beer fish was prepared by following the method described by Feng et al. (2021), with some modifications. In brief, for the purpose of the preparation of marinade solution contents, the fish's weight is 100%. To prepare the marinade, weigh 120% water, 5% green onion, and 4% ginger. After combining these materials, the mixture was thoroughly mixed using a high-speed wall breaker and then filtered. The filtrate was then supplemented with 30% beer, 1.5% balsamic vinegar, 2.5% light soy sauce, 0.6% pepper, and 12% salt, and then the ingredients were mixed thoroughly. A food bag was utilized to combine the marinade solution and tilapia fish, which were thereafter tightly sealed. The ultrasonic cleaner (SB25-12DTDN Ultrasonic Cleaner, Ningbo Xinzhi Biotechnology Co., Ltd.) was then adjusted to operate at an ultrasonic temperature of 20 °C, an ultrasonic power of 235 W, and an ultrasonic time of 27 min to produce ultrasonically pickled fish (UF). Then, the UF was collected, dried, and subsequently fried in the fryer (JKWS-9200 Fryer, Ruian Kaisheng Food Machinery Co., Ltd.) at 180 °C for 4 min to produce deep-fried fish (FF). The FF was submerged in the fish sauce for 30 min to produce the final beer fish (SF). The collection of samples occurred at several stages of processing, namely fresh fish (RF), ultrasonically pickled fish (UF), deep-fried fish (FF), and final beer fish (SF), to facilitate further analysis. The full procedure for the preparation of beer fish is depicted in Fig. S1.

2.5. Free amino acids (FAA) analysis

The FAAs were determined in beer fish at different processing stages by following the method described by Wang et al. (2018), with slight modification. The back muscle of the fish is finely chopped to create a homogeneous and uniform sample. A 1 g sample was carefully weighed, placed into a 10 mL centrifuge tube, and mixed with 5 mL hydrochloric acid (HCl, 0.01 M). The resulting mixture was then subjected to a 30-min boiling water bath. Following this, centrifugation at 12300*g* for 10 min. Finally, the supernatant was carefully collected. Subsequently, a volume of 2 mL of HCl (0.01 M) was added to the precipitate. The mixture was subjected to ultrasonic levitation for 5 min, followed by centrifugation. The supernatant obtained was carefully collected and mixed with the solution that had been previously obtained. After that, the total volume was adjusted to 10 mL and filtered using a 0.22 μm membrane for HPLC analysis. HPLC (Agilent1100, Agilent Technologies, Co., Ltd.) was used to analyze the FAAs. The HPLC conditions were determined as follows: the column used was ZORBAX Eclipse AAA (4.6 × 150 mm, 3.5 μm); the column temperature was set at 45 °C; the gradient elution settings for mobile phases A and B described in Table S1. Mobile phase A consisting of 40 mmol/L sodium phosphate monobasic (pH 7.8), and mobile phase B containing acetonitrile: methanol: water in a ratio of 45:45:10. The flow rate was set at 1.0 mL/min. The wavelengths for UV detection were adjusted at 338 nm (0–19 min) and 266 nm (19.01–25 min).

2.6. Nucleotide analysis

The nucleotides were determined in beer fish at different processing stages by following the method described by (Wang, Wu, et al., 2021), with slight modifications. In brief, a sample weighing 4.0 g was weighed,

and 20 mL of 10% perchloric acid solution (*v/v*) pre-cooled at 4 °C was added. The mixture was homogenized at 10000 *g* and 4 °C for 15 min, and the resulting supernatant was collected. The precipitate was washed with 20 mL of 5% perchloric acid, the mixture was centrifugated, and the resulting supernatant was collected. The supernatant was combined twice, and the pH was adjusted to 6.5 with 1 mol/L KOH solution. The solution was allowed to stand for 30 min and then diluted to 100 mL with double distilled water. The supernatant was filtered with a 0.22 μm filter membrane and tested. The HPLC procedure involved using the ODS-SPC18 column (4.6 mm × 250 mm, 5 μm) with mobile phase A consisting of 20 mmol/L citric acid, 40 mmol/L triethylamine, 0.1% glacial acetic acid (pH 4.8), and mobile phase B contains methanol. The gradient elution conditions of mobile phases are described in Table S2. The column temperature was 40 °C, the flow rate was 1.0 mL/min, the detecting wavelength was 260 nm, and the injection volume was 10 μL.

2.7. Electronic nose (E-nose) analysis

An Electronic Nose (PEN3 Electronic nose, Airsense, Germany) was used to distinguish the odor profile of the beer fish samples at different processing stages. This method was described by Zhang et al. (2023). A precise weight of a minced fish sample (5 g) was placed in a 10 mL headspace vial. An airflow rate of 400 mL/min was kept constant, and an analysis was performed for 80 s at 60 °C. The measurements and reports of the intensities of ten metal oxide sensors were conducted. The ten different sensors and their sensitivity for different compounds are shown in Table S3.

2.8. Electronic tongue (E-tongue) analysis

The taste characteristics of the beer fish samples at different processing stages were determined by E-tongue (TS-5000 Electronic Tongue, Japan) following the method described by (Zhang et al., 2023). A 15 g sample was weighed, and 150 mL of distilled water was added for homogenization. Subsequently, the mixture was centrifuged at 1970*g* for 10 min. The resulting solution was subsequently filtered through gauze and filter paper to provide a suitable solution for testing. A total of 6 sensors were selected for the test: AAE (umami), CT0 (salty), CA0 (sour), C00 (bitter), AE1 (astringency), and GL1 (sweetness).

2.9. HS-GC-IMS analysis

HS-GC-IMS (Flavor Spec® Gas Phase Ion Mobility Spectrometer, G.S. A., Germany) was used to analyze the volatile flavor components according to the method described by Li, Al-Dalali, et al. (2022), with slight modifications. An amount of 2 g of the weighed samples was minced and transferred into a 20 mL headspace vial and incubated at 60 °C for 15 min at a speed of 500 rpm. In the splitless injection mode, the temperature of the injection needle was 85 °C, and the injection volume was 500 μL. Volatiles were separated using an MXT-5 capillary column (15 m × 0.53 mm, 1 μm), and the column temperature was set at 60 °C. The high-purity nitrogen (99.999%) was used as carrier gas/drift gas. The initial flow rate of carrier gas was maintained at 2.0 mL/min for 2 min, linearly increased to 10.0 mL/min within 8 min, and linearly increased to 100.0 mL/min within 10 min. The flow rate of drift gas was set to 150 mL/min. The IMS parameters were as follows: the ionization source was tritium source (^{3}H); the length of the migration tube was 98 mm; the intensity of the electric field was 500 V/cm; the temperature of the migration tube was set to 45 °C; and the ionization process was carried out in positive mode. In order to qualify volatile flavors, the retention index (RI) was calculated after the injection of a mixture of *n*-ketones (C4-C9), and the drift times of RI were compared to those of the GC-IMS library standards and the NIST database. In addition, the area normalization method was applied to determine the relative content of volatile flavor components; the calculation was performed using eq. (1):

$$\text{Relative content of volatile component} = \frac{\textit{Peak area of volatile component}}{\textit{Total peak area of all volatile components}} * 100 \quad (1)$$

2.10. Calculation of taste active value (TAV)

TAV is calculated by dividing the content of each taste component by its threshold. A TAV value larger than 1 indicates that the substance contributes to taste, while a TAV value <1 indicates that the substance does not contribute to taste (Wang, Wu, et al., 2021). From this, the amino acids and nucleotides that are the main taste substances can be determined.

2.11. Data analysis

Laboratory analytical viewer (LAV) processing software, which includes GC-IMS library search and the reporter, was used to analyze the GC-IMS data. Three-dimensional (retention time, migration time, and peak intensity) and two-dimensional (retention time and migration time) top views of volatile compounds in samples could be automatically generated using LAV software. The related two-dimensional top view may more clearly depict the changes and differences of volatile compounds. The three-dimensional map directly shows the differences between volatile compounds in various samples. The gallery plot has been generated by the program's gallery plot plug-in. Three duplicates of each experiment were run. All results are shown as the average ± standard deviation (SD). Microsoft Excel 2010 was used to process the data preliminarily, and then SPSS Statistics17.0 was utilized to examine the significance of the data ($P < 0.05$). The beer fish samples were separated at different processing stages using multivariate statistical analysis, such as principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). Variable importance in projection (VIP) analysis was used to evaluate the significance of volatile components; compounds with VIP > 1 were determined to be statistically significant. Multivariate statistical analyses were carried out using SIMCA 14.1.

3. Results and discussion

3.1. Changes in FAAs during different processing stages of beer fish production

FAAs play a significant role as key taste components in fish meat, serving as a crucial indicator for assessing the taste of aquatic products. Among them, aspartic acid and glutamic acid were identified as umami taste contributors, while glycine, alanine, threonine, proline, and serine contributed to the sweet taste profile. These amino acids collectively enhance fish umami and mellow flavor characteristics (Kong et al., 2017). The data shown in Table 1 reveals that the total FAA content of the RF, UF, FF, and SF samples amounted to 551.71 ± 5.34, 523.67 ± 42.85, 501.17 ± 4.21, and 888.28 ± 18.80 mg/100 g, respectively. Significantly, there was a statistically significant difference in the concentration of FAA in the SF sample compared to the other fish samples at different stages ($P < 0.05$), with the SF sample exhibiting the highest FAA content. During the fish curing process, ultrasound promotes the material exchange between its muscles and the curing solution to dissolve FAAs, resulting in the reduction of its content, and ultrasound can also promote the Maillard reaction, leading to consuming part of the amino acids (Wang et al., 2018). In addition, the frying process leads to further degradation for FAAs, resulting in reduced contents. During the dipping process, the ethanol in the sauce has a detrimental effect on the protein structure, therefore facilitating the penetration of FAAs from light soy sauce, dark soy sauce, oyster sauce, and other seasonings into the fish. Consequently, this process led to increases in the total quantity of FAAs in the final beer fish (SF). The variation in umami amino acid

Table 1
Taste substances and their taste activity values during different stages of beer fish processing.

Free amino acids(mg/100 g)	Taste characteristics	Threshold [a](mg/100 g)	RF		UF		FF		SF	
			FAAs content	TAVs	FAAs content	TAVs	FAAs content	TAVs	FAAs content	TAVs
FAAs										
Asp*	umami/sour (+)	100	3.00 ± 0.17^{b}	0.03	3.40 ± 0.18^{b}	0.03	3.32 ± 0.09^{b}	0.03	8.23 ± 0.66^{a}	0.08
Glu*	umami/sour (+)	30	15.02 ± 0.03^{d}	0.50	22.23 ± 2.56^{b}	0.74	18.80 ± 0.26^{c}	0.63	52.08 ± 0.59^{a}	1.74
Ser**	sweet (+)	150	8.68 ± 0.17^{c}	0.06	11.97 ± 0.99^{a}	0.08	10.56 ± 0.15^{b}	0.07	11.64 ± 0.26^{a}	0.08
His	bitter (−)	20	23.71 ± 0.43^{b}	1.19	10.03 ± 1.65^{d}	0.50	21.25 ± 1.51^{c}	1.06	29.17 ± 0.77^{a}	1.46
Gly**	sweet (+)	130	198.18 ± 1.38^{a}	1.52	115.75 ± 4.02^{c}	0.89	182.20 ± 2.78^{b}	1.40	201.46 ± 1.30^{a}	1.55
Thr**	sweet (+)	260	9.38 ± 0.51^{c}	0.04	14.39 ± 1.76^{a}	0.06	8.86 ± 0.56^{c}	0.03	12.05 ± 0.61^{b}	0.05
Arg	sweet/bitter (+)	50	8.33 ± 0.14^{b}	0.17	10.20 ± 1.44^{a}	0.20	8.14 ± 0.00^{b}	0.16	10.96 ± 0.28^{a}	0.22
Ala**	sweet (+)	60	172.20 ± 0.71^{a}	2.87	178.79 ± 9.28^{a}	2.98	137.09 ± 3.59^{c}	2.28	160.54 ± 1.81^{b}	2.68
Tyr	bitter (−)	–	2.24 ± 0.04^{b}	–	7.54 ± 1.11^{a}	–	1.96 ± 0.09^{b}	–	2.68 ± 2.10^{b}	–
Cys	bitter/sweet/sulfurous (−)	–	0.39 ± 0.03^{b}	–	0.34 ± 0.01^{b}	–	0.67 ± 0.27^{b}	–	267.17 ± 9.59^{a}	–
Val	sweet/bitter (−)	40	5.84 ± 0.07^{c}	0.15	12.43 ± 1.28^{a}	0.31	6.63 ± 0.04^{bc}	0.17	7.57 ± 0.10^{b}	0.19
Met	bitter/sweet/sulfurous (−)	30	4.89 ± 0.08^{b}	0.16	6.00 ± 0.63^{a}	0.20	4.10 ± 0.02^{c}	0.14	5.13 ± 0.02^{b}	0.17
Trp	bitter (−)	–	62.04 ± 2.83^{ab}	–	64.60 ± 12.23^{a}	–	49.59 ± 2.33^{b}	–	64.44 ± 3.52^{a}	–
Phe	bitter (−)	90	2.83 ± 1.04^{b}	0.03	7.13 ± 0.71^{a}	0.08	2.92 ± 1.06^{b}	0.03	4.00 ± 1.28^{b}	0.04
Ile	bitter (−)	90	4.62 ± 0.80^{c}	0.05	10.15 ± 0.61^{a}	0.11	5.70 ± 0.86^{bc}	0.06	6.73 ± 0.96^{b}	0.07
Leu	bitter (−)	190	5.55 ± 0.06^{c}	0.03	14.86 ± 1.56^{a}	0.08	6.50 ± 0.06^{bc}	0.03	7.70 ± 0.01^{b}	0.04
Lys	sweet/bitter (−)	50	19.51 ± 0.74^{c}	0.39	36.47 ± 4.18^{a}	0.73	29.48 ± 0.25^{b}	0.59	27.31 ± 1.13^{b}	0.55
Pro**	sweet/bitter (+)	300	5.29 ± 1.51^{b}	0.02	6.41 ± 1.14^{ab}	0.02	3.41 ± 1.03^{b}	0.01	9.42 ± 3.05^{a}	0.03
TFAA			551.71 ± 5.34^{b}		523.67 ± 42.85^{bc}		501.17 ± 4.21^{c}		888.28 ± 18.80^{a}	
UAA			18.02 ± 0.18^{d}		25.64 ± 2.60^{b}		22.11 ± 0.31^{c}		60.31 ± 0.56^{a}	
SAA			393.74 ± 3.32^{a}		327.30 ± 16.82^{b}		342.10 ± 5.75^{b}		395.11 ± 6.64^{a}	
UAA/TFAA			3.27%		4.81%		4.41%		6.79%	
SAA/TFAA			71.37%		61.45%		68.26%		44.48%	
Nucleotides										
IMP	umami(+)	25	35.01 ± 1.22^{c}	1.40	11.00 ± 1.21^{d}	0.44	62.03 ± 2.33^{a}	2.48	51.01 ± 2.22^{b}	2.04
AMP	umami/sweet(+)	50	7.11 ± 1.34^{a}	0.14	7.08 ± 1.25^{a}	0.14	7.09 ± 1.11^{a}	0.14	6.03 ± 1.04^{b}	0.12

RF, fresh fish; UF, ultrasonic-cured fish; FF, deep-fried fish after marinating; SF, final beer fish; TFAA, total free amino acids; UAA, umami amino acids; SAA, sweet amino acids; [a] threshold values were collected from Chen and Zhang (2007); "*" indicates umami amino acids;"**" indicates sweet amino acids; "(+)" indicates a pleasant taste, and "(−)" indicates a bad taste; "-" indicates that the threshold is not found; different letter in the same row indicate a significant difference ($P < 0.05$).

content in the fish meat samples showed a statistically significant difference ($P < 0.05$). Notably, the SF sample displayed the highest umami amino acid content, followed by UF, FF, and RF. The concentrations of sweet amino acids in RF, UF, FF, and SF samples were measured to be 393.74 ± 3.32, 327.30 ± 16.82, 342.10 ± 5.75, and 395.11 ± 6.64 mg/100 g, respectively, with SF exhibiting a statistically significant higher sweet amino acid content compared to UF and FF ($P < 0.05$). In conclusion, beer fish products have a rich taste profile and superior quality (Table 1).

The taste activity value (TAV) is calculated by dividing the concentration of an amino acid in samples by its threshold value, which is usually determined in water or another matrix (Kong et al., 2017). The TAVs (mg/100 g) and taste perception of FAAs in water were obtained from previous research conducted by Chen and Zhang (2007). Compounds with a TAV >1 were recognized to be active in influencing the taste of food (Kong et al., 2017). The TAV indicator demonstrated significant application in evaluating the taste influence of these substances.

Based on the data shown in Table 1, the TAVs indicate that alanine is the primary umami amino acid in the UF sample, whereas glycine and alanine are the primary umami amino acids in the FF sample. The TAVs of glutamic acid, glycine, and alanine in the SF sample were found to be >1. The TAVs of alanine, glutamic acid, and glycine exhibited high values of 2.68, 1.74, and 1.55, respectively, suggesting that these three amino acids substantially impact the taste profile of the SF sample. The dipping process played a substantial role in influencing the taste of the fish.

Fig. S2 displays the clustering heat map of FAAs, enabling the observation of variations in amino acid composition in fish meat at different stages. The RF sample exhibited high levels of glycine and alanine, while other FAAs were present in low quantities. The UF sample demonstrated a significant amount of bitter amino acids. The majority of FAA content in the FF sample was low. Conversely, the SF sample exhibited high FAA levels, surpassing other stages. Generally, the taste quality of a product is directly correlated with its FAA content (Qi et al., 2017). In conclusion, the dipping process facilitates the generation of FAAs in fish meat, thereby positively influencing the taste of beer fish.

3.2. Nucleotide changes during different processing stages of beer fish production

The quantity of taste nucleotides present in aquatic products has a significant role in determining the taste of meat. In fish meat, the primary taste nucleotides identified are inosinate (IMP) and adenosine monophosphate (AMP) (Qiu et al., 2016). The degradation rate of IMP was relatively slow, resulting in a higher accumulation of IMP within the fish meat. Notably, IMP has a strong umami taste and synergistic effect when coexisting with glutamic acid (Li, Sun, et al., 2022). According to Li, Sun, et al. (2022), the presence of AMP in fish meat can reduce the perception of bitterness and simultaneously enhance the umami taste when combined with IMP. The results of Fig. 1 reveal a notable variation in the IMP content inside beer fish meat at different stages ($P < 0.05$). Specifically, the UF sample exhibited the lowest IMP content, while the FF sample had the greatest IMP level. Ultrasonic waves induce cavitation, which helps in the quick penetration of sodium chloride into fish tissue at a certain period of the curing process. According to Chen, Yan, et al. (2023), this occurrence leads to the loss of juice, increasing IMP loss, and a reduction in its total content. In addition, ultrasound can potentially modify the internal conditions of muscle tissue, induce the activation of nucleotidases, and accelerate the degradation of nucleotides in fish. According to Chen, Yan, et al. (2023), the phenomenon is characterized by a decrease in umami nucleotide IMP and an increase in bitter nucleotide Hx. Following the process of deep-frying, there was an observed rise in the levels of AMP and IMP, accompanied by a reduction in the levels of ATP and Hx. The study's findings revealed that subjecting fish to high-frying temperatures led to the breakdown of ATP, decreasing bitterness, and increasing umami perception. As shown in Table 1, except for the UF sample, the TAV of IMP in fish meat was >1 across all stages. The TAV of the FF sample had a high value of 2.48, signifying that IMP contributed to the umami taste of fresh fish, marinated fried fish, and beer fish. The frying process may significantly enhance the umami taste profile of fish meat.

3.3. E-tongue analysis during different processing stages of beer fish production

Taste perception is initiated when specific chemicals come into touch with the taste receptors situated on the tongue (Munekata et al., 2023). The E-tongue has superior selectivity, sensitivity, and multiplexing capacity when compared to the human tongue. The recent progress with modern biosensors allows the E-tongue to quickly and accurately evaluate the taste of samples. Consequently, it has gained recognition and utilization in various industries, such as pharmaceuticals, environmental monitoring, cosmetics, and food production (Munekata et al., 2023). Fig. 2A depicts the radar diagram of the E-tongue during the different processing stages of beer fish production. According to the reference solution's taste evaluation, all the fish samples tested had undetectable levels of sourness, astringency, bitterness aftertaste, and astringent aftertaste. The taste characteristics of umami, saltiness, and sweetness exhibited increased response values, indicating their importance and prevalence as taste markers. Subsequently, the attributes of richness and bitterness were also recognized as noteworthy characteristics. The observed changes in the taste of the fish meat among the different samples, as seen in Fig. 2B, might potentially be attributable to the influence of processing stages. The RF sample demonstrates pronounced sweetness and umami tastes, but the umami taste of the UF sample decreases, aligning with the taste characteristics of the SF sample. The intensity of the umami taste in the FF sample is the highest. The level of sweetness in the FF sample is similar to that of the UF sample, while the saltiness is close to that of the SF sample. The findings suggest that using ultrasonic curing has a notable impact on reducing the umami taste in fish. Additionally, the umami taste of fish meat is considerably improved following the frying process.

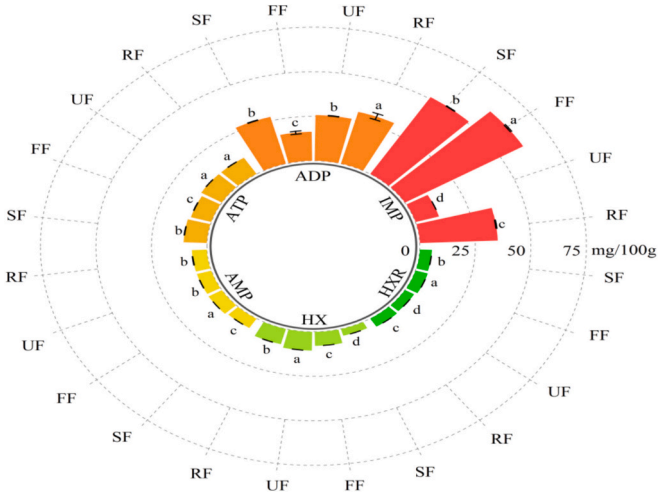

Fig. 1. Nucleotide changes during different stages of beer fish processing.

The results in Fig. 2C indicate that the PC1 and PC2 explained 83.6% of the total variance. The fish samples were scattered throughout distinct areas at each processing stage, demonstrating non-overlapping distributions. This observation suggests a notable variation in the overall taste of fish meat during different processing stages. According to Zhang et al. (2019), the distance between samples on a PCA plot can be used as a measure of how similar or different their tastes are. A smaller distance indicates more similarity, while a larger distance indicates more difference. The RF sample was observed to be positioned towards the left side of the PCA diagram, suggesting its distinct distribution from the other samples. This positioning implies an obvious change in the overall taste of the fish following processing. Furthermore, it is evident that the taste of the RF sample significantly deviates from that of the remaining three samples. The closeness of the distribution distance between the FF and SF samples suggests a greater degree of taste similarity between them (Fig. 2C).

3.4. E-nose analysis during different processing stages of beer fish production

The E-nose is a very efficient and speedy instrument to detect a product's smell without necessitating any particular sample preparation. E-nose metal oxide sensors imitate biological olfactory functions. Thus, they may distinguish meat products by simulating biological organisms' smell sense (Al-Dalali et al., 2022). Fig. 3A illustrates changes in the odor characteristics of beer fish during different processing stages. The figure illustrates notable variations in signal strength among the W2W, W2S, W1W, W1S, and W5S sensors, with response values exceeding 1. The observed variations, together with the sensor's particular responsive substances, indicate that the unique flavor might originate from aromatic chemicals such as inorganic sulfides, nitrogen oxides, methyl groups, alcohols, aldehydes, and ketones that are produced during the preparation of beer fish. Conversely, there appear to be no apparent differences in ammonia levels and certain aromatic compounds, hydrides, long-chain alkanes, and other substances. The study conducted by Liu et al. (2022) revealed that the predominant flavor profile of fried tilapia mostly originated from inorganic sulfides, nitrogen oxides, aldehydes, and esters. Wang, Wang, et al. (2021) demonstrated that beer fish products exhibited a high signal intensity of alcohols, aldehydes, and ketones aromatic compounds. These findings align with the results obtained in the present investigation. The observed response intensities of the samples followed the UF > SF > RF > FF sequence (Fig. 3A). Notably, the ultrasonic curing process yielded the greatest response values on W2W, W2S, W1W, W1S, W5S, and W6S. This finding suggests that ultrasonic curing may enhance the release of flavor components in fish.

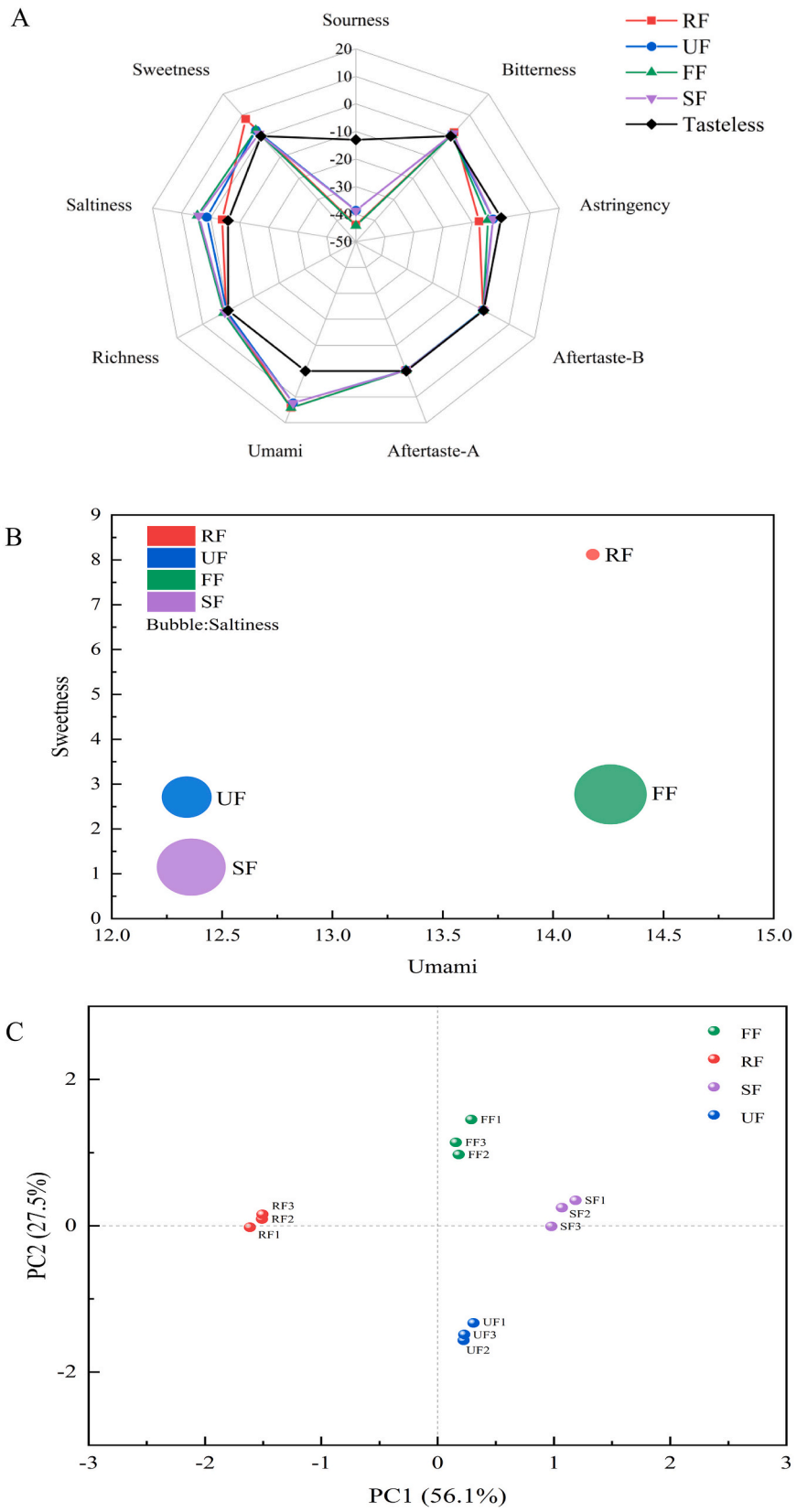

Fig. 2. Electronic tongue radar diagram during different stages of beer fish processing (A); bubble diagram of umami, sweet, and salty tastes of beer fish samples during different processing stages (B); and principal component analysis of E-tongue during different stages of beer fish processing (C).

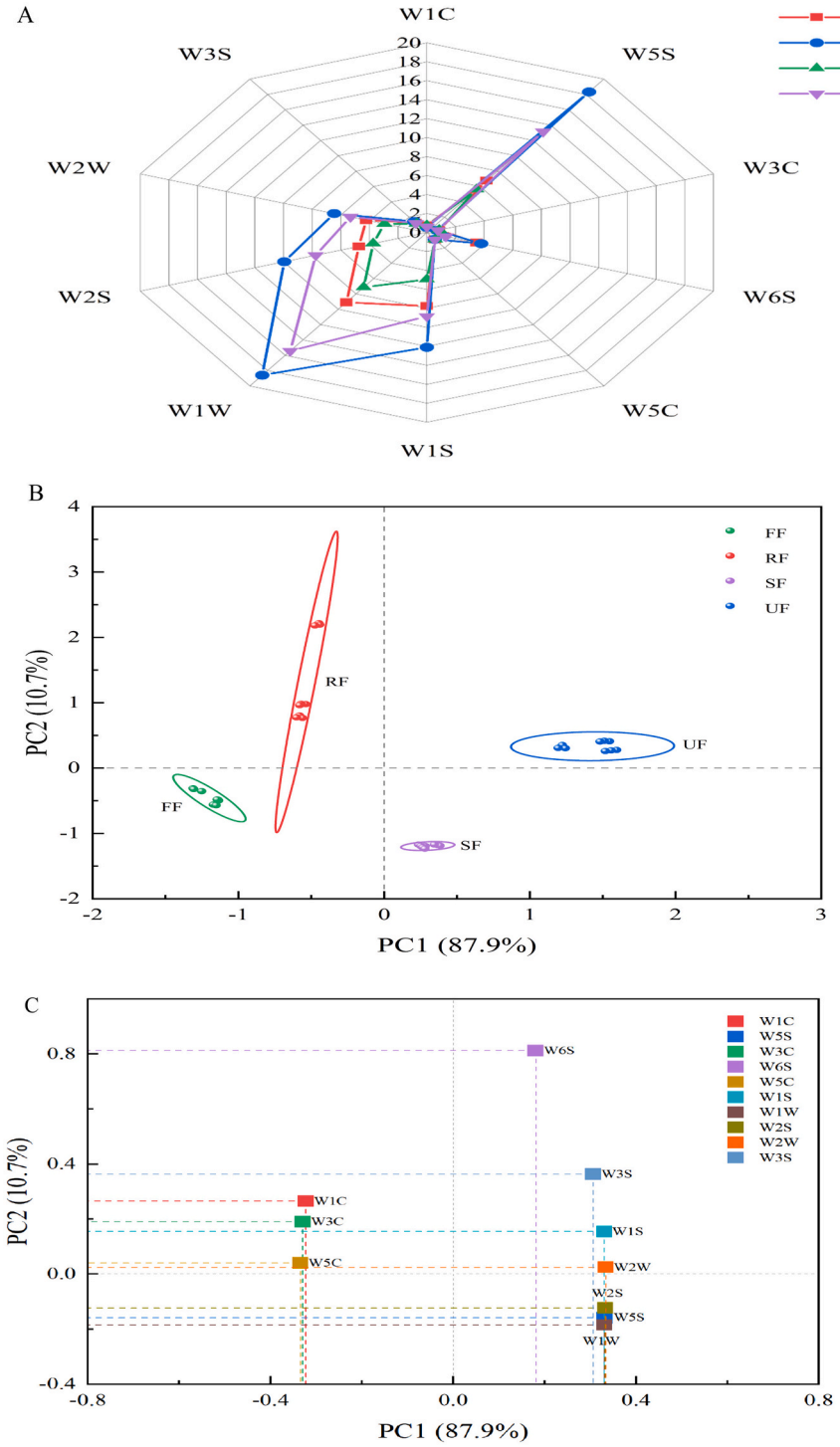

Fig. 3. Radar diagram of the electronic nose during different stages of beer fish processing (A); principal component analysis of electronic nose during different stages of beer fish processing (B); and the analysis of the contribution rate of the loading sensor (C).

The results depicted in Fig. 3B indicate that the E-nose can distinguish the unique fish aroma linked to various processing stages. PC1 and PC2 explained 98.6% of the overall variation. The results suggest that when the value of PC1 exceeds that of PC2, a larger difference is observed as the distance between the samples along the abscissa axis increases. Furthermore, there is a significant disparity between the FF and UF samples.

The loadings assay is associated with PCA, a statistical technique employed to assess the contribution of each sensor in distinguishing samples. This analysis helps to identify the important volatile substances that play an essential role in the process of sample discrimination (Gómez et al., 2006). As seen in Fig. 3C, the left side of the PCA diagram reveals the distribution of the W5C, W3C, and W1C sensors. This distribution indicates that these sensors contribute little to the discrimination of the samples. The remaining sensors are distributed on the right side of the PCA diagram, indicating that they have a large contribution rate to the first principal component, and the W6S sensor has the largest contribution rate on the second principal component, indicating that the second principal component is mainly hydrides.

3.5. Analysis of volatile flavor compounds during different processing stages of beer fish production

3.5.1. Differential analysis of volatile components in beer fish processing

In recent years, GC-IMS has gained popularity due to its accurate measurement of flavor components in food. This technique offers several advantages, including high sensitivity, good stability, fast response, time-saving, and visually distinguishing sample variations (Li, Al-Dalali, et al., 2022; Li, Sun, et al., 2022). Consequently, it provides a simplified technical approach for analyzing volatile components in aquatic products. Fig. 4A illustrates the three-dimensional (3D) spectrum of the volatile components included in the different stages of beer fish

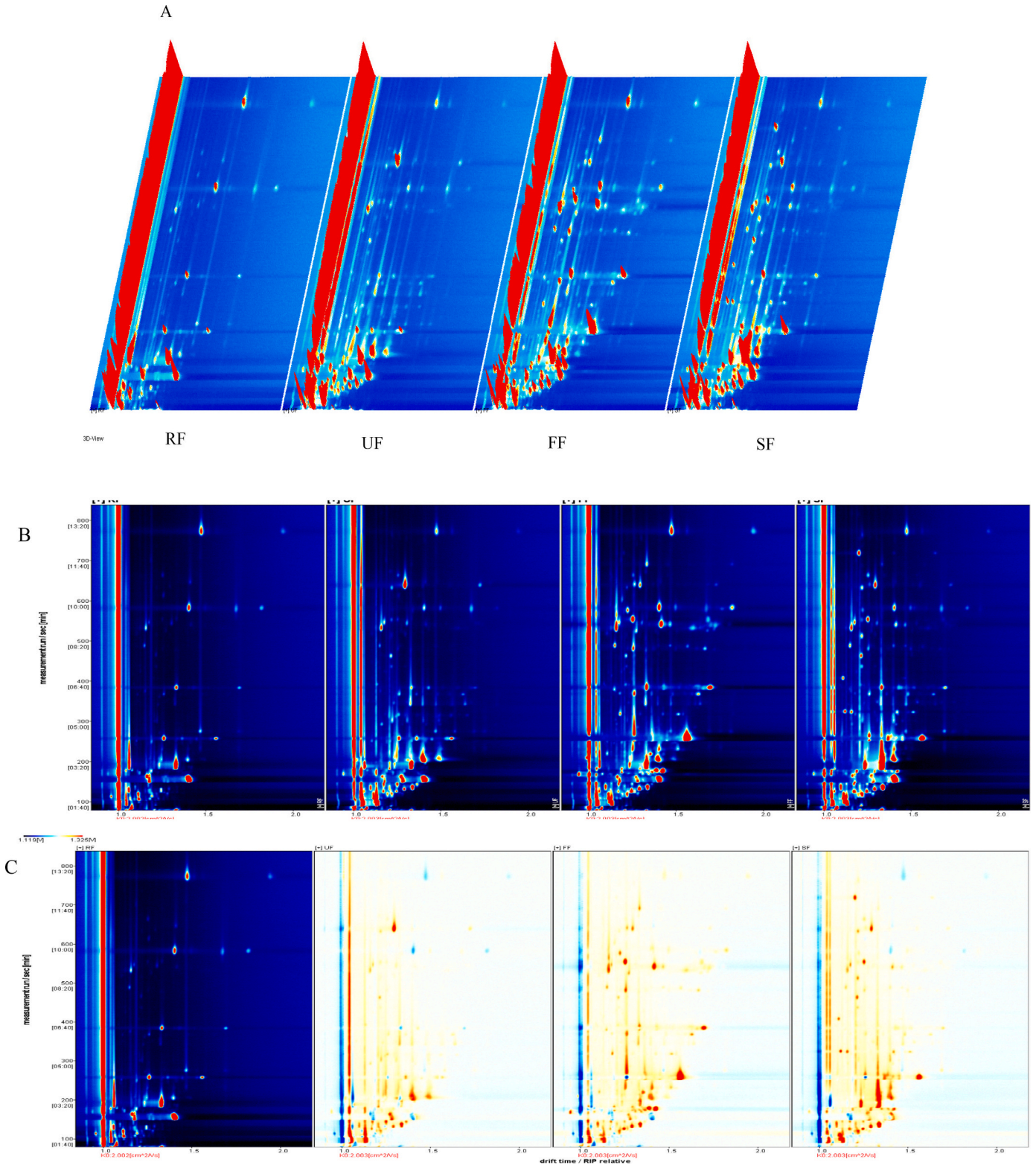

Fig. 4. Three-dimensional spectra of volatile components (A); GC-IMS two-dimensional spectra of volatile components (B); GC-IMS differential spectrum of volatile components (C); and Fingerprint of volatile components during different stages of beer fish processing (D).

D

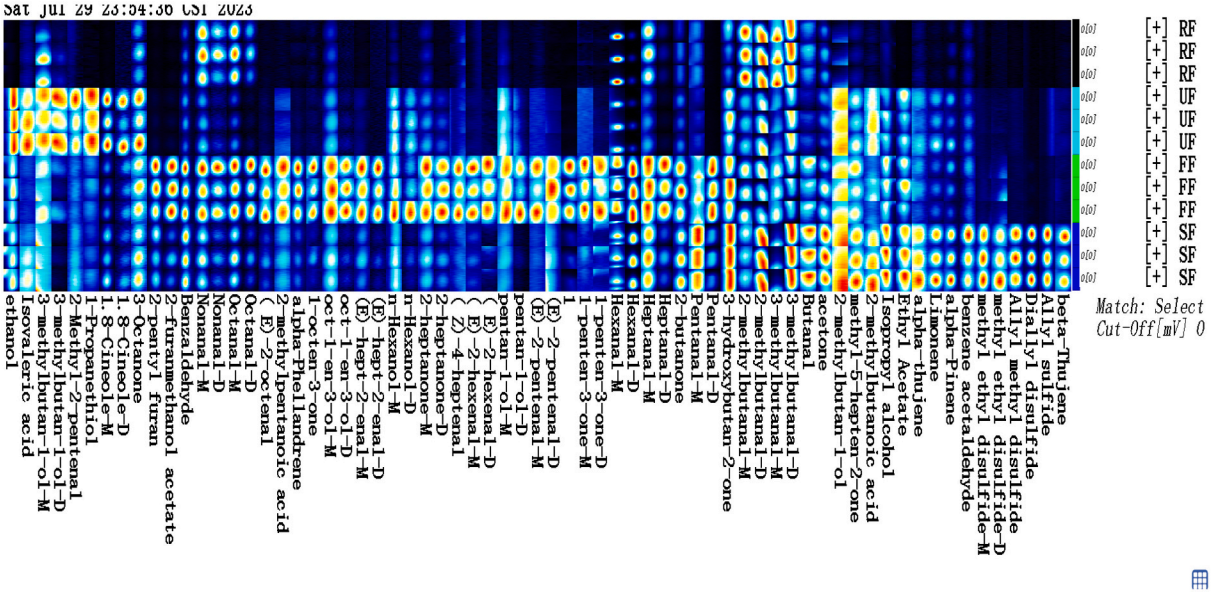

Fig. 4. (*continued*).

processing. The volatile organic compounds exhibit clear differences at different stages of the beer fish preparation. To provide a more comprehensive comparison of the variations in flavor components of beer fish at different stages of processing, we performed dimensionality reduction techniques to obtain the two-dimensional (2D) spectrum shown in Fig. 4B and the difference spectrum depicted in Fig. 4C. The 2D spectrum shows a blue background, while a red vertical line is observed at an abscissa value of 1.0, corresponding to the reactive ion peak (RIP). The ordinate of the graph represents the retention time (s) of the gas chromatography, while the *x*-axis represents the ion migration time (Wang et al., 2022). According to Wang et al. (2022), the RIP peak is represented by dots on both sides, where each dot corresponds to a volatile organic compound. The color of the dot signifies the concentration of the substance, with white indicating lower concentrations, red indicating higher concentrations, and darker colors indicating greater concentrations. The sample codes presented in Fig. 4 are arranged sequentially, moving from left to right, with the following sequence: RF, UF, FF, and SF. The observed data in Fig. 4B and C demonstrate notable variations in the types and concentrations of volatile compounds across the different samples. Moreover, the result demonstrates that the concentration of flavor components in the UF, FF, and SF samples exceeds that of the RF sample. This implies that the application of cooking and processing techniques can potentially improve the production of flavor components. The greater amount of volatile components determined in the FF and SF samples can be attributed to the proteolytic degradation that takes place during the processing of these samples, resulting in the production of more flavor components. Zou et al. (2018) observed a modest rise in the levels of ketones associated with fat oxidation following ultrasonic treatment. In order to provide a more thorough investigation of volatile components among several samples, signal peaks were specifically selected to conduct a fingerprint analysis.

Fig. 4D illustrates the volatile components, with the bright and dark regions within each bright spot representing different amounts of these components. Additionally, the graph displays numbers indicating that the specific compounds have not been identified. Visualization tools enable an in-depth investigation of the volatile components found in individual samples, as well as the variations seen among these samples. The figure illustrates the presence of various aldehydes, including 2-methylbutyraldehyde, 3-methylbutyraldehyde, hexanal, heptanal, octanal, and nonanal. Notably, hexanal, heptanal, and octanal are among the most common fishy compounds in aquatic products. These aldehydes primarily originate from the oxidation of monounsaturated fatty acids or omega-6 polyunsaturated fatty acids (Domínguez et al., 2019). According to Petričević et al. (2018), hexanal exhibits a grassy flavor when present in low quantities, whereas higher amounts result in a greasy flavor. According to Yarnpakdee et al. (2012), heptanal possesses a strong and unpleasant fatty aroma, thereby serving as a significant marker for the degradation of fish flavor. Octanal demonstrates a grassy and oily flavor and can produce both a pleasant and rancid odor when fats and fatty foods break down (Domínguez et al., 2019). The signal intensity of these substances exhibited attenuation in the UF sample. Conversely, ethanol, isovaleric acid, isoamyl alcohol (primarily derived from beer present in the marinade), 2-methyl-2-pentenal, 1-propanthiol (primary volatile components in green onions), eucalyptol, and 3-octanone demonstrated the highest concentration in the UF sample (Ji, 2021; Wang et al., 2008). According to Xi et al. (2023), the use of ultrasound has the ability to greatly enhance the alcohol content. Also, ultrasonic waves can cause the oxidation of alcohol, resulting in the formation of aldehydes and esters.

During the frying process, there is a significant increase in the concentration of aldehydes and ketones. This can be attributed to the oxidative degradation of fatty acids or the Strecker degradation reaction of amino acids. Some examples of these compounds include nonanal, octanal, valeraldehyde, hexanal, trans-2-octenal, trans-2-heptenal, cis-4-heptenal, trans-2-hexenal, 2-heptenone, 2-butanone, 1-octen-3-one, and 1-penten-3-one (see Fig. 4D). It is worth noting that nonanal and octanal are particularly responsible for the oily flavor observed in fried food, making them significant contributors to the overall flavor composition (Al-Dalali et al., 2022). The most prevalent compounds identified in the SF sample are allyl sulfide, diallyl disulfide, allyl methyl disulfide, methyl ethyl disulfide, methyl heptenone, limonene, α-pinene, β-thujene, butyraldehyde, acetone, 2-methyl-1-butanol, isopropanol, ethyl acetate, and phenylacetaldehyde. Among them, allyl sulfide, diallyl disulfide, allyl methyl disulfide, and other sulfur compounds predominantly originate from raw ingredients such as garlic, pickled peppers, and shallots commonly found in sauces (Wang et al., 2008).

Terpenoids possess aromatic fragrances, such as fruity, floral, and herbaceous odors, which contribute to the distinctive sensory attributes of food. These compounds mostly arise from the condiments and additives included in the final product (Ozkara et al., 2019). Ethyl acetate possesses a fruity fragrance and positively affects the flavor profile of beer fish.

3.5.2. Qualitative analysis of volatile components during different processing stages of beer fish production

The GC-IMS apparatus determined the volatile components in fish meat, and the qualitative spectra are shown in Fig. S3. A total of 67 volatile components, including monomers (M), dimers (D), and neutral, were determined in all fish samples, composed of 26 aldehydes, 11 alcohols, 10 ketones, 2 esters, 3 acids, 7 terpenoids, 6 sulfur-containing compounds, 1 furan, and 1 unidentified compound, and the details and relative contents of volatile components are shown in Table 2.

In order to efficiently investigate changes in various volatile compounds, the relative content of volatile components during the beer fish processing was converted based on the signal intensity of the molecules found on the fingerprint, as shown in Fig. S4. It can be seen from Fig. S4 that the volatile components in the different processing stages of beer fish are mainly aldehydes (37.98% ~ 71.67%), ketones (18.36% ~ 24.31%), alcohols (5.83% ~ 21.21%), in addition, there are also a small amount of terpenes, esters (2.20% ~ 14.82%), and trace amounts of acids, furans and sulfur-containing compounds (1.58% ~ 8.36%).

Aldehydes mostly arise from the oxidation of fats, and their presence in beer fish significantly influences its volatile flavor due to their low threshold. The relative content of aldehydes in the RF sample was significantly higher than in other fish stages. Decreasing content of aldehydes through the different processing stages of beer fish could be attributed primarily to the reduction of amino acids, and sugars present in the fish, marinade, and sauce during the curing and dipping process. Additionally, the enzymatic oxidation of fatty acids resulted in the conversion of aldehyde compounds to alcohols, further contributing to the reduction of aldehydes in the samples (Al-Dalali et al., 2022) of other processing stages. According to Uhm and Kim (2018), trans-2-octenal and trans-2-heptenal have a fatty and meaty odor. The concentration of these compounds is notably higher in the FF sample, mostly attributed to the oxidative degradation of fat during the Maillard reaction. Benzaldehyde has nutty and almond notes, whereas benzene acetaldehyde exhibits floral notes (Chang et al., 2020). Notably, both compounds were found in the highest concentrations in the SF sample. Additionally, benzaldehyde increased notably during frying, suggesting that the high temperature may facilitate its formation. According to Wu et al. (2014), 3-methylbutanal and 2-methylbutanal exhibit nutty odors. These compounds are found in significant quantities in fish meat throughout all stages, improving beer fish's flavor profile. During the curing process, ultrasonic-assisted meat products experienced the high-frequency breaking of water molecules. It increased the generation of oxygen free radicals and facilitated the oxidation of proteins and lipids. Ultrasonic treatment can enhance the TBARS value and carbonyl group content in meat products, hence promoting the synthesis of flavor compounds (Xi et al., 2023).

The primary sources of ketones, which have an odor similar to that of burned fat, are alcohol oxidation and the process of lipolysis. Their flavor contribution is little, and their thresholds are much higher than those of other chemical groups. After the RF sample was subjected to ultrasonic pickling and dipping processing, the relative concentration of ketones increased considerably. Zou et al. (2018) reported a rise in the levels of ketones was observed and associated with fat oxidation following ultrasonic treatment. Following ultrasonic curing, the concentrations of 3-hydroxy-2-butanone and 2-heptanone increased considerably, mostly as a result of the flavoring effect of the marinade. In contrast, the concentrations of 1-penten-3-one and acetone, which provide a pungent odor, declined. Such findings were in line with those reported by Xi et al. (2023). This decrease could be attributed to that the ultrasonic treatment may potentially decrease the activity of pyruvate decarboxylase during the process of curing or fermentation (Xi et al., 2023). 1-Octen-3-one and 2-heptanone exhibited a mushroom and fruity odor, which contributed significantly to the flavor of the FF sample, and the pungent odor of acetone was significantly reduced after fish frying.

Alcohols have pleasant fruity and floral odors and are mostly generated by the oxidative breakdown of lipids and reduced carbonyl compounds when heated (Al-Dalali et al., 2022). Most alcohols have high thresholds and contribute little to food flavor, except when they are present in high concentrations or unsaturated forms (Keenan et al., 2012). The RF sample had the lowest relative amount of alcohol; the UF sample had the greatest relative amount, followed by the FF and SF samples. The oxidative degradation of lipids, the reduction of aldehydes, amino acids, and sugars, and the formation of alcohols are all increased by the cavitation action of ultrasound. However, during frying and dipping, alcohols were decreased. This could be attributed to the oxidization of alcohols to generate aldehydes and ketones, or they are esterified with acids to form esters. Ethanol is a characteristic volatile substance of beer, and the high ethanol content is found in the UF sample due to the effective penetration of the marinade due to the mechanical effect of ultrasonic. The relative content of 1-octen-3-ol in the RF sample was 1.259%, showing a fishy odor, while its relative content in the FF sample was the highest (2.151%). At this time, 1-octen-3-ol was formed by the oxidation of linoleic acid, showing a mushroom odor, contributing to the overall flavor profile of beer fish products (Zhang et al., 2019).

Terpenoids mainly form from the homolysis of fatty acid alkoxy radicals, exhibiting floral, herbal, sweet, and fruity odors. These compounds possess high odor thresholds and provide little contribution to the overall flavor profile of food products. Terpenes can undergo chemical reactions under specific circumstances, forming chemicals like aldehydes and ketones. These chemical compounds can potentially influence the overall flavor characteristics of beer fish (Kolanowski et al., 2007). The terpene content notably increased compared to the RF sample throughout all processing stages. Moreover, the UF sample displayed the highest terpene content, followed by the SF sample. These findings suggest that the seasoning and additional ingredients present in the marinade and sauce played a crucial role in contributing to the hydrocarbon content.

Ester compounds are formed by lipid metabolism or the esterification of alcohols and carboxylic acids, resulting in a fruit-like or distinctive aromatic odor (Keenan et al., 2012). The concentration of esters in the FF sample exhibited a notable increase compared to earlier processing stages. This observation might be attributed to the potential occurrence of heat oxidation, wherein some aldehydes transform into acids, followed by further reactions between these acids and alcohols to provide esters (Keenan et al., 2012). This increase is in agreement with the concentration reduction of alcohols and aldehydes after the frying process, indicating that these compounds have been transformed into esters under the thermal process (Xi et al., 2023). The current investigation demonstrated a substantial decrease in the level of esters in the ultrasonic treatment group compared to the FF group. Lipids are very susceptible to oxidation and degradation during the preparation of meat products due to factors such as temperature, humidity, and microbes (Xi et al., 2023). Ultrasound deactivates bacteria, which hinders their metabolism throughout the entire curing process, leading to a reduced amount of esters (Xi et al., 2023). Acids can be cleaved from long-chain fatty acids. Its high threshold and low concentration make it only a slight flavor contributor.

Furans can be generated from the Maillard reaction between amino acids and reducing sugars and the pyrolysis reaction of amino acids and thiamine, which have a marked meaty odor and are present in cooked products (Domínguez et al., 2019). 2-Amylfuran is predominantly present in the FF sample, has a low threshold, and has a pleasant aroma with sweet and burnt.

Table 2
Volatile flavor compounds during different stages of beer fish processing by GC-IMS.

Serial number	Compound	CAS#	Formula	MW	RI	Rt[s]	Dt[ms]
Esters							
55	Ethyl Acetate	C141786	$C_4H_8O_2$	88.1	587.8	136.473	1.33764
8	2-Furanmethanol acetate	C623176	$C_7H_8O_3$	140.1	982.5	541.608	1.41264
Aldehydes							
1	Nonanal-M	C124196	$C_9H_{18}O$	142.2	1099.4	770.13	1.47636
15	Nonanal-D	C124196	$C_9H_{18}O$	142.2	1100.9	773.576	1.95116
6	Octanal-M	C124130	$C_8H_{16}O$	128.2	1002.1	581.815	1.40327
16	Octanal-D	C124130	$C_8H_{16}O$	128.2	1002.9	583.109	1.82164
27	Heptanal-M	C111717	$C_7H_{14}O$	114.2	896.6	385.297	1.33335
28	Heptanal-D	C111717	$C_7H_{14}O$	114.2	895.8	384.025	1.70075
34	Hexanal-M	C66251	$C_6H_{12}O$	100.2	784	257.58	1.26298
35	Hexanal-D	C66251	$C_6H_{12}O$	100.2	784.5	258.081	1.5636
60	Pentanal-M	C110623	$C_5H_{10}O$	86.1	690.6	177.894	1.1883
65	Pentanal-D	C110623	$C_5H_{10}O$	86.1	691	178.194	1.42359
50	2-Methylbutanal-M	C96173	$C_5H_{10}O$	86.1	662	164.921	1.16343
52	2-Methylbutanal-D	C96173	$C_5H_{10}O$	86.1	657.2	162.931	1.40066
51	3-Methylbutanal-M	C590863	$C_5H_{10}O$	86.1	631.7	152.674	1.17862
53	3-Methylbutanal-D	C590863	$C_5H_{10}O$	86.1	627.8	151.143	1.40843
14	(*E*)-Hept-2-enal-M	C18829555	$C_7H_{12}O$	112.2	953.5	482.719	1.25739
24	(*E*)-Hept-2-enal-D	C18829555	$C_7H_{12}O$	112.2	953.4	482.607	1.66971
62	(*E*)-2-Pentenal-M	C1576870	C_5H_8O	84.1	744.8	220.516	1.1088
63	(*E*)-2-Pentenal-D	C1576870	C_5H_8O	84.1	743.7	219.616	1.35483
42	(*E*)-2-Hexenal-M	C6728263	$C_6H_{10}O$	98.1	843.4	318.208	1.18168
43	(*E*)-2-Hexenal-D	C6728263	$C_6H_{10}O$	98.1	843.1	317.871	1.51653
12	Benzaldehyde	C100527	C_7H_6O	106.1	956.5	488.599	1.14909
3	Benzene acetaldehyde	C122781	C_8H_8O	120.2	1048.1	664.266	1.25522
19	(*E*)-2-Octenal	C2548870	$C_8H_{14}O$	126.2	1062.5	692.504	1.33107
38	2-Methyl-2-pentenal	C623369	$C_6H_{10}O$	98.1	820.5	293.396	1.16232
41	(*Z*)-4-Heptenal	C6728310	$C_7H_{12}O$	112.2	894	381.309	1.14715
58	Butanal	C123728	C_4H_8O	72.1	534.3	119.064	1.2807
Alcohols							
36	*n*-Hexanol-M	C111273	$C_6H_{14}O$	102.2	865.3	343.99	1.32917
39	*n*-Hexanol-D	C111273	$C_6H_{14}O$	102.2	864.2	342.737	1.64357
44	Pentan-1-ol-M	C71410	$C_5H_{12}O$	88.1	757.6	232.027	1.25213
45	Pentan-1-ol-D	C71410	$C_5H_{12}O$	88.1	758	232.389	1.51301
46	2-Methylbutan-1-ol	C137326	$C_5H_{12}O$	88.1	734.8	211.98	1.233
47	3-Methylbutan-1-ol-M	C123513	$C_5H_{12}O$	88.1	728.7	206.877	1.24827
48	3-Methylbutan-1-ol-D	C123513	$C_5H_{12}O$	88.1	730.1	208.029	1.4912
57	Isopropyl alcohol	C67630	C_3H_8O	60.1	529.3	117.563	1.22053
59	Ethanol	C64175	C_2H_6O	46.1	447.3	95.352	1.13136
9	Oct-1-en-3-ol-M	C3391864	$C_8H_{16}O$	128.2	978	531.937	1.15964
23	Oct-1-en-3-ol-D	C3391864	$C_8H_{16}O$	128.2	978.2	532.549	1.59662
Ketones							
18	3-Octanone	C106683	$C_8H_{16}O$	128.2	983.7	544.289	1.30527
22	1-Octen-3-one	C4312996	$C_8H_{14}O$	126.2	975	525.757	1.2716
29	2-Heptanone-M	C110430	$C_7H_{14}O$	114.2	884.1	367.795	1.26259
30	2-Heptanone-D	C110430	$C_7H_{14}O$	114.2	884.3	368.113	1.62999
49	3-Hydroxybutan-2-one	C513860	$C_4H_8O_2$	88.1	702.6	186.613	1.33139
56	2-Butanone	C78933	C_4H_8O	72.1	557.3	126.268	1.24632
61	Acetone	C67641	C_3H_6O	58.1	496	107.958	1.12062
66	1-Penten-3-one-M	C1629589	C_5H_8O	84.1	674.1	170.09	1.07979
67	1-Penten-3-one-D	C1629589	C_5H_8O	84.1	674.8	170.39	1.31078
10	Methyl-5-hepten-2-one	C110930	$C_8H_{14}O$	126.2	985.6	548.224	1.17464
Acids							
20	2-Methylpentanoic acid	C97610	$C_6H_{12}O_2$	116.2	1036.6	642.734	1.26271
37	Isovaleric acid	C503742	$C_5H_{10}O_2$	102.1	829.5	302.914	1.22161
40	2-Methylbutanoic acid	C116530	$C_5H_{10}O_2$	102.1	840.2	314.685	1.20644
Terpenoids							
5	Limonene	C138863	$C_{10}H_{16}$	136.2	1031.7	633.729	1.21961
21	*alpha*-Phellandrene	C99832	$C_{10}H_{16}$	136.2	999.4	577.297	1.22339
13	*alpha*-Pinene	C80568	$C_{10}H_{16}$	136.2	942.6	462.334	1.21441
25	*alpha*-Thujene	C2867052	$C_{10}H_{16}$	136.2	928.2	436.66	1.21561
11	*beta*-Thujene	C28634891	$C_{10}H_{16}$	136.2	968.4	512.088	1.21774
4	1.8-Cineole-M	C470826	$C_{10}H_{18}O$	154.3	1035.1	639.836	1.29832
17	1.8-Cineole-D	C470826	$C_{10}H_{18}O$	154.3	1035.3	640.319	1.728
Sulfur-containing compounds							
2	Diallyl disulfide	C2179579	$C_6H_{10}S_2$	146.3	1075.9	719.743	1.20462
26	Allyl methyl disulfide	C2179580	$C_4H_8S_2$	120.2	911.2	408.293	1.11142
31	Allyl sulfide	C592881	$C_6H_{10}S$	114.2	849.4	325.153	1.12244
32	Methyl ethyl disulfide-M	C20333395	$C_3H_8S_2$	108.2	848.3	323.88	1.15782
33	Methyl ethyl disulfide-D	C20333395	$C_3H_8S_2$	108.2	848	323.452	1.42156
54	1-Propanethiol	C107039	C_3H_8S	76.2	611.6	145.02	1.36334
Furans							
7	2-Pentyl furan	C3777693	$C_9H_{14}O$	138.2	987.7	552.805	1.25335
other							
64	1	unidentified	*	0	691.4	178.495	1.38921

Note: "1" is an unidentified volatile compound.

3.6. Multivariate statistical analysis of volatile flavor compounds by GC–IMS in the processing of beer fish

PCA was performed on the volatile composition data acquired from GC-IMS, and the results are displayed in Fig. 5A. The figure demonstrates that PCA1 (47.6%) and PCA2 (29.1%) collectively explain 76.7% of the total variance, providing an overview of the samples' overall characteristics. Each group was distributed in a relatively independent area, indicating that the volatile components of fish changed greatly at different processing stages. The distance between the FF sample and other samples is far, indicating that its volatile components are significantly different from other processing stages. In summary, PCA can effectively identify and distinguish fish flavors at different processing stages.

The supervised PLS-DA technique was used to evaluate the volatile flavor components to understand better the individual volatile components that contribute to the differences in flavor observed throughout different stages of beer fish processing. A PLS-DA model was constructed using 67 qualitative compounds as *x* variables and fish samples at different processing stages as *y* variables. The discriminant effect of the model is illustrated in Fig. 5B. The model's R^2X value of 0.956, R^2Y value of 0.984, and Q^2 value of 0.968 indicate that it is highly reliable and capable of accurately predicting and differentiating the flavor of beer fish at different stages of processing. Then, the permutation test was carried out to obtain $R^2 = (0.0,0.114)$, $Q^2 = (0.0, -0.52)$. A total of 2 groups of models were fitted and verified. All R^2 values were positioned above the horizontal axis at 0, and the Q^2 regression line had a negative slope, suggesting the model's reliability and lack of overfitting. Additionally, the model displayed a strong correlation (Fig. 5C). To better understand the main flavors contributing to the difference among the different stages of beer fish processing, we obtained a VIP value diagram. Taking VIP $>$ 1 as the standard, the higher the VIP value, the greater the contribution of the variable to the distinction between groups. Fig. 5D displays the screening results of 35 volatile compounds with VIP scores of $>$1. These compounds comprise 10 aldehydes, 8 alcohols, 6 terpenoids, 6 sulfur-containing compounds, 2 ketones, 2 acids, and 1 ester. 1-Propanol has a VIP value of 1.3, making it the most significant distinguishing compound. It is followed by isoamyl alcohol, ethanol, and eucalyptol, which all have VIP values of $>$1.2.

In addition, the 35 key compounds that were recognized by the VIP tool were analyzed for cluster analysis, and the results are shown in Fig. 5E. Based on Fig. 5E, region A in the RF sample contains the volatile components with significant content, such as nonanal, octanal, 3-methylbutanal, and 2-methylbutanal. Region B has the most significant quantity of volatile components in the UF sample, such as isovaleric acid, 2-methyl-butanol, 2-methyl-2-pentenal, 3-octanone, 1-propanethiol, and others. The volatile components included in region C of the FF sample are nonanal, octanal, 1-pentanol, n-hexanol, and 2-methyl-1-butanol, which contribute significantly to its odor. Region D represents the volatile flavor of the SF sample, mainly consisting of volatile compounds such as 2-methylbutanal, 2-methylbutanoic acid, α-pinene, α-thujene, limonene, methyl ethyl disulfide, and allyl methyl disulfide. The results above were in accordance with the GC-IMS fingerprint.

Lipids play a vital role in the synthesis of volatile flavor compounds in meat products, and the rate of lipid oxidation is strongly linked to the development of unique flavors (Al-Dalali et al., 2022). In this study, it was shown that eighteen out of the thirty-five substantial volatiles that have VIP>1 belong to aldehydes and alcohols were produced by lipid oxidation. Therefore, the pathway of lipid oxidation might be a critical factor in enhancing the flavor characteristics of beer fish through ultrasonic treatment. Lipid oxidation is comprised of autoxidation and enzymatic oxidation. Enzyme oxidation has been recognized as a crucial process for enhancing the flavor of meat products (Al-Dalali et al., 2022; Zhang et al., 2021). In addition, ultrasound can affect the functioning of naturally occurring enzymes through the mechanical force and cavitation effect on the muscles of animals (Zhang et al., 2021). The process of generating volatiles by enzymatic oxidation is as described below: lipids are initially degraded by lipases into free fatty acids (FFAs). The resulting FFAs are then subsequently oxidized into different volatiles, such as alcohols, aldehydes, and ketones, with the participation of Lipoxygenase (Zhang et al., 2021).

4. Conclusion

This study aimed to analyze the flavor changes of beer fish during various processing stages and identify the distinctive flavor components of the final beer fish products (SF sample). The findings of this study serve as a theoretical foundation for producing and processing fresh fish in terms of flavor chemistry. The study determined that frying and dipping are the primary procedures responsible for enhancing the taste of beer fish. The dipping process facilitated the generation of FAAs in fish meat. Frying caused the degradation of ATP into AMP and IMP, with the TAV value of IMP reaching the maximum. This led to a considerable enhancement of the fish's umami taste. The E-tongue and E-nose can accurately discriminate the taste and odor characteristics of beer fish at different stages of processing. Of all the samples, the umami taste in the FF sample is the most intense, resembling the taste of the SF sample. The FF stage had a significant effect on the odor of the SF sample, but the RF and UF stages had little influence on its odor. The GC-IMS analysis identified a total of 67 volatile compounds in all samples. These compounds included 26 aldehydes, 11 alcohols, 10 ketones, 2 esters, 3 acids, 7 terpenoids, 6 sulfur-containing compounds, 1 furan, and 1 unidentified substance. The relative content of aldehydes and ketones was found to be high, with aldehydes constituting $>$37% of the total compounds detected. After the application of ultrasonic pickling, the levels of fishy compounds such as hexanal, heptanal, and octanal reduced, while the levels of eucalyptol, 3-octanone, 3-hydroxy-2-butanone, and 2-heptanone increased. During the process of frying, the concentration of aldehydes and ketones with fat, mushroom, and fruity odors, such as *trans*-2-heptenal, trans-2-octenal, *cis*-4-heptenal, 1-octen-3-one, and 2-butanone, observed a substantial increase. This increase dramatically enhanced the overall flavor quality of the fish. In further investigations, the combination of gas chromatography–mass spectrometry, gas chromatography-olfactometry-mass spectrometry, metabolomics, and other technologies may be used to provide a scientific basis for understanding the flavor formation mechanism of beer fish and improve and enhance its flavor quality.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

CRediT authorship contribution statement

Yingying Liu: Writing – original draft, Methodology, Investigation, Conceptualization. **Sam Al-Dalali:** Writing – review & editing, Writing – original draft, Investigation. **Yan Hu:** Visualization, Formal analysis. **Dong Zhao:** Validation, Supervision. **Jinghan Wang:** Project administration, Data curation. **Zhigui He:** Writing – review & editing, Resources, Funding acquisition, 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

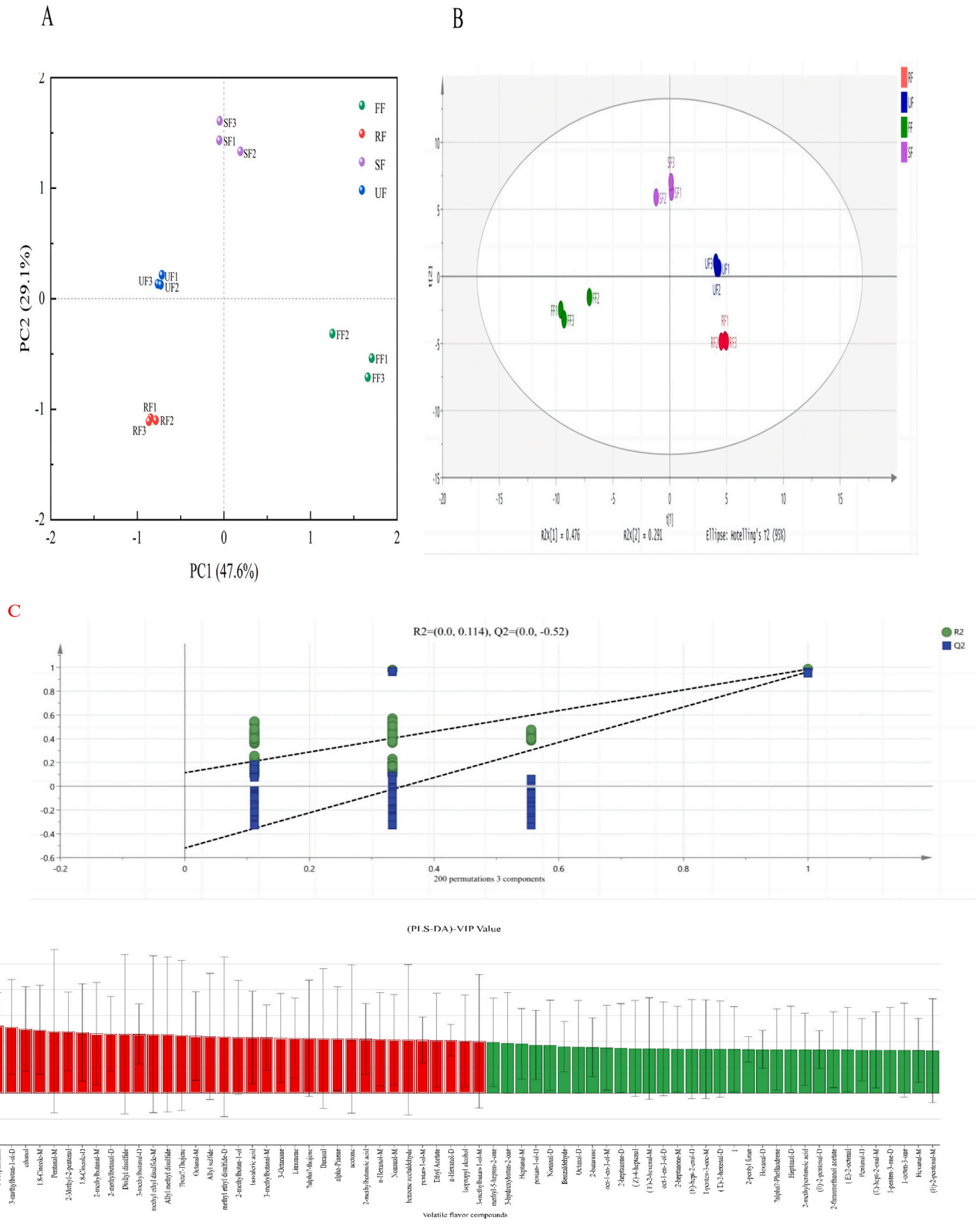

Fig. 5. PCA (A); PLS-DA (B); model cross-validation diagram (C); VIP score of the PLS-DA model (D); and cluster analysis of key compounds (E) of volatile flavor compounds during different stages of in beer fish processing. In Fig. 5D, the shading in red color indicates the flavor compounds that have VIPs>1, and the shading in green color indicates the flavor compounds that have VIPs<1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

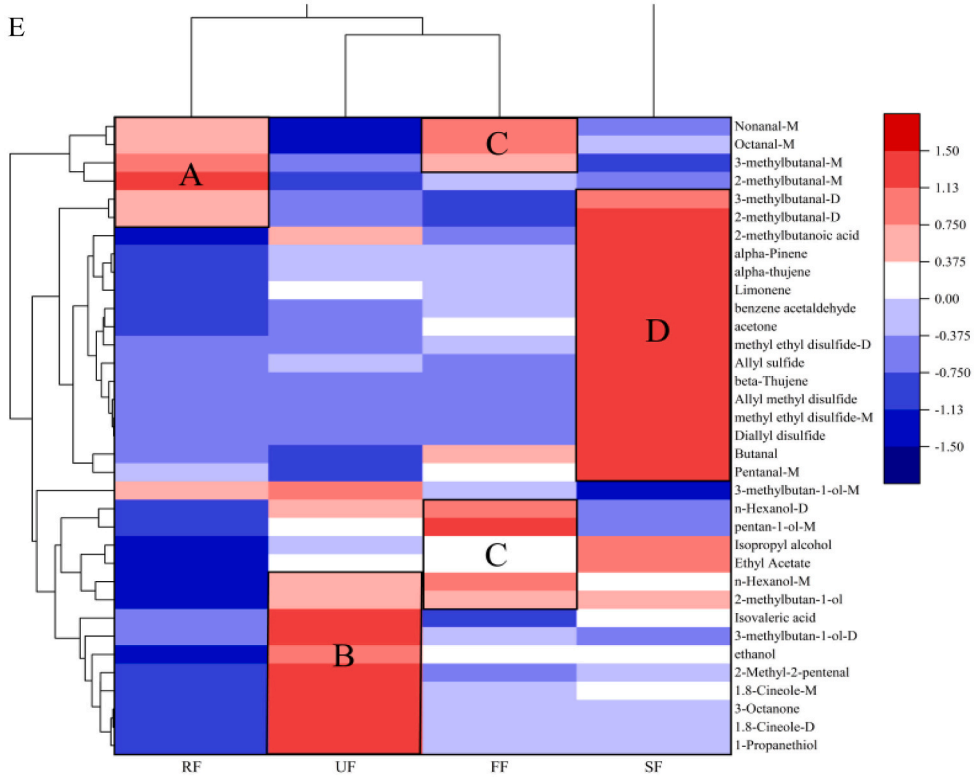

Fig. 5. (*continued*).

the work reported in this paper.

Data availability

Data will be made available on request.

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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.2024.101623.

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