

Identification of characteristic flavor profiles and reheat-induced warmed-over flavor production of bone soup-stewed pickled Chinese cabbage

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ABSTRACT

This study aimed to identify the composition of characteristic flavor profile in bone soup-stewed pickled Chinese cabbage (BSS), focusing on the effect of reheating-induced lipid oxidation on the warmed-over flavor (WOF) production. Results revealed that Alanine (Ala) and Asparagine (Asn) were the key flavor amino acids (FAAs), and the percentage of C18:1n9c reached 47.9 %. Eight substances including Benzaldehyde (M), 1-octen-3-ol, and 2-heptanone (D), etc. were identified as the key volatile organic compounds (VOCs) in BSS. Furthermore, six substances including Benzaldehyde (D) and 2-n-pentyl-furan, etc. were found to be associated with WOF after reheating (BSS-R). TG (16:1_18:1_18:1) + NH₄ and TG (16:0_18:2_18:3) + NH₄ were identified as the main flavor-forming lipids. 104 differential lipids were screened (VIP > 2, *P* < 0.05), of which PC (16:0_20:5) + H and PC (38:7) + H, were the crucial precursors in WOF formation. This study provides a reference for the mechanism of nutritional quality and flavor formation during thermal processing.

1. Introduction

Pickled Chinese cabbage is a fermented vegetable food with a long history in China, especially in the Northeast, and it is loved by most people (Du, Wu, Sun, & Yue, 2013). Pickled Chinese cabbage is a product made from cabbage through natural fermentation, using local cabbage as the processing raw material, fermented by lactic acid bacteria and other microorganisms in a low-salt environment, and the fermentation is completed after thirty days. Pickled Chinese cabbage not only retains the original nutrients of vegetables, but also contains functional microorganisms such as lactic acid bacteria (J. W. Wang et al., 2023). Therefore, pickled Chinese cabbage not only has a unique rich flavor but also has the functions of cooling and refreshing, quenching thirst, regulating the micro-ecology of the human intestinal tract, and lowering cholesterol.

In the Northeast, pickled Chinese cabbage is often cooked in a stew. Stewing is one of the traditional Chinese cooking techniques, through which the nutrients in the ingredients, such as proteins, peptides, flavorful nucleotides, and flavor amino acids (FAAs), are fully dissolved

into the soup (Yue et al., 2025). With the advent of food industrialization, the current market of industrialized traditional dishes faces a limited number and a relatively homogeneous variety of problems. Currently, there are research reports on the processing technology and industrialization of traditional Chinese dishes. Some scholars have investigated the processing technology and quality of beef stew (Jiang et al., 2024), roasted beef with potatoes (J. M. Liu et al., 2024), salt-baked chicken (Y. Xu et al., 2025), etc. The studies show that by optimizing the processing technology and production technology of the dishes, it is possible to produce dishes with rich nutrition, unique flavor, and good taste. Therefore, the development of pickled Chinese cabbage dishes into high-quality convenience foods is of great practical significance for the industrial upgrading and economic and social development of Chinese cuisine.

The production of volatile organic compounds (VOCs) is not only dependent on the original small molecules of free flavor presenting substances (small molecule clusters), but more critically, is formed by nutrients such as proteins and lipids through a series of chemical reactions, such as oxidation and degradation of fats, pyrolysis of amino

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acids and peptides, and decomposition of thiamine (Y. A. Lv et al., 2024). During the stewing process, proteins are broken down into small molecular peptides by heat, and these small molecular peptides are further hydrolyzed to produce FAA. During stewing, lipids are pyrolyzed to generate the free fatty acids (FFA), which are volatilized under heating conditions to form flavor substances. Unsaturated fatty acids (UFA) contain double bonds, which are further oxidized during the heating process, generating ketones, aldehydes, acids, and other flavor compounds that play an important role in the formation of volatile carbonyl compounds or participate in the Merad reaction to generate volatile heterocyclic compounds, which enhances the flavor of the meat (H. J. Wang et al., 2022).

While heat treatment promotes the formation of product flavor, it may lead to excessive lipid oxidation due to secondary heating of prepared foods, which in turn produces warmed-over flavor (WOF) and reduces their sensory quality. In addition, lipid over-oxidation may generate toxic compounds such as free radicals, peroxides, and malondialdehyde (Kim, Li, Lim, Kang, & Park, 2016). It was shown that second-heated, precooked beef stew exhibited significant WOF after reheating, as evidenced by diminished meat flavor and enhanced fat flavor (J. M. Liu et al., 2024).

In this study, the sensory indicators and flavor substances of BSS prepared food were determined, and the characteristic flavor profiles of BSS were identified. The key flavor substances and differential lipids of WOF flavor were screened by GC-IMS with lipidomics for reheated bone soup-stewed pickled Chinese cabbage (BSS-R) to reveal the pathways involved in WOF formation. The results of the study provide a theoretical basis for the flavor study of BSS.

2. Materials and methods

2.1. Materials

Fresh pork bone, spices, and salt were purchased from the Shenyang Food market (Shenyang, China). The pickled Chinese cabbage was purchased from Shifeng Sauerkraut Industrial Park Co., Ltd. (Shenyang, China). The lard was purchased from Henan Shuanghui Food Co., Ltd. (Luohe, China). The amino acid standard substances and 2-octanol (of analytical purity) were purchased from Shanghai Yuan Ye Biotechnology Co., Ltd. (Shanghai, China). Acetonitrile, isopropanol, methanol 14 % Bor trifluoride - methanol, and normal ketones, were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, Chian). Methyl tert-butyl ether, MTBE, was sourced from Sigma-Aldrich, Inc. (USA). Other analytical and chemical reagents were purchased from Macklin Biochemical Technology Co. Ltd. (Shanghai, China).

2.2. Sample preparation

The pork bones were soaked for 2 h to remove blood water, and 2.5 L of pure water and 500 g of pork bones were placed into the stockpot. After the mixture was brought to a boil over high heat, the scum was skimmed off. Then 2 g of pepper, 3 g of aniseed, and 2 g of ginger were added, and the soup continued to boil. The initial power of the electric clay was set to 2200 W until the soup boiled and then adjusted to 100–200 W to maintain a slight boiling state. After boiling for 3 h, a base of pork bone soup was obtained. The commercially available pickled Chinese cabbage was divided into two parts: the cabbage and the cabbage juice. The pickled Chinese cabbage was rinsed twice, drained, and mixed with 300 mL of water, 30 mL of cabbage juice, 4 % lard, and 0.3 % salt per 70 g. After boiling, it simmered for 1 h on low heat to make the water-stewed pickled Chinese cabbage, named CK. 70 g of pickled Chinese cabbage, 30 mL of cabbage juice, 4 % lard, and 0.3 % salt were added to 300 mL of bone soup. After boiling, it simmered for 1 h on low heat, creating bone soup-stewed pickled Chinese cabbage, named BSS. After BSS was cooled to room temperature, it was vacuum-packed in heat-resistant aluminum foil bags (each bag containing 400 g). It was

then frozen for 7 d. After freezing, the samples were taken out and heated in boiling water using an induction cooker with the aluminum foil packaging on for 25 min. The reheated bone soup-stewed pickled Chinese cabbage was then made into, named BSS-R.

2.3. Determination of soluble solids

The soluble solids content was performed using an Abbey refractometer (Shanghai INESA Physical Optics Co., LTD., China). The instrument was calibrated and zeroed using purified water. A 0.3 mL sample aliquot was transferred to the measurement cell. Prior to each subsequent measurement, the measurement cell was thoroughly cleaned and dried.

2.4. Color measurement

A CR-10plus colorimeter (Konica Minolta Holdings Inc., Japan) was utilized to measure the three-color components: L^* , a^* , and b^* . The sample cup was placed in the recessed area of the sample aperture plate after adding 20 mL of the sample and covered with a black cup cover. The whiteness (W) was calculated according to eq. (1).

$$W = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (1)$$

where L^* represents lightness; a^* represents red and green chromaticity; b^* represents yellow-blue degree; W represents whiteness.

2.5. Determination of soluble protein

The soluble protein was determined by bradford method, 5 mL of soup was taken in a test tube, 10 mL of G250 was added, and the mixture was homogenized. The absorbance was determined at 595 nm by UV spectrophotometer (Beijing Puxi Co.,Ltd).

2.6. Determination of lipid oxidation

Lipid oxidation was evaluated by determining the TBARS values and POV values using the methods described by Tikik, Haugen, Andersen, and Aaslyng (2008).

2.7. Determination of free amino acid (FAA) content

The sample 5 mL was gently mixed with 5 mL 10 % trichloroacetic acid for 20 min under room temperature, and then centrifuged at 10,000 rpm for 10 min. The sample was filtered through a 0.22 μ m water membrane, and 400 μ L of the supernatant was taken for injection. The FAA content was determined by HPLC (Agilent Technologies, Santa Clara, CA, USA), coupled with a C18 column (40 mm \times 125 mm, 5 μ m). The analytical conditions were as follows: the detection wavelength was 254 nm; the injection volume was 1 μ L; the flow rate was 1 mL/min; the column temperature was 40 $^{\circ}$ C; the flow rate was 1 mL/min. Quantification was carried out by utilizing standard curves of 17 amino acid standards under the same conditions.

2.8. Determination of free fatty acid (FFA) content

The FFA content of samples was determined as described by and Indrasti, Man, Mustafa, and Hashim (2010), using 1100 GC-MS (Agilent Technologies, Santa Clara, CA, USA) and the INNOWax capillary column (30 m \times 0.32 mm, 0.25 μ m). The analytical conditions were as follows: the starting temperature was 140 $^{\circ}$ C, held for 2 min, 200 $^{\circ}$ C at 6 $^{\circ}$ C/min and held for 2 min, then to 230 $^{\circ}$ C at 2 $^{\circ}$ C/min and held for 2 min, and finally to 250 $^{\circ}$ C at 4 $^{\circ}$ C/min and held for 2 min, flow rate of 1.0 mL/min.

2.9. Determination of volatile organic compounds (VOCs)

The GC-IMS (G.A.S., Dortmund, Germany) analysis was carried out as described by F. F. Wang et al. (2024). Sample 1 g was equilibrated at 60 °C for 15 min, then extracted (500 µL, 85 °C) by an automatic headspace sampling system. The MXT-5 column (15 m × 0.53 mm × 1.0 µm) conditions were set as follows: carrier gas was N₂, 2 mL/min carrier gas kept for 2 min, then linearly increased to 20 mL/min over 8 min, to 100 mL/min over 10 min, and 150 mL/min over 5 min. A calibration curve for the retention time and retention index was created after a mixture of n-ketones (C4 - C9) was analyzed. The GC retention index (NIST 2020) database, which is integrated into the VOCal program, and the IMS migration time database were searched and compared to determine the retention index of a substance, which was determined based on the target's retention time.

The relative odor activity value (ROAV) analysis is a method that combines the sensory thresholds of compounds to establish the key VOCs, is used to elucidate the contribution of individual compounds to the overall aroma characteristics of a sample, and is calculated according to Formula (2).

$$ROAV_i \approx \frac{C_{ri}}{C_{stan}} \times \frac{T_{stan}}{T_{ri}} \quad (2)$$

where C_{ri} - relative content of a compound (%); C_{stan} - relative content of the maximum compound (%); T_{stan} - maximum compound threshold (µg/kg); T_{ri} - threshold for a compound (µg/kg).

2.10. Lipidomics

Lipids were extracted following the method described by Y. Y. Zhang et al. (2018), with slight modifications. 200 µL water, 800 µL MTBE, and 240 µL pre-cooled methanol were added to the sample taken in an appropriate amount and vortexed in MP. Then, it was ultrasonicated in a cold water bath for 20 min, left at room temperature for 30 min, and centrifuged at 14000 g for 15 min at 10 °C. The upper organic phase was collected, dried with nitrogen gas, and analyzed by mass spectrometry. 200 µL of 90 % isopropanol/acetonitrile solution was added to the upper phase for re-dissolution and thorough vortexing. Then, 90 µL of the re-dissolved solution was taken and centrifuged at 14,000 rpm for 15 min at 10 °C, and the supernatant was taken for sample analysis.

The samples were separated on an ultra-performance liquid chromatography system (Thermo Fisher Scientific Inc., San Jose, CA, USA). An Acquity UPLC BEH C18 column (2.1 mm × 100 mm; 1.7 µm) was used, with a column temperature of 45 °C and a flow rate of 300 µL/min. The conditions were set as follows: 0–3.5 min, B was maintained at 40 %; 3.5–13 min, B was varied linearly from 40 % to 75 %; 13–19 min, B was varied linearly from 75 % to 99 %; 19–24 min, B was maintained at 40 % to 99 %; 19–24 min, B was maintained at 40 %.

Electrospray ionization (ESI) positive and negative ion modes were used for detection, respectively. The samples were separated by UHPLC and analyzed by mass spectrometry using a Q Exactive series mass spectrometer (Thermo Fisher Scientific, USA).

2.11. Statistical analysis

Data statistics were performed using Excel 2023, and analysis of variance (ANOVA) was performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA), with a significance level of $P < 0.05$. PLS-DA analyses were performed using MetaboAnalyst software, heatmaps were generated using TBtools, and other graphs were generated using Origin 2021 (OriginLab, Northampton, MA, USA) to generate.

3. Results and discussion

3.1. Sensory quality analysis of bone soup-stewed pickled Chinese cabbage

3.1.1. Solid content

Soups with high solids content are usually considered more nutritious and easier to digest and assimilate. As shown in Fig. 1 A, a significant difference ($P < 0.05$) was observed between the soluble solids content of CK and BSS. The lower solids of the CK samples were thought to be caused by the lack of bone broth supplementation, which was a more homogenous substance. In previous studies, micro- and nano-gelatinous particles have been found to be generated during the boiling process of pork bone broth. The components of the gelatinous particles have been identified as the main constituents of the bone broth solids, with the primary elements being amphiphilic molecules such as proteins (e.g., collagen), polysaccharides, lipids, and nucleotides (Rapoport, Goder, Heinrich, & Matlack, 2004).

3.1.2. Color change

Color is considered one of the key indicators of the quality of stewed soups, and white soup is usually preferred by consumers. As shown in Fig. 1B to 1E, no significant difference ($P > 0.05$) was observed in the a^* values between CK and BSS broths, while the L^* , b^* , and W values of BSS were found to be significantly higher than those of CK ($P < 0.05$). The highest brightness and whiteness values were recorded in BSS, and its broth color was perceived as the most creamy. This phenomenon was thought to be related to the gradual degradation of large molecules into small molecules in bone broth. Additionally, the boiling process of bone broth was believed to promote the full emulsification of fat and protein.

3.1.3. Soluble protein change

As shown in Fig. 1F, the soluble protein content in the CK group was significantly lower than that in the BSS group ($P < 0.05$). Since bone broth is rich in protein, prolonged high-temperature stewing causes macromolecular proteins to be converted into small-molecule peptides and amino acids. These changes collectively contribute to the increase in soluble protein content in the soup.

3.2. Characteristic flavor profiles

3.2.1. Free amino acid (FAA)

The role of FAA content in the taste of the soup is considered crucial, and the amino acid content of the soup is determined by the rate of dissolution of proteins, amino acids, and other nitrogen-containing substances during the cooking process (You, Yang, Song, Zhang, & Liu, 2019). FAAs are categorized into freshness, sweetness, and bitterness based on their taste characteristics. As shown in Table 1, higher total FAAs and individual FAA contents were observed in BSS compared to CK. Significant differences ($P < 0.05$) were found in the content of each FAA, as well as in the total amounts of fresh, sweet, bitter FAAs and total FAAs except cysteine between the two broths. These results suggest that BSS was more favorable for the solubilization of proteins, amino acids, and other nitrogen-containing substances.

Taste Activity Value (TAV) is defined as the ratio between the concentration of a taste substance and its threshold value. In both soups, the highest TAV value was observed in Asn (TAV > 1), indicating that the flavor of the pickled Chinese cabbage soup was most contributed by it, and a significant contribution to the flavor of the BSS boil was also made by Ala (TVA > 1). Although TAV values below 1 were exhibited by other FAA, their involvement in the enhancement of bitter as well as sweet flavors may still occur. A greater variety of bitter amino acids was contained in both soups, but the bitterness was not pronounced because the freshness and sweetness of the other flavor-presenting amino acids could be enhanced by bitter amino acids, such as phenylalanine and tyrosine when their levels below the threshold of flavor presentation (Y.

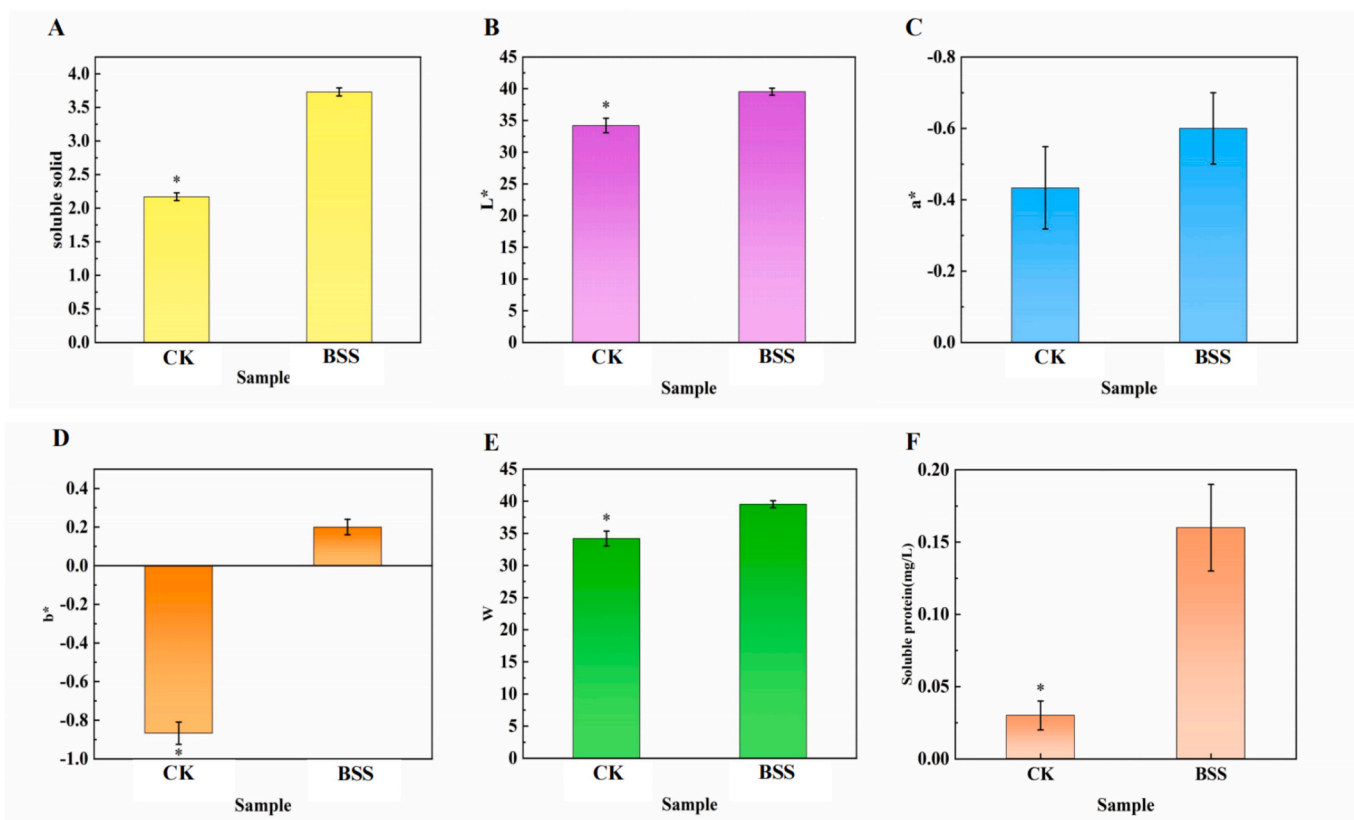


Fig. 1. Water-stewed pickled Chinese cabbage and bone soup-stewed pickled Chinese cabbage sensory quality analysis. (A) Difference in solids content, (B) Variations in the lightness component, (C) Variations in the red and green chromaticity components, (D) Variations in the yellow-blue chromaticity components, (E) Difference in W-values, (F) Soluble protein content.

A. Lv et al., 2024).

3.2.2. Free fatty acid (FFA)

The composition and content of FFA are considered to play an important role in the nutritional value of meat products, in addition to being recognized as one of the main sources of flavor substances in meat products. As shown in Table 2, a total of seven FFA were detected in both soups, with C18:0 found to be significantly higher in CK than in BSS samples ($P < 0.05$). The highest total content of monounsaturated fatty acid (MUFA) was observed in both pickled Chinese cabbage soups, followed by saturated fatty acid (SFA) and polyunsaturated fatty acid (PUFA). SFA are mainly composed of palmitic and stearic acids, and a higher content of UFA was detected in BSS compared to CK. UFA is regarded as an important flavor precursor, and its higher content is believed to contribute to the flavor formation of the soup (Enser et al., 1998). During stewing, oxidation of UFA leads to increased levels of volatiles such as aldehydes and alcohols in the samples (Bravo-Lamas, Barron, Farmer, & Aldai, 2018). It is demonstrated that the contents of SFA and UFA are positively correlated with the goodness of meat flavor. Oleic acid was found to account for 46.1 % and 47.9 % of the total FFA content in the CK and BSS samples, respectively, making it the most abundant FFA in the samples. Additionally, the UFA/SFA value of BSS samples was measured at 1.475, which was significantly higher than that of CK samples ($P < 0.05$), indicating higher nutritional value.

3.2.3. Volatile organic compounds (VOCs) analysis

3.2.3.1. Overall analysis. The VOCs in CK and BSS were analyzed using HS-GC-IMS. Further visualization and comparison of VOC differences across samples were performed. The spectra of CK samples were selected as the reference, and those of other samples were compared to obtain

differential comparison diagrams (Fig. 2A and B). VOCs were primarily detected within retention times of 100–600 s and drift times of 1.0–1.6 ms. A lower signal intensity was observed in CK compared to the BSS samples, suggesting that the BSS samples were more abundant in VOCs. A large number of red spots and a small number of blue spots were detected in the chromatogram of BSS, confirming that the concentration of VOCs was higher in the BSS samples than in CK.

As shown in Fig. 2C and D, a total of 69 VOCs were identified in the two samples, and aliphatic aldehydes, alcohols, ketones, esters, and acids produced by lipid oxidative degradation were detected in this experiment. The formation of dimers or multimers was caused by higher proton affinities or higher concentrations, and 19 VOCs were found to coexist as monomers and dimers. The presence of these dimers allows for more accurate and rapid characterization of the compounds (Chang et al., 2020). Significant differences were observed in the content of different types of VOCs between the CK and BSS samples ($P < 0.05$), with aldehydes and esters accounting for a higher percentage of the total content.

Cyclopentanone (pleasant odor), (E)-2-Heptenal (spicy, green vegetable, fresh), Butyl butanoate (fruity, tropical fruit), 2-furaldehyde (sweet, toasty), n-Pentanal (grassy, faint banana), 2-Furanmethanol, 5-methyl- (sweet caramel), (E)-2-Octenal (fresh cucumber, fatty), Acetic acid (spicy, fatty), and 1-Octen-3-one (pungent flavor) were detected in relatively high concentrations in the CK samples. Most of these VOCs were classified as aldehydes and ketones. Aldehydes are believed to be derived from oxidation and degradation products of fats during stewing, as well as from the production of volatile aldehydes through the Strecker amino acid reaction, where free amino acids are converted at higher temperatures. These compounds are considered a rich resource for the later formation of heterocyclic compounds such as pyrroles and furans. Ketones, which are associated with special flavors such as aromatic and

Table 1

FAA content and TAV values of water-stewed pickled Chinese cabbage and bone soup-stewed pickled Chinese cabbage.

Free amino acid		Flavor threshold (mg/L)	Free amino acid content (mg/L)		TAV	
			CK	BSS	CK	BSS
Delicate	Glu	300	54.27 ± 0.22*	104.79 ± 0.01	0.181 ± 0.000*	0.349 ± 0.000
	Asp	1000	16.70 ± 0.12*	41.88 ± 0.15	0.017 ± 0.000*	0.042 ± 0.000
	Total		70.96 ± 0.16*	146.67 ± 0.14		
Sweet	Gly	1300	155.10 ± 0.72*	233.10 ± 4.50	0.119 ± 0.000*	0.179 ± 0.003
	Ala	600	525.97 ± 2.03*	632.98 ± 2.59	0.877 ± 0.003*	1.055 ± 0.004
	Pro	3000	101.65 ± 0.24*	142.71 ± 1.72	0.034 ± 0.000*	0.048 ± 0.001
	Thr	2600	61.64 ± 0.09*	85.37 ± 0.06	0.024 ± 0.000*	0.033 ± 0.000
	Ser	1500	257.99 ± 0.21*	289.38 ± 1.11	0.172 ± 0.000*	0.193 ± 0.000
	Gln	2500	37.44 ± 1.41*	97.47 ± 14.44	0.015 ± 0.000*	0.039 ± 0.006
Total			1139.78 ± 3.47*	1481.01 ± 20.08		
	Val	400	219.09 ± 2.16*	241.62 ± 2.87	0.548 ± 0.005*	0.604 ± 0.007
	Leu	1900	149.80 ± 0.36*	175.36 ± 0.76	0.079 ± 0.000*	0.092 ± 0.000
	Ile	900	86.53 ± 0.18*	98.31 ± 0.39	0.096 ± 0.000*	0.109 ± 0.000
	Asn	100	182.95 ± 0.52*	221.48 ± 0.54	1.830 ± 0.005*	2.215 ± 0.005
	Met	300	33.83 ± 0.12*	40.52 ± 0.07	0.113 ± 0.000*	0.135 ± 0.000
	His	200	2.96 ± 0.37*	11.62 ± 0.47	0.015 ± 0.002*	0.058 ± 0.002
	Tyr	/	8.29 ± 0.42*	18.71 ± 0.21	/	/
	Lys	500	55.91 ± 0.30*	77.65 ± 0.39	0.112 ± 0.001*	0.155 ± 0.001
	Arg	500	50.39 ± 0.80*	324.30 ± 3.74	0.101 ± 0.002*	0.649 ± 0.007
	Trp	900	0.77 ± 0.02*	3.43 ± 0.32	0.001 ± 0.000*	0.004 ± 0.000
	Cys	/	2.27 ± 0.16	2.47 ± 0.81	/	/
	Phe	900	66.27 ± 0.25*	78.67 ± 0.29	0.074 ± 0.000*	0.087 ± 0.000
	Total		859.06 ± 3.99*	1294.13 ± 4.82		
	FAAs		2069.81 ± 5.65*	2921.82 ± 21.67		

Note: *Indicates significant difference in content, (p < 0.05).

Table 2

FFA composition of water-stewed pickled Chinese cabbage and bone soup-stewed pickled Chinese cabbage.

Fatty acids	Relative percentage content%	
	CK	BSS
<i>SFA</i>		
Myristic acid(C14:0)	/	0.011 ± 0.001
Methyl pentadecanoate(C15:0)	/	0.015 ± 0.004
Palmitic acid(C16:0)	0.267 ± 0.043	0.271 ± 0.058
Stearic acid(C18:0)	0.152 ± 0.002	0.118 ± 0.031*
<i>MUFA</i>		
Palmitoleic acid(C16:1)	0.022 ± 0.001*	0.026 ± 0.002
Oleic acid(C18:1n9c)	0.461 ± 0.035	0.479 ± 0.002
<i>PUFA</i>		
Linoleic acid(C18:2n6c)	0.094 ± 0.002	0.107 ± 0.015
SFA	0.419 ± 0.043	0.415 ± 0.022
MUFA	0.483 ± 0.036	0.505 ± 0.003
PUFA	0.094 ± 0.002	0.107 ± 0.015
UFA/SFA	1.377 ± 0.232*	1.475 ± 0.091

Note: SFA. saturated fatty acid, MUFA. monounsaturated fatty acid, PUFA. polyunsaturated fatty acid, UFA. unsaturated fatty acid, *Indicates significant difference in content, (p < 0.05).

fatty notes, may be formed through the pyrolysis of lipids, generating FFAs. These FFAs can be further oxidized under heating conditions, particularly when UFA are involved, contributing to an enhanced bloody flavor. Additionally, ketones are known to act synergistically, enhancing the overall richness of the flavor profile (Adams, Kitryte, Venskutonis, & De Kimpe, 2011).

Benzaldehyde (bitter almond, cherry, and nutty aroma), Hexanal (fresh, green aroma, fatty flavor), Heptanal (fresh, aldehydic, fatty), (E, E)-2,4-Heptadienal (fatty, oily, aldehydic), 1-Hexanol (fresh, fruity, alcoholic), 3-methyl-butanol (whisky aroma, banana fruity), 2-Heptanone (pear, banana-like fruity aroma, slightly medicinal aroma), 1-Octen-3-ol (mushroom, lavender, rose, and hay aroma), Propyl acetate (fruity, pear-like aroma), Ethyl 2-butenolate, and Ethyl 2-methylpropanoate (sweet, fruity, alcoholic flavor) were found at higher concentrations. The main components were identified as aldehydes and ketones, but an increase in the content of esters and alcohols was also observed. During processing, alcohols are primarily formed through lipid oxidation, as well as the decarboxylation and dehydrogenation of amino acids. Compared to aldehydes, alcohols are characterized by a higher odor threshold and lower abundance, resulting in a relatively minor direct impact on flavor. However, due to their botanical aroma, alcohols are considered important for the formation of overall flavor complexity and the enrichment of sensory hierarchy (Adams et al., 2011). Hexanol and isoamyl alcohol may be derived from intermediate products of fat oxidation. 1-Octen-3-ol, an unsaturated alcohol with a low aroma threshold, is produced through fat oxidation (Wu et al., 2023). The significant increase in 1-octen-3-ol content in the BSS sample suggested that accelerated lipid oxidation may contribute to the enhancement of the soup aroma. Esters are generally formed through reactions between alcohols and fatty acids generated by fat oxidation. These compounds are known for their fruity aroma and are recognized for their contribution to the flavor profile and overall taste of the soup.

3.2.3.2. Key volatile organic compounds (VOCs). In Fig. 2E, a heatmap was shown that indicates the key VOCs that were initially identified based on the criterion of ROAV > 1. From the analysis results, it was found that 18 and 16 key VOCs along with 4 and 5 modified VOCs were screened in the CK and BSS samples, respectively. Among the compounds with ROAV > 10, Benzaldehyde (M), 1-octen-3-ol, 2-heptanone (D), 2-heptanone (M), 2-n-pentyl-furan, n-hexanol, n-hexanal (D), and n-butanal were included, suggesting that these eight substances were considered to be the key VOCs in the BSS samples.

Among them, Butyraldehyde was found to contribute greatly to the overall flavor of the samples, with the highest ROAV value of 145.828

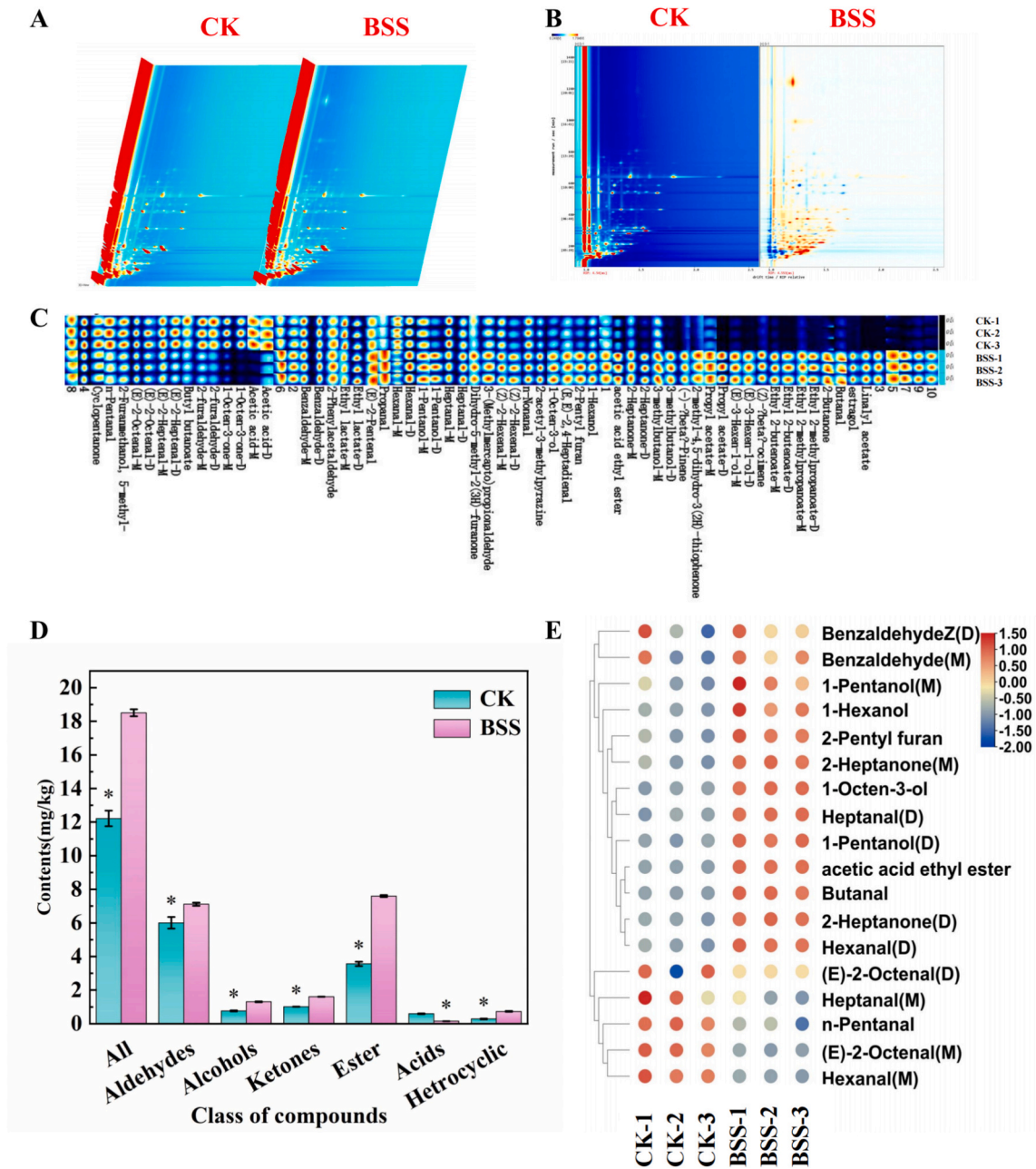


Fig. 2. VOCs identified by GC-IMS. (A) 3D topographic plot, (B) 2D Variance plot, (C) Gallery plot, (D) Concentration of VOCs in broad categories, (E) Heat map of VOCs with ROAV>1, “M” and “D” denote monomer and dimer, respectively. *Indicates significant difference in content, ($p < 0.05$).

being obtained in BSS. In addition, the key VOC species were shown to be the same in both samples except for (E)-2-heptenal and valeraldehyde. Aldehydes were determined to be the predominant volatiles in both the CK and BSS, followed by alcohols. In the study of Sandiao soup, it was reported by [Zhu et al. \(2025\)](#), that aldehydes, particularly nonanal and hexanal, were identified as the key VOCs affecting Sandiao's flavor. The detected 2-heptanone and 2-pentyl-furan can be produced through the oxidative degradation of linoleic acid and are known to possess certain aroma characteristics. 1-Octen-3-ol, which has been recognized as a common VOC in stewed meat products, has been identified as a key VOC in both beef broth powder ([Gao et al., 2024](#)) and crucian carp soup ([M. Zhang, Chen, & Xing, 2021](#)). In addition to the key VOCs, $0.1 \leq \text{ROAV} \leq 1$ was also found to have an important influence on flavor characteristics. The modified VOCs jointly identified in the CK and BSS samples were determined to be furfural and ethyl lactate.

3.3. Reheating quality and warm-over flavor production

3.3.1. Oxidation indicators

The industrial production of prepared meat products inevitably requires reheating, during which excessive fat oxidation is caused by secondary heating. As a result, WOF are generated, leading to flavor deterioration ([Chen et al., 2024](#)). Oxidation reactions are observed during the processing and storage of meat products, and the aldehydes generated by lipid oxidation are closely associated with off-flavors in meat products ([Al-Dalali, Li, & Xu, 2022](#)). As shown in [Fig. 3A](#), the highest peroxide value was detected in the BSS-R sample, followed by BSS, with the lowest value found in CK. A significant difference in peroxide values was observed ($P < 0.05$), indicating that fat oxidation was enhanced after reheating. The TBARS value, which is recognized as an important indicator of fat oxidation degree, was measured as shown in [Fig. 3B](#). The TBARS values of CK, BSS, and BSS-R samples were

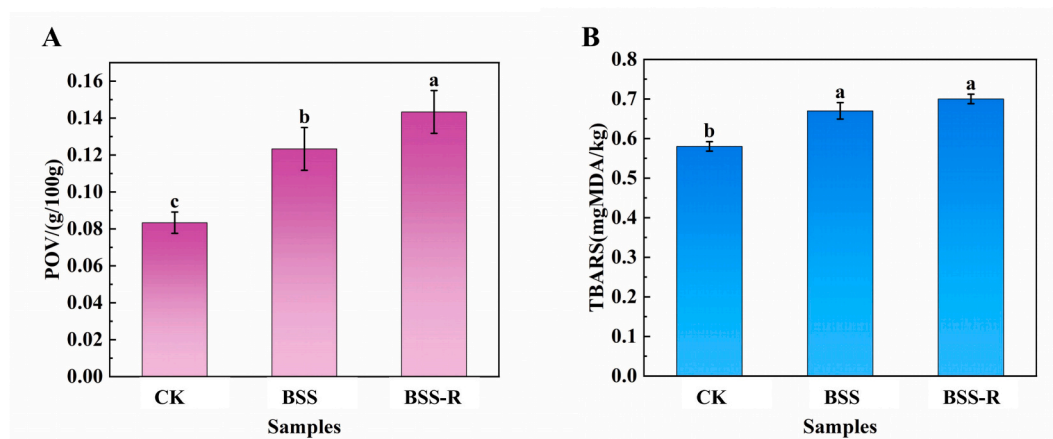


Fig. 3. Differences in oxidation indicators. (A) POV variance, (B) TBARS variance.

determined to be 0.58, 0.67, and 0.7 mg MDA/kg, respectively. No significant difference in TBARS values was found between BSS-R and BSS samples ($P > 0.05$), but an increase in TBA content was still noted, suggesting accelerated lipid oxidation and quality deterioration in BSS. Lipid oxidation is accelerated by raw meat processing techniques such as marinating, stirring, and heat treatment (Al-Dalali et al., 2022). Not only is the characteristic aroma of meat products generated by lipid oxidation but peroxide accumulation is also caused, which is significantly aggravated by heating. Furthermore, reheating after refrigeration promotes the decomposition of peroxides into alcohols, aldehydes, and ketones, which contribute to WOF formation (Zang et al., 2020).

3.3.2. Lipidomics overall analysis

Lipids are recognized as playing an important role in biological processes and are considered major components of biological membrane structures (including cell membranes, mitochondria, endoplasmic reticulum, and other subcellular structures), while also being involved in signaling and energy storage (Lou, Wang, & Xue, 2013). A total of 1937 lipid metabolites were detected across 12 samples from the three groups, encompassing six major lipid categories (Fig. 4A).

The GL class was the most numerous with 1029 species, followed by SP 428, GP 414, ST 34, Lipid FA 8, and PR 4. These lipids were divided into 40 subclasses, mainly consisting of 742 triglycerides (TG), 263 diglycerides (DG), 215 ceramides (Cer), 111 phosphatidylcholines (PC), 95 hexosylceramides (Hex1Cer), 88 phosphatidylethanolamines (PE), and 64 phosphatidylglycerols (PG). TG, DG, Cer, PC, Hex1Cer, PE, and PG accounted for 38.3 %, 13.58 %, 11.10 %, 5.73 %, 4.90 %, 4.54 %, and 3.30 %, respectively, of the total lipid quantity, which accounted for more than 80 % of the total lipid, while the remaining categories of lipids were less abundant (Fig. 4B). After reheating, the type and number of lipid molecules remained unchanged, with the GL class being the most abundant, of which TG and DG were the main components, in agreement with previous studies (L. Liu et al., 2019). As shown in Fig. 4C, the content of TG, PG, and DG showed an upward trend after reheating, indicating that they may be closely related to the flavor formation after reheating. PC is the most abundant class of GP and the predominant glycerophospholipid in animal skeletal muscle cells (Chao et al., 2020). SP sphingolipids had the highest amount of Cer, while other lipid types were less abundant.

The lipid profiles of CK, BSS, and BSS-R were differentiated by PLS-DA analysis, and the two-dimensional scores, as shown in Fig. 4D, showed that the lipid compositions of the three types of samples were significantly varied, with BSS and BSS-R samples having tightly clustered lipids, which were distinctly different from those of CK. CK lipid components were concentrated in quadrants 2 and 4, whereas bone soup-stewed pickled Chinese cabbage before and after reheating were aggregated in quadrants 1, 2, and 4, and the addition of bone broth

caused lipid components to migrate from quadrant 2 to quadrant 1. Fig. 4E showed the PLS-DA scatter plot, indicating that lipids were mainly clustered near the zero point, with labeling concentrated in quadrants 1 and 4. CK had lower lipid subclass content than the BSS stewed samples, and the addition of bone broth resulted in the massive migration of lipid molecules into SM, LPC, and TG. Notably, the MG, DG, and TG contents of BSS-R were higher than those of fresh samples, highlighting the difference in fat composition between fresh and reheated.

Lipids were classified into nine categories by k-means clustering analysis (Fig. S1). GP, SP, and GL were the main lipid types in all clusters, with clusters 5 and 9 enriched in GP and SP, while clusters 4 and 6 were dominated by GL. Clusters 5 and 9 peaked and then declined in the BSS samples, indicating that GP and SP contents were highest in the BSS samples, and both acted similarly. Clusters 4 and 6 were highest in group BSS-R. Since TG accounts for 98 % of GL, changes in GL were mainly driven by TG. The results showed that the lipid composition of the three groups varied dynamically, and the differences might originate from differences in the degree of lipid oxidation, backbone cleavage, side chain modification, or lipolysis (J. Liu et al., 2025).

3.3.3. Correlation of key volatile organic compounds (VOCs) and key lipids

The fingerprints of the VOCs of BSS-R are shown in Fig. 5A. In the BSS-R samples, Propanal (pungent, grassy odor), 3-(Methylmercapto) propionaldehyde (onion, meaty, fruity), n-Nonanal (rose, citrus, etc., with a strong greasy odor), 2 phenylacetaldehyde (hyacinth aroma, fruity sweetness, almond), 2-pentyl furan (bean, fruity, earthy), 2-acetyl-3-methylpyrazine (nutty, roasted hazelnut, toasted grain), (E)-3-Hexen-1-ol (mossy, fresh), linalyl acetate (lily of the valley), Estragol (sweet, green, herbaceous), (E)-2-Pentenal (potato, pea) were higher, with aldehydes being the highest in the BSS-R fraction, a phenomenon that was consistent with the results of POV and TBARS. Furan is a VOC with a strong roasted caramel aroma, mainly produced by lipid over-oxidation and degradation of secondary products of the Melad reaction (F. Zhang et al., 2022). Meng et al. (2022) found that 2-n-pentyl-furan contributed significantly to the flavor of bone broth, whereas nonanal, a common product, was generated mainly by the oxidation of UFA and was prevalent in other stewed broths. Pyrazine is a product of carbonyl compounds that are produced through a Meladic reaction during the stewing process, where as estragol is derived from the addition of natural flavors such as ginger and clove.

Fifteen WOF associated with VOCs were screened according to ROAV >1, and six of them increased after reheating: Benzaldehyde (M), Benzaldehyde (D), 2-n-pentylfuran, n-pentanol (M), n-butyraldehyde, and ethyl acetate, which were determined to be the key VOCs sources of WOF of bone soup-stewed pickled Chinese cabbage. O'Sullivan, Byrne, Jensen, Andersen, and Vestergaard (2003) showed that pentane, 2-n-

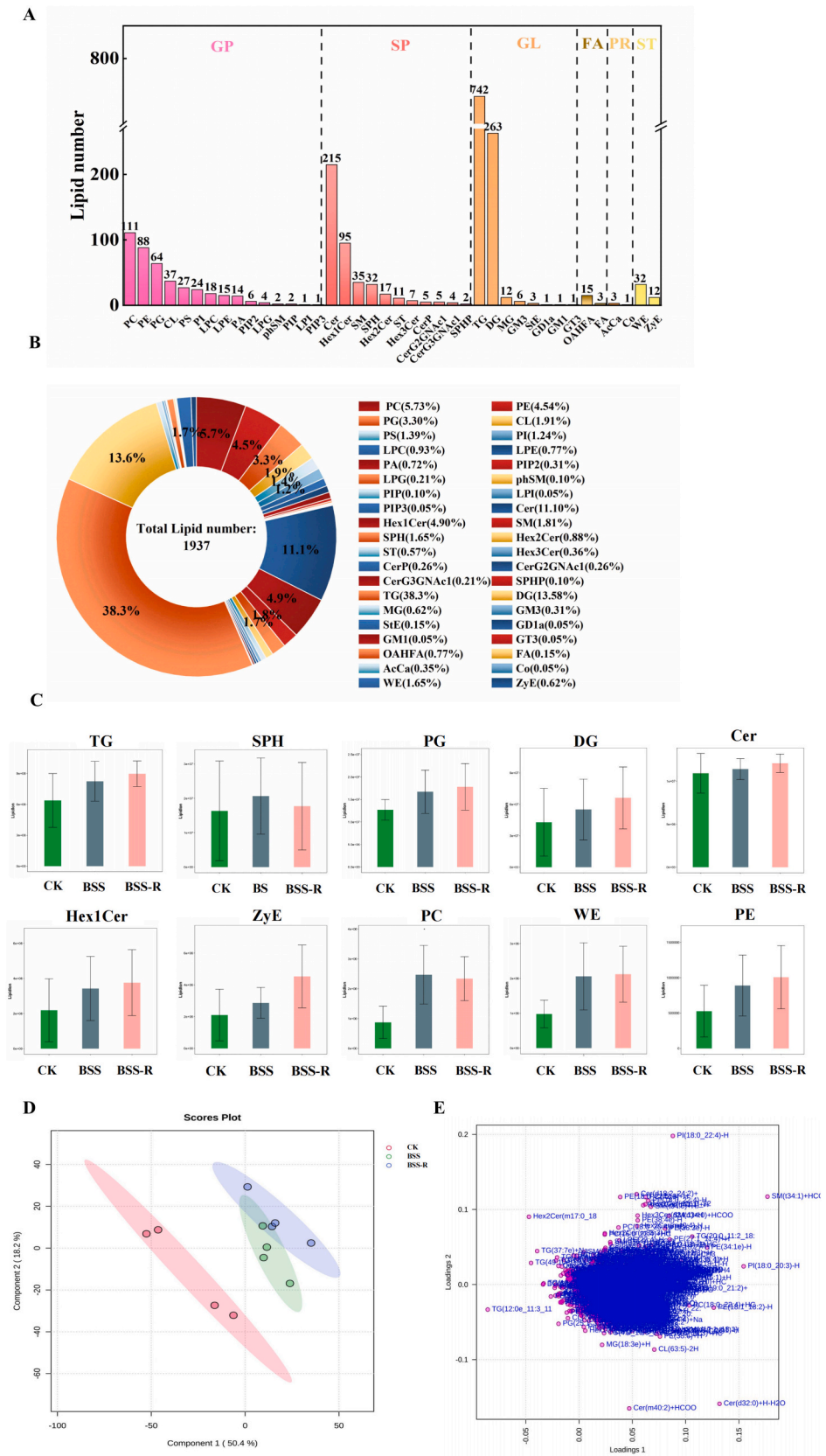


Fig. 4. (A)Statistical chart of the number of lipid subclasses and lipid molecules, (B) Percentage of lipid subclasses identified, (C) Important lipid subclasses, (D) PLS-DA scoring chart, (E) PLS-DA scatterplot.



pentylfuran, octane, nonanal, 1-octen-3-ol, and hexanal were the major VOCs of WOF in pork. Kim et al. (2016) utilized CSA data and employed Partial Least Squares Regression (PLSR) to establish a predictive model related to TBARS, valeraldehyde, hexanal, and heptanal, which were associated with WOF. This model can effectively predict the progression of WOF. Dang, Li, You, Xiong, and An (2024) study on the WOF of surimi gels showed that heptanal, decanal, 2-furfuryl alcohol, (E)-2-octanal, (E)-2-nonanal, and (E,E)-2,4-decenal were the key WOF factors. The present study showed that aldehydes contributed significantly to the WOF and were the main cause of WOF in reheated meat products, consistent with previous studies (An et al., 2022).

Lipids are considered to be the most effective volatile retention agents compared to proteins and carbohydrates (H. Liu et al., 2022). A total of 104 differential lipid molecules were screened by PLS-DA analysis in combination with the screening conditions of VIP > 2 and $P < 0.05$. Differential analyses showed that only GP lipids were significantly changed before and after reheating, such as PC (20:2e_16:1) + H, PE (8:1e_12:3) + H, and PC (26:1_10:3) + H, and these molecules were identified to be the distinguishing samples as potential markers. Phospholipids have been shown to be key in differentiating meat products, e.g. PC (30:6) and PC (28:3) were identified as potential biomarkers for charcoal-grilled lamb (H. Liu et al., 2022).

The correlation between lipid oxidative degradation and WOF flavor substances was explored. 104 differential lipid molecules were used to correlate with 15 aroma compounds (ROAV > 1), and Pearson correlation heat maps were drawn. A strong correlation between differential lipids and key VOCs was demonstrated in Fig. 5B. 70 potential lipid markers showed a significant negative correlation ($r < 0$, $P < 0.05$) with key VOCs, suggesting that a decrease in these lipids may lead to an increase in the concentration of certain VOCs. TG molecules containing 16:0 and 18:1 FFA, as well as PC and PE molecules containing 18:1, 18:2, and 20:4 FFA, were susceptible to degradation during cold storage (J. Liu et al., 2025). TG may be oxidized or hydrolyzed to DG during refrigeration-reheating to produce VOCs (Guo et al., 2022). During refrigeration, lipids are continuously hydrolyzed to produce FFA, which are oxidized by free radical chain reactions, similar to thermal oxidation processes, to produce short-chain aldehydes, ketones, and alcohols. These substances contribute to odor production due to low odor thresholds and accumulation over time.

3.3.4. Key lipid precursors of warmed-over flavor (WOF)

The flavor of meat products is decisively influenced by lipid composition, whereas WOF is caused by excessive lipid oxidation. The lipids with the greatest impact on flavor are TG and PC, and their correlation was explored, as shown in Fig. 6A and B. TG had the highest species and content, which may contribute significantly to the production of WOF in BSS-R. TG (16:1_18:1_18:1) + NH₄, TG (18:0_16:0_22:4) + NH₄, TG (16:0_18:1_22:5) + NH₄, TG (18:0_10:0_18:1) + NH₄, TG (16:0_18:2_18:3) + NH₄, etc., were found to be high in the three samples, particularly TG (16:1_18:1_18:1) + NH₄ and TG (16:0_18:2_18:3) + NH₄ remained stable throughout the refrigeration-reheating process without any significant change in concentration, which may be the key to the binding of VOCs (Fig. 6C and D). Changes in TG content may lead to changes in the levels of aroma compounds. The increase in TG content in BSS-R may be related to the destruction of fat globules by secondary heating and the release of TG. At the same time, a significant increase in VOCs, such as aldehydes (e.g., hexanal and heptanal), was observed in BSS-R. Additionally, the elevation of the lipid content is considered to improve the retention of aromas (H. Liu et al., 2022).

In BSS, lipid oxidation is mainly caused by the oxidation or hydrolysis of triglycerides in lard and the leaching of yellow bone marrow from bone broth. Reis and Spickett (2012) showed that esterified unsaturated FAAs were more susceptible to free radical attack and oxidation reactions, such as PC, PE and other phospholipids were more susceptible to oxidation. UFA can be broken down by oxidation or as hydrolysis products of glycerophospholipids and glycerolipids. After

refrigeration and reheating, the PC content of BSS-R was lower than that of BSS, in which the PC (16:0_20:5) + H of BSS-R was significantly lower than that of other PC molecules and was significantly negatively correlated with Hexanal (M). PC (38:7) + H content was second only to PC (16:0_20:5) + H, and both had similar trends, suggesting that they are key lipid precursors for WOF formation (Fig. 6E and F). The decrease in PE is usually accompanied by an increase in LPC, LPE, and FFA as a result of phospholipid down-regulation due to phospholipase hydrolysis and up-regulation of its hydrolysis products (J. Lv et al., 2023). PC, PE, etc. are key precursors for the formation of VOCs in meat products because they are rich in UFA, such as soft fat oleic acid (16:1), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), and arachidonic acid (C20:4), which can be oxidized to form volatiles such as hexanal and nonanal (C20:4) (Calkins & Hodgen, 2007). Lipid molecules can be interconverted, e.g., LPC can produce glycerophosphatidylcholine by acylation, PE can be formed by decarboxylation of PS, and sphingomyelin can be converted from ceramide by sphingomyelinase (J. Lv et al., 2023). In addition, spoilage bacteria metabolically decompose FFA to produce ketones, aldehydes, hydrocarbons, and alcohols, which further contribute to the elevation of TBARS and exacerbate the changes in meat off-flavors and flavors (Guo et al., 2022). Oxidation of linoleic acid produces characteristic aldehydes such as hexanal, whereas alkanals, 2-enals, and alkanols may originate from the autoxidation of linoleic acid (C18:2n-6) and oleic acid (C18:1n-9), and 1-octen-3-ol may be produced by oxidation of linoleic acid and arachidonic acid. The significant increase in TG content, especially TG (22:5_14:4_21:1) + NH₄, after refrigerated reheating could be attributed to the reheating process that further disrupts the cell membrane and leads to TG release (H. Liu et al., 2023).

In addition, GP can be converted to TG by oxidation (Guo et al., 2022). At the same time, aldehydes such as glutaraldehyde, hexanal, and heptanal were significantly increased in aroma compounds, which may be related to the oxidative breakdown of lipids. The elevated TG levels in BSS-R may stem from oxidative degradation during storage. TG levels were significantly increased in BSS compared to BSS-R and CK, indicating that the rate of oxidation was lower than the rate of production. These results suggest that TG plays a key role in the formation and development of WOF in BSS-R.

Lipids in foods can be oxidized through catalysis by light, heat, and metal ions (e.g., copper, iron), with intermediates such as free radicals being generated that subsequently trigger photo-, thermo-, or auto-oxidation processes. Among them, autoxidation is a key process in WOF formation (Barden & Decker, 2016). As shown in Fig. 7, during cooking, phospholipids and triglycerides are broken down by lipoproteases to FFA, which subsequently enter the mitochondria and undergo free radical chain reactions. In the initial phase, lipid molecules are catalyzed by light, heat, or metals to generate free radicals, followed by the propagation and termination phases of the chain reaction. Generation of non-radical compounds by intergroup reactions and ultimately formation of hydroperoxides by enzymatic action. Hydroperoxides, with their weak and thermally unstable O—O bonds, decompose to produce VOCs such as aldehydes, ketones, carboxylic acids, alcohols, lactones, and alkyl furans, which collectively comprise the characteristic flavor of cooked meat. Further breakdown into small molecules such as aldehydes, ketones, alcohols, and acids (Chen et al., 2024). As these substances accumulate in lipid oxidation, the levels of certain aldehydes, ketones, and other undesirable flavor substances increase, leading to WOF.

4. Conclusion

This study elucidated the characteristic flavor of BSS and the formation pathway of WOF in reheated sample. In BSS, Ala and Asn were the main FAAs, with UFFAs oleic acid accounting for a high proportion. Key VOCs include Benzaldehyde (M), 1-octen-3-ol, 2-heptanone (D), 2-heptanone (M), 2-n-pentyl-furan, n-hexanol, n-hexanal (D), and n-

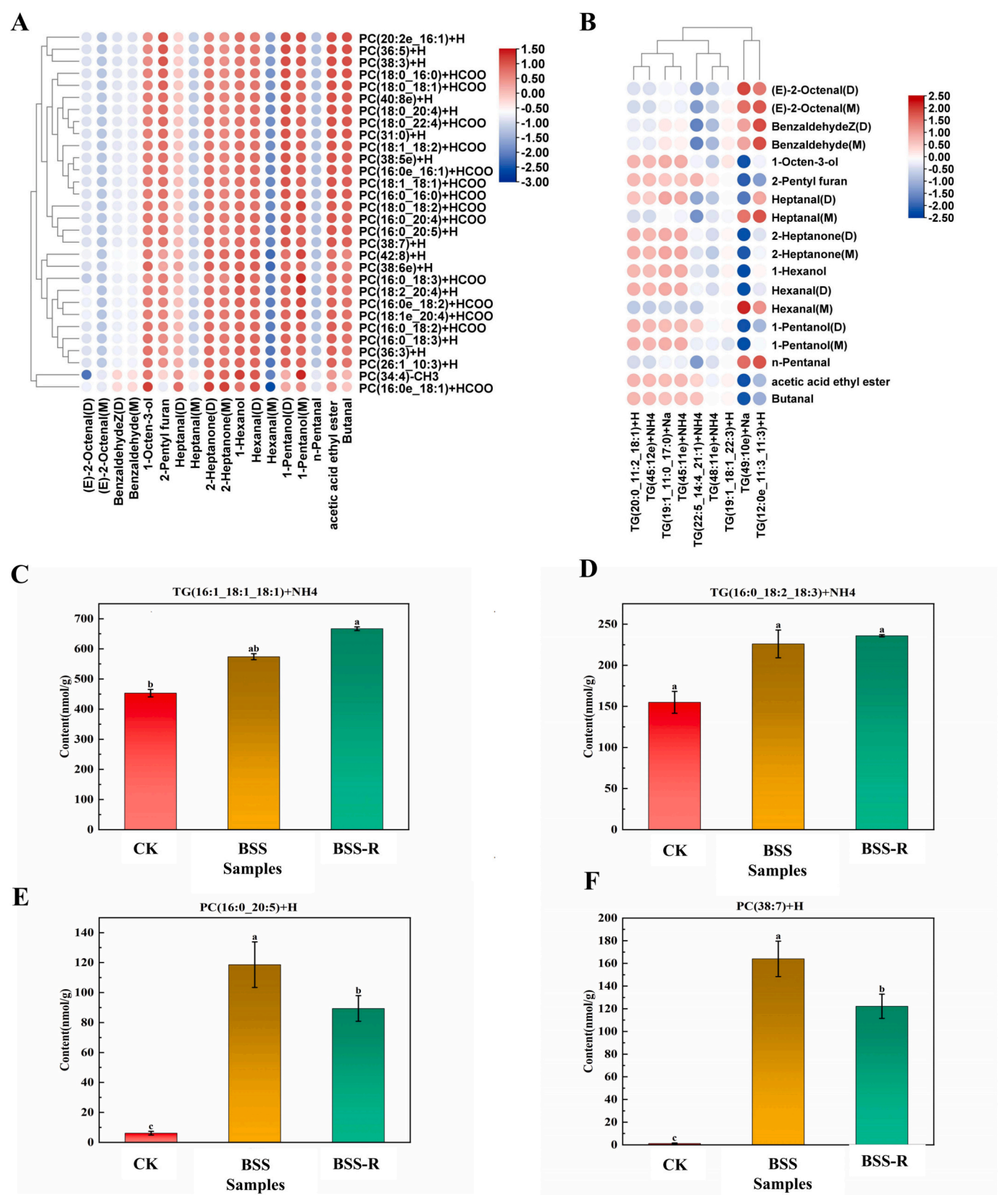


Fig. 6. (A) Heat map of differential lipids of PC subclasses, (B) TG subclass differential lipid thermogram, (C–D) Changes in the content of TG-focused lipid molecules, (E–F) Changes in the content of PC-focused lipid molecules.

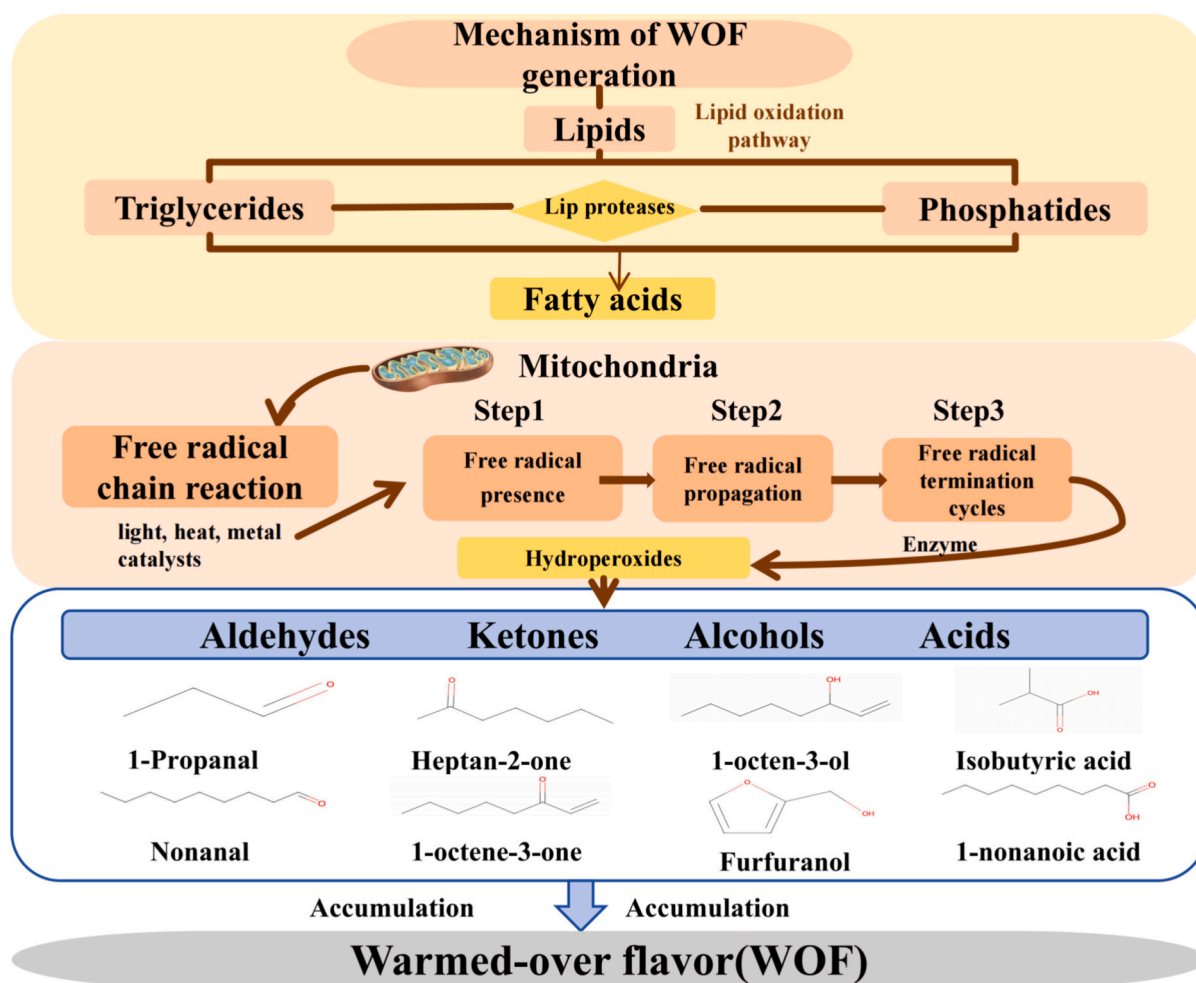


Fig. 7. Mechanism of lipid autooxidation in wormed-over flavors (WOF).

butanal. Reheating intensified fat oxidation and hydrolysis, leading to the accumulation of unpleasant odors. Based on ROAV >1, Benzaldehyde (M), Benzaldehyde (D), 2-n-pentylfuran, n-pentanol (M), n-butyraldehyde and ethyl acetate were identified as the main contributors to the WOF in the reheated samples. Lipomics indicated that TG (16:1_18:1_18:1) + NH₄ and TG (16:0_18:2_18:3) + NH₄ were the major flavor forming lipids. 104 different lipids were screened, and it was found that PC (16:0_20:5) + H and PC (38:7) + H were the key precursors for the formation of WOF. This study provides theoretical and technical support for flavor control and quality improvement of pre-fabricated pickled Chinese cabbage products. Therefore, future research can focus on the micro interface localization of oxidation mechanisms, clarify the differences and contributions of lipids in the cellular structure during the oxidation process, and achieve more accurate WOF correction.

CRediT authorship contribution statement

Cairong Jin: Writing – original draft, Validation, Software, Methodology, Data curation, Conceptualization. **Yali Zhou:** Software, Investigation. **Yuxian Wang:** Methodology, Formal analysis. **Honglin Jiang:** Formal analysis, Data curation. **Bin Li:** Project administration. **Ce Liu:** Resources. **Yang Ji:** Methodology. **Xu Si:** Writing – review & editing, Project administration, Conceptualization. **Yanwei Zhang:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2025.103124>.

Data availability

Data will be made available on request.

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