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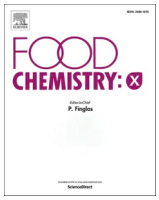

Flavor formation and phospholipids degradation of crayfish meat treated by boiling combined air-frying during accelerated storage

Mingzhu Zhou [a,b,1], Dongyin Liu [b,c,1], Hongyuan Tan [b,d], Chao Wang [a], Wei Yu [b], Guangquan Xiong [b], Lan Wang [b], Wenjin Wu [b], Yu Qiao [b,*]

[a] Key Laboratory of Fermentation Engineering (Ministry of Education), Hubei Key Laboratory of Industrial Microbiology, Hubei Provincial Cooperative Innovation Center of Industrial Fermentation, Hubei University of Technology, Wuhan 430068, China
[b] Key Laboratory of Agricultural Products Cold Chain Logistics, Ministry of Agriculture and Rural Affairs, Institute of Agro-Products Processing and Nuclear agricultural Technology, Hubei Academy of Agricultural Sciences, Wuhan 430064, China
[c] Key Laboratory of Food Nutrition and Functional Food of Hainan Province, School of Food Science and Engineering, Hainan University, Haikou 570228, China
[d] School of Environmental Ecology and Biological Engineering, Wuhan Institute of Technology, Wuhan 430205, China

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ABSTRACT

Thermal treatment is an essential processing method in crayfish processing. This study analyzed the changes in lipids and volatile compounds in crayfish muscle subjected to three thermal processes: boiling (BO), air-frying (AF), and boiling combined air-frying (BO-AF). Aldehydes and heterocyclic compounds were found to be the predominant volatile compounds in crayfish muscle during thermal processing and storage. The intensity of lipid oxidation (POV, TBARS and *p*-AnV) was greatest in AF, and was notably lower in BO-AF. the total concentration of free fatty acids (FFAs) was highest in the AF group (4.14 mg/g) after processing, followed by BO (3.26 mg/g) and BO-AF (2.04 mg/g). During storage, the FFAs content gradually decreased, with generally lower levels observed at 65 °C compared to 45 °C. A total of 383 phospholipid species were identified, phosphatidylethanolamine being the primary difference lipid type in BO (26.7 %) and AF (36.7 %), while fatty acids were the main differential lipid types in BO-AF group, under the comparison between processed and stored. Overall, the BO-AF method improved the flavor sensory and decreased lipid oxidation, compared to the other two methods. These findings provide valuable insights into the effects of different thermal processing and storage methods on the quality and safety of crayfish muscle.

1. Introduction

Crayfish (*Procambarus clarkii*) is rich in nutrients since crayfish meat contains high-quality proteins (approximately 20 %) and suitable fatty acids (nearly 50 % unsaturated fatty acid). In 2022, crayfish production reached 2.89 million tons, of which 1.21 million tons were processed. Thermal treatment is the normal method to process crayfish, which is beneficial in sterilization, storage, and transport (Li et al., 2023). However, thermal treatment could promote lipid oxidation. Excessive oxidation of lipid was closely related to quality fission, such as rancidity, rancid taste, reduced nutritional value, and low consumer acceptance (Xu et al., 2025).

The oxidation decomposition of phospholipids in lipids is one of the primary sources for forming flavor compounds in meat products (Whitfield, 1992). Phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS) played an essential role in the formation of key aroma compounds when pork was treated by different roasting methods (Liu et al., 2024). Lysophosphatidylcholine (LPC) produced by PC hydrolysis also increased significantly during the chicken thermal process, and lipid oxidation occurred (Zhang et al., 2023). Nevertheless, different thermal-treated meat products have different oxidation models of phospholipids due to the treatment temperature, treatment methods, and meat species. Therefore, monitoring the changes of phospholipid molecules in different thermal processed methods and storage is necessary.

Real-time and accelerated storage tests are commonly used to

* Corresponding author at: Institute of Agro-Products Processing and Nuclear Agricultural Technology, Hubei Academy of Agricultural Sciences, Wuhan 430064, China.
E-mail address: qiaoyu412@sina.com (Y. Qiao).
[1] These authors contributed equally to this work and should be considered co-first authors.

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evaluate the shelf life of food products. Accelerated storage of dried clams at 50 °C and 65 °C resulted in lipid loss (Xie et al., 2018). Lipid profiles of hot-air-dried (HD) and freeze-dried (FD) Vanabin shrimp were investigated during the storage period using accelerated storage tests. It was found that lipid components such as triacylglycerol (TAG), PC, and PE, and fatty acid content decreased to a lesser extent after the FD shrimp storage, which indicated that FD shrimp had better quality stability during the storage process (Li et al., 2019). However, the changes in flavor and phospholipids of crayfish meat during storage after being prepared with BO, AF, and BO-AF are rarely reported. The effects of two single processing methods (BO and AF) and compound processing (BO-AF) on the flavor of crayfish storage are still unclear.

This study investigated the flavor and lipid changes in crayfish during accelerated storage under three processing methods: boiling, air-frying, and a combination of boiling followed by air-frying. Special attention was paid to the impact of pre-cooking crayfish via boiling before air-frying on its flavor. Additionally, the study unveiled the correlation mechanism between flavor and lipids in crayfish subjected to these three thermal processing methods. This research provides a theoretical foundation and methodological guidance for developing different processing methods for crayfish ready-to-eat products.

2. Materials and methods

2.1. Sample preparation and thermal treatments

150 crayfish (total weight 4.5 kg) were purchased from Baishazhou Aquatic Products Market in Wuhan, Hubei, China. Fresh crayfish were placed on crushed ice and transported to the laboratory within 20 min. Crayfish were decapitated, de-shelled to obtain the meat. Then, the meat was rinsed with pure water, and separated into three portions (50 crayfish meat per portions). The first group was boiled (BO), the second group was air-fried (AF), and the third group was first boiled and then air-fried (BO-AF). All crayfish processing was performed by the Animal Care and Use Committee Guidelines.

For the BO group, 2000 mL of ultrapure water was added to a household electric cooker. Once the water was boiled, the crayfish meat was added and boiled for 3 min. For the AF group, the air fryer was preheated to 170 °C. Once the temperature was reached, the crayfish meat was placed inside and fried for 8 min, with the meat being turned over after 7 min. The BO-AF group follows a sequence where the BO processing is carried out before AF processing.

The samples were dried in a vacuum freeze dryer (FD-1000, Tokyo Physical and Chemical Instruments Co., Japan). After drying, the samples were placed in an incubator and stored at 45 °C and 65 °C for 0, 3, 6, 9, 12, 15, and 18 d. The samples were then taken out for measurement.

2.2. Determination of volatile compounds

Volatiles of samples were collected, separated, and detected with solid-phase microextraction (SPME)-GC–MS systems (7890 A-5975C, Agilent, USA) (**Figure S2**). Approximately 2.0 g of sample and 50 μL of 2-octanol (0.86 μg/mL) were introduced in a 20 mL headspace bottle and sealed. Sealed vials were equilibrated at 60 °C for 15 min. SPME fibers (50/30 μm DVB/CAR/PDMS, Supelco, USA) were adsorbed in a sample vial at 60 °C for 40 min. Desorb at 250 °C for 5 min.

Volatile compounds were segregated in a DB-WAX capillary column (60 m × 250 μm × 0.25 μm, Agilent, USA). The GC initial column temperature was 40 °C and then raised to 100 °C at a rate of 5 °C/min. and raised to 140 °C at a rate of 2 °C/ min, and kept for 2 min, next raised to 250 °C at a rate of 8 °C/min, and kept for 2 min. Mass spectrometry conditions: electron energy is 70 eV, full scan mode, no solvent delay, mass scan range *m/z* 35 to 350, ion source temperature is 230 °C, quadrupole temperature is 150 °C, detector temperature is 250 °C, and transmission line temperature is 260 °C. Mass spectrometry library (NIST 08 MS database) and n-alkane (C7-C30) standards were used for volatile compound characterization. The relative quantification of volatile compounds was performed using 2-octanol as an internal standard.

2.3. Lipid extraction

Total lipids were extracted from crayfish meat according to the method of Xie et al. (2019) and Folch, Lees, and Sloane Stanley (1957). A mixture of sample, chloroform-methanol (2:1, v: v) and deionized water (1:3.25:2, w: v: v) was prepared and stood for 2 h and filtered, and the extracts were added with saline. After centrifuging at 7800 g for 10 min at 4 °C, the lower organic phase was collected, and the lipids were obtained by nitrogen blowing. The extracted lipids were stored at −80 °C for subsequent use.

2.4. Determination of lipid oxidation

The peroxide value (POV) of the crayfish meat was measured according to the titration method of the American Oil Chemists Society (2003). Oil sample (2.00 g) added 30 mL chloroform-glacial acetic acid (30.00 mL) and saturated potassium iodide solution (1.00 mL), then shaken and placed in the dark for 3 min. Titration was performed with sodium thiosulfate standard solution. When the solution appeared pale yellow, starch indicator (1 mL) was added and continued to drop until the blue solution disappeared as the titration endpoint.

The method for determining thiobarbituric acid reactants (TBARS) was referred to by GB 5009.181–2016. Briefly, 1.00 g of crayfish meat was mixed with 20 mL of 10 % (*w/v*) trichloroacetic acid (TCA) solution, and then 20 mL of distilled water was added. After thorough mixing, the mixture was left to stand at 4 °C for 1 h. Subsequently, it was centrifuged at 8000 rpm for 10 min at 4 °C. Next, 5 mL of the supernatant was mixed with 5 mL of 0.02 M thiobarbituric acid solution. The mixture was then placed in a boiling water bath for 20 min. The absorbance was measured at 532 nm (722 N, INESA, China) after cooling to room temperature. The standard curve was created using the standard substance 1,1,3,3-tetramethoxypropane. Each sample was repeated three times.

The *p*-anisidine value (*p*-AnV) of the crayfish meat was measured using the method described by Xie et al. (2019). Briefly, 0.5 g of lipid was filled with isooctane to 25 mL. 1.0 mL mixture was mixed with 1 mL of 0.0025 g/mL *p*-anisidine solution, and then the shading reaction time was 10 min at 23 ± 3 °C. The *p*-AnV was recorded at 350 nm by a spectrophotometer.

2.5. Determination of free fatty acid (FFA) content

Fatty acid methyl esterification was referred to a literature with slightly modified (García Regueiro, Gibert, & Díaz, 1994). The 5 mL of 0.5 mol/L sodium hydroxide-methanol solution was added to the extracted total lipids and heated in a boiling water bath for 5 min. Then, 2 mL of 14 % mass fraction boron trifluoride-methanol solution was added and boiled in a water bath for 5 min. Finally, 1 mL of n-hexane and ultrapure water were added, respectively. The solution was oscillated for 1 min, and then the upper layer of the liquid was aspirated after the stratification of the extracts was allowed to settle and then filtered through a 0.22 μm organic micropore filter membrane, and added 25 μL of methyl nineteen-alkanoate (5 mg/mL) was added as the internal standard. Hexane was used as the internal standard. Then, the sample was diluted to 1 mL for gas chromatographic analysis.

Fatty acids were analyzed by an Agilent 7890 A gas chromatography-flame ionization detector equipped with a DM-2560 capillary column (100 m × 0.25 mm × 0.2 μm). The initial oven temperature was set at 100 °C for 13 min, next increased at 10 °C/min to 180 °C and maintained for 6 min, then further increased at 1 °C/min to 200 °C and maintained for 20 min, finally increased at 4 °C/min to 230 °C and maintained for 10.5 min. Fatty acids were qualitative analyzed by comparing them with mixed fatty acid standards (GLC #463, NuChek. Prep, Minnesota, USA). The content of free fatty acids is calculated by its ratio to the internal

standard peak area.

2.6. Determination of phospholipidomics

Phospholipidomics determination was referred to literature with slightly modified (Zhou et al., 2023). The 120 mg freeze-dried crayfish meat, 60 μL standard solution (10 μg/mL), and 2 mL methanol were put into a tube. The mixed samples were left for 15 h to allow the proteins to be precipitated. Then, 2 mL of dichloromethane was added and mixed for 1 h. The mixture was added to 2 mL of dichloromethane and 1.6 mL of ultrapure water before being vortexed again and centrifuged at 4 °C (5000 rpm, 5 min). After the supernatant was removed, the lipid was obtained by nitrogen blowing. The lipids were redissolved by 0.6 mL of isopropyl alcohol and filtered through a 0.22 μm microporous filter membrane. Finally, the filtrate was used for UPLC-Q-TOF-MS/MS assay. The sample was quantified using internal standard.

2.7. Statistical analysis

The one-way analysis of variance (ANOVA) was performed to determine significant differences using data processing system (DPS) software. Data were plotted using Origin 2021 software (Microcal Software, Inc., Northampton, MA, USA). Principal components analysis (PCA), orthogonal partial least squares-discriminant analysis (OPLS-DA), and correlation analysis were conducted using the Metware Cloud (https://cloud.metware.cn). In analyzing differential substances for volatile compounds, differential substances were identified by screening VIP (VIP $\geq$ 1). Three sample repeats were performed for each index.

3. Results and discussion

3.1. Volatile compounds

3.1.1. Constitution of volatile compounds

Flavor changes in crayfish treated with BO, AF, and BO-AF at 45 °C and 65 °C for 18 d of storage are shown in Fig. 1A. The total volatile compound content was higher after accelerated storage compared to the beginning of the storage period. Furthermore, the higher the storage temperature was led to the higher the total volatile compound content. This change was mainly reflected in the elevated content of aldehydes, alcohols, ketones, heterocycles, and hydrocarbons in the samples. At the same time, the oxidative degradation of lipids, degradation of proteins, and amino acids produces aldehydes, alcohols, ketones, heterocycles, and hydrocarbons. Among the identified compounds, aldehydes, alcohols, and ketones contributed more to the flavor of the samples due to their low thresholds. Heterocyclic compounds are a typical source of aroma for fried foods, presenting a characteristic roasted aroma (Chang, Wu, Zhang, Jin, & Wang, 2020). Hydrocarbons did not contribute significantly to the flavor of the samples due to the high threshold (Zhang et al., 2021). Consequently, the aldehydes, alcohols, ketones, and heterocyclic compounds present in the samples were the primary volatile compounds during the processing and accelerated storage of dried crayfish meat.

3.1.2. Analysis of differential volatile compounds

In order to clarify the characteristic flavor components of dried crayfish meats during accelerated storage, the differential substances were explored by statistical analysis. Volatile compounds were analyzed using the OPLS-DA model for the 0 d VS 18 d-45 °C group, the 0 d VS 18 d-65 °C group, and the 18 d-45 °C VS 18 d-65 °C group. The cumulative contributions of these three groups were 52.1 %, 66.4 %, and 59.7 %, respectively **(Figure S1)**. No overlap existed in the comparison groups, and the flavors were different. In addition, a 200-permutation test was performed for each of the above models, and none of the models were found to be overfitted, and the results were plausible. Ultimately, a total of 46 volatile compounds had VIP values greater than 1, which may represent differences between samples during accelerated storage **(**Fig. 1B**)**. The 21, 36, and 32 volatile compounds with VIP $>$ 1 were found in the 0 d VS 18 d-45 °C group, the 0 d VS 18 d-65 °C group, and the 18 d-45 °C VS 18 d-65 °C group, respectively.

3-Methyl-butanal, pyrazine, trimethyl-pyrazine, and 3-methyl-thiophene were differential substances specific to the 0 d VS 18 d-45 °C group. 3-Methyl-butanal is an aldehyde found only during drying, formed by degrading leucine and isoleucine, and provides nutty, cheesy, and savory flavors (Zhang et al., 2020). Pyrazines are generated by Strecker degradation between α-dicarbonyl compounds and amino acids and the dimerization of α-amino ketones (Shen et al., 2023). Those two compounds could contribute to a roasted aroma. The contents of 3-methyl-butanal, pyrazine, and trimethyl-pyrazine were higher at 18 d-45 °C than at 0d. The BO-AF had higher 3-methyl-butanal, pyrazine, and trimethyl-pyrazine contents than the BO and AF groups at 0 d. While 3-methyl-butanal and trimethyl-pyrazine contents were lower than those of the AF group at 18 d-45 °C, compared with BO-AF group. 3-Methyl-thiophene, produced by the interaction of lipid oxidation products and the Maillard reaction products, was detected only at 18 d of accelerated storage (45 °C) with BO-AF. The above results suggest that BO-AF-treated crayfish meat had aromas such as nutty and roasted flavors and that the BO-AF group may retard the degradation of free amino acids as accelerated storage (45 °C) proceeds.

Hexanal, 3-methylthio-propanal, benzyl alcohol, acetophenone, 1–2-pyridinyl-ethanone, and 2,6-dimethyl-pyrazine were the differential substances specific to the 0 d VS 18 d-65 °C group. Hexanal is mainly produced by linoleic acid oxidation. Methionine degradation produces 3-methylthio-propanal and has a boiled potato odor. Benzyl alcohol is formed mainly from the oxidation of linoleic acid and arachidonic acid. Acetophenone has a fruity aroma. 2,6-Dimethyl-pyrazine has a nutty and roasted grain aroma. The content of these six specific differential substances was higher at 18 d-65 °C than at 0 d. BO-AF had the lowest levels of hexanal and the highest levels of 3-Methylthio-propanal and 2,6-dimethyl-pyrazine at 0 d. This suggests that BO-AF-treated crayfish meat delays the oxidation of certain fatty acids while providing aromas such as nutty. With accelerated storage (65 °C), hexanal content was higher in the BO-AF group than in the AF group, while 3-methylthio-propanal and 2,6-dimethyl-pyrazine were lower than in the AF group. Benzyl alcohol, acetophenone, and 1–2-pyridinyl-ethanone were detected only at 18 d-65 °C and were lower in the BO-AF group than in the AF group.

3-Methyl-2-butenal, benzeneacetaldehyde, alpha-ethyl, 5-methyl-2-thiophenecarboxaldehyde, 3(2H)-furanone, dihydro-2-methyl-, 2-ethyl-furan, and 4,5-dimethyl-thiazole were differential substances specific to the 18 d-45 °C VS 18 d-65 °C group. 3-Methyl-2-butenal is mainly derived from the oxidation of unsaturated fatty acids. The mercaptoacetaldehyde, acetaldehyde, and 2-butenal may be crucial intermediates for the formation of 5-methyl-2-thiophenecarboxaldehyde (Liu et al., 2023). Oxygen-containing heterocyclic compounds (e.g., 3(2H)-furanone, dihydro-2-methyl-) originate from the skeleton of reducing sugars. Furans are mainly derived from lipid oxidation. Thiazoles can be produced by the interaction of lipid autoxidation products and simple intermediates of the Maillard reaction (Mottram & And, 2002). 3-Methyl-2-butenal was only detected in the AF group at 18 d-45 °C. Benzeneacetaldehyde, alpha-ethyl, 5-methyl-2-thiophene carboxaldehyde, and 2-ethyl-furan were detected only in the BO-AF group at 18 d-65 °C. 3(2H)-Furanone, dihydro-2-methyl- was detected only in the BO-AF group at 18 d-45 °C and was lower in the BO-AF than the AF. 4,5-Dimethyl-thiazole was detected only at 18 d-65 °C and had a higher BO-AF content than AF.

In addition, a total of 13 shared differential substances were detected in the 0 d VS 18 d-45 °C, 0 d VS 18 d-65 °C, and 18 d-45 °C VS 18 d-65 °C groups ((E)-2-methyl-2-butenal, nonanal, benzaldehyde, 1-penten-3-ol, 1-octen-3-ol, ethanol, 2-octanone, 2-ethyl-5-methyl-pyrazine, ethyl-pyrazine, 2,5-dimethyl-pyrazine, methyl-pyrazine, pyridine, and pyrrole), which were of differential. The primary sources of these

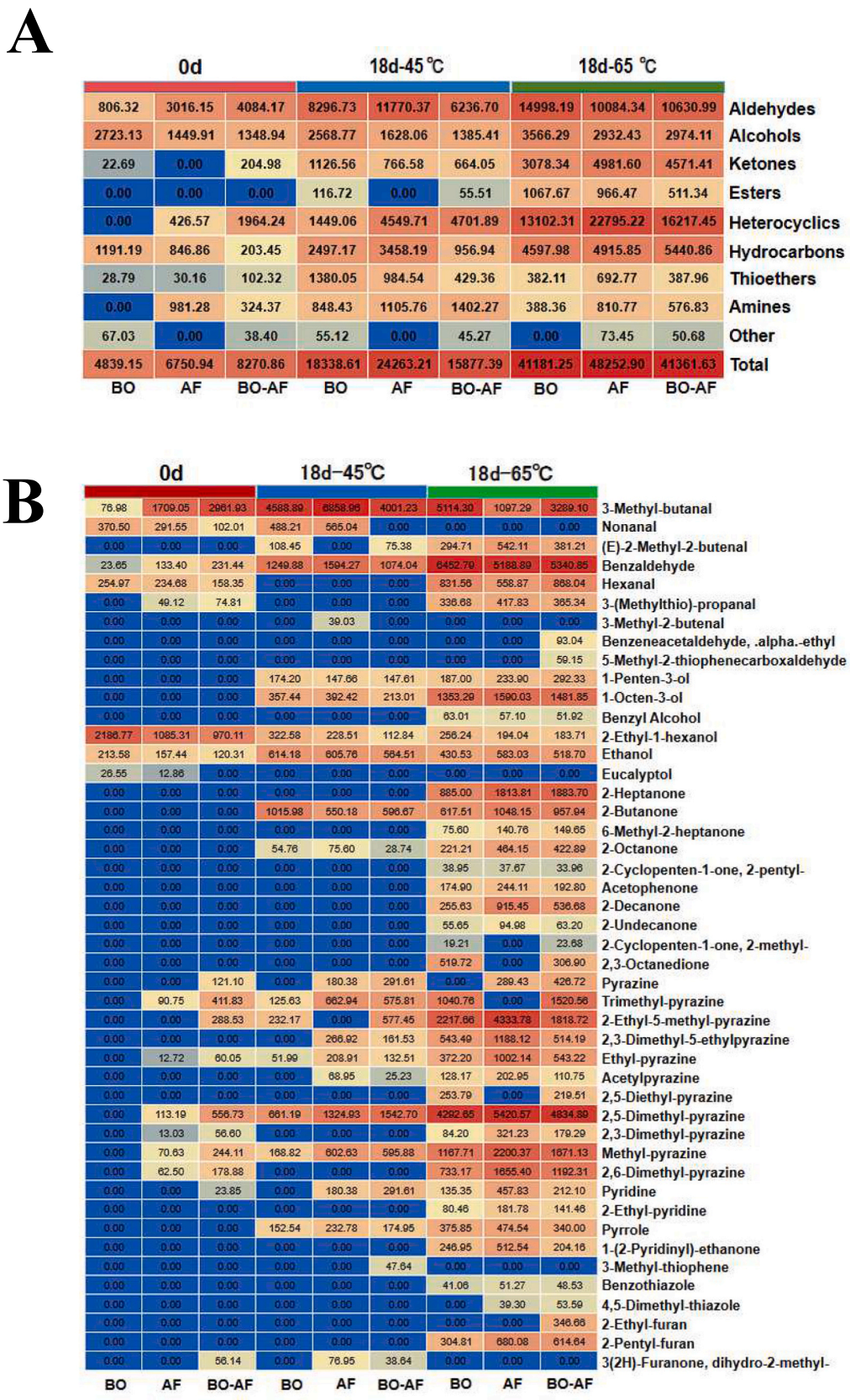

Fig. 1. Changes of volatile compounds during different thermal treatment groups. (A) the contents of volatile compounds in three thermal treated methods during two temperature storage. (B) changes in sample differential substance content throughout accelerated storage. Values in the figure are average values, $n = 3$.

differences are lipid oxidation, Maillard reactions, amino acid degradation, and the interaction of their products. Aldehydes produced by lipid degradation can facilitate the incorporation of unstable dihydropyrazine compounds to produce longer-chain pyrazines (Huang, Bruechert, Hartman, Rosen, & Ho, 1987). The thermal interaction of valine and linoleic acid produces pyridines (Henderson & Nawar, 1981). Except for nonanal and ethanol, the content of all differential substances increased with storage time and temperature. At 0 d and 18 d-45 °C, BO-AF-treated crayfish meat contained lower levels of nonanal than the AF group; nonanal was not detected at 18 d-65 °C, probably due to interactions with the products of the Maillard reaction. In all groups, (E)-2-methyl-2-butenal, 1-penten-3-ol, 1-octen-3-ol, and 2-octanone were almost always less in the BO-AF group than in the AF group, while benzaldehyde, 2-ethyl-5-methyl-pyrazine, 2-ethyl-5-methyl-pyrazine, ethyl-pyrazine, 2,5-dimethyl-pyrazine, methyl-pyrazine, pyridine and pyrrole were higher than that of the AF group in the pre-storage period, but decreased with accelerated storage. In summary, the results showed that the BO-AF group retarded lipid oxidation and imparted aromas such as roasted aroma to the samples in the pre-storage period, but the content of compounds imparting aromas such as roasted aroma decreased with accelerated storage. The substances resulting from the interaction of lipid oxidation and the products of the Maillard reaction also imparted aromas such as nutty aromas to the samples.

3.2. Lipid oxidation analysis

Thermal processing and accelerated storage affect the oxidation of lipids in crayfish meat, while the oxidation of lipids is a complex reaction with many products. Therefore, three indices were used to measure lipids' oxidation degree: POV, TBARS, and *p*-AnV (Fig. 2). Hydroperoxides (ROOH) are the primary products of lipid oxidation, which uses POV values to indicate its content (Kontogianni & Gerothanassis, 2022). As shown in Fig. 2A, POV values increased with storage time in 0–12 d (65 °C) storage. After 12 d of storage time, the POV values decreased significantly, indicating that the decomposition rate of ROOH exceeded its production rate, and deep oxidation was entered at this stage. At 45 °C, the three thermal processing methods showed a maximum POV value at 15 d storage. Higher temperatures could accelerate the primary oxidation of lipids in crayfish meat. POV maximum values were similar in both storage temperatures. At the beginning of storage (0–9 d), the AF group had the highest POV values than the other two groups. However, the BO group had the largest peak value of POV among the three groups.

Aldehydes and ketones are oxidation products of unsaturated fatty acids, which react with thiobarbituric acid to form colored substances (Ghani, Barril, Bedgood, & Prenzler, 2017). Therefore, TBARS was also used to measure the degree of oxidation of lipids. As shown in Fig. 2B, the peak values of TBARS for all three thermal treatments appeared at 12 d for both temperature conditions. The maximum value of TBARS was twice as different between the two temperatures, and the AF group had the largest TBARS value. The study of silver carp mince found a significant positive correlation between TBARS and off-flavor (Siddaiah, Sagar Reddy, Raju, & Chandrasekhar, 2001). This suggested that the AF group may have had more obvious odor changes during storage.

The *p*-AnV responds to the degree of oxidation of lipids by characterizing the number of compounds, such as aldehydes and ketones, in the lipid (Zhang et al., 2022). As shown in Fig. 2C, the trend was similar to the previous two lipid oxidation metrics. The *p*-AnV was more intense at the temperature condition of 65 °C. The AF treatment group exhibited the highest *p*-AnV in both temperature storage. The *p*-AnV values tended to increase all the time as storage progressed. In summary, AF had the highest intensity of lipid oxidation, and BO-AF may prevent lipid oxidation.

3.3. Changes of free fatty acids

The degree of lipid oxidation is related to the type of fatty acid in the

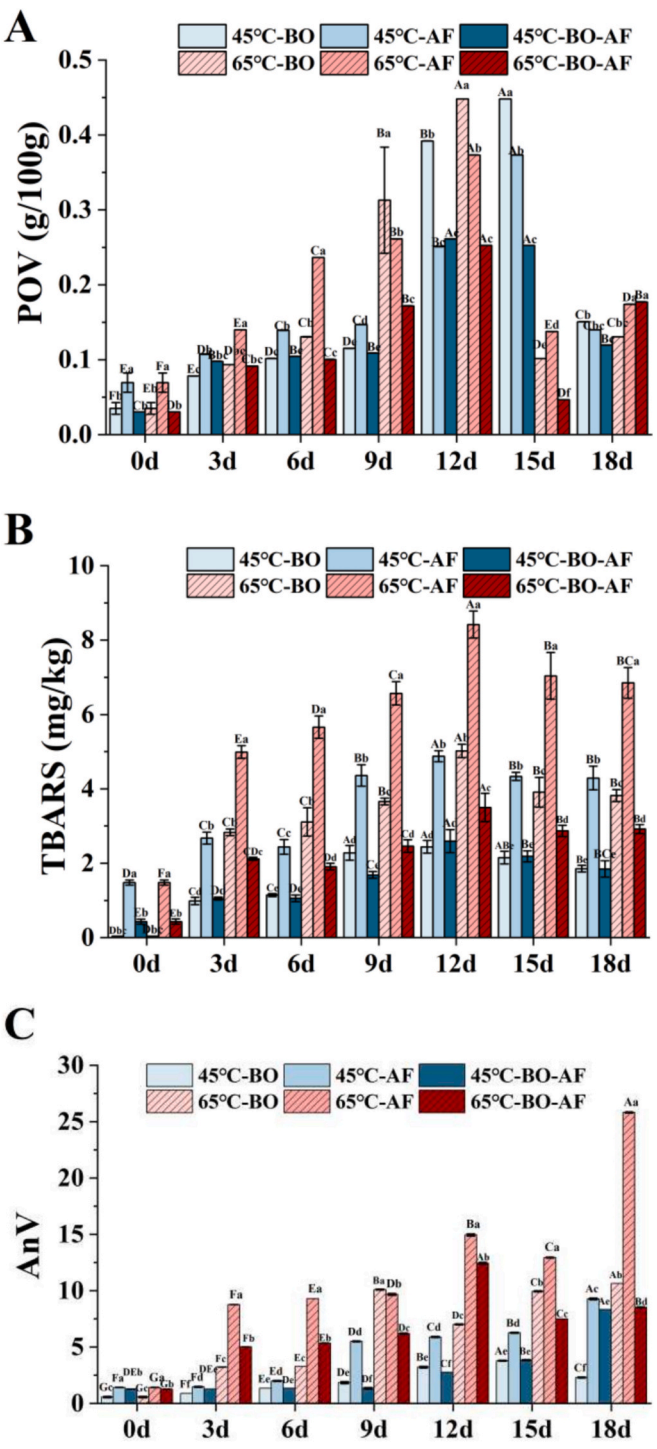

Fig. 2. Oxidation of lipid in different groups. (A) POV values. (B) TBARS values. (C) *p*-AnV values. $^{a\text{-}f}$ represent the significance of the difference between different treatment groups on the same day. $^{A\text{-}F}$ represent the significance of the difference between different days in the same treatment group.

food (degree of unsaturation, position of double bond, cis-trans isomerism, etc.) (Arshad et al., 2018). Under higher temperature conditions, both saturated and unsaturated fatty acids undergo thermal decomposition reactions to produce ROOH compounds, which further produce hydrocarbons, aldehydes, ketones, and other compounds (Quesada-Medina & Olivares-Carrillo, 2011). As shown in Fig. 3, free fatty acids were determined for the three thermal processing methods in storage at two temperatures. 28 free fatty acids were identified, including 10

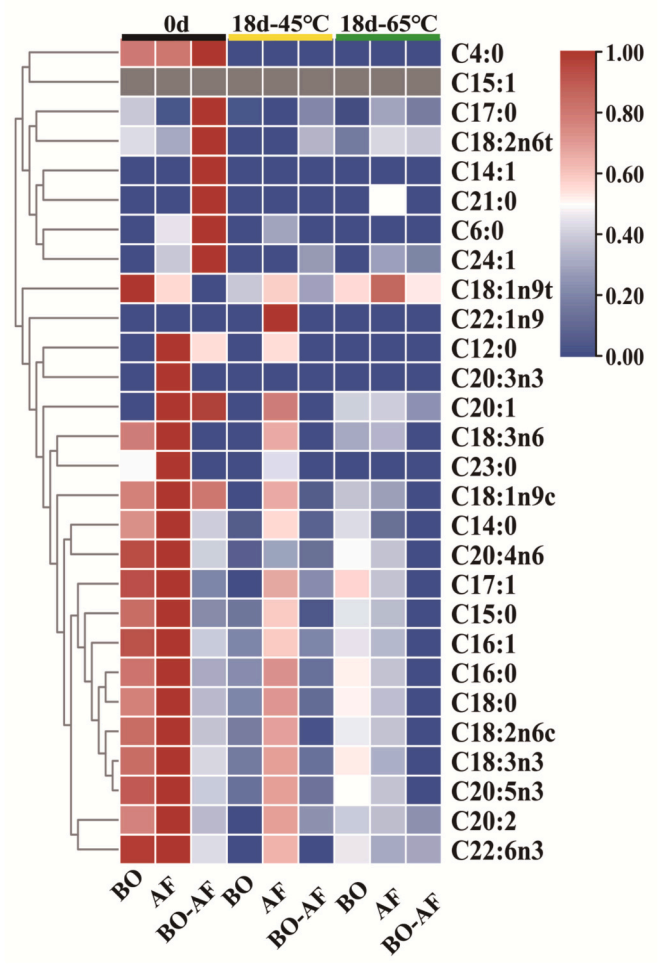

Fig. 3. The contents of free fatty acid in different groups after processing and during storage.

saturated and 18 unsaturated fatty acids. Free fatty acids and phospholipids are often regarded as surface-active compounds, which are the active site of lipid oxidation (Budilarto & Kamal-Eldin, 2015). The total concentration of free fatty acids in BO, AF, and BO-AF were 3.26 mg/g, 4.14 mg/g, and 2.04 mg/g, respectively. The ratio of unsaturated free fatty acid/saturated free fatty acid (USF/SF) in BO-AF was 1.20, while BO and AF were 1.29 and 1.26, respectively. The lower ratio of USF/SF in BO might also lead to flavor differences compared with the other two methods. Regarding food composition, as unsaturated lipids are more prone to oxidation, the levels of unsaturated lipids components may determine the oxidative stability of foods (Wu, Richards, & Undeland, 2022). At the beginning of processing (0 d), the highest total free fatty acid content was found in the AF group, and some of the free fatty acids (C4: 0, C17: 0, C18: 2n6t, C14: 1, C21: 0, C6: 0, and C24:1) exhibited higher contents in the BO-AF group. A study has reported that the oxidation and degradation of unsaturated fatty acids such as oleic acid (C18:1n9c) and linoleic acid (C18:2n6c) have a significant effect on the odor deterioration of tilapia during cold storage (Cheng, Mei, & Xie, 2024). Oleic acid and linoleic acid can be oxidized to produce short-chain aldehydes, such as octanal, nonanal, and hexanal (Xu et al., 2022). In our study, the free fatty acid content gradually decreased as storage progressed and was generally lower at 65 °C than at 45 °C, consistent with the results of volatile compounds and lipid oxidation.

3.4. Lipid profiles of phospholipid in three thermal processing

3.4.1. Lipid constitution of phospholipid

As shown in Fig. 4A, 383 molecular species of phospholipids were detected in phospholipids. Their classification included PE (40.21 %), PC (14.88 %), MAG (1.83 %), lysophosphatidylethanolamine (LPE, 4.44 %), LPC (4.96 %), hexosylceramide (HexCer, 4.7 %), fatty acid (FA, 5.48 %), ceramide (Cer, 14.36 %), sphingomyelin (SM, 1.83 %), PS (0.78 %), phosphatidylinositol (PI, 3.39 %), phosphatidylglycerol (PG, 3.13 %). The PE class had the most percentage of lipid molecules, followed by the two types of lipid molecules, Cer and PC. PC had the highest concentration of all, although not as many molecular species as PE.

After the thermal processing, the LPC and LPE concentration in the BO-AF group were larger than those in the other two groups (Fig. 4B). This indicated that the BO-AF group may reduce the production of some volatile compounds by delaying the further oxidative degradation of LPC and LPE. LPC is also widely reported to some bioactive such as anti-atherogenic properties and anti-inflammation (Liu et al., 2017), which suggested that crayfish cooked by BO-AF could not only improve the flavor sensory and decrease lipid oxidation but also contain more bioactive lipids for consumers, compared to the other two methods. Cer and HexCer are the essential structural compounds of the lipid bilayer of cell membranes, and these lipids are also reported to play an essential role in maintaining rice storage quality (Zhang, Duan, Shang, Hong, & Sun, 2021). In our study, the concentration of HexCer was significantly higher in the BO-AF compared to the other two methods.

During the accelerated storage, significant changes in the phospholipids occurred at either 45 °C or 65 °C. Among the PC lipid molecules, the AF group showed the greatest reduction in storage, from 0 d (32,484.23 μg/g) to 45 °C (32,085.77 μg/g) and 65 °C (27,654.83 μg/g), respectively. The lipid content of PE in all three treatments also showed a greater reduction at 65 °C. On the contrary, there was a greater increase in the lipid molecular content of FA, HexCer, LPC, and LPE at 65 °C. Overall, higher temperatures (65 °C) had a greater effect on the changes in lipids during storage.

3.4.2. Differential lipid profiles among three thermal processing

Given that the changes in lipids in crayfish meat from the three thermal methods at 65 °C were more pronounced when compared with 0 d, a subsequent two-by-two comparison was made between BO, AF, and BO-AF at 0 d and 18 d-65 °C (Fig. 4C). The different lipid molecules showed the top 15 lipid molecules (Fold change >2, $p < 0.05$). The AF group had the largest variety of differential lipid molecules, and it seems that AF had a greater effect on the lipid changes in storage. BO-AF, a composite of the two treatments, instead resulted in fewer lipid changes in storage. Notably, the largest variety of differential lipid molecules in both the BO and AF groups were of the PE class, whereas only 10.2 % of the differential lipid molecules in the BO-AF group were of the PE class. FA (21.6 %), LPC (20.5 %), and HexCer (20.5 %) were the main differential lipid molecular species in the BO-AF group. This implies that the two composite processing methods of boiling and frying altered the pattern of lipid changes in food products during storage. Although there were also differences between the two single processing methods of BO and AF, the main lipid molecular species (PE, LPC, and HexCer) that changed during storage were consistent.

In addition, a unique lipid molecular species Cer (60:9;4O|Cer 42:7;3O (FA 18:1)) was identified under the BO treatment, which was not present in the other two treatments. There were no MAG-type differential lipid molecules in the BO group, no PG-type differential lipid molecules in the AF group, and no PC differential lipid molecules in the BO-AF group.

3.5. Association analysis of phospholipids and free fatty acids on the formation of volatile compounds in dried crayfish

AF has some advantages in aroma compound formation and without

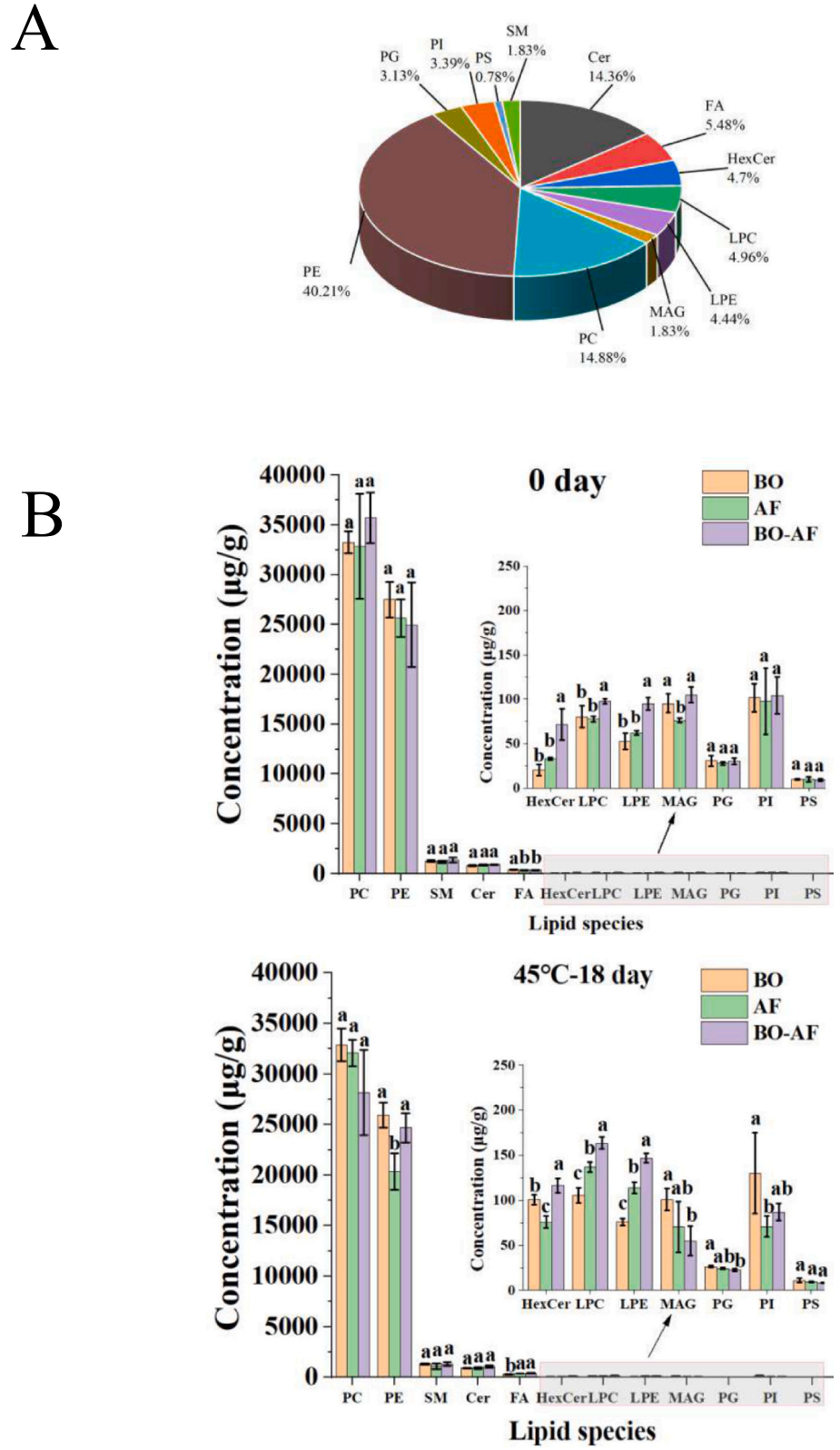

Fig. 4. Effects of different heat treatments on phospholipid molecules during storage. (A) Class of 383 phospholipid molecules. (B) The peak area of phospholipid molecules in crayfish meat was changed by storage time and temperature. (C) Different kinds of lipid molecules stored at 65 °C for 18d and 0d by three thermal treatment methods.

oil addition, while the elevated temperature combined with oxygen exposure provides a prooxidant environment for lipid oxidation (Ferreira et al., 2017). BO, as a traditional thermal method for meat, also promotes the generation of lipid oxidation products and hydroperoxides (Rasinska, Rutkowska, Czarniecka-Skubina, & Tambor, 2019). In our study results, BO combined with AF could delay lipid oxidation during

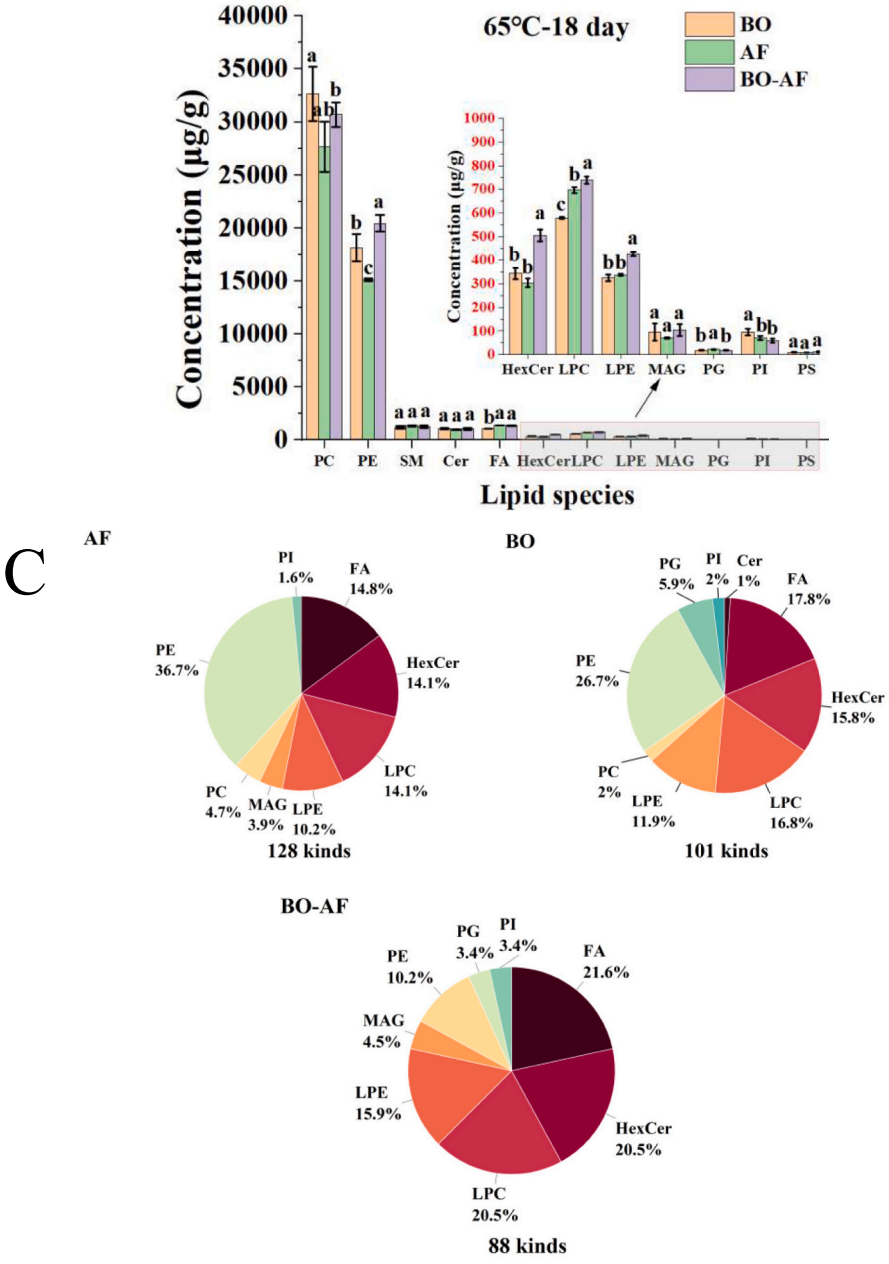

Fig. 4. (*continued*).

processing and storage and provide a pleasant flavor from AF treatment compared with AF-treated crayfish. Thus, this section aimed to elucidate the differences in lipid oxidation and flavor among the three methods, focusing on the association of lipids and flavor (Fig. 5).

The phospholipids such as PE and PC can lose one fatty acyl group to generate LPE and LPC, respectively, and these fatty acyl groups will oxidaze and degrade to produce volatile compounds such as aldehydes, ketones, alcohols, and heterocyclic compounds (Liu et al., 2023). The phospholipid data of three methods in 0 days were analyzed, and 11 kinds of phospholipid molecules with the largest VIP value were obtained. These phospholipids' content changes were calculated after thermal processing and storage at 18 d-65 °C. The results suggested that BO-AF had the highest content increasing of these phospholipids, and AF was the lowest. This was the reason why the content of some volatile compounds increased during processing and storage in our study. In addition, phospholipids such as LPE, LPC, and HexCer can also continue to degrade and oxidize, which are finally decomposed into small molecular compounds, especially volatile compounds (Liu, Nie, et al., 2023). The 11 types of phospholipids in AF and BO had a relatively lower content increase during storage compared with BO-AF. The reason for this phenomenon may be a higher degradation level of these phospholipids in AF and BO groups leading to less content accumulating, thus more volatile compounds would be obtained, which was consistent with the result of volatile compounds increasing during storage. The phospholipid is the predominant composition of cell membranes, and BO-AF may promote cell membranes' degradation, resulting in a content increase of phospholipid molecules. Hence, the lipid oxidation products produced from the above chemical reaction result in changes in the lipid oxidation index (POV, TBARS, and *p*-AnV), which was lowest in the BO-AF group, compared with BO and AF groups.

The content changes of free fatty acids differed from phospholipids, of which unsaturated fatty acids were the main free fatty acids that occurred in oxidation. Aldehydes are common secondary oxidation products in the AF samples, such as hexanal and heptanal, mainly

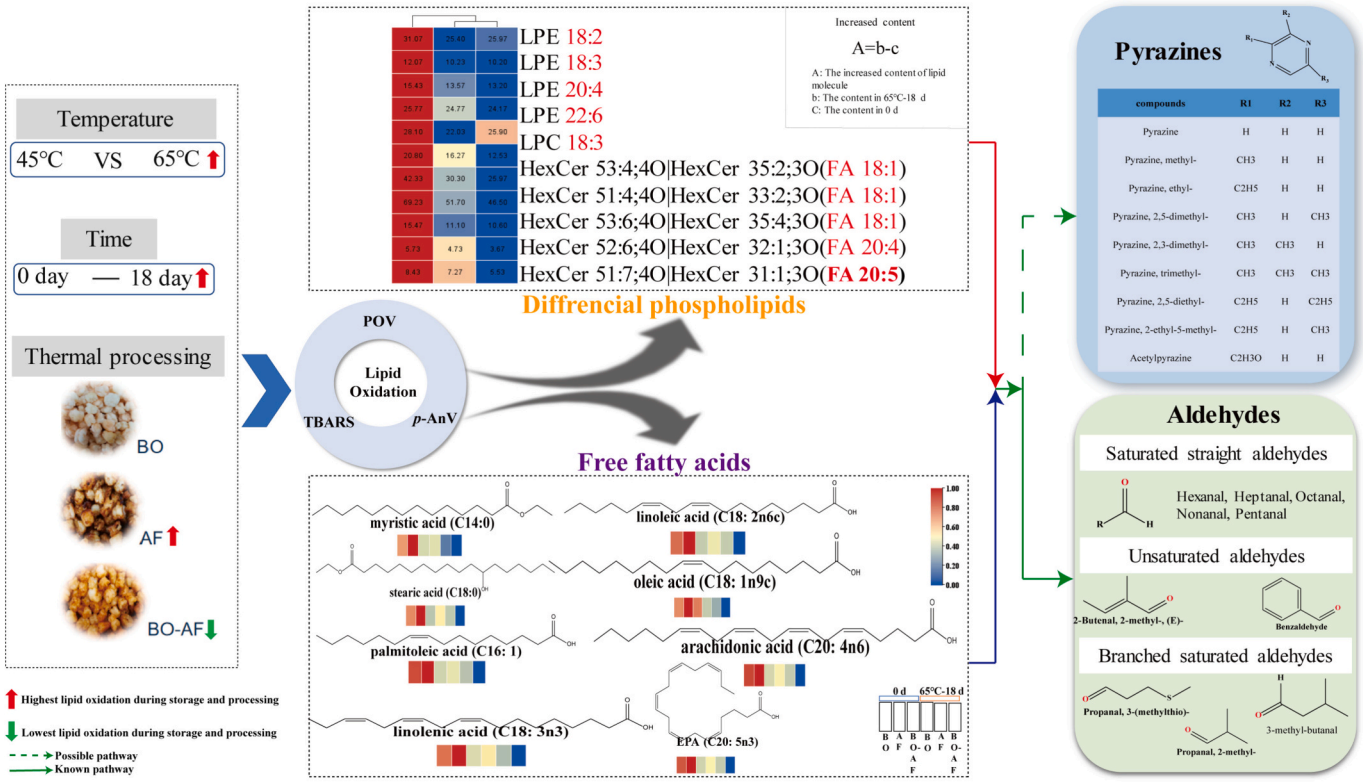

Fig. 5. Possible partial potential mechanism for the formation of volatile compounds by oxidative decomposition of phospholipids and free fatty acids. The data in the heat map was treated with Min-Max Normalization (0–1). Values in the figure are average values, n = 3.

formed by oxidizing linoleic acid and arachidonic acid, respectively (Ding et al., 2022). Among the three treatment groups, the content of free fatty acids in the AF group after processing was the highest, and they were greatly oxidized and degraded in storage, while the BO-AF group had a relatively low content of free fatty acids, which may be one of the reasons for the low lipid oxidation degree and volatile compound concentration in BO-AF group during storage.

In addition, processing factors, such as the heat transfer rate of cooked meat and raw meat during AF processing, oxygen concentration in meat, and changes in meat substrate state (de Oliveira et al., 2022), may contribute to the low oxidation of BO-AF lipid. The cooked crayfish meat may delay the spontaneous reaction of lipids with oxygen during the air frying process of crayfish meat, and may also pre-inactivate the pro-oxidation elements in food, such as myoglobin, hemoglobin, metal ions, etc. (Maqsood, Benjakul, Abushelaibi, & Alam, 2014). In summary, LPE (18:2, 18:3, 20:4, 22:6), LPC (18:3), and five types of HexCer generated a large number of flavor compounds through oxidative degradation pathways during storage. These flavor compounds included unsaturated aldehydes, saturated aldehydes, branched-chain aldehydes, and pyrazine compounds. The lipid oxidation intermediate products involved in the formation pathways of these flavor compounds led to significant changes in three indicators representing different states of

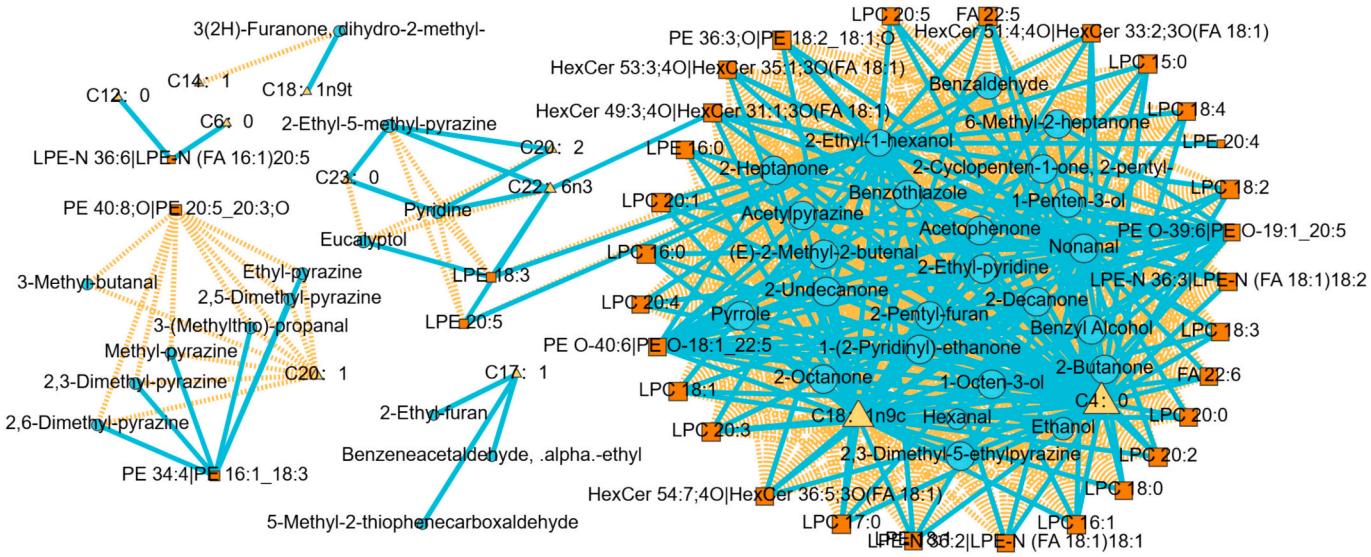

Fig. 6. Correlation analysis between differential lipids, free fatty acids and differential volatile flavor substances.

lipid oxidation products: TBARS, POV, and *p*-AnV. In addition, more free fatty acids were released during thermal processing, such as C18:1n9 and C18:2n6. Previous studies have demonstrated that these free fatty acids are the main precursors of lipid-derived volatile compounds (Zhou et al., 2024).

In order to better elucidate the relationship between phospholipid molecules and free fatty acids and volatile compounds, we performed correlation analyses on the 35 differential lipids, 28 free fatty acids, and 46 differential volatiles screened above (Fig. 6). The effect of phospholipids on the flavor of dried shrimp was related to the degree of unsaturation of the fatty acids that make up the phospholipids. The oxidative stability of phospholipids depends on the structure of sn-1 and sn-2 on the fatty acids, thus leading to significant differences in thermal degradation products (Wu & Wang, 2019). Unsaturated fatty acids exhibit higher oxidative sensitivity, and the cleavage of unsaturated double bonds catalyzed by heat and oxygen is more likely to produce carbonyl compounds (Choe & Min, 2006).

37 volatiles showed significant negative correlations with differential lipids and free fatty acids. Among them, ketones (8), pyrazines (8), and other nitrogen- and oxygen-containing compounds (10) were negatively correlated with PE lipids (PE O-19:1_20:5, PE O-18:1_22:5) as well as C4:0 and C18:1n9c, whereas aldehydes (5) and alcohols (6) showed significant negative correlations with FA (FA 22:6), HexCer (HexCer FA 18:1), LPC, LPE (LPE-N (FA 18:1)18:1, LPE-N (FA 18:1)18:2, LPE 18:1), PE (PE 18:2_18:1; O, PE O-19:1_20:5, PE O-18:1_22:5), C4:0, C18:1n9c, showed a significant negative correlation; which suggests that PE differential lipids (PE O-19:1_20:5, PE O-18:1_22:5) and C4:0, C18:1n9c may be the main flavor precursors that constitute the flavor profile of dried crayfish during processing as well as storage. A study also demonstrated that PE(C18:1/C18:1), PE(C18:0/C18:0) significantly promoted the production of pyrazines and aroma compounds such as benzaldehyde (Zheng et al., 2022). In addition, C4:0, C18:1n9c with FA (FA 22:5, FA 22:6,), LPC, HexCer (HexCer FA 18:1), LPE (LPE 16:0, LPE 18:1, LPE-N (FA 18:1)18:1, LPE-N (FA 18:1)18:2, PE 18:2_18:1; O) showed a highly negative correlation; C4:0 showed a highly positive correlation with PE O-19:1_20:5 and PE O-18:1_22:5. The formation of C4:0 and C18:1n9c was associated with these phospholipids.

4. Conclusions

In summary, the thermal processing resulted in changes in the volatile compounds and lipids of the crayfish meat. Aldehydes and heterocyclic compounds were the main volatile compounds. Lipid oxidation occurred continuously during storage, and the peak values of POV and TBARS in the three treatment groups were during 12–15 days. AF treatment had the most effects on lipid oxidation and increased the concentration of volatile compounds in crayfish meat during processing and storage, while BO-AF was the opposite. The content of free fatty acids, especially unsaturated fatty acids, was the highest after AF treatment, which provided the precursor substance for lipids oxidation and volatile compounds production (aldehydes, ketones, alcohols, and heterocycles) during storage. In the analysis of phospholipid species, the change pattern of phospholipid molecules in the AF and BO groups was similar during storage, but the types of differential lipids in the BO-AF group were different. Finally, the changes of different phospholipids before and after storage were compared, and it was found that the BO-AF group may reduce lipid oxidation and volatile compound generation by delaying the further degradation of LPE, PLC, HexCer, and other phospholipids during storage. This composite method of BO-AF was precuring by BO (relatively low processing temperature) and then processing at higher temperatures, which seems to be more conducive to the maintenance of crayfish quality.

CRediT authorship contribution statement

Mingzhu Zhou: Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Dongyin Liu:** Writing – review & editing, Writing – original draft, Methodology. **Hongyuan Tan:** Methodology, Data curation. **Chao Wang:** Supervision. **Wei Yu:** Supervision. **Guangquan Xiong:** Supervision. **Lan Wang:** Supervision. **Wenjin Wu:** Supervision. **Yu Qiao:** Writing – review & editing, Supervision, Investigation, 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 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.2025.102406.

Data availability

No data was used for the research described in the article.

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