**Lipid oxidation and flavor changes in saturated and unsaturated fat fraction from chicken fat during thermal process**

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**Abstract:** Chicken fat, due to its rich fatty acids (FAs), is more prone to lipid oxidation and the production of volatile compounds. This aim of the present study was to investigate the oxidative characteristics and flavor changes of saturated (SFF) and unsaturated fat fraction (USFF) from chicken fat induced by heating (140 °C at 70 rpm/min for 1 h and 2 h: SFF1, USFF1, SFF2 and USFF2). The FAs and volatile compounds were analyzed by gas chromatography-mass spectrometry (GC-MS) and two-dimensional gas chromatography time of flight mass spectrometry (GC × GC-ToFMS), respectively. The results showed that higher contents of unsaturated fatty acids (UFAs) were found in USFF compared to SFF, whereas USFF showed lower levels of saturated fatty acids (SFAs). With the extension of heating time, the SFAs/UFAs in USFF and SFF significantly increased (*p* < 0.05), and more aldehydes, alcohols, ketones, and lactones were formed. Moreover, the odor activity values of 23 important compounds in USFF1-2 were significantly higher (*p* < 0.05) than those in SFF1-2. As revealed by principal component analysis (PCA) and cluster analysis (CA), it was obviously observed that all samples were divided into four clusters (USFF-SFF, USFF1-SFF1, USFF2, and SFF2). According to correlation analysis between FAs and volatile compounds, C18:2 ω6, C18:3 ω6 and C18:3 ω3 were significantly associated with dodecanal, (*Z*)-3-hexenal, (*E*)-2-decenal, 2-undecenal, (*E*)-2-dodecenal, (*E,E*)-2,4-nonadienal and (*E,E*)-2,4-decadienal, 2-decanone, γ-octalactone and γ-nonalactone. Our data elucidated the fat fraction from chicken fat with varying degrees of saturation could impart different flavor characteristics during thermal process.

**Keywords:** Oxidative properties, Heating time, Different fat fractions, Volatile compounds, Fatty acid composition

# Introduction

As an essential component in meat products, animal fat affects meat flavor and palatability, and contributes to the species distinctive flavor after reacting with other components.1,2 According to reports, the main fatty acids (FAs) in chicken fat are palmitic acid (C16:0), oleic acid (C18:1 ω9) and linoleic acid (C18:2 ω6), and the contents of unsaturated fatty acids (UFAs) in chicken fat is higher than that in other animal fats,3 making it a potential ingredient in the elaboration of meat products for improving nutritional value.4 Additionally, due to the oxidation characteristics of UFAs, it has also been found that chicken fat can be used to produce or enhance meat flavors in different processed-meat flavorings or meat processing.

Chicken fat plays a crucial role in forming species-specific flavors, and the oxidation of lipid during heating is the main factor responsible for volatile organic compounds, such as aldehydes, ketones, alcohols, esters and aliphatic compounds.5,6 It has been reported that the Maillard reaction process was noticeably enhanced by producing more aliphatic aldehydes and alcohols (green/fatty/fruity notes) after addition of chicken fats, especially with the addition of oxidized chicken fat.7-9 Whereas, it is also well-known that lipid oxidation to a certain extent produces off-flavors, known as “warmed-over flavor”. For example, high concentrations of hexanal, octanal, and nonanal may contribute rancid, pungent and other undesirable flavor characteristics to meat.8 Thus, the oxidation reaction of lipid would be significant for the formation of a special flavor during thermal treatment.

Oxidative susceptibility of lipids is correlated with FA compositions, especially the degree of unsaturation of lipids. It is widely accepted that UFAs are more prone to oxidation.10 Evidence has shown that phospholipids are more critical in developing volatile compounds during the cooking of meat than triacylglycerols.11 This is attributed to a higher proportion of UFAs, especially arachidonic acid (C20:4) in phospholipids.12 Also, a previous study has shown that long-chain ployunsaturated fatty acids (PUFAs) of ω3 FAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have direct and beneficial effects on healthy.13 It has also been reported that the meat products considered functional foods by adding UFAs.14 For instance, the ω3 PUFAs and balance of ω3 to ω6 FAs (approximately 2:1) in human diet can effectively reduce the incidence of lifestyle disease like coronary artery diseases, hyper tension and diabetes.13,15,16 Besides, a recent study revealed that FAs are flavor precursors of lipid hydrolysis, and the UFAs produce various volatile compounds by oxidation treatments.17 The volatile compounds are negatively associated with the content of saturated fatty acids (SFAs).18 Due to different lipid content and FA compositions, including various UFAs and SFAs, they would notably affect the volatile compounds of duck products.19 Currently, the above studies regarding FAs have mainly focused on human health and characteristic volatile compounds induced by oxidation. However, there is a systematic lack of study about the impact of the fat fractions with varying degrees of saturation during thermal process on flavor characteristics, and the available literature has limited information on the relationship between special FAs and volatilome in different fat fractions from chicken fat.

In the present study, yellow-feathered chicken fat was fractionated by step-wise dry fractionation process to obtain saturated triglycerides-enriched fractions and unsaturated triglycerides-enriched fractions.20 We exploited the headspace solid phase microextraction (HS-SPME) combined with two-dimensional gas chromatography time of flight mass spectrometry (GC × GC-ToFMS) to compare volatile compounds of saturated and unsaturated fat groups from chicken fat, and then to quantify how these volatile compounds vary with thermal induced oxidation process. Simultaneously, the quantitative relationship between special FAs and volatile compounds is also clarified by partial least squares regression (PLSR). This work was expected to provide important information to improve the flavor in processed-meat flavorings or meat processing.

# Materials and methods

## Chemicals and materials

A C7-C40 n-alkane mixture was obtained from Sigma-Aldrich (St. Louis, MO, USA) to determine linear retention indices. 2-Methyl-3-heptanone (99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The chicken fat was obtained from a commercial broiler processing plant in Urumchi city, Xingjiang Province, China. Here, chicken fat refers to the abdominal fat of yellow-feathered chickens, often sold as a by-product of the company. Three independent batches of chicken fat on different days were used in this study.

## Fractionation of chicken fat

In each batch, frozen chicken fat (-20 °C) was thawed at 4 °C overnight and cut into small cubes (approximately 0.5🞨0.5🞨0.5 cm3). Around 1000 g of chicken fat was placed in a 2-L beaker and melted in water bath (HWS-12, Yiheng Scientific Instrument Co., Ltd, Shanghai, China) at 100 °C for 30 min to separate chicken fat from fat tissues. The obtained oil was separated from solid impurities by two layers of medical gauze and stored at 4 °C overnight.

The fractionation process was designed on the basis of step-wise dry fractionation, using a modification of the procedure described by Liu et al. (2018) (Fig. S1).10 Firstly, the chicken fat was heated into liquid in a water bath at 60 °C for 30 min. Then, the chicken oil was cooled in water bath to 24 °C and incubated overnight. Chicken oil was centrifuged at 10,000 g for 1 h at 24 °C. The solid and liquid fraction were obtained using a benchtop centrifuge (Allegra 64R, Beckman Coulter Inc., Brea, California, USA) and stored at 4 °C overnight. The above different fractions were turned into oil in a water bath at 60 °C. Subsequently, the solid fraction was cooled to 30 °C in water bath and centrifuged at 10,000 g for 1 h at 30 °C. The obtained solid layer was used as the saturated fat fraction (SFF). Similarly, the liquid fraction was further fractionated at 20 °C and centrifuged at 10,000 g for 1 h at 20 °C. The obtained liquid layer was used as the unsaturated fat fraction (USFF). Consequently, these two fat fractions were collected and stored at -80 °C.

## Preparation of the oxidized chicken fat

The oxidized chicken fat was prepared by the heating-induced process. Twenty-five milliliters of SFF or USFF was placed in a 250 mL three-necked round bottom flask (Kastmer Technology Development Co., Ltd, Beijing, China). The necks of the flask were connected to a reflux condenser ([i-Quip-R3439](http://www.dkmchem.hk/product/274201.html), Aladdin Biochemical Technology Co., Ltd, Shanghai, China) and one glass vent pipe ([i-Quip-R3399](http://www.dkmchem.hk/product/274201.html), Aladdin Biochemical Technology Co., Ltd, Shanghai, China) to supply compressed air at a rate of 60 mL/min. The oxidization reaction of SFF and USFF was performed at 140 °C using oil-bath (Du-20, Yiheng Scientific Instrument Co., Ltd, Shanghai, China) through a hydrothermal method with magnetic stirring (RCT Basic, IKA®-Werke GmbH & CO., Staufen, Germany) at 70 rpm/min for different time (1 h: SFF1 and USFF1; 2 h: SFF2 and USFF2).

## Fatty acids composition

The FAs composition of different chicken fat samples was determined following methylation with some modifications based on Al-Dalali, Li, and Xu (2022)21 and Liu et al. (2018).10 In a test tube, 50 mg of the fat fraction was added to 1.5 mL of 0.5 M NaOH in methanol. The tube was placed in boiling water for 5 min. After cooling, 2 mL of 14% (w/v) boron trifluoride methanol solution (BF3-CH3OH) was added, and the mixture was heated in the boiling water for another 5 min. After cooling at room temperature, 5 mL of heptane and 2 mL of saturated NaCl solution were added to the tube, which was then shaken on a vortex-type mixer for 1 min. The mixture was separated into two layers after standing for 10 min. The upper heptane layer was transferred to a new test tube and dried with nitrogen. The obtained fatty acid methyl esters were stored at -20 °C until chromatographic analysis.

Chromatographic separation was performed using an Agilent Technologies 7890N gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) with a flame-ionization detector and a DB-23 fused silica capillary column (60 m, 0.25 mm i.d., 0.25 μm film thickness, Agilent, USA). Chromatographic conditions were as follows: initial oven temperature of 50 °C (held for 5 min), first ramp at 20 °C/min to 175 °C (held for 3 min), second ramp at 3.5 °C/min to 200 °C, third ramp at 1 °C/min to 210 °C, and final ramp at 1.5 °C/min to final temperature of 230 °C (held for 13 min). The temperature of the injector and detector was maintained at 250 °C. Helium was used as carrier gas at a constant flowrate of 1.2 mL/min. One microliter of solution was injected in split mode (1:50). Identification and quantification of FAs were performed by comparison of the retention times and standard curve with standards (Supelco™ 37 Component FAME Mix, Supelco, Bellefonte, PA, USA). The concentration of individual FA was expressed as g/100 g of chicken fat, and summarized as SFA, monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), UFA. The ratio of SFA to UFA was calculated.

## Thiobarbituric acid reactive substance (TBARS)

The TBARS of different chicken fat was measured as reported by Bao, and Ertbjerg (2015).22 The TBARS values, expressed as mg malonaldehyde (MDA)/kg, were calculated as follows:

(1)

where *A532* is the absorbance of the solution, *Ws* is the chicken fat weight (g), and “9.48” is a constant derived from the dilution factor and the molar extinction coefficient (152000 M-1 cm-1) of the red thiobarbituric acid reaction product.

## Volatile compounds of chicken fat with different heating time

### *2.6.1. Extraction of volatile compounds*

Volatile compounds were isolated from different fat fractions following a previously described method.23 A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (df 50/30 μm, 2 cm) fiber (Supelco, Belletonte, PA, USA) was employed for the extraction of volatile compounds. The automation of HS-SPME process was performed using a MPSFF2 multipurpose sampler (Gerstel, Mülheim an der Ruhr, Germany) equipped with an agitator, and SPME fiber conditioning station was installed on the GC × GC-MS system. Two milliliters of sample oil and 2 μL internal standard (2-methyl-3-heptanone, 0.816 μg/μL in methanol) were placed in a 20-mL headspace glass vial. Before the extraction, the samples were incubated at 60 °C for 10 min. During the extraction, the vial was agitated at 100 rpm for 3 s every four seconds. Extraction was carried out at 60 °C for 40 min.

### *2.6.2. GC × GC-ToFMS analysis*

After the extraction, the SPME fiber was automatically inserted into the GC × GC-ToFMS injection port at 250 °C and kept for 10 min for desorption. The working condition of GC **×** GC-ToFMS in this study was modified according to the methods by Shi, Zhu, Zhang, Lin, and Lv (2020).24 LECO Pegasus 4D (LECO, St. Joseph, MI, USA) GC **×** GC-ToFMS system consisted of an Agilent GC 7890B gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), with a cold-jet modulator and a high-resolution time of flight mass spectrometry (Zoex Corp., NE, USA). A DB-Wax (30 m **×** 0.25 mm I.D., 0.25 μm film thickness) was used as the first dimension (1st D) column and a DB-17 ms (1.78 m **×** 0.1 mm I.D., 0.1 μm thickness) was used as the second dimension (2 nd D) column. Ultra-high purity (99.9999%) helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The primary oven temperature was maintained at 40 °C for a further 4 min, and the temperature was raised at 3 °C/min to 160 °C, then at 20 °C/min to 240 °C (12 min). The secondary oven temperature was kept at 5 °C offset (above the primary oven temperature). The modulator temperature was kept at 5 °C offset (above the secondary oven temperature). The transfer line was set at 270 °C. The modulation period was 5 s with the hot jet widths of 300 ms. The mass spectrometer was operated in electron impact mode with an electron energy of 70 eV. The ion source was maintained at 220 °C. The mass spectrometer scanned from m/z 20 to 500 at 100 scans/s, and the voltage was 1640 V.

### *2.6.3. Identification and quantification of volatile compounds*

The volatile compounds were tentatively identified by comparing the similarity of mass spectrometric information of each chromatographic peak with NIST (Version 2.0, National Institute of Standards and Technology, Gaithersburg, USA) mass spectra library. Also, the similarity matching threshold and reverse match threshold should be greater than 850. Later, the identified compounds were further confirmed by comparing their retention index (RI) values with those of published values. The experimental retention index (RIexp) in 1st D was calculated after the injection of the liquid sample solution of n-alkanes (C7-C40) under the same conditions of the GC × GC-ToFMS analysis (injection volume of 1 μL, injection rate 20 μL/s). ﻿A compound was identified if the 1st D RIexp and reported RI did not differ by more than 50 units.

Concentration of the volatile compounds was measured by comparison of their peak areas with that of the 2-methyl-3-heptanone internal standard (IS).19 The equation can be written as follows:

(2)

Where Conc stands for the concentration of detected volatile compound, and V (IS) represent the volume of added internal standard (2 μL).

The odor activity value (OAV) was calculated using the following equation:

(3)

where Ci is the concentration of a compound in fat fraction from chicken fat and OTi is the odor threshold in water. OTi was obtained from the online database (http://www.odour.org.uk).

## 2.7. Statistical analysis

The experiment data were expressed as the mean ± standard deviation. Significant differences of means were determined by analysis of variance (ANOVA), and Duncan’s multiple range tests (*p* < 0.05) were performed by SPSS 19.0 (IBM, Armonk, NY, USA). Multivariate statistical analysis, including principal component analysis (PCA) and clustering analysis (CA) were conducted using the software XLSTAT (2016) from Addinsoft (Barcelona, Spain). The heatmap of the correlation data of partial least squares-discriminant analysis (PLS-DA) and partial least square regression (PLSR) were applied by R v3.2.2 (R Studio Team, 2012).

# Results and discussion

## Fatty acids and thiobarbituric acid reactive substance values in saturated and unsaturated chicken fat fractions during thermal process

FAs are considered as important flavour precursors in chicken fat because the oxidation process generates abundant volatile compounds through various pathways.7 The changes in FAs composition may be ascribed to the lipolysis of triglycerides and phospholipids.19 In this study, a total of fifteen FAs were identified, including five SFAs, four MUFAs and six PUFAs, of which the dominant FAs in chicken fat samples were palmitic acid (C16:0), oleic acid (C18:1 ω9) and linoleic acid (C18:2 ω6), which corroborate those found by Santos, Lima, Madruga, and Silva (2020) (Table 1).25 Significant differences (*p* < 0.05) in FA compositions were observed in SFF and USFF from chicken fat. The content of ∑SFA (P = 0.016) and percentage of ∑SFA/∑UFA (P = 0.000) in SFF were significantly higher than those in USFF, while the contents of ∑MUFA (P = 0.026), ∑PUFA (P = 0.006) and ∑UFA (P = 0.008) presented relatively low levels in SFF. This result showed that there are differences in FA components between saturated and unsaturated fat fractions extracted. Similar results were found by Liu et al. (2018),10 who obtained fat fractions from lard differing in FA compositions. In addition, regardless of heating for 1 or 2 h, the content of UFAs in USFF was significantly greater (*p* < 0.05) than that in SFF, and this result was related to the composition of the extracted fat fraction. Furthermore, with increased heating time, all PUFAs except for C20:2 had no significant difference in SFF or USFF, whereas the overall trend is decreasing. This might be attributed to lipid oxidation, which induced the formation of a larger number of volatile compounds.26 It was also found that there was no significant difference for SFAs and MUFAs in USFF, USFF1 and USFF2. Except for C20:1 ω9, the contents of SFAs and MUFAs in SFF first decreased and then increased (*p* < 0.05) during the heating process. This may be that the SFAs and MUFAs in triacylglycerols treated for a short time were involved to produce more volatile compounds through chemical reactions. Subsequently, a long heating time would induce a high release of the neutral lipids containing more abundant SFAs and MUFAs.27

The TBARS value is a suitable indicator for evaluating the extent of lipid oxidation in meat products.28 The initial TBARS values of USFF and SFF were 1.18 mg MDA/kg fat and 0.21 mg MDA/kg fat, respectively. Also, USFF has significantly higher (*p* < 0.05) levels of TBARS than SFF after 1 and 2 h heat treatment (Fig. 1). This may be due to the higher content of UFAs in USFF (Table 1), which was more prone to oxidation reaction under heating conditions. Additionally, the TBARS values of both saturated and unsaturated fat groups increased significantly (*p* < 0.05) with the extension of heating time, indicating that heating time greatly influenced lipid oxidation.

## Volatile organic compounds profiling in saturated and unsaturated fat fractions from chicken fat during thermal process

Volatile compounds from the different oxidized fat samples were detected by GC **×** GC-ToFMS, and the results are presented in Table S1 and Table 2. A total of 150 volatile compounds have been identified in different oxidized fat fractions, namely aldehydes (29), ketones (30), alcohols (26), hydrocarbons (18), phenols (2), esters (14), acids (7), and O-, N-, S-containing compounds (24). These compounds were mainly from thermal oxidation and degradation of lipids, as well as further interactions among protein, peptides and free amino acids.3,6 Among them, the contents of aldehydes, ketones, alcohols, esters, acids, O-, N- and S-containing compounds in different fat fractions after heating treatment (USFF1, USFF2, SFF1 and SFF2) were higher than those in unheated fat fractions (USFF and SFF), however, the hydrocarbons in USFF1, USFF2, SFF1 and SFF2 have showed the lower contents. This may be due to the fact that thermal treatment could accelerate the development of lipid oxidation to generate flavor contributors,19 and hydrocarbons can be used as important intermediates to participate in the formation of heterocyclic compounds.26

It was found that there were 45, 97, 115, 50, 101 and 108 volatile compounds in USFF, USFF1, USFF2, SFF, SFF1 and SFF2, respectively. Besides, the concentration of volatile compounds constantly increased for both USFF and SFF during heating process. These results indicated that the prolonged high-temperature treatment resulted in more types of volatile compounds as well as their concentrations. The quantities and contents of volatile compounds in USFF was significantly lower (*p* < 0.001) than those of SFF. However, it was found that the amount and contents of volatile compounds in USFF2 were significantly higher (*p* < 0.05) than those in SFF2. This showed that the fat fraction with more unsaturated components was more likely to produce volatile compounds during heating.

### *Aldehydes*

Aldehydes are regarded as the major volatile compounds of lipid oxidation in various meat or meat products because of low odor thresholds.5 Five alkanals (pentanal, hexanal, heptanal, octanal and nonanal), three alkenals ((*E*)-2-pentenal, (*E*)-2-heptenal and (*E*)-2-octenal) and benzaldehyde could be detected in all fat fraction samples. It has been reported that alkanals and alkenals were mainly generated from oxidation of UFAs like C18:1 ω9, C18:2 ω6 and C18:3 ω3,29,30 and benzaldehyde was derived from phenylglycine through the Strecker degradation pathway6 or linolenic acid through oxidative degradation pathway.31 Meanwhile, the contents of these compounds, except for pentanal and octanal, increased significantly (*p* < 0.01) with the extension of heating time in both fat fractions. Furthermore, the decanal, undecanal, (*E*)-4-heptenal, (*E*)-2-nonenal, 2-undecenal, (*E,E*)-2,4-hexadienal, (*E,E*)-2,4-heptadienal, (*E,E*)-2,4-octadienal, (*E,E*)-2,4-nonadienal and (*E,E*)-2,4-decadienal were found in the oxidized fat fractions groups (USFF1, USFF2, SFF1 and SFF2), with higher levels observed in USFF2 and SFF2. These results indicated that the longer the heating time, the more favorable the formation of aldehydes. In addition, when heated for 1 or 2 h, almost all aldehydes in USFF were more abundant than those in SFF, indicating that the degree of lipid oxidation in USFF is greater than that in SFF.

### *Ketones*

Ketones are formed by lipid oxidation and usually have a peculiar odor in food.32 The contents of some ketones, such as 1-penten-3-one, 1-octen-3-one, (*E*)-3-octen-2-one and (*E*)-3-nonen-2-one, in heat-treated fat fraction samples (USFF1, USFF2, SFF1 and SFF2) were markedly higher (*p* < 0.05) than those in the unheated fat fraction samples (USFF and SFF). They were considered as the most contribution to the oxidized fat fractions from chicken fat (Table 2). For 2-ketones, 2-hexanone, 2-heptanone, 2-octanone, 2-nonanone and 2-decanone, they could impart more fruity/sweet aroma to the fat fraction samples, and come from lipid oxidation.33 However, 2-undecanone less contributed to the flavor of fat fraction samples due to its lower content, and derived from Maillard reaction.34 The methyl ketones, like 5-methyl-3-heptanone and 2-methylcyclopentanone, could be produced from β-keto acid decarboxylation35 or β-oxidation of SFAs.36 Additionally, after heating with USFF and SFF for 1 and 2 h, the content of ketones in USFF1-2 was significantly higher (*p* < 0.05) than that in SFF1-2, and the certain ketones (6-undecanone, 3-hexen-2-one, (*E,E*)-3,5-octadien-2-one and 2-ethylcyclopentanone) were only detected in USFF2, but 3-ethyl-2-cyclopenten-1-one was only detected in SFF2. It can be seen that the ketones produced by the two fats with different levels of saturation during heating were different.

### *Alcohols*

Alcohols could provide pleasant fruity and floral aroma,37 and generally are not thought of as important contributors owing to their high threshold.38 The identified alcohols were generated through the degradation of secondary hydroperoxides of FAs.39 The contents of butanol, pentanol, octanol, heptanol, nonanol, 1-penten-3-ol and 1-octen-3-ol in USFF and SFF increased significantly (*p* < 0.05) with the extension of the heating. Similar results were found that there was an increase in alcohols content with heat treatment.6 Nonanol, 3-heptanol, 4-octanol, 3-octanol, (*Z*)-3-penten-1-ol, (*Z*)-2-penten-1-ol and (*E*)-2-octen-1-ol were detected in USFF1, USFF2, SFF1 and SFF2, while 3-hexanol, 4-heptanol and (*E*)-2-penten-1-ol were exclusively present in USFF2, and 2-hexanol and (*E*)-2-hexen-1-ol were only found in SFF2. Furthermore, the branched alcohols, especially 1-ethoxypropan-2-ol and 1-propoxypropan-2-ol, were observed in USFF and SFF. This could be due to the fact that these two compounds can be used as important intermediates to participate in the formation of esters.40

### *Hydrocarbons, Esters and acids*

As shown in Table 2, the contents of undecane, tridecane and decene significantly increased (*p* < 0.05) with the extension of heating time. It is probably because the thermal degradation of lipid or autoxidation of long-chain FAs8 produced some aromatic and aliphatic hydrocarbons. While the contents of toluene, ethylbenzene, p-xylene, o-xylene and styrene have an opposite trend. It may be attributed to the fact that these compounds are more prone to chemical reactions with other compounds under high temperature conditions. A large number of esters were found in USFF2 and SFF2, whereas only small amounts of esters were observed in USFF and SFF. This suggested that long-time thermal treatment led to a marked increase of esters (*p < 0.05*). It was found that eight typical lactones are fat-derived volatile compounds, including six 5-memberd rings (γ-valerolactone, γ-butyrolactone, γ-caprolactone, γ-heptalactone, γ-octalactone and γ-nonalactone) and two 6-memberd rings (δ-hexalactone and δ-valerolactone), respectively.41,42 The contents of typical lactones in USFF1 were apparently higher than those in SFF1, and similar results were also detected in USFF2 and SFF2. It may be that USFF was more beneficial for the formation of lactones. Seven acids were detected, most of which had high thresholds, which had a synergistic effect on the flavor of the oxidized fat samples. For instance, formic acid, hexanoic acid and nonanoic acid are formed through the hydrolysis of triglycerides and contributed to fatty flavors.43

### *Heterocyclic compounds*

It can be seen from Table 2, in terms of content, the following trend was observed: O-containing compounds > N-containing compounds > S-containing compounds. The O-containing compounds with high content in fat fraction samples were 2-ethylfuran, 2-propylfuran, 2-butuylfuran, 2-pentyluran, 2-heptylfuran, 2(5H)-furanone and 2H-pyran-2-one. Which are mainly derived from the oxidation and degradation of lipid.7 For instance, the 2-ethylfuran and 2-pentylfuran are noncarboxylic compounds generated from the C10 hydroperoxide of linolenate and linoleate respectively by the singlet oxygen oxidation.1 Four N-containing heterocyclic compounds were detected, of which pyrazine is usually formed at high temperature and provides the unique nutty, meaty and popcorn-like aroma.44 After heating for 1 h, the content of pyridine and 3-ethylpyridine in different fat fractions significantly increased (*p* < 0.05), but decreased significantly (*p* < 0.05) after heating for 2 h. This may be due to the formation of lipid oxidation and degradation products (pyridine and 3-methylpyridine) at the beginning of heating, which participate in the Maillard reaction with the increase of heating time.

It is worth noting that the content of 2-formylthiophene in SFF significantly increased (*p* < 0.05) during thermal process. It may be formed after the products of lipids oxidative decomposition take part in Maillard reaction.7

## Odor activity values of odor-active compounds in saturated and unsaturated fat fractions from chicken fat during thermal process

To assess the flavor contributions of volatile compounds, the OAVs were applied to screen odorants in different fat fractions.45 The compounds with OAVs > 1 were considered as the odor-active compounds, all of which are shown in Table 3. A total of 42 odor-active compounds were detected in six fat fraction samples. Among them, the OAVs of alkanals, like pentanal (fruity aroma), hexanal (green and grass aroma), heptanal (fatty and putty aroma), octanal (fatty and pungent aroma), nonanal (fatty and floral aroma) and decanal (orange peel and soapy aroma) in USFF1, USFF2, SFF1 and SFF2 were significantly higher (*p* < 0.05) than those in USFF and SFF, which might be the main reason that the heating could efficiently improve the volatile organic compounds profile of fat fraction samples. For alkenals ((*Z*)-3-hexenal, (*Z*)-4-heptenal, (*E*)-2-heptenal, (*E*)-2-octenal, (*E*)-2-nonenal and (*E*)-2-decenal) and alkadienals ((*E,E*)-2,4-nonadienal and (*E,E*)-2,4-decadienal), there was the higher level of OAVs in USFF2 and SFF2 than USFF1 and SFF1, indicating that prolonged heating treatments could promote the increase of olefin aldehydes. Additionally, the OAV of aldehydes in USFF1 was significantly higher (*p* < 0.05) than that of SFF1, and the same results were also observed for USFF2 and SFF2, which has shown that USFF with more UFAs could contribute to more fatty, grassy, fruity and sweet aroma at high temperature.

Additionally, the ketones (except for 2-heptanone and 2,3-pentanedione) presented the high OAVs in USFF1, USFF2, SFF1 and SFF2, while they were not found in USFF and SFF. It was shown that these ketones, especially 2-decanone and 1-octen-3-one, contributed fruity/floral/cheese notes to thermal oxidative fat samples. Due to their higher OAVs, 1-octen-3-ol and (*E*)-2-octen-1-ol could provide more intense mushroom and green apple aroma to USFF1, USFF2, SFF1 and SFF2. In particular, it has been reported that 1-octen-3-ol was one of the sources of characteristic flavor of chicken soup.46 The OAVs level of the long-chain esters (γ-octalactone and γ-nonalactone) with fatty notes was quite high, whereas the butyl butyrate with pineapple notes was showed in lower OAVs level. Regarding 2 furans, 2-ethylfuran and 2-pentylfuran might give the oxidized chicken fat the rich rubber and sweet flavor, respectively. Overall, 23 odor-active compounds, pentanal, hexanal, heptanal, octanal, nonanal, decanal, (*Z*)-3-hexenal, (*Z*)-4-heptenal, (*E*)-2-heptenal, (*E*)-2-octenal, (*E*)-2-nonenal, (*E*)-2-decenal, 2-undecenal, (*E,E*)-2,4-nonadienal, (*E,E*)-2,4-decadienal, 2-decanone, 1-octen-3-one, 1-octen-3-ol, (*E*)-2-octen-1-ol, γ-octalactone, γ-nonalactone, 2-ethylfuran and 2-pentylfuran, with a relatively high OAV in USFF1, USFF2, SFF1 and SFF2 samples, were known as important volatile compounds due to the significant contributions to the overall aroma of oxidized chicken fat.

## PCA, CA and PLS-DA of odor-active compounds

PCA was applied in the present study to better visualize the distribution of odor-active compounds in different fat fraction samples. As shown in Fig. 2A, the first two principal components (PC1 and PC2) were able to explain 75.14% and 11.78% of the data variance, respectively. The cumulative variance contribution rate was > 85%, indicating that most of odor characteristics of different fat fraction samples could be reflected by PC1 and PC2. Six fat fraction samples were clearly distinguished on PC1-2, and had their own aroma regions at different heating time stages. The sample dot of USFF2 was located on the positive side of PC2, whereas the sample dot of SFF2 was on the opposite side. It has been showed that significant differences were exhibited in odor-active compounds of the fat fraction samples with different UFA composition after 2 h of heating. The sample dot of USFF1 and SFF1 were distributed in the upper left side, which were associated with styrene, benzeneacetaldehyde and 2-ethylfuran. The sample points of USFF and SFF were clustered together, meaning that there was a similar odor profile.

Moreover, a heatmap has also been produced to display the differences in odor-active compounds among different oxidized fat fractions (Fig. 2B). Regarding the fat fraction samples, it was obviously observed that all samples were grouped into four clusters (USFF-SFF, USFF1-SFF1, USFF2, and SFF2). This result was consistent with the result of PCA. In terms of odor-active compounds, they were obviously distributed in five different regions. In zone I, the high OAVs of octanal and benzaldehyde were exhibited in SFF1 and SFF2. In zone II, the OAVs levels of 3-penten-2-one, nonanal, 2-heptanone, 2-pentylfuran, butyl butyrate, (*E*)-2-pentenal and (*E*)-2-heptenal in SFF2 and USFF2 were higher, while (*Z*)-4-heptenal, 1-penten-3-ol, hexanal and 1-penten-3-one with high OAVs were only present in SFF2. In zone III, the increase in 23 odor-active compounds showed a similar trend, after SFF and USFF was heated for 0 to 2 h. In zone IV, the OAVs of pentanal, (*E*)-2-hexenal and 3-ethyl-2-hydroxy-2-cyclopenten-1-one were the highest in USFF2, whereas in zone V, styrene, benzeneacetaldehyde and 2-ethylfuran were the highest in USFF1.

The correlation coefficients between odor-active compounds and different fat fraction samples were shown in Fig. 2C. According to the results, almost all compounds, except for styrene, represented a negative correlation with USFF and SFF. Benzeneacetaldehyde and 2-ethylfuran were significantly positively correlated with USFF1, as well as, pentanal and octanal had a strong positive influence on SFF1. Additionally, the significant positive effect of (*E*)-2-hexenal, (*E*)-2-hepetnal, 3-ethyl-2-hudroxy-2-cyclopenten-1-one and butyl butyrate on USFF2, while more compounds, such as 7 alkanals, 4 alkenals, 4 alkadienals, 7 ketones, 3 alcohols and 2 esters were highly relevant to SFF2, indicating that the volatile compounds formed were significant differences after 2 h of heat treatment of fat fractions with different FAs composition.

## Relationship analysis between fatty acids and odor-active compounds

The correlation analysis was performed to investigate the associations between FAs and odor-active compounds. The results indicated that all involved FAs had the positive and negative effects on odor-active compounds of different chicken fat samples (Fig. 3). The thermal oxidation of FAs creates classed of compounds, such as aldehydes, alcohols, ketones, esters, and furans, similar to those formed during lipid autoxidation.31,47 The SFAs of C16:0 and C18:0 were significantly positively associated with dodecanal, (*Z*)-3-hexenal, 2-undecenal, (*E,E*)-2,4-nonadienal, 2-decenone and γ-nonalactone (*p* < 0.05), and it was also found that C14:0 had significantly affected the content of (*E,E*)-2,4-decadienal, γ-octalactone and γ-nonalactone. However, the content of pentanal has showed a significant negative correlation with C20:0 in different chicken fat samples. It indicated that SFAs of C16:0, C18:0, C14:0 and C20:0 was responsible for the generation of aldehydes and volatile oxygen compounds.48 For MUFAs, C16:1 and C18:1 ω9 showed a strong positive correlation with dodecanal, (*Z*)-3-hexenal, γ-nonalactone, (*E*)-2-decenal and (*E*)-2-dodecenal. Meanwhile, C20:1 ω9 was observed that more effects on volatile compounds of different chicken fat samples than C16:1 and C18:1 ω9. Moreover, PUFAs of C18:2 ω6, C18:3 ω6 and C18:3 ω3 exhibited positive correlations with seven aldehydes (dodecanal, (*Z*)-3-hexenal, (*E*)-2-decenal, 2-undecenal, (*E*)-2-dodecenal, (*E,E*)-2,4-nonadienal and (*E,E*)-2,4-decadienal), one ketone (2-decanone) and two esters (γ-octalactone and γ-nonalactone). However, the content of C20:3 ω6 and C20:4 ω6 did not correlate with odor-active compounds. These analyses concluded that C18:0, C20:1 ω9, C18:2 ω6, C18:3 ω6 and C18:3 ω3 were confirmed as the key potential flavor precursors for the enhancement of overall flavor in different fat fraction samples. Additionally, C14:1, C20:0 and C20:2 was negatively related to the formation of pentanal, (*E*)-2-hexenal and styrene.

# Conclusions

As mentioned above, a total of 150 volatile compounds were identified in different fat fractions from chicken fat. Among them, more aldehydes, alcohols, ketones and lactones were produced after heating. Moreover, the contents of these compounds in USFF1 were higher significantly (*p* < 0.05) than those in SFF1, and similar results were also found in USFF2 and SFF2. Which indicated that the unsaturated fat groups were more susceptible to lipid oxidation, resulting in the production of volatile organic compounds. Based on PCA and CA, it was also found that six fat fraction samples were clearly distinguished on PC1-2, and had their own aroma regions at different heating time stages. The C18:0, C20:1 ω9, C18:2 ω6, C18:3 ω6 and C18:3 ω3 were confirmed as the key potential flavor precursors for enhancing of overall flavor in different fat groups with varying degrees of saturate. This study can be concluded that volatile compounds induced by lipid oxidation or degradation were influenced by the heating time and FAs composition. The next work will further explore the formation mechanism of volatile compounds in fat fractions from chicken fat.

**Ethical Approval**

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The authors declared to have bought the fat as a by-product. No animals were used at all.

**CRediT authorship contribution statement**

**Dong Han:** Date curation, Formal analysis, Writing - original draft. **Siyang Deng:** Date curation, Formal analysis, Writing - original draft. **Hang Wang:** Methodology, Investigation. **Feng Huang:** Formal analysis, Writing - review & editing. **Marie-Laure Fauconnier:** Formal analysis, Writing - review & editing. **Hong Li:** Investigation, Validation. **Jian Zheng:** Investigation, Writing - review & editing. **Linchun Meng:** Formal analysis. **Chunhui Zhang:** Funding acquisition, Supervision, Project administration. **Xia Li:** Funding acquisition, Supervision, Project administration.

**Declaration of Competing Interest**

All authors declare that they have no conflict of interest.

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