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Lipid oxidation and flavor changes in saturated and unsaturated fat fractions from chicken fat during a thermal process†

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Chicken fat, due to its rich fatty acids (FAs), is more prone to lipid oxidation and the production of volatile compounds. The aim of the present study was to investigate the oxidative characteristics and flavor changes of saturated (SFF) and unsaturated fat fractions (USFF) from chicken fat induced by heating (140 °C at 70 rpm min^{-1} 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 that in SFF, whereas USFF showed lower levels of saturated fatty acids (SFAs). With the extension of heating time, the SFA/UFA ratio 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, (*E*,*E*)-2,4-decadienal, 2-decanone, γ-octalactone and γ-nonalactone. Our data elucidated that fat fractions from chicken fat with varying degrees of saturation could impart different flavor characteristics during a thermal process.

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1. 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 are higher than those 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 the production of 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 the addition of chicken fat, especially with the addition of oxidized chicken fat.[7–9] However, 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 impart rancid, pungent and other undesirable flavor characteristics to meat.[8] Thus, the oxidation reaction of lipids would be significant for the formation of a special flavor during thermal treatment.

The oxidative susceptibility of lipids is correlated with FA compositions, especially the degree of unsaturation of lipids.

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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 polyunsaturated fatty acids (PUFAs) of ω3 FAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have direct and beneficial effects on health.[13] It has also been reported that meat products can be considered functional foods by adding UFAs.[14] For instance, the ω3 PUFAs and the balance of ω3 to ω6 FAs (approximately 2 : 1) in the human diet can effectively reduce the incidence of lifestyle diseases like coronary artery disease, hypertension 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 contents and FA compositions, including various UFAs and SFAs, the volatile compounds of duck products would be notably affected.[19] Currently, the above studies regarding FAs have mainly focused on human health and characteristic volatile compounds induced by oxidation. However, there is a lack of systematic studies on the impact of fat fractions with varying degrees of saturation during a thermal process on flavor characteristics, and the available literature has limited information on the relationship between special FAs and the volatilome in different fat fractions from chicken fat.

In the present study, yellow-feathered chicken fat was fractionated by a step-wise dry fractionation process to obtain saturated triglyceride-enriched fractions and unsaturated triglyceride-enriched fractions.[20] We exploited 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 quantify how these volatile compounds vary with a thermally-induced oxidation process. Simultaneously, the quantitative relationship between special FAs and volatile compounds is also clarified by partial least squares regression (PLSR). This work is expected to provide important information to improve the flavor in processed meat flavorings or meat processing.

2. Materials and methods

2.1. Chemicals and materials

A C_7–C_{40} *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). 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.

2.2. 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 cm^3). Around 1000 g of chicken fat was placed in a 2 L beaker and melted in a 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 a 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 fractions 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 a 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.

2.3. Preparation of oxidized chicken fat

Oxidized chicken fat was prepared by a heat-induced process. Twenty-five milliliters of SFF or USFF were placed in a 250 mL three-necked round-bottomed flask (Kastmer Technology Development Co., Ltd, Beijing, China). The necks of the flask were connected to a reflux condenser (i-Quip-R3439, Aladdin Biochemical Technology Co., Ltd, Shanghai, China) and one glass vent pipe (i-Quip-R3399, Aladdin Biochemical Technology Co., Ltd, Shanghai, China) to supply compressed air at a rate of 60 mL min^{-1}. The oxidation reaction of SFF and USFF was performed at 140 °C using an oil bath (Du-20, Yiheng Scientific Instrument Co., Ltd, Shanghai, China) through a hydrothermal method under magnetic stirring (RCT Basic, IKA®-Werke GmbH & CO., Staufen, Germany) at 70 rpm min^{-1} for different times (1 h: SFF1 and USFF1; 2 h: SFF2 and USFF2).

2.4. Fatty acid composition

The FA 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 (BF_3-CH_3OH) was added, and the mixture was heated in boiling water for another 5 min. After cooling to room temperature, 5 mL of heptane and 2 mL of saturated NaCl solution were added to the tube, which was then shaken

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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^{-1} to 175 °C (held for 3 min), second ramp at 3.5 °C min^{-1} to 200 °C, third ramp at 1 °C min^{-1} to 210 °C, and final ramp at 1.5 °C min^{-1} to a 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 a carrier gas at a constant flow rate of 1.2 mL min^{-1}. 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 per 100 g of chicken fat and summarized as SFA, monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), and UFA. The ratio of SFA to UFA was calculated.

2.5. Thiobarbituric acid reactive substance (TBARS)

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

$$\mathrm{TBARS}(\mathrm{mg\,MDA\,kg^{-1}}) = \frac{A_{523}}{W_s} \times 9.48 \quad (1)$$

where A_{532} is the absorbance of the solution, W_s is the chicken fat weight (g), and 9.48 is a constant derived from the dilution factor and the molar extinction coefficient (152 000 M^{-1} cm^{-1}) of the red thiobarbituric acid reaction product.

2.6. Volatile compounds of chicken fat with different heating times

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) (d_f 50/30 μm, 2 cm) fiber (Supelco, Belletonte, PA, USA) was employed for the extraction of volatile compounds. The automation of the HS-SPME process was performed using a MPSFF2 multipurpose sampler (Gerstel, Mülheim an der Ruhr, Germany) equipped with an agitator, and the SPME fiber conditioning station was installed on the GC × GC-MS system. Two milliliters of sample oil and 2 μL of the internal standard (2-methyl-3-heptanone, 0.816 μg μL^{-1} 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 conditions of GC × GC-ToFMS in this study were modified according to the methods by Shi, Zhu, Zhang, Lin, and Lv (2019).[24] The 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 spectrometer (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 (2nd D) column. Ultra-high purity (99.9999%) helium was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. The primary oven temperature was maintained at 40 °C for a further 4 min, and the temperature was raised at a rate of 3 °C min^{-1} to 160 °C, and then at 20 °C min^{-1} 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 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 per 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 the mass spectrometric information of each chromatographic peak with the NIST (Version 2.0, National Institute of Standards and Technology, Gaithersburg, USA) mass spectra library. Also, the similarity matching threshold and reverse matching threshold should be greater than 850. Later, the identified compounds were further confirmed by comparing their retention index (RI) values with the published values. The experimental retention index (RI_{exp}) in the 1st D was calculated after the injection of the liquid sample solution of n-alkanes (C_7–C_{40}) under the same conditions as the GC × GC-ToFMS analysis (injection volume of 1 μL, injection rate 20 μL s^{-1}). A compound was identified if the 1st D RI_{exp} and reported RI did not differ by more than 50 units.

The 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:

$$\mathrm{Conc}(\mu g\,L^{-1}) = \frac{\mathrm{Peak\ area\ ratio(volatile/IS)} \times 0.816\,\mu g\,\mu L^{-1}\,V(\mathrm{IS})}{2\,\mathrm{mL(chicken\ fat)}} 1000 \quad (2)$$

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

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

$$\mathrm{OAV}_i = \frac{C_i}{\mathrm{OT}_i} \quad (3)$$

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

2.7. Statistical analysis

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

3. Results and discussion

3.1. Fatty acid and thiobarbituric acid reactive substance values in saturated and unsaturated chicken fat fractions during a 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 FA 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 the 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 SFFs. 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 the FA composition. 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 in 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 because the SFAs and MUFAs in

Table 1 Contents (g per 100 g) of fatty acid composition in saturated and unsaturated fat fractions after heating for 0, 1, and 2 h

	Unsaturated			Saturated			*P* value heating time[a]		
Fatty acids	0 h	1 h	2 h	0 h	1 h	2 h	0 h	1 h	2 h
C14:0	0.59 ± 0.06^{a}	0.60 ± 0.07^{a}	0.57 ± 0.05^{a}	0.59 ± 0.03^{x}	0.55 ± 0.09^{y}	0.61 ± 0.04^{x}	0.951	0.373	0.190
C14:1	0.26 ± 0.03^{a}	0.24 ± 0.04^{a}	0.24 ± 0.02^{a}	0.22 ± 0.02^{x}	0.20 ± 0.02^{y}	0.22 ± 0.02^{x}	0.034	0.072	0.121
C15:0	0.29 ± 0.02^{a}	0.29 ± 0.01^{a}	0.30 ± 0.02^{a}	0.29 ± 0.01^{y}	0.28 ± 0.02^{y}	0.31 ± 0.02^{x}	0.814	0.768	0.286
C16:0	9.53 ± 1.07^{a}	9.94 ± 1.02^{a}	9.55 ± 0.51^{a}	10.93 ± 0.79^{xy}	10.26 ± 2.26^{y}	11.58 ± 0.83^{x}	0.027	0.760	0.000
C16:1	3.70 ± 0.37^{a}	3.81 ± 0.45^{a}	3.60 ± 0.26^{a}	2.97 ± 0.19^{xy}	2.76 ± 0.70^{y}	3.04 ± 0.22^{x}	0.003	0.011	0.002
C18:0	6.01 ± 0.71^{a}	6.28 ± 0.61^{a}	6.08 ± 0.35^{a}	7.35 ± 0.60^{xy}	6.89 ± 1.40^{y}	7.85 ± 0.54^{x}	0.005	0.360	0.000
C18:1 ω9	16.80 ± 2.71^{a}	18.56 ± 2.65^{a}	17.32 ± 1.02^{a}	14.17 ± 1.08^{xy}	13.26 ± 2.88^{y}	14.96 ± 1.65^{x}	0.052	0.008	0.014
C18:2 ω6	25.15 ± 3.11^{a}	25.76 ± 2.17^{a}	23.40 ± 1.66^{a}	20.37 ± 1.34^{x}	18.79 ± 3.86^{x}	19.38 ± 0.67^{x}	0.006	0.003	0.000
C18:3 ω6	0.40 ± 0.06^{a}	0.37 ± 0.03^{a}	0.38 ± 0.03^{a}	0.39 ± 0.03^{x}	0.34 ± 0.02^{y}	0.35 ± 0.02^{y}	0.610	0.067	0.040
C18:3 ω3	1.00 ± 0.10^{a}	0.99 ± 0.06^{a}	0.92 ± 0.06^{a}	0.89 ± 0.06^{x}	0.87 ± 0.10^{x}	0.85 ± 0.03^{x}	0.038	0.031	0.019
C20:0	0.22 ± 0.01^{a}	0.22 ± 0.02^{a}	0.22 ± 0.01^{a}	0.22 ± 0.01^{x}	0.22 ± 0.01^{x}	0.23 ± 0.01^{x}	0.581	0.950	0.154
C20:1 ω9	0.79 ± 0.06^{a}	0.79 ± 0.03^{a}	0.82 ± 0.03^{a}	0.74 ± 0.02^{x}	0.74 ± 0.07^{x}	0.76 ± 0.05^{x}	0.124	0.130	0.026
C20:2	0.51 ± 0.02^{ab}	0.51 ± 0.03^{a}	0.47 ± 0.03^{b}	0.49 ± 0.02^{x}	0.48 ± 0.06^{x}	0.44 ± 0.01^{y}	0.263	0.250	0.052
C20:3 ω6	0.58 ± 0.03^{a}	0.56 ± 0.02^{a}	0.56 ± 0.03^{a}	0.56 ± 0.03^{x}	0.54 ± 0.02^{x}	0.53 ± 0.02^{x}	0.333	0.163	0.130
C20:4 ω6	0.35 ± 0.04^{a}	0.34 ± 0.03^{a}	0.32 ± 0.01^{a}	0.32 ± 0.01^{x}	0.32 ± 0.02^{x}	0.32 ± 0.04^{x}	0.130	0.129	0.877
∑SFA	16.64 ± 1.83^{a}	17.33 ± 1.71^{a}	16.73 ± 0.91^{a}	19.38 ± 1.41^{xy}	18.21 ± 3.77^{y}	20.58 ± 1.42^{x}	0.016	0.612	0.000
∑MUFA	21.54 ± 2.99^{a}	23.41 ± 3.10^{a}	21.98 ± 1.24^{a}	18.10 ± 1.24^{xy}	16.96 ± 3.65^{y}	18.99 ± 1.82^{x}	0.026	0.008	0.008
∑PUFA	28.00 ± 3.27^{a}	28.53 ± 2.29^{a}	26.05 ± 1.76^{a}	23.03 ± 1.38^{x}	21.34 ± 4.01^{x}	21.87 ± 0.69^{x}	0.006	0.003	0.000
∑UFA	49.53 ± 5.62^{a}	51.94 ± 5.23^{a}	48.03 ± 2.87^{a}	41.13 ± 2.56^{x}	38.30 ± 7.65^{x}	40.86 ± 2.40^{x}	0.008	0.005	0.001
∑SFA/∑UFA	0.34 ± 0.02^{ab}	0.33 ± 0.004^{b}	0.35 ± 0.01^{a}	0.47 ± 0.01^{y}	0.47 ± 0.01^{y}	0.50 ± 0.01^{x}	0.000	0.000	0.000

∑SFA, sum of saturated fatty acids; ∑MUFA, sum of monounsaturated fatty acids; ∑PUFA, sum of polyunsaturated fatty acids; ∑UFA, sum of unsaturated fatty acids. Data are shown as mean ± standard deviation ($n = 6$). The different superscript letters (a, b) and (x, y) indicate significant differences ($p < 0.05$) among different heating times within the saturated and unsaturated fat fractions, respectively. [a] *P* value means the result of the significance analysis of unsaturated and saturated fat fractions heated for 0, 1 and 2 h.

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triacylglycerols treated for a short time were involved in producing more volatile compounds through chemical reactions. Subsequently, a long heating time would induce a high release of 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^{-1} fat and 0.21 mg MDA kg^{-1} fat, respectively. Also, USFF has significantly higher ($p < 0.05$) levels of TBARS than SFF after 1 and 2 h of heat treatment (Fig. 1). This may be due to the higher content of UFAs in USFF (Table 1), which is more prone to oxidation reactions 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.

3.2. Volatile organic compound profiling in saturated and unsaturated fat fractions from chicken fat during the 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 resulted mainly from thermal oxidation and degradation of lipids, as well as further interactions among proteins, peptides and free amino acids.[3,6] Among them, the contents of aldehydes, ketones, alcohols, esters, acids, and 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 showed 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 in the formation of heterocyclic compounds.[26]

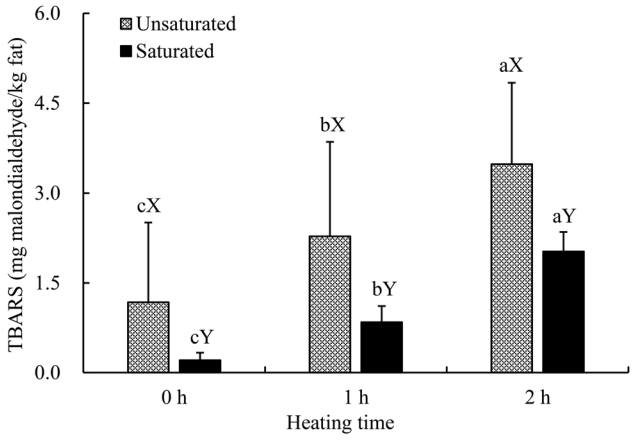

Fig. 1 Thiobarbituric acid-reactive substances (TBARS) of the saturated or unsaturated fat fractions heated for 0, 1 and 2 h. Means with standard deviation ($n = 6$) are shown. The different lowercase letters (a, b, c) indicate significant differences ($p < 0.05$) in the means among heating times within the same fat fraction group; different uppercase letters (X, Y) indicate significant differences ($p < 0.05$) in the means between saturated and unsaturated fat fractions at the same heating time.

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 the heating process. These results indicated that the prolonged high-temperature treatment resulted in more types of volatile compounds and their concentrations. The quantities and contents of volatile compounds in USFF were significantly lower ($p < 0.001$) than those in 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.

3.2.1. Aldehydes. Aldehydes are regarded as the major volatile compounds of lipid oxidation in various types of meat or meat products because of their 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 the 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 pathway[6] or linolenic acid through the 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, 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 fraction 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.

3.2.2. 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 the largest contributors to the oxidized fat fractions from chicken fat (Table 2). 2-Ketones, 2-hexanone, 2-heptanone, 2-octanone, 2-nonanone and 2-decanone could impart a more fruity/sweet aroma to the fat fraction samples and come from lipid oxidation.[33] However,

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Table 2 Quantification of volatile compounds of saturated and unsaturated fat fractions from chicken fat during the thermal process using GC × GC-ToFMS (μg L^{-1})

		Unsaturated			Saturated			Sign. heating time		
No.	Compounds	0 h	1 h	2 h	0 h	1 h	2 h	0 h	1 h	2 h
	Aldehydes (29)	286.3 ± 5.5^{c}	$18\,504.6 \pm 293.3^{b}$	$36\,971.8 \pm 940.3^{a}$	408.8 ± 2.9^{z}	$12\,954.8 \pm 257.0^{y}$	$28\,732.3 \pm 874.1^{x}$	***	***	***
1	Pentanal	14.4 ± 1.1^{c}	2685.2 ± 209.4^{a}	321.1 ± 73.1^{b}	19.0 ± 0.8^{z}	1655.8 ± 53.4^{y}	2396.7 ± 152.9^{x}	**	**	***
2	2-Ethylbutanal	0.0^{b}	0.0^{b}	60.5 ± 1.4^{a}	0.0^{y}	5.9 ± 0.9^{x}	0.0^{y}	NS	**	***
3	Hexanal	114.0 ± 0.1^{c}	2648.2 ± 46.0^{b}	4246.5 ± 161.5^{a}	231.0 ± 4.8^{z}	2432.0 ± 94.6^{y}	2958.3 ± 85.0^{x}	***	*	***
4	Heptanal	5.9 ± 0.6^{c}	494.5 ± 14.7^{b}	1521.3 ± 23.9^{a}	12.1 ± 0.8^{z}	424.4 ± 12.3^{y}	1146.2 ± 35.3^{x}	***	**	***
5	Octanal	13.3 ± 0.3^{c}	1088.2 ± 39.1^{a}	532.0 ± 21.1^{b}	1.6 ± 0.4^{z}	180.1 ± 11.1^{y}	342.5 ± 11.6^{x}	***	***	***
6	Nonanal	12.5 ± 2.0^{c}	1583.6 ± 11.3^{b}	3315.0 ± 80.1^{a}	25.2 ± 2.3^{z}	1220.5 ± 94.8^{y}	2859.3 ± 47.8^{x}	**	**	**
7	Decanal	0.0^{c}	62.0 ± 1.3^{b}	152.4 ± 9.1^{a}	0.0^{z}	34.8 ± 5.1^{y}	100.4 ± 14.1^{x}	NS	**	**
8	Undecanal	0.0^{c}	4.4 ± 0.3^{b}	30.0 ± 2.0^{a}	0.0^{z}	3.9 ± 0.7^{y}	12.3 ± 0.9^{x}	NS	NS	***
9	Dodecanal	0.0^{b}	0.0^{b}	7.5 ± 0.5^{a}	0.0^{y}	0.0^{y}	6.7 ± 0.9^{z}	NS	NS	NS
10	(*E*)-2-Butenal	0.0^{c}	2.5 ± 0.4^{a}	1.4 ± 0.2^{b}	18.9 ± 2.0^{x}	3.2 ± 1.6^{z}	5.5 ± 0.6^{y}	***	NS	***
11	(*E*)-2-Pentenal	8.4 ± 2.5^{c}	471.0 ± 18.1^{b}	770.8 ± 10.9^{a}	0.0^{z}	439.4 ± 10.5^{y}	732.6 ± 22.5^{x}	**	NS	NS
12	(*Z*)-3-Hexenal	0.0^{b}	0.0^{b}	7.6 ± 1.6^{a}	0.0^{y}	0.0^{y}	6.0 ± 0.9^{x}	NS	NS	NS
13	(*E*)-2-Hexenal	0.0^{b}	4.5 ± 2.2^{a}	4.6 ± 1.3^{a}	3.4 ± 0.2^{z}	75.3 ± 19.1^{y}	108.2 ± 11.6^{x}	**	**	***
14	(*Z*)-4-Heptenal	0.0^{c}	166.7 ± 7.6^{b}	414.0 ± 56.4^{a}	0.0^{z}	364.2 ± 9.2^{x}	260.5 ± 25.0^{y}	NS	***	*
15	(*E*)-2-Heptenal	28.2 ± 2.0^{c}	4606.0 ± 152.2^{b}	6611.6 ± 490.6^{a}	39.2 ± 2.3^{z}	4370.9 ± 113.1^{y}	8081.5 ± 213.9^{x}	**	NS	**
16	(*E*)-2-Octenal	6.7 ± 0.6^{c}	914.6 ± 78.7^{b}	2696.7 ± 156.0^{a}	2.2 ± 0.4^{z}	761.1 ± 33.8^{y}	1839.3 ± 167.8^{x}	***	*	**
17	(*E*)-2-Nonenal	0.0^{c}	293.0 ± 8.8^{b}	765.1 ± 44.7^{a}	0.0^{z}	157.8 ± 7.9^{y}	526.5 ± 11.2^{x}	NS	***	**
18	(*Z*)-4-Decenal	0.0^{b}	0.0^{b}	30.6 ± 3.6^{a}	0.0^{z}	0.0^{y}	20.1 ± 1.9^{x}	NS	NS	*
19	(*E*)-2-Decenal	0.0^{b}	0.0^{b}	975.2 ± 16.4^{a}	0.0	0.0	0.0	NS	NS	***
20	2-Undecenal	0.0^{c}	76.7 ± 6.1^{b}	532.1 ± 28.4^{a}	0.0^{z}	32.5 ± 2.8^{y}	308.8 ± 55.8^{x}	NS	***	**
21	(*E*)-2-Dodecenal	0.0^{b}	0.0^{b}	4.2 ± 0.8^{a}	0.0	0.0	0.0	NS	NS	*
22	(*E*,*E*)-2,4-Hexadienal	0.0^{b}	45.7 ± 2.5^{b}	117.5 ± 5.3^{a}	0.0^{z}	34.3 ± 1.2^{y}	67.0 ± 9.2^{x}	NS	**	**
23	(*E*,*E*)-2,4-Heptadienal	0.0^{c}	2771.8 ± 52.7^{b}	3087.0 ± 203.0^{a}	0.0^{z}	525.0 ± 34.3^{y}	3413.9 ± 356.9^{x}	NS	***	NS
24	(*E*,*E*)-2,4-Octadienal	0.0^{c}	47.9 ± 1.4^{b}	190.7 ± 6.4^{a}	0.0^{z}	15.5 ± 1.6^{y}	84.5 ± 6.4^{x}	NS	***	***
25	(*E*,*E*)-2,4-Nonadienal	0.0^{c}	44.9 ± 2.6^{b}	299.1 ± 21.2^{a}	0.0^{z}	13.4 ± 1.8^{y}	164.9 ± 9.5^{x}	NS	***	**
26	(*E*,*E*)-2,4-Decadienal	0.0^{c}	370.6 ± 33.8^{b}	$10\,134.3 \pm 439.2^{a}$	0.0^{z}	122.0 ± 18.0^{y}	3194.2 ± 621.6^{x}	NS	***	***
27	Benzaldehyde	81.6 ± 3.8^{c}	121.3 ± 7.1^{b}	138.5 ± 6.9^{a}	56.2 ± 2.5^{z}	76.3 ± 3.1^{y}	93.9 ± 9.4^{x}	**	**	**
28	3-Methylbenzaldehyde	0.0^{c}	1.1 ± 0.2^{b}	4.6 ± 0.4^{a}	0.0^{y}	0.0^{y}	2.6 ± 0.1^{x}	NS	*	**
29	Benzeneacetaldehyde	1.2 ± 0.1^{a}	0.0^{b}	0.0^{b}	0.0^{y}	6.7 ± 1.1^{x}	0.0^{y}	**	**	NS
	Ketones (30)	29.2 ± 2.0^{c}	2123.9 ± 75.1^{b}	6147.4 ± 94.1^{a}	14.1 ± 2.1^{z}	1440.5 ± 31.9^{y}	4235.5 ± 173.0^{x}	**	***	***
30	3-Hexanone	0.0^{b}	0.0^{b}	5.9 ± 0.6^{a}	0.0^{y}	4.3 ± 0.3^{x}	0.0^{y}	NS	***	**
31	2-Hexanone	0.0^{c}	75.4 ± 2.5^{b}	158.7 ± 2.3^{a}	0.0^{z}	52.0 ± 2.6^{y}	78.8 ± 0.4^{x}	NS	***	***
32	3-Heptanone	0.0^{c}	15.0 ± 2.8^{b}	34.4 ± 2.1^{a}	0.0^{y}	0.0^{y}	28.1 ± 1.3^{x}	NS	**	*
33	2-Heptanone	2.8 ± 0.3^{c}	185.6 ± 2.8^{b}	500.1 ± 37.4^{a}	2.6 ± 0.1^{z}	184.2 ± 2.1^{y}	481.2 ± 8.7^{x}	NS	NS	NS
34	4-Octanone	0.0^{c}	4.3 ± 0.2^{b}	14.2 ± 1.3^{a}	0.0^{z}	4.6 ± 1.0^{y}	10.2 ± 0.5^{x}	NS	NS	**
35	2-Octanone	0.0^{c}	116.9 ± 2.9^{b}	367.3 ± 25.7^{a}	0.0^{z}	85.7 ± 0.7^{y}	268.5 ± 8.7^{x}	NS	***	**
36	2-Nonanone	0.0^{c}	22.4 ± 0.4^{b}	119.5 ± 10.9^{a}	0.0^{z}	15.3 ± 0.4^{y}	58.3 ± 4.9^{x}	NS	***	**
37	2-Decanone	0.0^{c}	74.5 ± 2.3^{b}	446.1 ± 12.3^{a}	0.0^{z}	32.6 ± 2.2^{y}	251.0 ± 30.8^{x}	NS	***	**
38	6-Undecanone	0.0^{b}	0.0^{b}	4.8 ± 0.5^{a}	0.0	0.0	0.0	NS	NS	**
39	2-Undecanone	0.0^{b}	0.0^{b}	7.6 ± 1.5^{a}	0.0^{y}	0.0^{y}	7.3 ± 1.5^{x}	NS	NS	NS
40	5-Methyl-3-heptanone	0.0^{c}	34.4 ± 1.8^{b}	79.3 ± 4.4^{a}	0.0^{z}	26.1 ± 3.7^{y}	77.9 ± 10.2^{x}	NS	*	NS
41	1-Hydroxy-2-propanone	13.5 ± 1.4^{b}	0.0^{c}	77.1 ± 6.0^{a}	10.6 ± 2.2^{y}	8.3 ± 0.8^{y}	83.3 ± 4.5^{x}	NS	**	NS
42	1-Hydroxy-2-butanone	0.0^{c}	10.7 ± 1.3^{b}	35.4 ± 2.2^{a}	0.0^{z}	8.5 ± 0.3^{y}	19.9 ± 3.7^{x}	NS	NS	**
43	1-Penten-3-one	0.0^{c}	365.8 ± 29.4^{b}	474.3 ± 35.9^{a}	0.0^{z}	253.4 ± 2.9^{y}	289.9 ± 10.8^{x}	NS	*	**
44	3-Penten-2-one	0.0^{c}	5.1 ± 0.0^{b}	10.4 ± 1.5^{a}	0.0^{z}	4.0 ± 0.2^{y}	12.2 ± 1.2^{x}	NS	**	***
45	3-Hexen-2-one	0.0^{b}	0.0^{b}	75.5 ± 7.4^{a}	0.0	0.0	0.0^{y}	NS	NS	**
46	1-Octen-3-one	0.0^{c}	485.1 ± 55.1^{b}	1071.8 ± 5.3^{a}	0.0^{z}	302.4 ± 14.7^{y}	820.8 ± 119.9^{x}	NS	**	NS

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Table 2 *(Contd.)*

No.	Compounds	Unsaturated			Saturated			Sign. heating time		
		0 h	1 h	2 h	0 h	1 h	2 h	0 h	1 h	2 h
47	2,3-Pentanedione	11.9 ± 0.7^{b}	12.9 ± 0.9^{b}	33.1 ± 1.8^{a}	0.0^{y}	13.2 ± 1.6^{x}	17.3 ± 4.8^{x}	***	NS	**
48	(*E*)-3-Octen-2-one	0.0^{c}	222.1 ± 10.4^{b}	661.3 ± 22.6^{a}	0.0^{z}	180.4 ± 6.6^{y}	746.6 ± 7.9^{x}	NS	**	**
49	(*E*)-3-Nonen-2-one	0.0^{c}	424.8 ± 10.2^{b}	1840.3 ± 38.0^{a}	0.0^{z}	184.7 ± 12.0^{y}	790.4 ± 2.8^{x}	NS	***	***
50	(*E*,*E*)-3,5-Octadien-2-one	0.0^{b}	0.0^{b}	7.8 ± 0.3^{a}	0.0	0.0	0.0	NS	NS	***
51	Cyclopentanone	0.0^{c}	3.8 ± 0.3^{b}	7.1 ± 1.1^{a}	0.0^{z}	3.3 ± 0.3^{y}	5.7 ± 0.7^{x}	NS	NS	NS
52	2-Methylcyclopentanone	0.0^{c}	30.7 ± 3.5^{b}	45.5 ± 3.2^{a}	0.0^{z}	19.8 ± 0.9^{y}	51.0 ± 5.0^{x}	NS	**	NS
53	2-Ethylcyclopentanone	0.0^{a}	0.0^{a}	3.4 ± 0.7^{a}	0.0	0.0	0.0	NS	NS	*
54	Cyclohexanone	0.0^{c}	17.3 ± 0.9^{b}	37.9 ± 3.0^{a}	0.0^{z}	9.8 ± 0.1^{y}	28.3 ± 2.7^{x}	NS	**	*
55	2-Cyclopentenone	0.0^{c}	6.2 ± 0.5^{b}	13.9 ± 0.6^{a}	0.0^{z}	8.9 ± 0.4^{y}	13.4 ± 0.9^{x}	NS	**	NS
56	3-Ethyl-2-cyclopenten-1-one	0.0	0.0	0.0	0.0^{y}	0.0^{y}	6.7 ± 0.2^{x}	NS	NS	***
57	3-Methylcyclopentane-1,2-dione	0.0	0.0	0.0	0.0^{z}	1.9 ± 0.1^{y}	6.4 ± 0.5^{x}	NS	**	**
58	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	0.0^{c}	8.5 ± 0.9^{b}	14.6 ± 1.8^{a}	0.0^{z}	35.2 ± 2.0^{y}	77.8 ± 6.8^{x}	NS	***	**
59	Acetophenone	1.0 ± 0.1^{b}	2.4 ± 0.2^{a}	0.0^{c}	0.9 ± 0.2^{z}	1.9 ± 0.3^{y}	4.5 ± 0.5^{x}	NS	NS	**
	Alcohols (26)	109.5 ± 8.8^{c}	7693.9 ± 265.8^{b}	$16\,977.3 \pm 1313.7^{a}$	419.9 ± 47.9^{z}	6477.0 ± 124.6^{y}	9626.5 ± 397.5^{x}	**	**	**
60	Butanol	3.8 ± 0.1^{c}	75.3 ± 3.3^{b}	128.2 ± 16.0^{a}	7.0 ± 0.6^{z}	69.0 ± 5.9^{y}	129.5 ± 2.8^{x}	**	NS	NS
61	Pentanol	26.5 ± 4.3^{c}	2194.4 ± 142.6^{a}	1876.4 ± 101.3^{b}	61.3 ± 6.8^{z}	1808.9 ± 49.7^{y}	2584.1 ± 302.1^{x}	**	*	*
62	Glycerin	0.0^{b}	136.1 ± 23.6^{a}	0.0^{b}	272.4 ± 34.6^{x}	0.0^{y}	0.0^{y}	**	**	NS
63	Octanol	1.5 ± 0.3^{c}	168.3 ± 2.5^{b}	488.2 ± 36.9^{a}	1.9 ± 0.7^{z}	118.0 ± 7.6^{y}	360.8 ± 32.7^{x}	NS	***	*
64	Heptanol	1.5 ± 0.3^{c}	186.5 ± 11.8^{b}	407.3 ± 85.8^{a}	1.4 ± 0.9^{z}	173.0 ± 15.1^{y}	393.5 ± 34.8^{x}	NS	NS	NS
65	Nonanol	0.0^{c}	11.3 ± 0.5^{b}	102.8 ± 0.8^{a}	0.0^{z}	5.3 ± 0.6^{y}	38.0 ± 4.6^{x}	NS	***	***
66	Cyclohexanol	0.0	0.0	0.0	0.0^{y}	16.7 ± 0.9^{x}	0.0^{y}	NS	***	NS
67	3-Hexanol	0.0^{b}	0.0^{b}	6.3 ± 0.6^{a}	0.0	0.0	0.0	NS	NS	***
68	2-Hexanol	0.0	0.0	0.0	0.0^{y}	0.0^{y}	22.9 ± 0.8^{x}	NS	NS	***
69	4-Heptanol	0.0^{b}	0.0^{b}	3.2 ± 0.6^{a}	0.0	0.0	0.0	NS	NS	**
70	3-Heptanol	0.0^{c}	5.6 ± 0.1^{b}	18.1 ± 1.5^{a}	0.0^{z}	7.3 ± 0.1^{y}	14.7 ± 0.2^{x}	NS	***	NS
71	2-Heptanol	0.0^{c}	12.4 ± 0.4^{b}	66.5 ± 1.6^{a}	0.0	0.0	0.0	NS	***	***
72	4-Octanol	0.0^{c}	3.4 ± 0.3^{b}	20.2 ± 1.0^{a}	0.0^{z}	3.9 ± 0.8^{y}	10.6 ± 0.6^{x}	NS	NS	***
73	3-Octanol	0.0^{c}	8.6 ± 0.2^{b}	43.9 ± 2.4^{a}	0.0^{z}	5.9 ± 0.6^{y}	33.3 ± 0.8^{x}	NS	**	**
74	2-Ethylhexanol	5.0 ± 0.4^{b}	9.0 ± 3.1^{a}	0.0^{c}	6.7 ± 0.5^{x}	6.7 ± 2.0^{x}	0.0^{y}	**	NS	NS
75	1-Penten-3-ol	4.2 ± 1.0^{c}	555.5 ± 8.6^{b}	713.0 ± 8.3^{a}	4.5 ± 1.0^{z}	634.0 ± 30.2^{x}	555.8 ± 3.2^{y}	NS	*	***
76	(*Z*)-3-Penten-1-ol	0.0^{c}	12.5 ± 0.7^{b}	17.5 ± 2.0^{a}	0.0^{z}	10.4 ± 0.2^{y}	17.7 ± 2.5^{x}	NS	**	NS
77	(*E*)-2-Penten-1-ol	0.0^{b}	0.0^{b}	240.6 ± 21.2^{a}	0.0	0.0	0.0	NS	NS	**
78	(*Z*)-2-Penten-1-ol	0.0^{c}	30.6 ± 2.2^{b}	46.5 ± 3.4^{a}	0.0^{z}	28.2 ± 0.8^{y}	38.4 ± 1.5^{x}	NS	NS	*
79	(*E*)-2-Hexen-1-ol	0.0	0.0	0.0	0.0^{y}	0.0^{y}	10.4 ± 2.4^{x}	NS	NS	**
80	1-Octen-3-ol	17.9 ± 2.8^{c}	4176.3 ± 101.6^{b}	$12\,480.1 \pm 1531.1^{a}$	13.1 ± 2.6^{z}	3516.6 ± 46.6^{y}	5229.6 ± 186.8^{x}	NS	**	**
81	(*E*)-2-Octen-1-ol	0.0^{c}	108.2 ± 5.6^{b}	$314.0.\pm 6.2^{a}$	0.0^{z}	73.2 ± 2.3^{y}	187.2 ± 11.4^{x}	NS	**	***
82	(*Z*)-3-Nonen-1-ol	0.0^{b}	0.0^{b}	4.5 ± 0.8^{a}	0.0	0.0	0.0	NS	NS	**
83	1-Ethoxypropan-2-ol	14.7 ± 0.5^{a}	0.0^{b}	0.0^{b}	9.4 ± 0.9^{x}	0.0^{y}	0.0^{y}	**	NS	NS
84	1-Propoxypropan-2-ol	34.3 ± 1.9^{a}	0.0^{b}	0.0^{b}	25.9 ± 1.3^{x}	0.0^{y}	0.0^{y}	**	NS	NS
85	Diethylene glycol	0.0	0.0	0.0	16.3 ± 4.3^{x}	0.0^{y}	0.0^{y}	*	NS	NS
	Hydrocarbons (18)	979.1 ± 66.4^{a}	388.0 ± 5.3^{c}	863.3 ± 21.7^{b}	1006.6 ± 30.3^{x}	426.6 ± 15.8^{z}	649.3 ± 51.7^{y}	NS	*	**
86	Decane	0.0^{c}	44.7 ± 5.8^{b}	86.0 ± 8.1^{a}	0.4 ± 0.1^{y}	0.0^{z}	80.9 ± 4.1^{x}	*	**	NS
87	Undecane	0.0^{c}	36.6 ± 3.6^{b}	180.0 ± 4.2^{a}	0.0^{z}	54.4 ± 2.7^{y}	113.8 ± 5.2^{x}	NS	**	***
88	Dodecane	0.0	0.0	0.0	33.8 ± 0.9^{y}	0.0^{z}	147.7 ± 19.1^{x}	***	NS	***
89	Tridecane	0.0^{c}	91.0 ± 4.3^{b}	159.8 ± 3.9^{a}	26.7 ± 0.3^{z}	92.7 ± 6.3^{y}	206.2 ± 28.7^{x}	***	NS	***
90	Tetradecane	0.0^{b}	0.0^{b}	242.3 ± 16.0^{a}	0.0	0.0	0.0	NS	NS	***
91	Decene	0.0^{c}	22.1 ± 3.6^{b}	126.4 ± 12.2^{a}	0.0^{y}	0.0^{y}	72.7 ± 0.4^{x}	NS	**	*
92	Decyne	0.0	0.0	0.0	19.1 ± 4.7^{x}	0.0^{y}	0.0^{y}	*	NS	NS

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Table 2 *(Contd.)*

No.	Compounds	Unsaturated			Saturated			Sign. heating time		
		0 h	1 h	2 h	0 h	1 h	2 h	0 h	1 h	2 h
93	Toluene	253.3 ± 7.6^{a}	0.0^{b}	0.0^{b}	261.2 ± 5.8^{x}	0.0^{y}	0.0^{y}	NS	NS	NS
94	Ethylbenzene	28.5 ± 1.6^{a}	22.0 ± 0.5^{b}	0.0^{c}	123.0 ± 8.5^{x}	12.7 ± 1.4^{y}	0.0^{z}	***	***	NS
95	*p*-Xylene	326.8 ± 50.4^{a}	88.7 ± 4.3^{b}	0.0^{c}	223.2 ± 15.0^{x}	61.4 ± 0.3^{y}	0.0^{z}	*	**	NS
96	*o*-Xylene	41.9 ± 0.5^{a}	13.7 ± 2.5^{c}	7.1 ± 0.6^{c}	37.2 ± 0.6^{x}	16.7 ± 1.3^{y}	5.4 ± 0.2^{z}	**	NS	*
97	(−)-Limonene	0.0	0.0	0.0	1.7 ± 0.2^{y}	3.3 ± 0.6^{x}	3.8 ± 0.9^{x}	***	**	*
98	Propylbenzene	4.5 ± 1.0^{c}	8.4 ± 1.3^{b}	20.1 ± 0.6^{a}	0.0^{y}	0.0^{y}	16.3 ± 1.4^{x}	**	**	*
99	Isopropylbenzene	2.9 ± 0.1^{b}	59.8 ± 3.4^{a}	0.0^{c}	1.8 ± 0.9^{x}	0.0^{y}	0.0^{y}	NS	**	NS
100	Styrene	27.5 ± 4.6^{a}	0.0^{b}	0.0^{b}	237.6 ± 46.7^{x}	184.4 ± 8.8^{y}	0.0^{z}	*	***	NS
101	1,3,5,7-Cyclooctatetraene	242.2 ± 34.4^{a}	0.0^{c}	35.4 ± 0.9^{b}	0.0	0.0	0.0	***	NS	***
102	3-Ethylstyrene	0.0^{b}	0.0^{b}	3.5 ± 1.0^{a}	0.0	0.0	0.0	NS	NS	**
103	Azulene	0.7 ± 0.0^{b}	0.0^{c}	2.7 ± 0.2^{a}	1.2 ± 0.2^{y}	1.2 ± 0.1^{y}	2.5 ± 0.1^{x}	**	*	NS
	Phenols (2)	25.4 ± 1.4^{a}	0.0^{b}	0.0^{b}	19.9 ± 2.5^{x}	0.0^{y}	0.0^{y}	*	NS	NS
104	Butylated Hydroxytoluene	24.8 ± 1.5^{a}	0.0^{b}	0.0^{b}	19.9 ± 2.5^{x}	0.0^{y}	0.0^{y}	*	NS	NS
105	*p*-Cresol	0.6 ± 0.2^{a}	0.0^{b}	0.0^{b}	0.0	0.0	0.0	*	NS	NS
	Esters (14)	41.3 ± 1.6^{c}	534.0 ± 14.9^{b}	2167.0 ± 39.2^{a}	15.7 ± 1.0^{z}	369.5 ± 15.7^{y}	1433.6 ± 85.1^{x}	***	***	***
106	Amyl acetate	0.0^{c}	1.2 ± 0.1^{b}	2.8 ± 0.4^{a}	0.0^{z}	1.7 ± 0.0^{y}	3.4 ± 0.1^{x}	NS	**	**
107	Butyl butyrate	27.3 ± 1.1^{c}	60.7 ± 0.4^{b}	138.9 ± 5.8^{a}	0.0^{z}	84.0 ± 1.6^{y}	159.7 ± 3.7^{x}	**	***	**
108	Hexyl formate	0.0^{c}	9.3 ± 1.5^{b}	26.7 ± 6.8^{a}	0.0^{z}	4.4 ± 0.9^{y}	20.7 ± 0.6^{x}	NS	**	NS
109	Dimethyl oxalate	0.0^{c}	13.2 ± 0.9^{b}	18.2 ± 0.7^{a}	0.0^{z}	5.7 ± 0.7^{y}	11.5 ± 0.4^{x}	NS	***	***
110	Pentyl hexanoate	0.0^{b}	0.0^{b}	13.2 ± 3.3^{a}	0.0^{y}	0.0^{y}	3.4 ± 0.4^{x}	NS	NS	**
111	Octyl formate	0.0^{b}	0.0^{b}	2.5 ± 0.9^{a}	0.0	0.0	0.0	NS	NS	*
112	γ-Valerolactone	0.0^{c}	32.5 ± 2.3^{b}	112.5 ± 14.8^{a}	0.0^{z}	18.2 ± 1.1^{y}	47.5 ± 1.6^{x}	NS	**	*
113	γ-Butyrolactone	12.4 ± 1.1^{c}	57.3 ± 1.6^{b}	167.3 ± 17.9^{a}	14.4 ± 1.5^{z}	58.9 ± 1.4^{y}	135.8 ± 0.2^{x}	NS	NS	*
114	γ-Caprolactone	1.5 ± 0.4^{c}	233.7 ± 8.2^{b}	768.5 ± 26.8^{a}	1.3 ± 0.6^{z}	142.7 ± 7.5^{y}	597 ± 27.4^{x}	NS	***	**
115	δ-Hexalactone	0.0^{c}	22.3 ± 0.9^{b}	138.3 ± 11.4^{b}	0.0^{z}	15.3 ± 1.2^{y}	64.5 ± 4.4^{x}	NS	**	***
116	δ-Valerolactone	0.0^{c}	53.1 ± 1.7^{b}	254.8 ± 16.1^{a}	0.0^{z}	28.1 ± 3.3^{y}	166.7 ± 7.2^{x}	NS	***	**
117	γ-Heptalactone	0.0^{c}	28.1 ± 2.2^{b}	200.8 ± 20.9^{a}	0.0^{z}	10.4 ± 1.5^{y}	66.6 ± 5.8^{x}	NS	***	***
118	γ-Octalactone	0.0^{c}	22.5 ± 1.1^{b}	196.4 ± 21.0^{a}	0.0^{y}	0.0^{y}	76.2 ± 7.6^{x}	NS	**	**
119	γ-Nonalactone	0.0^{c}	0.0^{b}	126.0 ± 13.5^{a}	0.0^{y}	0.0^{y}	80.7 ± 29.4^{x}	NS	NS	NS
	Acids (7)	266.0 ± 30.5^{c}	1435.0 ± 118.8^{b}	8711.1 ± 443.2^{a}	1012.1 ± 42.7^{z}	2236.0 ± 121.3^{y}	7624.3 ± 287.5^{x}	***	**	*
120	Acetic acid	254.0 ± 29.6^{a}	72.2 ± 13.2^{c}	123.0 ± 8.4^{b}	950.6 ± 43.2^{y}	669.6 ± 16.8^{z}	1802.8 ± 121.5^{x}	***	***	**
121	Formic acid	0.0^{c}	538.5 ± 23.3^{b}	5289.1 ± 196.7^{a}	0.0^{z}	730.8 ± 34.2^{y}	3211.5 ± 99.7^{x}	NS	**	***
122	Propanoic acid	12.0 ± 1.6^{c}	28.1 ± 3.0^{b}	48.8 ± 6.9^{a}	61.5 ± 1.1^{y}	40.4 ± 8.9^{z}	72.6 ± 2.3^{x}	***	NS	**
123	Pentanoic acid	0.0^{c}	256.2 ± 25.4^{a}	62.1 ± 16.4^{b}	0.0^{z}	67.2 ± 8.4^{y}	142.2 ± 6.9^{x}	NS	***	**
124	Hexanoic acid	0.0^{c}	288.9 ± 78.4^{b}	2662.3 ± 300.9^{a}	0.0^{z}	465.0 ± 62.7^{y}	1645.0 ± 73.2^{x}	NS	*	**
125	Heptanoic acid	0.0^{b}	0.0^{b}	14.8 ± 2.7^{a}	0.0	0.0	0.0	NS	NS	*
126	Nonanoic acid	0.0^{c}	251.1 ± 13.9^{b}	510.9 ± 33.7^{a}	0.0^{z}	263.0 ± 15.4^{y}	750.2 ± 10.8^{x}	NS	NS	***
	O-, N- and S-Containing compounds (24)	73.4 ± 5.9^{c}	1445.3 ± 38.5^{b}	3321.8 ± 152.0^{a}	58.5 ± 5.7^{z}	1623.5 ± 14.4^{y}	3355.3 ± 334.1^{x}	**	***	NS
127	2-Ethylfuran	0.0	0.0	0.0	0.0^{y}	116.1 ± 8.6^{x}	0.0^{y}	NS	**	NS
128	2-Propylfuran	0.0^{c}	39.4 ± 2.2^{b}	71.3 ± 5.4^{a}	0.0^{y}	61.3 ± 5.1^{x}	65.8 ± 4.8^{x}	NS	**	NS
129	2-Butylfuran	0.0^{c}	24.2 ± 0.9^{b}	64.4 ± 5.7^{a}	0.0^{z}	14.5 ± 1.3^{y}	44.9 ± 2.9^{x}	NS	***	**
130	2-Pentylfuran	39.4 ± 5.3^{c}	1313.3 ± 43.6^{b}	2929.5 ± 134.0^{a}	0.0^{z}	1342.0 ± 14.9^{y}	3046.5 ± 317.9^{x}	**	NS	NS
131	Tetrahydro-2-furanmethanol	0.0^{b}	0.0^{b}	9.5 ± 1.4^{a}	0.0	0.0	0.0	NS	NS	**
132	2-Hexylfuran	0.0^{c}	14.1 ± 1.3^{b}	54.0 ± 7.8^{a}	0.0^{z}	8.5 ± 1.1^{y}	33.6 ± 2.3^{x}	NS	**	*
133	3-Furaldehyde	0.0	0.0	0.0	4.3 ± 0.4^{x}	0.0^{y}	0.0^{y}	***	NS	NS
134	2-Heptylfuran	0.0^{b}	0.0^{b}	30.0 ± 4.4^{a}	0.0^{y}	0.0^{y}	9.5 ± 0.4^{x}	NS	NS	**
135	Furfural	1.3 ± 0.2^{c}	5.8 ± 0.2^{b}	16.9 ± 0.3^{a}	14.8 ± 3.8^{x}	13.0 ± 1.1^{x}	18.8 ± 1.6^{x}	*	**	NS
136	2-Octylfuran	0.0	0.0	0.0	0.0^{y}	0.0^{y}	13.0 ± 0.8^{x}	NS	NS	**

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Table 2 *(Contd.)*

No.	Compounds	Unsaturated			Saturated			Sign. heating time		
		0 h	1 h	2 h	0 h	1 h	2 h	0 h	1 h	2 h
137	5-Methyl-2(5*H*)-furanone	0.0^{c}	1.8 ± 0.2^{b}	5.1 ± 0.2^{a}	0.0^{z}	0.8 ± 0.1^{y}	2.1 ± 0.1^{x}	NS	**	***
138	3,4-Dimethyl-2,5-furandione	0.0^{b}	0.0^{b}	19.7 ± 1.5^{a}	0.0^{y}	0.0^{y}	16.0 ± 1.7^{x}	NS	NS	NS
139	2(5*H*)-Furanone	0.0^{c}	16.3 ± 1.8^{b}	67.0 ± 3.9^{a}	1.0 ± 0.4^{z}	13.9 ± 1.0^{y}	43.8 ± 9.1^{x}	*	NS	*
140	2*H*-Pyran-2-one	0.0^{c}	7.5 ± 0.4^{b}	54.5 ± 2.1^{a}	0.0^{z}	4.3 ± 1.1^{y}	31.6 ± 2.0^{x}	NS	*	***
141	Pyridine	14.4 ± 2.4^{b}	17.9 ± 2.1^{a}	0.0^{c}	15.2 ± 1.1^{z}	24.8 ± 0.9^{x}	22.8 ± 1.1^{y}	NS	**	**
142	Pyrazine	0.0	0.0	0.0	0.0^{y}	2.9 ± 0.9^{x}	0.0^{y}	NS	*	NS
143	3-Ethylpyridine	0.0^{b}	1.9 ± 0.4^{a}	0.0^{b}	0.0^{z}	8.1 ± 0.7^{x}	3.9 ± 0.9^{y}	NS	***	*
144	Pyrrole	1.2 ± 0.1^{a}	0.0^{b}	0.0^{b}	0.0	0.0	0.0	***	NS	NS
145	Benzonitrile	4.6 ± 0.2^{a}	3.2 ± 0.1^{b}	0.0^{c}	3.4 ± 0.2^{x}	3.5 ± 0.2^{x}	0.0^{y}	**	NS	NS
146	Pyrrolidinecarboxaldehyde (N,O)	0.0	0.0	0.0	10.4 ± 1.0^{x}	0.0^{y}	0.0^{y}	**	NS	NS
147	2-Piperidinone (N,O)	0.0	0.0	0.0	2.1 ± 0.1^{x}	0.0^{y}	0.0^{y}	**	NS	NS
148	2-Propylthiophene	0.0	0.0	0.0	6.7 ± 1.8^{x}	0.0^{y}	0.0^{y}	*	NS	NS
149	2-Formylthiophene	0.0	0.0	0.0	0.7 ± 0.1^{z}	2.0 ± 0.3^{y}	2.9 ± 0.3^{x}	**	***	**
150	Dimethyl sulfone	12.5 ± 1.1^{a}	0.0^{b}	0.0^{b}	0.0^{y}	8.0 ± 0.9^{x}	0.0^{y}	**	***	NS
	Total	1810.2 ± 30.7^{c}	$32\,124.7 \pm 198.9^{b}$	$75\,159.6 \pm 232.6^{a}$	2955.5 ± 90.2^{z}	$25\,527.9 \pm 242.1^{y}$	$55\,656.8 \pm 1631.4^{x}$	***	***	***

The different superscript letters (a, b, c) and (x, y, z) indicate significant differences ($p < 0.05$) among heating times within the saturated and unsaturated fat fractions. The stars denote significance levels between saturated and unsaturated fat fractions at the same heating time (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$; NS, not significant).

2-undecanone contributed less to the flavor of fat fraction samples due to its lower content, and is derived from the Maillard reaction.[34] Methyl ketones, like 5-methyl-3-heptanone and 2-methylcyclopentanone, could be produced from β-keto acid decarboxylation[35] 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 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.

3.2.3. Alcohols. Alcohols could provide a pleasant fruity and floral aroma,[37] and they are generally 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 heating. Similar results showed that there was an increase in alcohol 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, 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 in the formation of esters.[40]

3.2.4. 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 lipids or autoxidation of long-chain FAs[8] produced some aromatic and aliphatic hydrocarbons. However, 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-term 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-membered rings (γ-valerolactone, γ-butyrolactone, γ-caprolactone, γ-heptalactone, γ-octalactone and γ-nonalactone) and two 6-membered 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 found in USFF2 and SFF2. It may be because 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

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formed through the hydrolysis of triglycerides and contribute to fatty flavors.[43]

3.2.5. Heterocyclic compounds. As 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 a high content in fat fraction samples were 2-ethylfuran, 2-propylfuran, 2-butylfuran, 2-pentylfuran, 2-heptylfuran, 2(5*H*)-furanone and 2*H*-pyran-2-one, which are mainly derived from the oxidation and degradation of lipids.[7] For instance, 2-ethylfuran and 2-pentylfuran are noncarboxylic compounds generated from the C_{10} hydroperoxide of linolenate and linoleate respectively by singlet oxygen oxidation.[1] Four N-containing heterocyclic compounds were detected, of which pyrazine is usually formed at high temperatures and provides a 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 the thermal process. It may be formed after the products of lipid oxidative decomposition take part in the Maillard reaction.[7]

3.3. Odor activity values of odor-active compounds in saturated and unsaturated fat fractions from chicken fat during the 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 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 compound profile of the 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), a higher level of OAVs was found 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 a more fatty, grassy, fruity and sweet aroma at high temperatures.

Additionally, ketones (except for 2-heptanone and 2,3-pentanedione) presented 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/cheesy notes to thermally-oxidized fat samples. Due to their higher OAVs, 1-octen-3-ol and (*E*)-2-octen-1-ol could provide a more intense mushroom and green apple aroma to USFF1, USFF2, SFF1 and SFF2. In particular, it has been reported that 1-octen-3-ol is one of the sources of the characteristic flavor of chicken soup.[46] The OAV level of the long-chain esters (γ-octalactone and γ-nonalactone) with fatty notes was quite high, whereas butyl butyrate with pineapple notes showed a lower OAV level. Regarding 2 furans, 2-ethylfuran and 2-pentylfuran might give the oxidized chicken fat a rich rubbery 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 their significant contributions to the overall aroma of oxidized chicken fat.

3.4. 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 the 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 shown that significant differences were exhibited in the odor-active compounds of the fat fraction samples with different UFA compositions after 2 h of heating. The sample dots of USFF1 and SFF1 were distributed on 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 OAVs of octanal and benzaldehyde were high in SFF1 and SFF2. In zone II, the OAV levels of 3-penten-2-one, nonanal, 2-heptanone, 2-pentylfuran, butyl butyrate, (*E*)-2-pentenal and (*E*)-2-heptenal in SFF2 and

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Table 3 Odor-active compounds in saturated and unsaturated fat fractions after heat treatment for 0, 1 and 2 h

Compounds	Odor threshold (μg kg^{-1})[a]	Odor descriptions[b]	Unsaturated			Saturated			Sign. heating time		
			0 h	1 h	2 h	0 h	1 h	2 h	0 h	1 h	2 h
Pentanal	9	Fruity	1.6^{c}	298.4^{a}	35.7^{b}	2.1^{z}	184.0^{y}	266.3^{x}	**	**	***
Hexanal	5	Green, grass	22.8^{c}	529.6^{b}	849.3^{a}	46.2^{z}	486.4^{y}	591.7^{x}	***	*	***
Heptanal	3	Fatty, putty	2.0^{c}	164.8^{b}	507.1^{a}	4.0^{z}	141.5^{y}	382.1^{x}	***	**	**
Octanal	0.578	Fatty, pungent	23.0^{c}	1882.7^{a}	920.4^{b}	2.7^{z}	311.6^{y}	592.5^{x}	***	***	***
Nonanal	1	Fatty, floral, wax	12.5^{c}	1583.6^{b}	3315.0^{a}	25.2^{z}	1220.5^{y}	2859.3^{x}	**	**	**
Decanal	2	Orange peel, soapy	0.0^{c}	31.0^{b}	76.2^{a}	0.0^{z}	17.4^{y}	50.2^{x}	NS	**	**
Dodecanal	2	Herbaceous, fatty	0.0^{b}	0.0^{b}	3.7^{a}	0.0^{y}	0.0^{y}	3.4^{x}	NS	NS	NS
(*E*)-2-Pentenal	150	Strawberry, fruity	0.06^{c}	3.1^{b}	5.1^{a}	0.0^{z}	2.9^{y}	4.9^{x}	**	NS	NS
(*Z*)-3-Hexenal	0.25	Leaf, green	0.0^{b}	0.0^{b}	30.3^{a}	0.0^{y}	0.0^{y}	23.9^{x}	NS	NS	NS
(*E*)-2-Hexenal	40	Apple, green	0.0^{b}	0.1^{a}	0.1^{a}	0.09^{z}	1.9^{y}	2.7^{x}	**	**	***
(*Z*)-4-Heptenal	0.04	Biscuit, cream	0.0^{c}	4167.4^{b}	10 349.9^{a}	0.0^{z}	9106.0^{x}	6511.4^{y}	NS	***	*
(*E*)-2-Heptenal	10	Soap, fatty, almond	2.8^{c}	460.6^{b}	661.2^{a}	3.9^{z}	437.1^{y}	808.2^{x}	**	NS	**
(*E*)-2-Octenal	3	Burdock, fatty	2.2^{c}	304.9^{b}	898.9^{a}	0.7^{z}	253.7^{y}	613.1^{x}	***	*	**
(*E*)-2-Nonenal	1	Cardboard, cucumber	0.0^{c}	293.0^{b}	765.1^{a}	0.0^{z}	157.8^{y}	526.5^{x}	NS	***	**
(*E*)-2-Decenal	0.4	Fatty, green	0.0^{b}	0.0^{b}	2438.0^{a}	0.0	0.0	0.0	NS	NS	***
2-Undecenal	0.78	Wax, fatty	0.0^{c}	98.3^{b}	682.2^{a}	0.0^{z}	41.6^{y}	395.9^{x}	NS	***	**
(*E*)-2-Dodecenal	1.4	Green, fatty, sweet	0.0^{b}	0.0^{b}	3.0^{a}	0.0	0.0	0.0	NS	NS	*
(*E*,*E*)-2,4-Hexadienal	30	Green	0.0^{c}	1.5^{b}	3.9^{a}	0.0^{z}	1.1^{y}	2.2^{x}	NS	**	**
(*E*,*E*)-2,4-Octadienal	150	Green, seaweed	0.0^{c}	0.3^{b}	1.3^{a}	0.0^{z}	0.1^{y}	0.6^{x}	NS	***	***
(*E*,*E*)-2,4-Nonadienal	0.16	Fatty, green	0.0^{c}	280.8^{b}	1869.4^{a}	0.0^{z}	83.8^{y}	1030.7^{x}	NS	***	**
(*E*,*E*)-2,4-Decadienal	0.07	Fatty, deep-fried	0.0^{c}	52 938.4^{b}	1 447 759.4^{a}	0.0^{z}	17 424.5^{y}	456 316.1^{x}	NS	***	***
Benzaldehyde	41.7	Bitter, almond	2.0^{c}	2.9^{b}	3.3^{a}	1.3^{z}	1.8^{y}	2.3^{x}	**	**	**
Benzeneacetaldehyde	4	Hawthorne, honey	0.3^{a}	0.0^{b}	0.0^{b}	0.0^{y}	1.7^{x}	0.0^{y}	**	**	NS
3-Heptanone	8	Fruity, sweet	0.0^{c}	1.9^{b}	4.3^{a}	0.0^{y}	0.0^{y}	3.5^{x}	NS	**	*
2-Heptanone	140	Soap	0.02^{c}	1.3^{b}	3.6^{a}	0.02^{z}	1.3^{y}	3.4^{x}	NS	NS	NS
2-Octanone	40	Soap, gasoline	0.0^{c}	2.9^{b}	9.2^{a}	0.0^{z}	2.1^{y}	6.7^{x}	NS	***	**
2-Decanone	7.94	—	0.0^{c}	9.4^{b}	56.2^{a}	0.0^{z}	4.1^{y}	31.6^{x}	NS	***	**
1-Penten-3-one	1	Fish, pungent	0.0^{c}	2.6^{b}	3.4^{a}	0.0^{z}	1.8^{y}	2.1^{x}	NS	*	**
3-Penten-2-one	1.5	Fruity	0.0^{c}	3.4^{b}	6.9^{a}	0.0^{z}	2.6^{y}	8.2^{x}	NS	**	NS
1-Octen-3-one	0.01	Mushroom, metal	0.0^{c}	48 510.4^{b}	107 183.7^{a}	0.0^{y}	30 244.3^{z}	82 078.9^{x}	NS	**	NS
2,3-Pentanedione	5	Cream, butter	2.4^{c}	2.6^{b}	6.6^{a}	0.0^{z}	2.6^{y}	3.5^{x}	***	NS	**
3-Ethyl-2-hydroxy-2-cyclopenten-1-one	52	—	0.0^{c}	0.2^{b}	0.3^{a}	0.0^{z}	0.7^{y}	1.5^{x}	NS	***	**
Octanol	110	Metal, burnt	0.01^{c}	1.5^{c}	4.4^{a}	0.02^{z}	1.1^{y}	3.3^{x}	NS	***	*
1-Penten-3-ol	400	Butter, pungent	0.01^{c}	1.4^{b}	1.8^{a}	0.01^{z}	1.6^{x}	1.4^{y}	NS	*	***
1-Octen-3-ol	2	Mushroom	8.9^{c}	2088.2^{b}	6240.0^{a}	6.5^{z}	1758.3^{y}	2614.8^{x}	NS	**	**
(*E*)-2-Octen-1-ol	3	Fruity, green apple	0.0^{c}	36.1^{b}	104.7^{a}	0.0^{z}	24.4^{y}	62.4^{x}	NS	**	***
Styrene	65	Herbaceous, fatty	0.4^{a}	0.0^{b}	0.0^{b}	3.7^{x}	2.8^{y}	0.0^{z}	*	**	NS
Butyl butyrate	100	Fruity, pineapple	0.3^{c}	0.6^{b}	1.4^{a}	0.0^{z}	0.8^{y}	1.6^{x}	**	***	**
γ-Octalactone	7	Coconut	0.0^{c}	3.2^{b}	28.1^{a}	0.0^{y}	0.0^{y}	10.9^{x}	NS	***	**
γ-Nonalactone	7	Coconut, peach	0.0^{b}	0.0^{b}	18.0^{a}	0.0^{y}	0.0^{y}	11.5^{x}	NS	NS	NS
2-Ethylfuran	2.3	Rubber, pungent	0.0	0.0	0.0	0.0^{y}	50.5^{x}	0.0^{y}	NS	**	NS
2-Pentylfuran	6	Pungent, sweet	6.6^{c}	218.9^{b}	488.2^{a}	0.0^{z}	223.7^{y}	507.8^{x}	***	NS	NS
Total			87.9^{c}	113 926.1^{b}	1 585 339.3^{a}	96.5^{z}	62 198.1^{y}	556 336.8^{x}	*	**	***

The different superscript letters (a, b, c) and (x, y, z) indicate significant differences ($P < 0.05$) among heating times within the saturated and unsaturated fat fractions. The stars denote significance levels between saturated and unsaturated fat fractions at the same oxidation time (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$; NS, not significant). [a] Odor thresholds were mainly obtained from the literature and an online database, with water applied as the matrix: **https://www.flavornet.org**, **https://www.odour.org.uk**. [b] Odor descriptions were mainly gathered from the following literature and an online database: **https://www.flavornet.org**.

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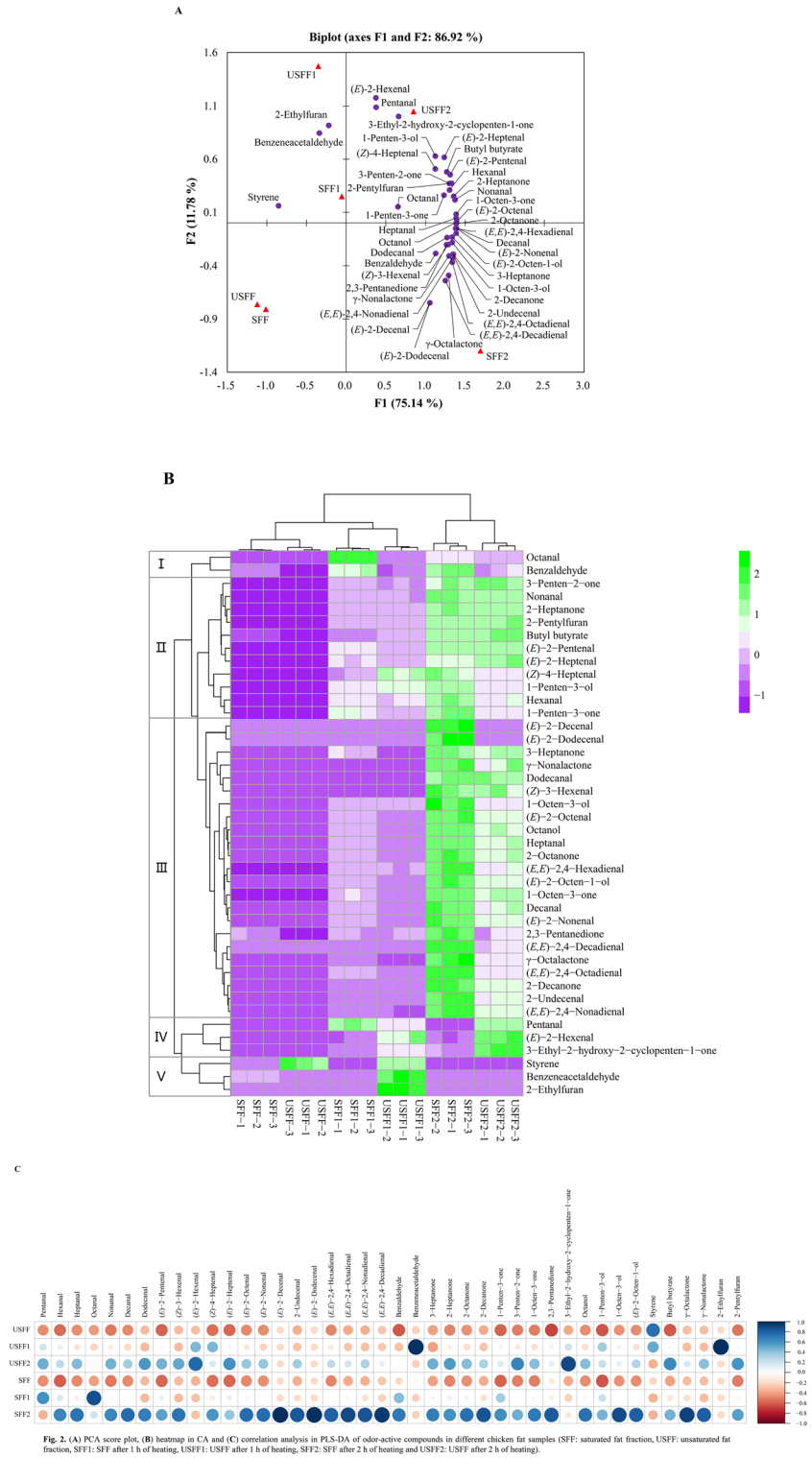

Fig. 2. (A) PCA score plot, (B) heatmap in CA and (C) correlation analysis in PLS-DA of odor-active compounds in different chicken fat samples (SFF: saturated fat fraction, USFF: unsaturated fat fraction, SFF1: SFF after 1 h of heating, USFF1: USFF after 1 h of heating, SFF2: SFF after 2 h of heating and USFF2: USFF after 2 h of heating).

Fig. 2 (A) PCA score plot, (B) heatmap in CA and (C) correlation analysis in PLS-DA of odor-active compounds in different chicken fat samples (SFF: saturated fat fraction, USFF: unsaturated fat fraction, SFF1: SFF after 1 h of heating, USFF1: USFF after 1 h of heating, SFF2: SFF after 2 h of heating and USFF2: USFF after 2 h of heating).

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 were 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

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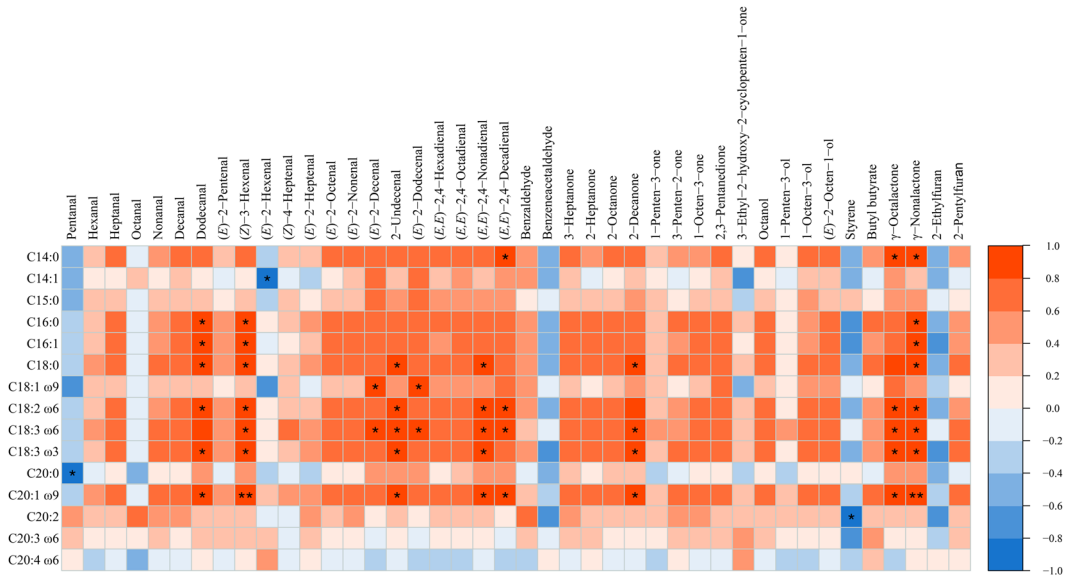

Fig. 3 The correlation analysis in PLSR of odor-active compounds in different chicken fat samples (SFF: saturated fat fraction, USFF: unsaturated fat fraction, SFF1: SFF after 1 h of heating, USFF1: USFF after 1 h of heating, SFF2: SFF after 2 h of heating and USFF2: USFF after 2 h of heating).

USFF2, whereas in zone V, the OAVs of styrene, benzeneacetaldehyde and 2-ethylfuran were the highest in USFF1.

The correlation coefficients between odor-active compounds and different fat fraction samples are 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, and pentanal and octanal had a strong positive influence on SFF1. Additionally, there was a significant positive effect of (*E*)-2-hexenal, (*E*)-2-heptenal, 3-ethyl-2-hydroxy-2-cyclopenten-1-one and butyl butyrate on USFF2, while more compounds, including 7 alkanals, 4 alkenals, 4 alkadienals, 7 ketones, 3 alcohols and 2 esters were highly relevant to SFF2, indicating that the volatile compounds formed showed significant differences after 2 h of heat treatment of fat fractions with different FA compositions.

3.5. 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 positive and negative effects on odor-active compounds of different chicken fat samples (Fig. 3). The thermal oxidation of FAs creates classes 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 shown 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 were 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 to have 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 were negatively related to the formation of pentanal, (*E*)-2-hexenal and styrene.

4. 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 significantly higher ($p < 0.05$) 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

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different heating time stages. 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 the overall flavor in different fat groups with varying degrees of saturation. In this study, it can be concluded that volatile compounds induced by lipid oxidation or degradation were influenced by the heating time and FA 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.

Author contributions

Dong Han: data curation, formal analysis, and writing – original draft. Siyang Deng: data curation, formal analysis, and writing – original draft. Hang Wang: methodology and investigation. Feng Huang: formal analysis and writing – review & editing. Marie-Laure Fauconnier: formal analysis and writing – review & editing. Hong Li: investigation and validation. Jian Zheng: investigation and writing – review & editing. Linchun Meng: formal analysis. Chunhui Zhang: funding acquisition, supervision, and project administration. Xia Li: funding acquisition, supervision, and project administration.

Conflicts of interest

All authors declare that they have no conflict of interest.

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