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# Volatile metabolite profiling of linolenic acid oxidation in the heating process

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**Abstract**

Linolenic acid is an important fatty acid, and volatiles generated by its oxidation are the major components of food flavor. In this study, volatile components generated from linolenic acid during heating were detected and analyzed by using thermal desorption cryo-trapping system coupled with gas chromatography-mass spectrometry. A total of 52 volatile compounds were identified, including aldehydes (18), ketones (12), alcohols (6), furans (4), acids (6) and aromatic compounds (6). The forming temperature of each volatile compound was also determined. It was found that most volatile compounds with shorter carbon chains were mainly generated at lower temperatures, while volatile compounds with longer carbon chains were mainly produced at higher temperatures. Results of principal component analysis show that most of the identified volatiles were considered as the characteristic ones of the high temperature points. Potential thermal oxidation mechanism of linolenic acid was also given at the same time.

**Keywords:** linolenic acid; flavor; volatile compounds; heating; GC-MS.

**Practical Application:** By controlling the heating temperature, the types and contents of volatile flavor substances generated by the oxidation of linolenic acid residues in lipids were regulated during heating.

## 1 Introduction

Flavor is one of the most important factors affecting the quality of food, which mainly originates from the oxidation of lipids during the thermal processing of food. Lipids are mainly composed of triacylglycerols (Rivas-Cañedo et al., 2021). It is found that the oxidation of lipids mainly occurs in the fatty acids contained in their molecules, especially the oxidation of unsaturated fatty acids is significantly higher than that of saturated fatty acids, and it is enhanced with the increase of their unsaturation (Oliveira et al., 2020). Vegetable oil is currently the main edible lipid and is the main source of fat intake for humans (Lima et al., 2021; Yuenyong et al., 2021). It contains about 50%-60% linoleic acid (C18:2n-6), 10%-40% linolenic acid (C18:3), 22%-30% oleic acid (C18:1n-9), 7%-10% palmitic acid (C16:0), 5%-9% stearidonic acid (C18:3n-3) and small amounts of n-eicosanoic acid (C20:0) (Zhang et al., 2015). Common vegetable oils such as olive oil, canola oil and soybean oil are widely used in food industry (Chen et al., 2017; Nikzad et al., 2021). During heating of these vegetable oils, the thermal oxidation products of fatty acids are considered to be the main source of flavor in foods (Eckl & Bresgen, 2017), especially the aroma of fried foods. Therefore, exploring the composition and content of volatiles produced from fatty acids is important to investigate the mechanisms of flavor production during heating.

Linolenic acid is an ω-3 fatty acid with three double bonds, and is one of the important fatty acids that constitute fats and oils in the form of glycerides (Babiszewska, 2020; Almas et al., 2022). At the same time, linolenic acid as essential fatty acids, is only through food intake for the normal growth and development of the human body (Rincón-Cervera et al., 2016). Linolenic acid can be used in the body to promote fat metabolism, composition of body cells, synthesis of prostaglandins and related to vision, brain and other aspects of behavioral development (Khan et al., 2020; Bourourou et al., 2016). In our previous study, we analyzed and identified the volatile compounds produced during the heating process of oleic and linoleic acids, and found that these two fatty acids produced a large number of volatile substances during heating, such as volatile aldehydes, alcohols, ketones, acids and furans (Bao et al., 2022). Meanwhile, it is further demonstrated that fatty acids are the main source of food flavor during thermal processing.

Generally, 18-carbon unsaturated fatty acids are the main components of fats and oils, and their oxidation process is one of the important sources of food flavor. In order to further investigate the flavor formation mechanism of lipid oxidation, this study analyzed the volatile components produced by the oxidation of linolenic acid during heating, explored the effect of temperature on the formation of volatiles, and determined the temperature at which each volatile are formed.

The results of this study will serve the food industry in the following aspects: 1. artificial regulation of flavor during thermal processing. 2. theoretical guidance for the development of optimal processes during processing. 3. control of undesirable flavor production while increasing the content of aroma substances by controlling the process technology during thermal processing.

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## 2 Materials and methods

### 2.1 Chemicals

Standard chemicals: linolenic acid and C4–C20 n-alkanes (analytical grade) were purchased from Sigma-Aldrich (Shanghai, China).

### 2.2 Extraction and collection of volatile compounds from linolenic acid during heating by using a thermal desorption cryo-trapping system

Simulated heating was carried out in a qualified micro-chamber/thermal extractor (M-CTE, M-CTE250 Markes International, UK). M-CTE250 is a quality control tool for screening volatile and semi-volatile organic compounds in organic materials. Before using the M-CTE, it is important to clean it and ensure that it is free of any residues or contaminants to ensure the accuracy of the experiment. First, the M-CTE chamber, o-ring and the sample holders were rinsed with ethanol and cyclohexane. Then, a 20 mL volume of glassware was placed in the sample holder and 20 μL of linolenic acid was added directly into the glassware. Subsequently, the lid of the sample holder was quickly closed. Three sample racks were used simultaneously for parallel experiments (one sample per chamber) and heated at 30, 60, 90, 120, 150, 180 and 210 °C for 30 minutes. Finally, volatile compounds were extracted from the samples by opening the air valve at a flow rate of 100 mL/min for 30 minutes. These compounds were captured in 250 mg Tenax TA absorber tubes (60-80 mesh). As soon as these compounds were trapped the volatile compounds were thermally desorbed from the sorbent tubes using an automated thermal desorber (TD100-xr Markes International) at 280 °C for 5 minutes and cryogenically focused in a cold trap at 10 °C. This was followed by flash heating at 280 °C for 3 minutes to desorb from the cold trap onto a GC-MS column with a flow path temperature of 200 °C.

### 2.3 GC-MS analysis

Volatile compounds desorbed from the cold trap were analyzed using an Agilent 6890/5975C GC-MS (Santa Clara, CA, USA) system equipped with an HP-5 MS column (30 m × 0.25 mm ID, 0.25 μm film thickness; J&W Scientific). The carrier gas was helium in a non-split mode, delivered at a linear rate of 1 mL/min. The desorption time in the injection port was 5 minutes at a temperature of 250 °C. The temperature was programmed to be held at 35 °C for 3 minutes and increased to 280 °C at a rate of 5 °C/min. The mass selective detector was operated in electron collision ionization mode at 70 eV with a scan range of m/z 40-400. The interface temperature was 230 °C and the retention times for each volatile were converted to Kovats retention indices using n-alkanes (Sigma, Co.) as a reference. Volatile compounds were initially identified by matching mass spectra to spectra of reference compounds from the Wiley Mass Spectrometry (MS) library (version 8) and the NIST/EPA/NIH MS library (version 2014) and validated against mass spectra and Kovats retention indices obtained from the literature compared to those reported in the literature. Finally, identification accuracy was determined by separating relevant standard compounds by GC-MS analysis under the same conditions (Chai et al., 2019).

### 2.4 Statistical analysis

All experiments were conducted in triplicate. The measured data were analyzed using PCA by the MetaboAnalyst 3.0 (Qin et al., 2020). Significance between related samples was analyzed according to the one-way ANOVA test at the level of 0.05 ($p < 0.05$) using the SPSS 18.0 (Chicago, IL, USA).

## 3 Results and discussion

### 3.1 Composition of volatile compounds from linolenic acid during heating

A total of 52 volatile substances were identified during the heating of linolenic acid, including aldehydes (18), ketones (12), alcohols (6), furans (4), acids (6) and aromatic compounds (6). The volatile compounds detected were dominated by aldehydes, followed by ketones, alcohols, furans, acid esters, aromatic and furans, and the generation of aldehydes was detected throughout the heating process. The results show that aldehydes were the main oxidation products of linolenic acid during heating, and that there were up to 18 species. Most of the aldehydes start to be generated at 90 °C, with individual ones being formed at lower temperatures. Aldehydes are usually considered to be the main product of lipid oxidation (Vandemoortele et al., 2021; Zhang et al., 2022). Low concentrations of volatile aldehydes usually contribute a cereal, cream and caramel aroma, while higher concentrations have a strong solvent odor, which is often referred to as 'rancid' (Xiao et al., 2020). Throughout the heating process, 4 alkyl aldehydes (acetaldehyde, hexanal, nonanal, and decanal) from C2 to C10 were identified, as well as a large variety of alkenals and alkadienals. Acrolein is one of the more representative aldehydes detected in linolenic acid and is inherently toxic to human respiratory and physical health (Kishimoto & Kashiwagi, 2018).

During heating, 12 ketones were identified from linolenic acid. They were the second largest proportion of volatile substances. Volatile ketones are important flavor components in fried foods (Du et al., 2020). High concentrations of volatile ketones provide a strong solvent flavor, while low concentrations of ketones usually contribute a nut flavor (Jackson & Penumetcha, 2018). Ketones can be obtained directly from the oxidation of the linolenic acid carbon chain or from the oxidation of the corresponding alcohols. Current research indicates that the majority of volatile ketones are 2-ketones, such as 3-penten-2-one, 2-hexanone, 3-hexen-2-one, 6-octen-2-one, (E,E)-3,5-octadien-2-one and 6-methyl-3,5-hepten-2-one (Table 1).

Six alcohols from C2 to C12 were identified from linolenic acid. These alcohols include both saturated and unsaturated alcohols. Among them, ethanol, and 2-butyl-1-octanol are saturated alcohols; 1-penten-3-ol, (E)-2-penten-1-ol, 2-methyl-1,3-pentanediol and 2-methyl-1-penten-3-ol are unsaturated alcohols. The alcohols are mainly derived from the reduction of carbonyl compounds, and they usually contribute a soft or vegetal odor (Jackson & Penumetcha, 2018).

Furans usually impart a certain sweetness. During heating, 4 furans, including 2-pentylfuran, were identified from linolenic acid oxidation. This result was consistent with the results reported

**Table 1**. Volatile compounds from linolenic acid oxidation in the heating process.

| No. | Compounds | RI | Identification method | Peak area ($\times 10^6$) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 30 °C | 60 °C | 90 °C | 120 °C | 150 °C | 180 °C | 210 °C |
| Aldehydes | | | | | | | | | | |
| 1 | Acetaldehyde | 382 | RI, MS, STD | nd | nd | 13.2 ± 2.07b | 15.41 ± 2.38a | nd | nd | nd |
| 2 | 2- Acrolein | 456 | RI, MS | nd | 79.51 ± 3.77b | 34.04 ± 5.07d | 34.85 ± 2.31d | 59.11 ± 5.06c | 104.26 ± 8.04a | 52.71 ± 9.25c |
| 3 | 2-Butenal | 606 | RI, MS, STD | 39.05 ± 4.2d | 148.36 ± 22.55c | nd | 203.81 ± 28.45b | 361.98 ± 47.44a | 376.83 ± 19.06a | 334.95 ± 42.39a |
| 4 | (E)-2-Butenal | 629 | RI, MS | nd | nd | 104.74 ± 5.64a | nd | nd | nd | nd |
| 5 | 2-Methyl-2-butenal | 632 | RI, MS, STD | nd | nd | nd | 180.7 ± 26.96a | nd | nd | nd |
| 6 | (E)-2-Methyl-2-butenal | 745 | RI, MS | nd | nd | nd | nd | 263.5 ± 50.6a | 5.86 ± 5.86b | 2.1 ± 0.73b |
| 7 | (E)-2-Pentenal | 754 | RI, MS, STD | 3.43 ± 1.57e | 28.26 ± 1.29d | 101.41 ± 3.86c | 8.14 ± 2.43e | 160.8 ± 17.65b | 282.39 ± 11.91a | 324.25 ± 64.47a |
| 8 | 2-Methyl-4-pentenal | 793 | RI, MS | nd | 13.06 ± 0.43b | nd | 99.57 ± 12.63a | nd | 1.53 ± 0.17c | 1.41 ± 0.46c |
| 9 | Hexanal | 800 | RI, MS | nd | nd | nd | nd | 75.49 ± 12.72b | 67.55 ± 23.94b | 106.37 ± 23.08a |
| 10 | Furfural | 833 | RI, MS, STD | nd | nd | nd | nd | 5.21 ± 2.03b | 42.86 ± 11.04a | 44.14 ± 3.63a |
| 11 | (E)-2-Hexenal | 851 | RI, MS, STD | nd | nd | nd | nd | nd | 59.74 ± 5.72a | nd |
| 12 | (Z)-4-heptenal | 900 | RI, MS, STD | nd | nd | nd | 8.92 ± 2.67b | 36.75 ± 11.75b | 21.28 ± 11.82c | 74.34 ± 7.27a |
| 13 | (E,E)-2,4-Hexadienal | 911 | RI, MS, STD | nd | 7.85 ± 1.79d | 26.04 ± 5.23c | 125.85 ± 7.55a | 108.24 ± 20.1b | nd | nd |
| 14 | (Z)-2-heptenal | 958 | RI, MS, STD | nd | nd | nd | 16.56 ± 2.14b | 14.19 ± 4.18b | 15.15 ± 2.54b | 23.96 ± 4.25a |
| 15 | (E,E)-2,4-heptadienal | 1012 | RI, MS, STD | 7.2 ± 1.7c | 330.76 ± 34.78b | 4.45 ± 0.04c | 435.11 ± 60.74b | nd | 1999.72 ± 152.31a | nd |
| 16 | (Z,Z)-3,6-nonadienal | 1100 | RI, MS, STD | nd | nd | 7.34 ± 1.94d | 23.68 ± 4.17c | 33.08 ± 6.68b | 19.7 ± 9.24c | 78.84 ± 9.34a |
| 17 | Nonanal | 1104 | RI, MS, STD | nd | nd | nd | 38.12 ± 5.81b | 25.26 ± 2.37c | 9.7 ± 0.65d | 73.1 ± 8.31a |
| 18 | Decanal | 1211 | RI, MS, STD | nd | nd | nd | 88.38 ± 18.33a | nd | nd | nd |
| Alcohols | | | | | | | | | | |
| 19 | Ethanol | 427 | RI, MS, STD | nd | nd | 11.08 ± 1.33b | 15.57 ± 2.45a | 17.32 ± 1.34a | nd | 8.45 ± 1.58c |
| 20 | 1-penten-3-ol | 684 | RI, MS, STD | 11.71 ± 3.6c | 32.23 ± 2.86b | 31.18 ± 3.33b | 49.04 ± 6.46b | 206.47 ± 42.18a | 163.65 ± 76.39a | 49.3 ± 18.92b |
| 21 | (E)-2-Penten-1-ol | 769 | RI, MS, STD | nd | nd | 8.54 ± 1.72c | 13.24 ± 2.38c | 95.21 ± 23.6a | 51.01 ± 22.98b | 45.55 ± 5.11b |
| 22 | 2-Methyl-1-penten-3-ol | 845 | RI, MS | nd | nd | 26.79 ± 7.26a | nd | nd | nd | nd |
| 23 | 2-Methyl-1,3-pentanediol | 1005 | RI, MS | nd | nd | nd | 16.21 ± 3.15a | nd | nd | nd |
| 24 | 2-Butyl-1-octanol | 1393 | RI, MS, STD | nd | 8.4 ± 0.96a | nd | nd | nd | nd | nd |
| Ketones | | | | | | | | | | |
| 25 | Acetone | 475 | RI, MS, STD | 102.67 ± 14.37a | nd | nd | nd | nd | nd | nd |
| 26 | 3-Penten-2-one | 657 | RI, MS, STD | nd | nd | nd | 26.72 ± 3.83a | nd | nd | nd |
| 27 | 1-penten-3-one | 681 | RI, MS, STD | nd | 98.37 ± 8.32bc | 66.32 ± 7.68c | 133.38 ± 18.38bc | 382.53 ± 50.79a | 80.36 ± 80.36bc | 140.53 ± 15.88b |
| 28 | 1-Hydroxy-2-butanone | 739 | RI, MS | nd | nd | 8.25 ± 1.62a | nd | nd | nd | nd |
| 29 | 2-Hexanone | 761 | RI, MS, STD | 4.07 ± 0.6a | 1.5 ± 0.17b | nd | nd | nd | nd | nd |
| 30 | 5-Methyl-4-hexen-3-one | 792 | RI, MS | nd | nd | 20.2 ± 0.96b | 125.59 ± 12.99a | nd | nd | nd |
| 31 | 3-Hexen-2-one | 845 | RI, MS, STD | nd | nd | 3.16 ± 1.89d | 21.41 ± 1.72c | 79.42 ± 14.27a | 43.8 ± 2.38b | 30.93 ± 5.96c |
| 32 | 3-Heptanone | 887 | RI, MS, STD | nd | nd | nd | nd | 12.47 ± 3.95b | 9.33 ± 4.84b | 34.16 ± 6.41a |
| 33 | 6-octen-2-one | 985 | RI, MS, STD | nd | nd | nd | 35.07 ± 6.47a | 24.42 ± 12ab | 22.49 ± 8.06b | 26.26 ± 4.65b |
| 34 | (E,E)-3,5-octadien-2-one | 1073 | RI, MS, STD | nd | 45.09 ± 5.59b | 33.78 ± 4.83b | 158.51 ± 22.47a | 28.96 ± 5.14bc | 17.01 ± 4.51c | 35.25 ± 2.27b |
| 35 | 6-Methyl-3,5-hepten-2-one | 1107 | RI, MS | nd | nd | nd | nd | 23.12 ± 10.09a | 24.68 ± 15.49a | 10.46 ± 2.81b |
| 36 | Dihydro-3-methylene-5-methyl-2-furanone | 2048 | RI, MS | nd | 54.7 ± 5.3b | 30.4 ± 1c | 503.0 ± 35.2a | nd | nd | nd |

nd = Compound not detected in the sample. MS = Identification by MS spectra, RI = Kovat's retention indexes, STD = Comparison with a standard compound; Peak area is the mean ± standard error of three replicates; "a,b, c, d" indicates a significant difference ($p < 0.05$).

**Table 1**. Continued...

| No. | Compounds | RI | Identification method | Peak area (×10⁶) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 30 °C | 60 °C | 90 °C | 120 °C | 150 °C | 180 °C | 210 °C |
| Furans | | | | | | | | | | |
| 37 | 2-Ethylfuran | 672 | RI, MS, STD | nd | nd | 8.44 ± 0.07d | 139 ± 11.98c | 118.88 ± 3.9c | 228.47 ± 3.78a | 187.94 ± 29.39b |
| 38 | cis-2-(2-Pentenyl)-furan | 767 | RI, MS | nd | nd | nd | nd | 1740.61 ± 424.37a | 1135.91 ± 257.67b | 1293.18 ± 149.04b |
| 39 | 2-pentylfuran | 977 | RI, MS, STD | nd | nd | nd | 17.26 ± 3.18c | 32.14 ± 12.05b | 38.04 ± 2.31ab | 43.56 ± 7.14a |
| 40 | 5-Methyl-2-ethyl-furan | 990 | RI, MS | nd | nd | nd | nd | 9.4 ± 3.24c | 28.96 ± 7.97b | 40.24 ± 6.86a |
| Acids and esters | | | | | | | | | | |
| 41 | Formic acid | 526 | RI, MS, STD | nd | nd | nd | 228.94 ± 18.58a | 69.01 ± 23.41c | 158.91 ± 47.01b | 190.8 ± 13.58a |
| 42 | Acetic acid | 610 | RI, MS, STD | nd | nd | nd | 547 ± 83.32a | 99.9 ± 33.06d | 209.31 ± 43.23c | 305.34 ± 72.55b |
| 43 | Propionic acid | 702 | RI, MS, STD | nd | nd | 4.26 ± 0.39b | 9.81 ± 0.01a | nd | nd | nd |
| 44 | 3-Methylbutyric acid | 863 | RI, MS | nd | nd | nd | nd | 2.01 ± 0.67b | 4.17 ± 0.57a | 4.11 ± 0.69a |
| 45 | Heptanoic acid | 1078 | RI, MS, STD | nd | nd | nd | nd | 89.55 ± 8.53a | 19.92 ± 10.87b | 12.58 ± 2.01b |
| 46 | Octanoic acid | 1180 | RI, MS, STD | nd | nd | 36.06 ± 6.4a | 36.72 ± 4.64a | 27.61 ± 2.12a | 18.92 ± 1.92b | 33.32 ± 5.25a |
| Aromatic compounds | | | | | | | | | | |
| 47 | Toluene | 760 | RI, MS, STD | 25.69 ± 1.14a | nd | nd | nd | nd | nd | nd |
| 48 | Ethylbenzene | 855 | RI, MS, STD | 20.12 ± 0.32c | nd | nd | nd | 8.68 ± 2.87d | 30.27 ± 0.98b | 59.44 ± 9.58a |
| 49 | p-Xylene | 865 | RI, MS, STD | nd | 60.62 ± 3.17a | 6.6 ± 0.05b | nd | nd | nd | nd |
| 50 | Propylbenzene | 992 | RI, MS | 32.2 ± 0.11a | nd | nd | nd | nd | nd | nd |
| 51 | Benzyl alcohol | 1036 | RI, MS | nd | nd | nd | nd | 26.35 ± 6.07a | 20.54 ± 1.02b | 15.41 ± 3.01c |
| 52 | 3-Ethylbenzaldehyde | 1168 | RI, MS | nd | nd | nd | nd | 68.87 ± 13.8b | 27.65 ± 6.79c | 344.13 ± 57.27a |

nd = Compound not detected in the sample. MS = Identification by MS spectra, RI = Kovat's retention indexes, STD = Comparison with a standard compound; Peak area is the mean ± standard error of three replicates; "a,b, c, d" indicates a significant difference ($p < 0.05$).

by Becalski and Seaman that furans are oxidation products of polyunsaturated fatty acids (Becalski et al., 2005). In addition, amino acids and α, β-unsaturated aldehydes can be catalyzed into alkyl furans by the Maillard reaction (Adams et al., 2011). During heating, six acids were identified from linolenic acid and all of them were saturated. The saturated acids usually producing sweetness and fruit aromas (Wahman et al., 2020). They are also considered to be oxidation products of the corresponding aldehydes (Wu et al., 2021). Six aromatic compounds were identified, and aromatic compounds are usually considered as solvent residues. However, the analysis of aromatic compounds represented by ethylbenzene showed that the substance appeared at 30 °C and could not be detected at 60 °C, 90 °C and 120 °C.This substance was detected again after 150 °C and showed a clear trend of increasing content with the temperature increased. This phenomenon suggested that the aromatic compounds as solvent residues were almost completely volatilized at 30 °C, while the aromatic analogues detected at 150 °C were obtained from the oxidation of linolenic acid. However, the forming mechanism of these aromatic compounds was still unknown.

### *3.2 Characterization of volatile compounds forming from linolenic acid oxidation during heating*

The heating process of linolenic acid is a complex thermal oxidation reaction, with many reactions occurred. One of the important parameters in this complex process is the temperature, which directly affects the degree of oxidation and final product composition (Semenov et al., 2011). In this study, a simulated heating process was carried out in which seven temperature points (30 °C, 60 °C, 90 °C, 120 °C, 150 °C, 180 °C and 210 °C) were selected to investigate the effect of temperature on the volatile compounds generated from linolenic acid. Total ion current chromatogram based on GC/MS of linolenic acid at different temperature was shown in Figure 1.

Figure 2 visualizes the formation temperatures of the different volatiles from linolenic acid oxidation during heating and the composition of volatiles in each temperature points. The general trends indicate that most volatiles with short carbon chains are generated at low temperatures, and the forming temperatures of volatiles in descending order are volatile ketones, alcohols, aldehydes, acids, and furans. As shown in Figure 2, 9 volatile compounds (aldehydes (3), alcohols (1), ketones (2), and aromatic compounds (3)) were generated at 30 °C, including 2-butenal, (E)-2-pentenal, (E, E)-2,4-heptadienal, 1-penten-3-ol, acetone, 2-hexanone, toluene, ethylbenzene and propyl benzene. Eight volatile compounds (aldehydes (3), alcohols (1), ketones (3), aromatic compounds (1)) were produced at 60 °C, including 2-acrolein, (E,E)-2,4-hexadienal, 2-methyl-4-pentenal, 2-butyl-1-octanol, 1-penten-3-one, (E,E)-3,5-octadien-2-one, dihydro-3-methylene-5-methyl-2-furanone and p-xylene. This phenomenon indicated that linolenic acid is very unstable and could be oxidated under lower temperature conditions during heating.

In order to see the variation tendency of compounds more intuitively at different temperatures, a heatmap is drawn to assess the changing trend of the volatile compounds at different temperatures. The heatmap shows the amount of volatile compounds during heating by the warm and cold color, where the cold colors represent low levels and the warm colors represent high levels. As shown in Figure 3, the color of most volatile compounds gradually changes from cold to warm as the temperature increased.

With the heating temperature increased, 12, 11 and 11 volatile compounds were detected at 90 °C, 120 °C and 150 °C, respectively. It is obviously that a large number of new volatile compounds were generated at 90 °C and 150 °C, accounting for nearly 65% or more of the total volatile compounds, and that the volatiles are mainly composed of volatile aldehydes and ketones, which are usually key flavor substances in foods (Wang et al., 2021; Cao et al., 2021). However, few new volatile compounds were generated at 180 °C and 210 °C, and the content of most volatiles dramatically increased at these two temperature points (Figure 3), such as 2-butenal, (E)-2-pentenal, (Z, Z)-3,6-nonadiene,

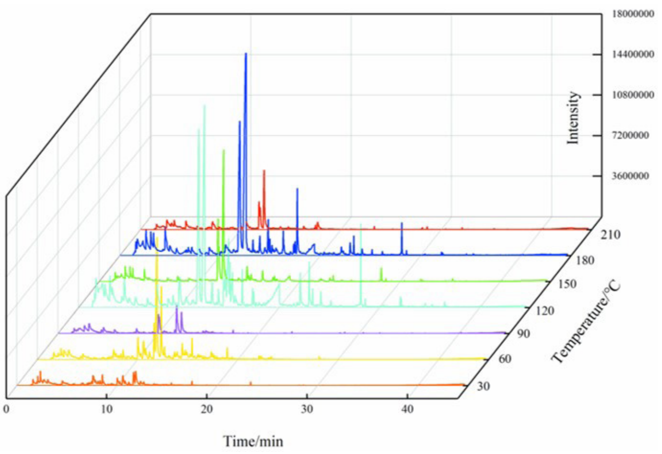

**Figure 1**. Total ion current chromatogram based on GC/MS at different temperature during linolenic acid heating.

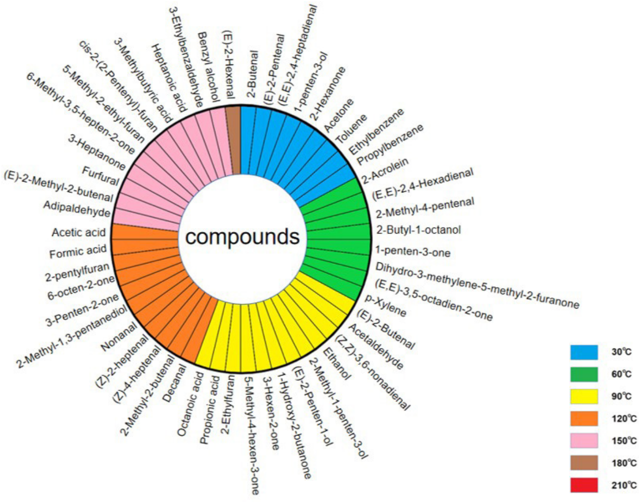

**Figure 2**. Formation temperatures of different volatile compounds from linolenic acid oxidation during heating.

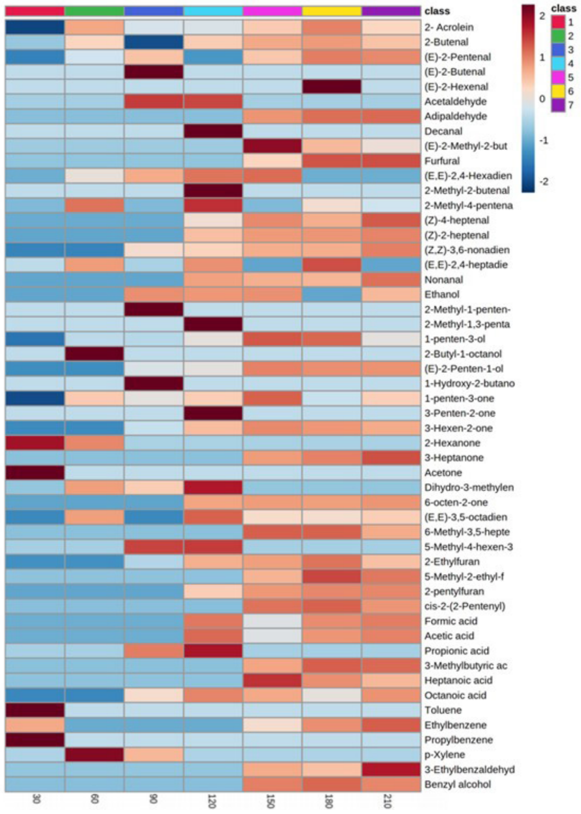

**Figure 3**. Heatmaps of volatile compounds from linolenic acid oxidation during heating.

3-ethylbenzaldehyde, 3-hexen-2-one 2-ethylfuran, 2-pentylfuran and 6-octen-2-one, because the linolenic acid oxidation becomes more intense at higher temperature conditions. By comparing with our previous experimental data on heating oleic and linoleic acids, we found that the amount of volatiles generated by thermal oxidation of linolenic acid was the highest (Bao et al., 2022). Meanwhile, the number of products containing carbon-carbon unsaturated double bonds is also the highest for linolenic acid, especially for the two unsaturated degrees. Moreover, linoleic acid has the highest number of lower temperatures products, followed by linolenic acid, and oleic acid has the least. This phenomenon indicates that linoleic and linolenic acid residues are more susceptible to oxidation compared to oleic acid residues during the thermal oxidation of lipids.

In order to better understand the composition and changing characteristics of the volatile compounds of linolenic acid during heating, a principal component analysis was performed by normalizing the peak area of each compound. The scoring scatter plots and loading scatter plots are shown in Figure 4A and Figure 4B, respectively. In the Figure 4A, it can be seen that the cumulative contribution of PC1 (48.2%) and PC2 (20.6%) is 68.8%. Furthermore, the scoring points at different temperatures can be clearly separated. This result indicates that volatile components and changing characteristics of linolenic acid at each heating temperature points are different from each other. It is obviously that lower temperature scoring points (30 °C, 60 °C and 90 °C) were distributed in the 3rd quadrant of scoring plot, while the high temperature scoring points (150 °C, 180 °C and 210 °C) were distributed in the 4th quadrant of scoring plot, indicating that the volatiles produced at lower temperature points or higher temperature points were relatively similar. Corresponding to the loading plot (Figure 4B), it is easy to see that compounds such as p-xylene and 2-hexanone contribute more for the lower temperatures. The score points at 120 °C located in the 2nd quadrant of scoring plot, separately. And compounds such as 2-methyl-1,3-pentanediol, decanal, 3-penten-2-one, and 5-methyl-4-hexen-3-one contribute more to this temperature point (Figure 4B). As can be seen from Figure 4A, the scoring points at 150, 180 and 210 °C are located in the positive loading region of PC1, which is significantly different from the distribution of scoring points at low temperature conditions. At the same time, the distribution of scoring points at these three temperatures is relatively concentrated, indicating that the composition of

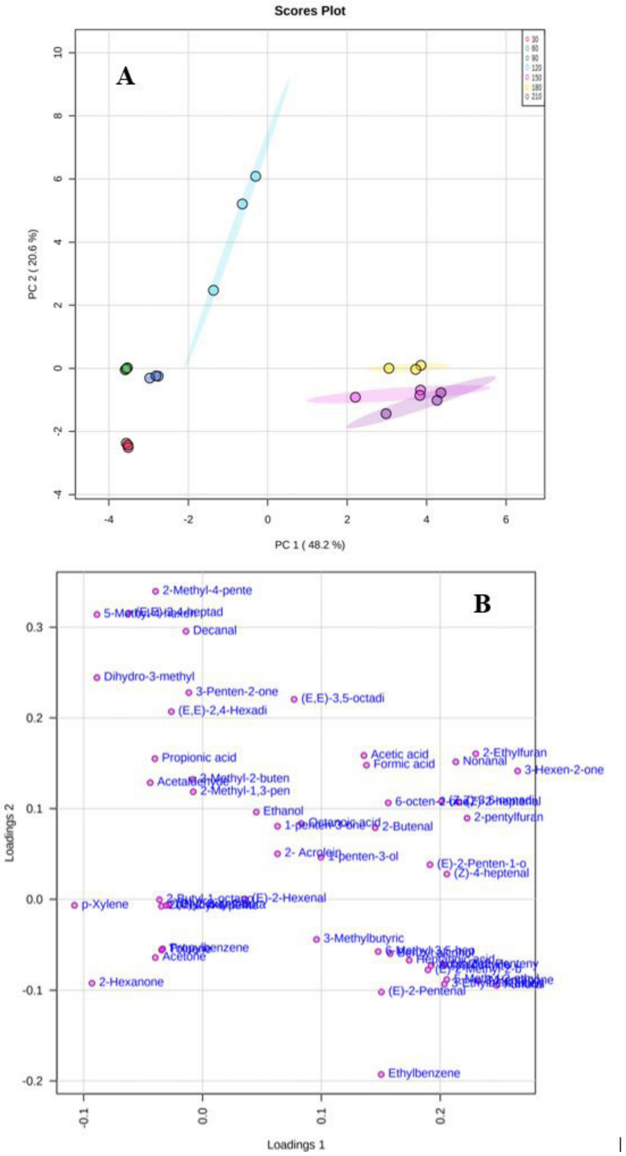

**Figure 4**. PCA plots of volatile compounds from linolenic acid oxidation during heating. (A) Scoring scatter plot; (B) loading scatter plot.

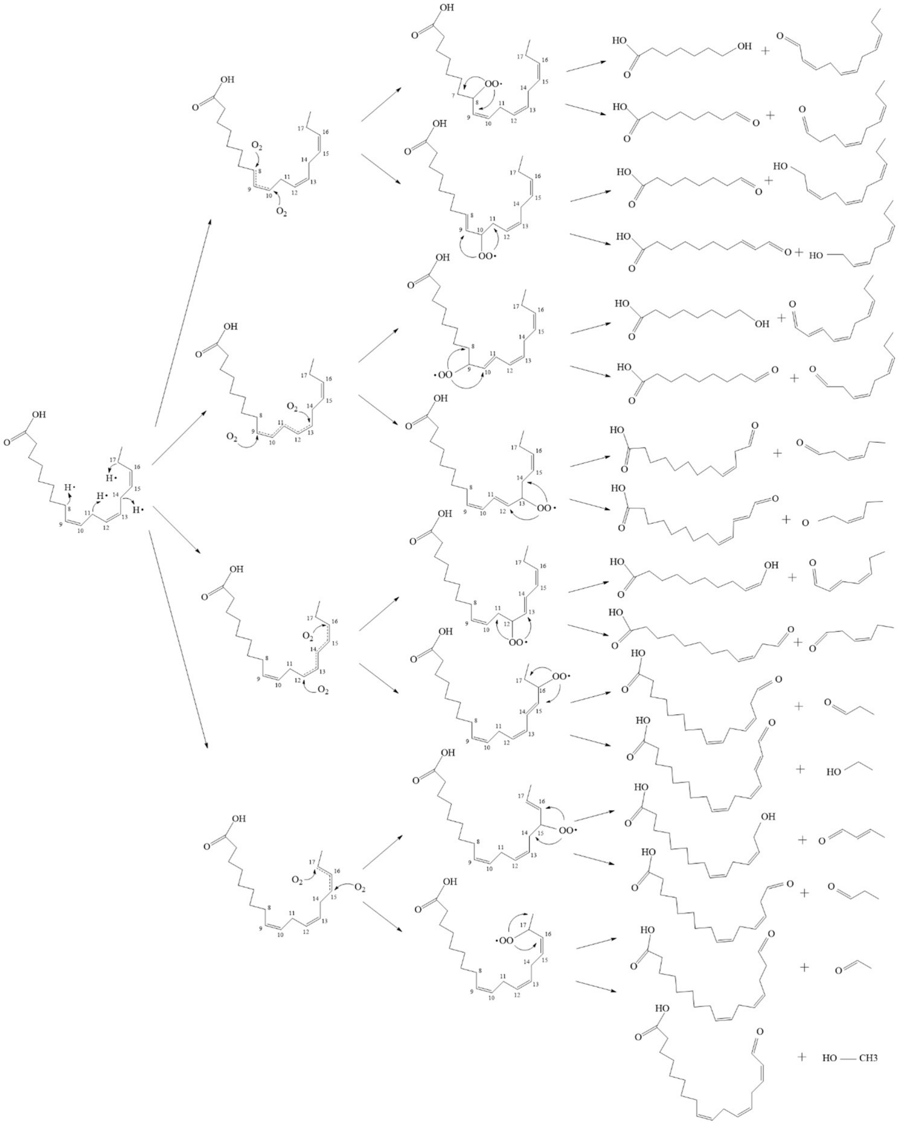

**Figure 5**. Potential oxidative mechanism of linolenic acid.

volatiles at 150 °C, 180 °C and 210 °C is very similar. As shown in Figure 4B, most of volatile compounds have a big contribution to these higher temperature points, such as (E)-2-pentenal, (E)-2-hexenal and ethylbenzene.

### *3.3 Mechanism of linolenic acid oxidation in the heating process*

As shown in Figure 5, during heating, due to the electron absorption of the carbon-carbon unsaturated double bond in the linolenic acid molecule, the electron density of the hydrogen atoms on both sides of the α-carbon reduced, which makes it very reactive and susceptible to oxidation by other electron-rich groups, such as hydrogen atoms on the 8,11,14 and 17 carbon atoms (Jackson & Penumetcha, 2018). During the heating process, the hydrocarbon bonds at these carbons firstly absorb energy and break, forming the corresponding olefin radicals and hydrogen radicals. The olefin radicals and the double bonds on either side of them form a conjugated π-bond system, which results in the rearrangement of the double bonds of the carbon chain. Specifically, the carbon at position 8 forms the C8-C9-C10 π-bond system, the carbon at position 11 forms the C9-C10-C11-C12-C13 π-bond system, the carbon at position 14 forms the C12-C13-C14-C15-C16 π-bond system, and the carbon at position 17 forms the C15-C16-C17 π-bond system. The positions of the double bonds on the carbon chain after rearrangement are shown in Figure 5. The olefin radical is then attacked by oxygen to form a peroxide radical. Finally, the peroxide radical continues to attack the carbon atoms on both sides leading to the breakage of the carbon chain, and form the volatile aldehydes.

## 4 Conclusion

Thermal desorption coupled with gas chromatography-mass spectrometry is an effective method for studying the forming mechanism of volatile compounds from linolenic acid oxidation during heating. Temperature is the most important parameters in this complex thermal oxidation system and plays an essential role. It is possible for us to regulate the production of food flavor during heating by controlling the heating temperature. Meanwhile, during the thermal oxidation of lipids, linoleic and linolenic acid residues are more susceptible to oxidation than oleic acid residues.

## Conflict of interest

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

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## References

Adams, A., Bouckaert, C., van Lancker, F., De Meulenaer, B., & De Kimpe, N. (2011). Amino acid catalysis of 2-alkylfuran formation from lipid oxidation-derived α,β-unsaturated aldehydes. *Journal of Agricultural and Food Chemistry*, 59(20), 11058-11062. http://dx.doi.org/10.1021/jf202448v. PMid:21928831.

Almas, M., Moldir, Y., Gulsim, A., Gulim, T., & Dina, D. (2022). Effects of ω-3 fatty acids and ratio of ω-3/ω-6 for health promotion and disease prevention. *Food Science and Technology*, 42, e58321. http://dx.doi.org/10.1590/fst.58321.

Babiszewska, M. (2020). Effects of energy and essential fatty acids content in breast milk on infant's head dimensions. *American Journal of Human Biology*, 32(6), e23418. http://dx.doi.org/10.1002/ajhb.23418. PMid:32307819.

Bao, Y., Du, J., Xu, C., Wang, M., Wang, B., Xiao, L., Cheng, K., & Dong, L. (2022). Detailed temperature-dependent study of linoleic acid oxidative decomposition into volatile compounds in the heating process. *Journal of Food Processing and Preservation*, 46(4), e16445. http://dx.doi.org/10.1111/jfpp.16445.

Becalski, A., Forsyth, D., Casey, V., Lau, B. P., Pepper, K., & Seaman, S. (2005). Development and validation of a headspace method for determination of furan in food. *Food Additives and Contaminants*, 22(6), 535-540. http://dx.doi.org/10.1080/02652030500129170. PMid:16019826.

Bourourou, M., Heurteaux, C., & Blondeau, N. (2016). Alpha-linolenic acid given as enteral or parenteral nutritional intervention against sensorimotor and cognitive deficits in a mouse model of ischemic stroke. *Neuropharmacology*, 108, 60-72. http://dx.doi.org/10.1016/j.neuropharm.2016.04.040. PMid:27133376.

Cao, Y., Zhu, S., Zhang, L., Cui, Q., & Wang, H. (2021). Qualitative and quantitative determination of trace aldehydes and ketones in food preservative propionic acid for quality improvement. *Analytical Methods*, 13(26), 2989-2996. http://dx.doi.org/10.1039/D1AY00742D. PMid:34124731.

Chai, D., Li, C., Zhang, X., Yang, J., Liu, L., Xu, X., Du, M., Wang, Y., Chen, Y., & Dong, L. (2019). Analysis of volatile compounds from wheat flour in the heating process. *International Journal of Food Engineering*, 15(10). http://dx.doi.org/10.1515/ijfe-2019-0252.

Chen, Y., Zhou, Z., Xu, K., Zhang, H., Thornton, M., Sun, L., Wang, Z., Xu, X., & Dong, L. (2017). Comprehensive evaluation of malt volatile compounds contaminated by fusarium graminearum during malting. *Journal of the Institute of Brewing*, 123(4), 480-487. http://dx.doi.org/10.1002/jib.453.

Du, W., Zhao, M., Zhen, D., Tan, J., Wang, T., & Xie, J. (2020). Key aroma compounds in Chinese fried food of youtiao. *Flavour and Fragrance Journal*, 35(1), 88-98. http://dx.doi.org/10.1002/ffj.3539.

Eckl, P. M., & Bresgen, N. (2017). Genotoxicity of lipid oxidation compounds. *Free Radical Biology & Medicine*, 111, 244-252. http://dx.doi.org/10.1016/j.freeradbiomed.2017.02.002. PMid:28167130.

Jackson, V., & Penumetcha, M. (2018). Dietary oxidised lipids, health consequences and novel food technologies that thwart food lipid oxidation: an update. *International Journal of Food Science & Technology*, 54(6), 1981-1988. http://dx.doi.org/10.1111/ijfs.14058.

Khan, M. R., Batool, M., Amir, R. M., Shabbir, A., Siddique, F., Adil, R. M., Ameer, K., Din, A., Rakha, A., Riaz, A., & Faiz, F. (2020). Ameliorating effects of okra (*Abelmoschus esculentus*) seed oil on hypercholesterolemia. *Food Science and Technology*, 41(1), 113-119. http://dx.doi.org/10.1590/fst.38919.

Kishimoto, N., & Kashiwagi, A. (2018). Reducing the formation of acrolein from linolenate-rich oil by blending with extra virgin olive oil during repeated frying of food at high temperatures. *Food Science and Technology Research*, 24(6), 1017-1020. http://dx.doi.org/10.3136/fstr.24.1017.

Lima, T. L. S., Costa, G. F., Cruz, G. R. B., Araújo, Í. B. S., Ribeiro, N. L., Ferreira, V. C. S., Silva, F. A. P., & Beltrão, E. M. Fo. (2021). Effect of storage time on colorimetric, physicochemical, and lipid oxidation parameters in sheep meat sausages with pre-emulsified linseed oil. *Food Science and Technology*, 42, e24721. http://dx.doi.org/10.1590/fst.24721.

Nikzad, N., Ghavami, M., Seyedain-Ardabili, M., Akbari-Adergani, B., & Azizinezhad, R. (2021). Effect of deep frying process using sesame oil, canola and frying oil on the level of bioactive compounds in onion and potato and assessment of their antioxidant activity. *Food Science and Technology*, 41(3), 545-555. http://dx.doi.org/10.1590/fst.35819.

Oliveira, V. S., Cháves, D. W. H., Gamallo, O. D., Sawaya, A. C. H. F., Sampaio, G. R., Castro, R. N., Torres, E. A. F. S., & Saldanha, T. (2020). Effect of *aroeira* (*Schinus terebinthifolius* Raddi) fruit against polyunsaturated fatty acids and cholesterol thermo-oxidation in model systems containing sardine oil (*Sardinella brasiliensis*). *Food Research International*, 132, 109091. http://dx.doi.org/10.1016/j.foodres.2020.109091. PMid:32331636.

Qin, L., Gao, J. X., Xue, J., Chen, D., Lin, S. Y., Dong, X. P., & Zhu, B. W. (2020). Changes in aroma profile of shiitake mushroom (Lentinus edodes) during different stages of hot air drying. *Foods*, 9(4), 444-455. http://dx.doi.org/10.3390/foods9040444. PMid:32272549.

Rincón-Cervera, M. A., Valenzuela, R., Hernandez-Rodas, M. C., Barrera, C., Espinosa, A., Marambio, M., & Valenzuela, A.. (2016). Vegetable oils rich in alpha linolenic acid increment hepatic n-3 LCPUFA, modulating the fatty acid metabolism and antioxidant response in rats. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, 111, 25-35. http://dx.doi.org/10.1016/j.plefa.2016.02.002. PMid:26995676.

Rivas-Cañedo, A., Martínez-Onandi, N., Gaya, P., Nuñez, M., & Picon, A. (2021). Effect of high-pressure processing and chemical composition on lipid oxidation, aminopeptidase activity and free amino acids of Serrano dry-cured ham. *Meat Science*, 172, 108349. http://dx.doi.org/10.1016/j.meatsci.2020.108349. PMid:33120177.

Semenov, K. N., Charykov, N. A., Keskinov, V. A., Piartman, A. K., Gruzinskaya, E. G., & Krokhina, O. A. (2011). Temperature dependence of light fullerenes solubility in oleic, linoleic and linolenic acids.

*Fullerenes, Nanotubes, and Carbon Nanostructures*, 19(4), 300-308. http://dx.doi.org/10.1080/15363831003721831.

Vandemoortele, A., Heynderickx, P. M., & Meulenaer, B. D. (2021). Kinetic modeling of malondialdehyde reactivity in oil to simulate actual malondialdehyde formation upon lipid oxidation. *Food Research International*, 140, 110063. http://dx.doi.org/10.1016/j.foodres.2020.110063. PMid:33648286.

Wahman, R., Graßmann, J., Sauvêtre, A., Schröder, P., & Letzel, T. (2020). Lemna minor studies under various storage periods using extended-polarity extraction and metabolite non-target screening analysis. *Journal of Pharmaceutical and Biomedical Analysis*, 188, 113362. http://dx.doi.org/10.1016/j.jpba.2020.113362. PMid:32526623.

Wang, F., Gao, Y., Wang, H., Xi, B., He, X., Yang, X., & Li, W. (2021). Analysis of volatile compounds and flavor fingerprint in Jingyuan lamb of different ages using gas chromatography-ion mobility spectrometry (GC-IMS). *Meat Science*, 175, 108449. http://dx.doi.org/10.1016/j.meatsci.2021.108449. PMid:33550158.

Wu, H., Xiao, S., Yin, J., Zhang, J., & Richards, M. P. (2021). Mechanisms involved in the inhibitory effects of free fatty acids on lipid peroxidation in turkey muscle. *Food Chemistry*, 342, 128333. http://dx.doi.org/10.1016/j.foodchem.2020.128333.

Xiao, L., Li, C., Chai, D., Chen, Y., Wang, Z., Xu, X., Wang, Y., Geng, Y., & Dong, L. (2020). Volatile compound profiling from soybean oil in the heating process. *Food Science & Nutrition*, 8(2), 1139-1149. http://dx.doi.org/10.1002/fsn3.1401. PMid:32148821.

Yuenyong, J., Pokkanta, P., Phuangsaijai, N., Kittiwachana, S., Mahatheeranont, S., & Sookwong, P. (2021). GC-MS and HPLC-DAD analysis of fatty acid profile and functional phytochemicals in fifty cold-pressed plant oils in Thailand. *Heliyon*, 7(2), e06304. http://dx.doi.org/10.1016/j.heliyon.2021.e06304. PMid:33665454.

Zhang, L., Chen, J., Zhang, J., Sagymbek, A., Li, Q., Gao, Y., Du, S., & Yu, X. (2022). Lipid oxidation in fragrant rapeseed oil: Impact of seed roasting on the generation of key volatile compounds. *Food Chemistry: X*, 16, e100491. http://dx.doi.org/10.1016/j.fochx.2022.100491. PMid:36339322.

Zhang, W., Li, N., Feng, Y., Su, S., Li, T., & Liang, B. (2015). A unique quantitative method of acid value of edible oils and studying the impact of heating on edible oils by UV-vis spectrometry. *Food Chemistry*, 185, 326-332. http://dx.doi.org/10.1016/j.foodchem.2015.04.005. PMid:25952875.