Oil Crop Institute of Chinese Academy
of Agricultural Sciences

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GLOBAL CROP - GOLDEN OPPORTUNITIES

# A novel comprehensive analysis approaches based on liquid chromatography-mass spectrometry for lipid oxidation of rapeseed oil

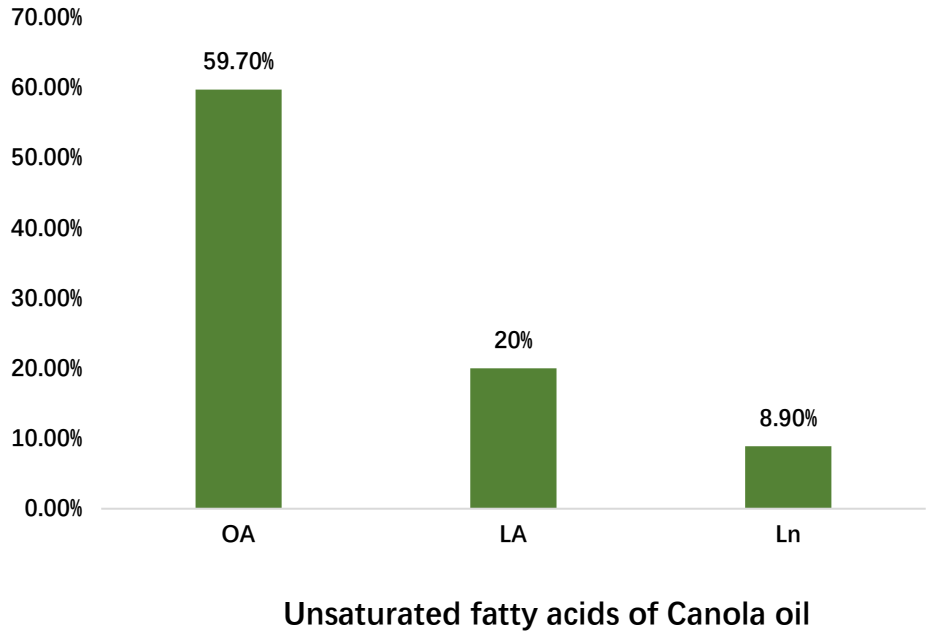

**Chen Hong**

**Oil Crops Research Institute**

**Chinese Academy of Agriculture Science**


**2023-09-27**

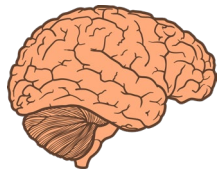

# Background and Objective


Rapeseed (Canola) oil is widely used around the world. Rapeseed oil is naturally low in saturated fat and high in monounsaturated fatty acid (MUFA) and polyunsaturated fatty acids (PUFA) , which are excellent for our health. As research result shown that there are more double bonds fatty acid unsaturated causing easier oxidation

[1]Xie Ya,Wei Fang, Lv Xin, Dong Xuyan, Chen Hong, Analysis of Triacylglycerols in Cold-Pressed Rapeseed Oil by Shotgun Electrospray Ionization Tandem Mass Spectrometry, Journal of Chinese Institute of Food Science and Technology, 2018,18。292-300

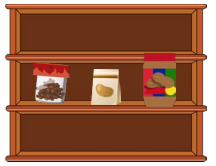

Oil oxidation



Cardiovascular disease

Nutrition and healthy

Food quality and safety



Storage and preservation

Oxidative deterioration of rapeseed oils is of great importance in the food industry.

Oxidation results in loss of nutrients, reduction of shelf life, and production of off-flavors.

**Initiation:**

$$R_1H \xrightarrow{\text{initiator}} R_1^{\bullet} + H^{\bullet}$$

*and/or*

$$O_2 \xrightarrow{\text{initiator}} O_2^{\bullet -} \longrightarrow HOO^{\bullet} \xrightarrow{R_1H} R_1^{\bullet} + H_2O_2$$

**Propagation:**

$$R_1^{\bullet} \xrightarrow{O_2} R_1OO^{\bullet} \xrightarrow{R_2H} R_2^{\bullet} \xrightarrow{O_2} R_2OO^{\bullet} \xrightarrow{R_3H} R_3^{\bullet} \xrightarrow{O_2} R_3OO^{\bullet} \xrightarrow{R_nH} R_n^{\bullet} \text{ .....etc.}$$

$R_1OOH$ (heat, UV light) → $R_1O^{\bullet} + {}^{\bullet}OH$ → $R_mH$ → $R_m^{\bullet}$; $R_xH$ → $R_x^{\bullet}$

$R_2OOH$ (oxidizing metals) → $R_2OO^{\bullet} + H^+$ → $R_yH$ → $R_y^{\bullet}$; $H_2O$

$R_3OOH$ (reducing metals) → ${}^{-}OH + R_3O^{\bullet}$ → $R_zH$ → $R_z^{\bullet}$

**Termination:**

$$\left.\begin{matrix} ROO^{\bullet} \\ RO^{\bullet} \\ R^{\bullet} \end{matrix}\right\} \longrightarrow \text{Non-radical products (R-R, ROR, ROOR, etc.)}$$

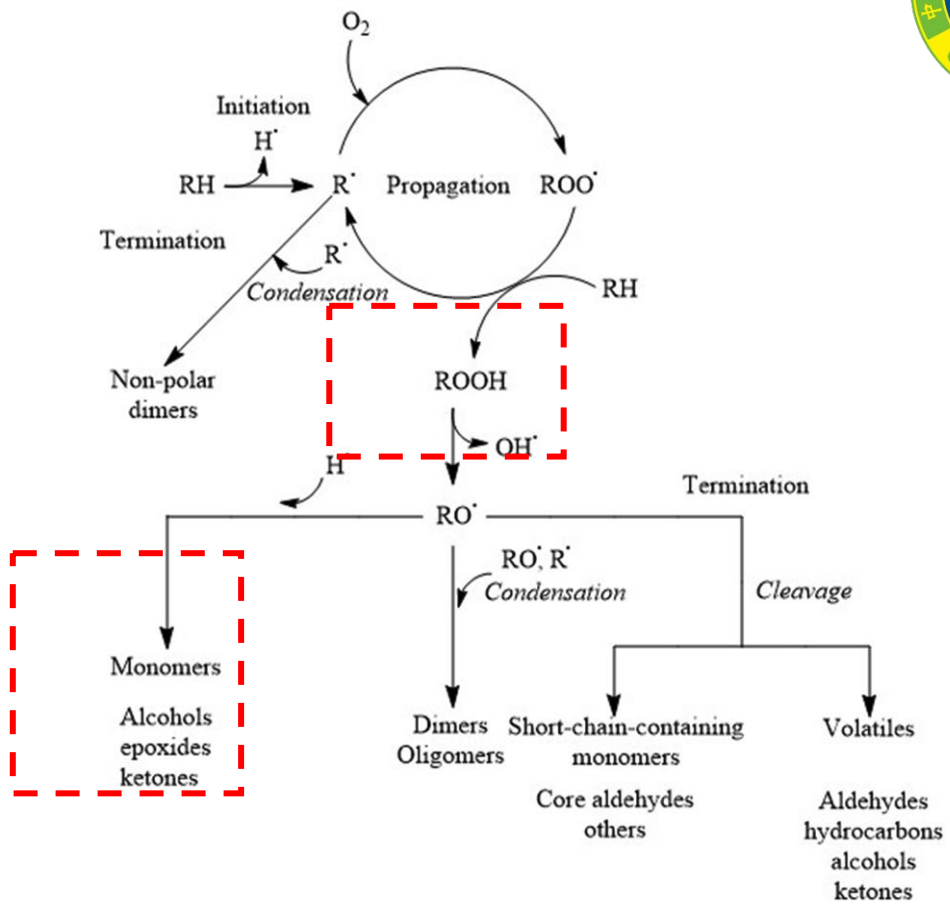
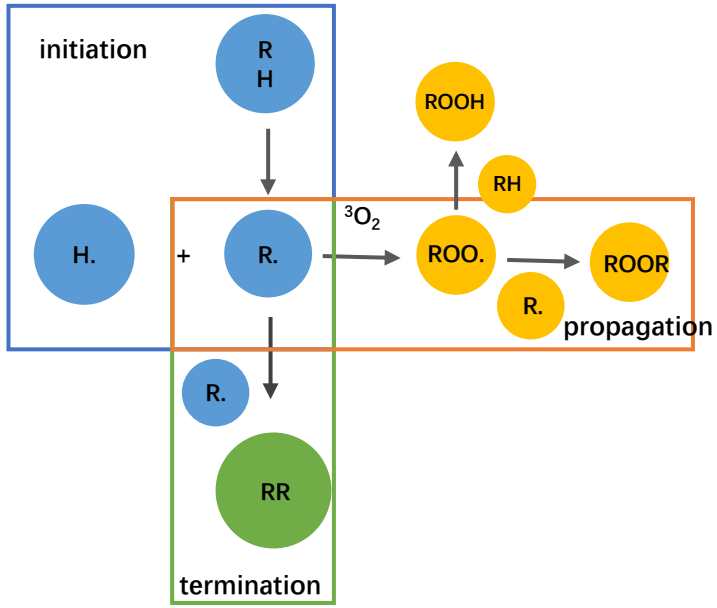



**Fig. 1.** Major steps in oxidation.

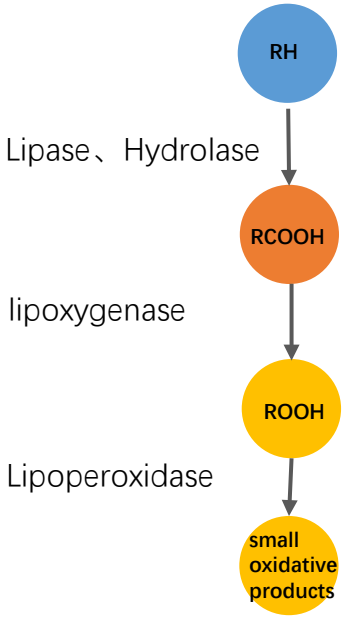
## Pathways of Lipid Oxidation

[1] Indelicato S , Bongiorno D , Pitonzo R , et al. Triacylglycerols (TAGs) in edible oils: determination, characterization, quantitation, chemometric approach and evaluation of adulterations[J]. Journal of Chromatography A, 2017:1-16.

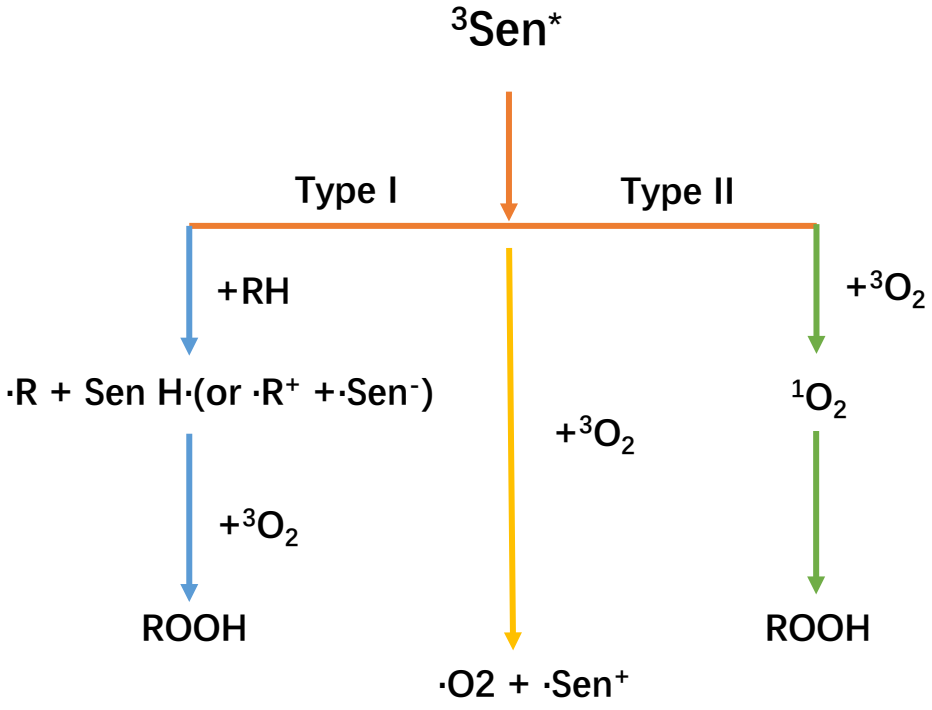
# Three Types of Lipid Oxidation




1、Autoxidation



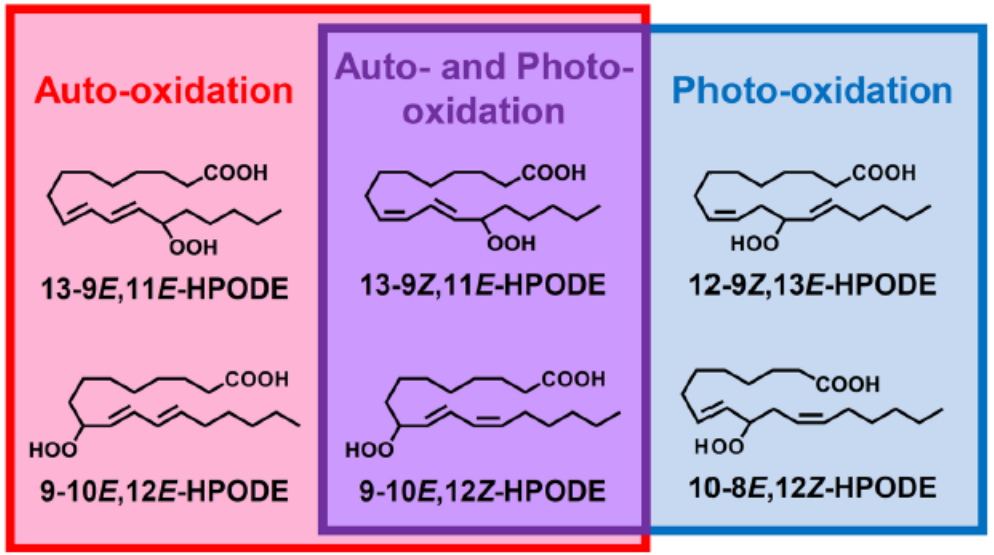

2、Enzymatic oxidation




3、Photooxidation

[1]Shahidi F, Zhong Y. Lipid oxidation and improving the oxidative stability. Chemical Society Reviews , 2010, 4067 4079

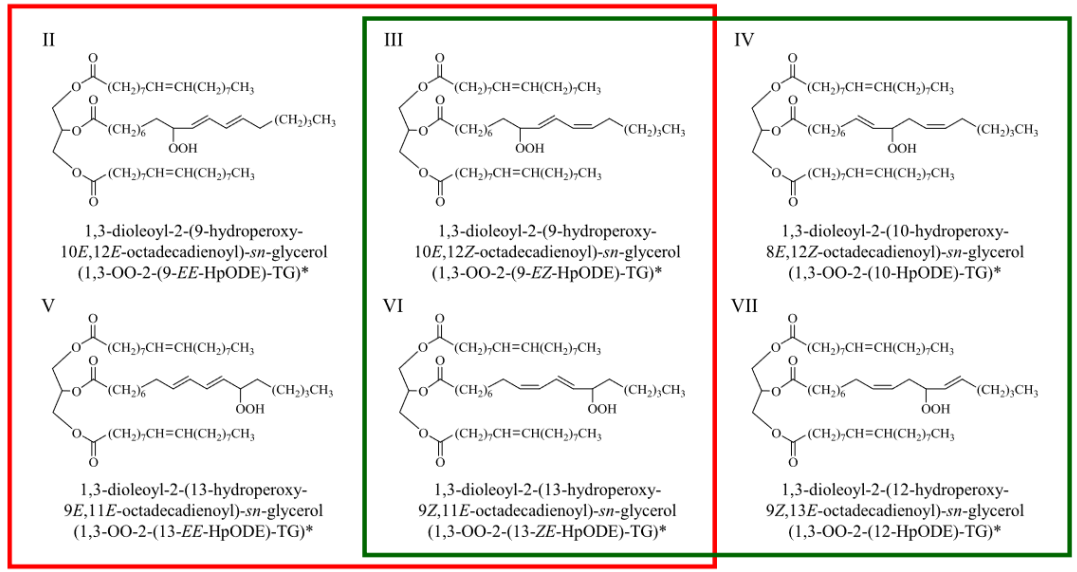
# Characteristic Chemical Structures of Oxidatice products from different Types of Oxidation




Oxitive products of fatty acid of different Types of Oxidation

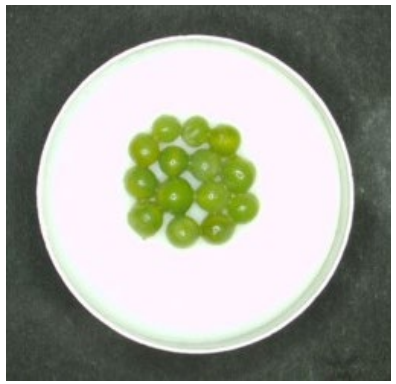
Auto/Thermal oxidation

Photo oxidation

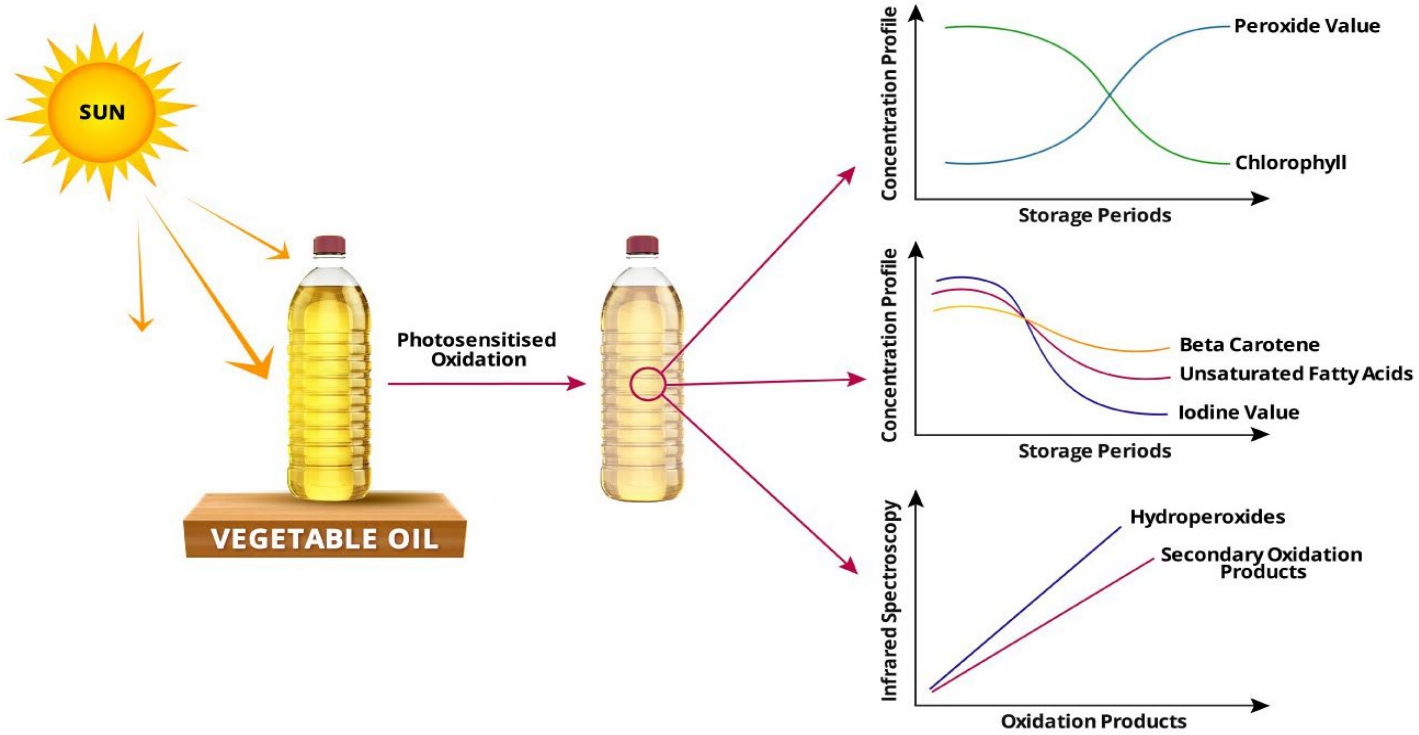



oxTAGs products of different Types of Oxidation

Ito J, Shimizu N, Kobayashi E, Hanzawa Y, Otoki Y, Kato S, Hirokawa T, Kuwahara S, Miyazawa T, Nakagawa K. A novel chiral stationary phase LC-MS/MS method to evaluate oxidation mechanisms of edible oils. Scientific reports 2017, 7(1): 10026








Chlorophyll pigments present in rapeseed oil are important quality factors because they impart undesirable color to vegetable oils and can promote oxidation in the presence of light

*Daniel Dodoo,et al. Journal of Oleo Science, 2022, 795-811*
*Xue Li, Ruinan Yang, Chunling Lv, Lin Chen, Liangxiao Zhang, Xiaoxia Ding, Wen Zhang, Qi Zhang, Chundi Hu, Peiwu Li,Effect of Chlorophyll on Lipid Oxidation of Rapeseed Oil,European Journal of Lipid Science and Technology,2018, 121(4) ,1800078*

## Conventional Methods for Analyzing Edible Oil Oxidation

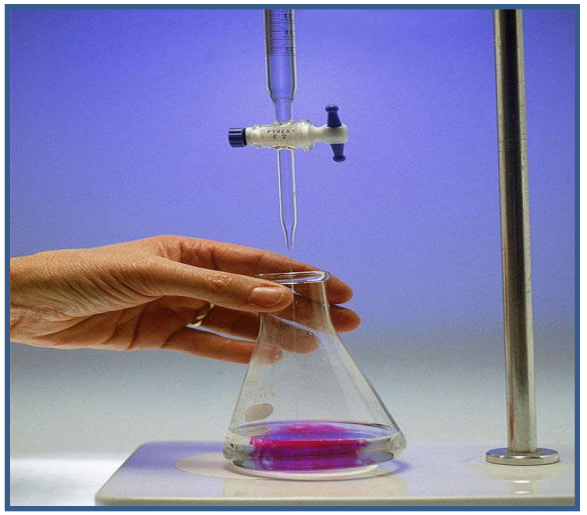
Steps of lipid oxidation

| Initiation | Propagation | Termination | Decomposition and aggregation |
|---|---|---|---|
| Raw edible oil | lipid peroxidation | lipid peroxidation<br>Polymers, small volatile molecules | Polymers,<br>small volatile molecules |
| Iodine value | (Peroxide value)<br>(Conjugated diene value ) | (Peroxide value)<br>(Conjugated diene value)<br>(Total polar compounds) | (P-Anisidine value)<br>(TBA)<br>(Conjugated diene value)<br>(Free fatty acids) |

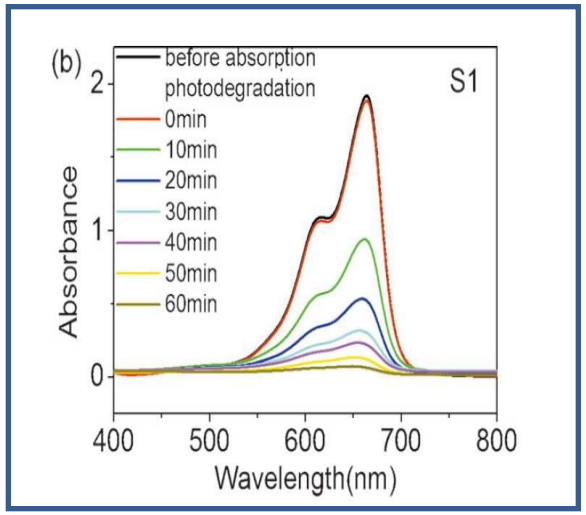
[1] Indelicato S , Bongiorno D , Pitonzo R , et al. Triacylglycerols (TAGs) in edible oils: determination, characterization, quantitation, chemometric approach and evaluation of adulterations[J]. Journal of Chromatography A, 2017:1-16.

## ●Current status

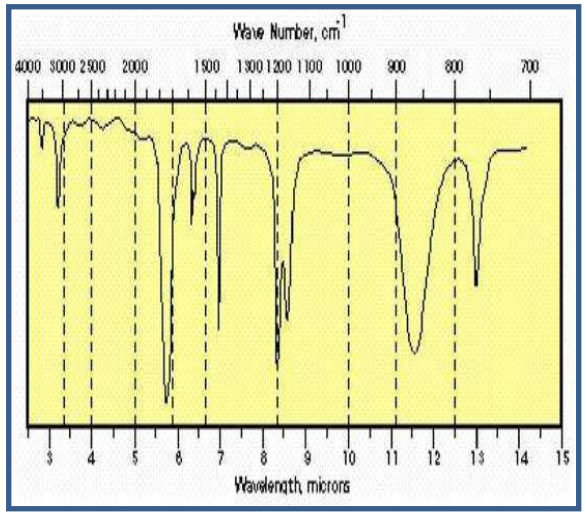
Although conventional methods for analyzing oxidative products in oils can monitor changes in oxidative stability, they are limited in providing information on the different types and composition of oxidation products generated through thermal oxidation and photosensitive oxidation pathways, as well as differences in chemical reaction pathways. This limitation hinders precise quality monitoring of vegetable oils from the perspective of oxidative mechanisms.



Chemical titration analysis




ultraviolet spectroscopy




infrared spectroscopy

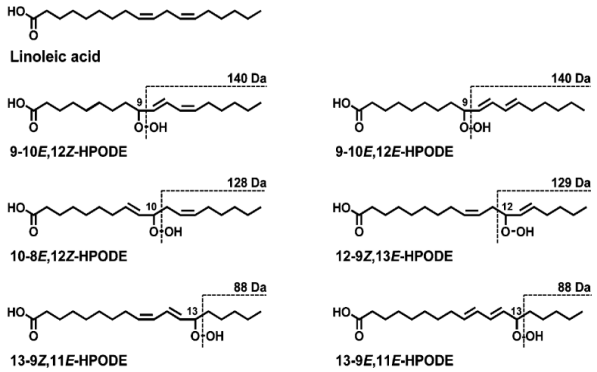
# Analysis Approaches Based on Liquid Chromatography-Mass Spectrometry for Lipid Oxidation of Rapeseed Oil

HPLC-MS/MS-Qtrap4000

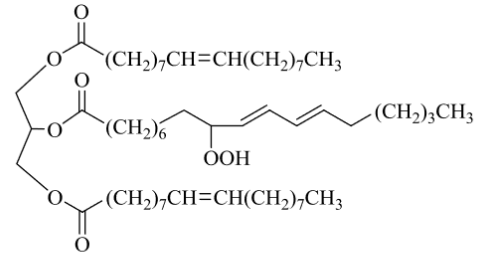
Targeted analysis: high sensitivity, such as SRM, MRM mode.
Suitable for profiling of trace small molecules with extremely low limits of LOD and LOQ.
The MRM mode is the "gold standard" for the analysis of oxidative products of lipid.

UPLC-MS/MS-Q-tof 6600

Non-targeted analysis: high coverage and high resolution, such as DIA, DDA mode.
By using UPLC-QTOF- MS/MS combined with MS/MS library to profiling oxidative products of lipid.




Analysis of oxidive of fatty acids

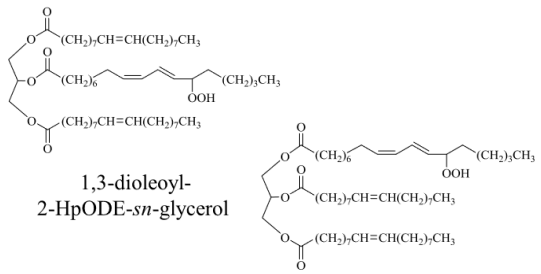


1,3-dioleoyl-2-(9-hydroperoxy-10*E*,12*E*-octadecadienoyl)-*sn*-glycerol (1,3-OO-2-(9-*EE*-HpODE)-TG)*

Analysis of Ox-TAG

[1] Indelicato S , Bongiorno D , Pitonzo R , et al. Triacylglycerols (TAGs) in edible oils: determination, characterization, quantitation, chemometric approach and evaluation of adulterations[J]. Journal of Chromatography A, 2017:1-16.

# Difficulties




1

- Huge amount of oxidative products of lipids with complex structures
- Difficult to analysis by quantify and quantitive

2

- Difficulty to reveal mechanisms of lipid oxidation
- Lack of comprehensive methods for profling of lipid oxidative products

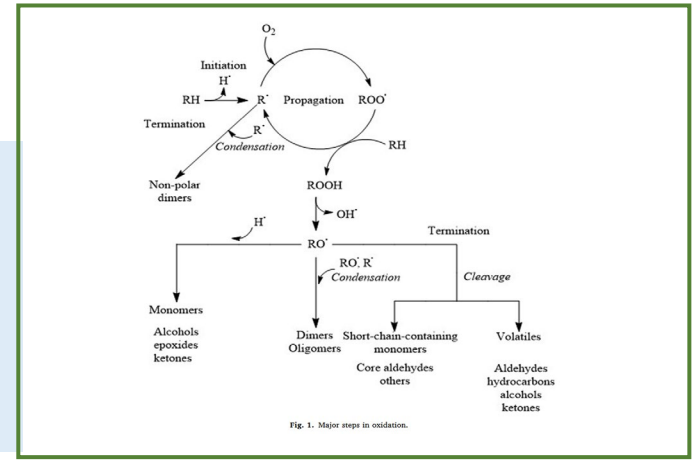
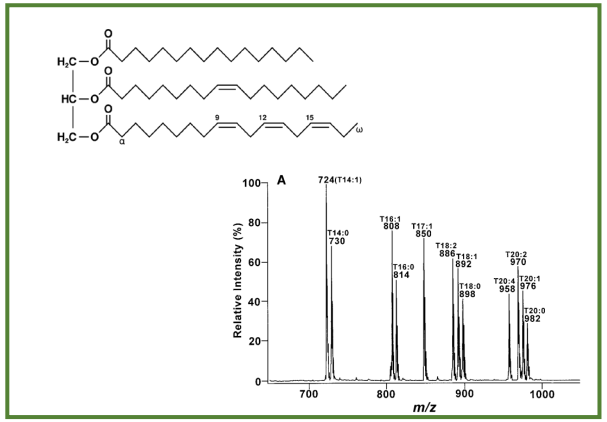


Fig. 1. Major steps in oxidation.




3

- Lack commercial standard substances for quantitative analysis of lipid oxidative products.

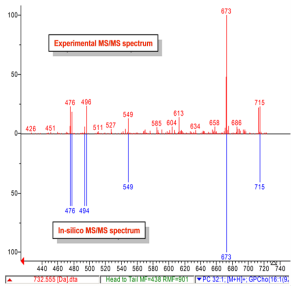
4

- Without MS database for profiling lipid oxidative products due to fragmentation rule of MS of lipid oxidative products

MassBank High Quality Mass Spectral Database

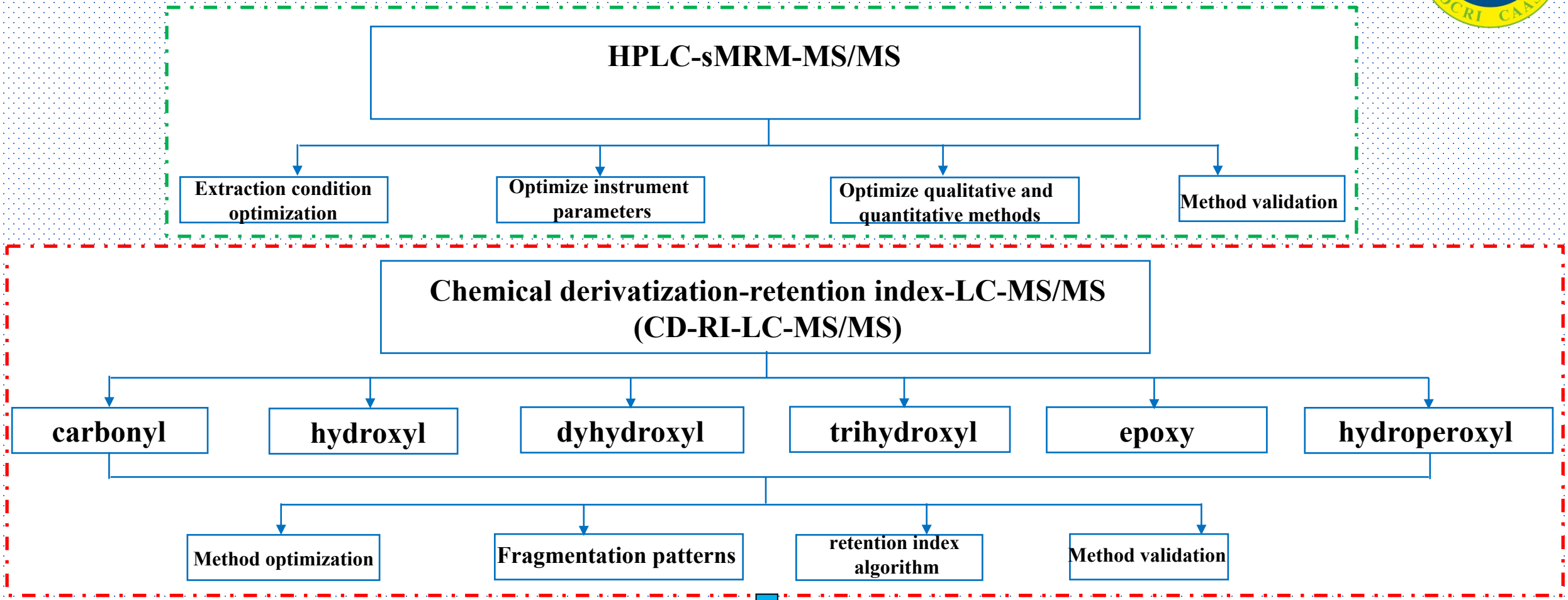
FOODB

hmdb The Human Metabolome Database




[1] Indelicato S , Bongiorno D , Pitonzo R , et al. Triacylglycerols (TAGs) in edible oils: determination, characterization, quantitation, chemometric approach and evaluation of adulterations[J]. Journal of Chromatography A, 2017:1-16.

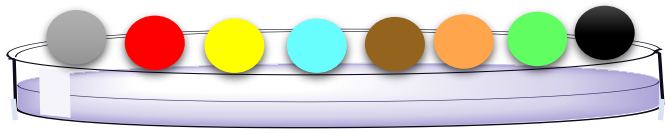

# Methods



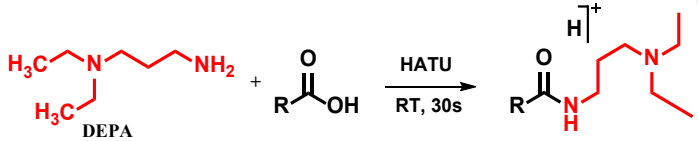

**Establishing the comprehensive analysis approaches based on liquid chromatography-mass spectrometry for lipid oxidation of rapeseed oil .**

# Technical Route

Carboxylated metabolites



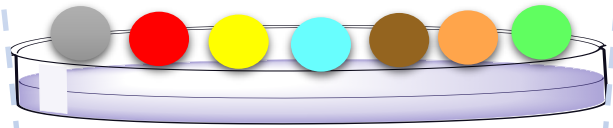
DEPA derivatization




significantly improves detection sensitivity

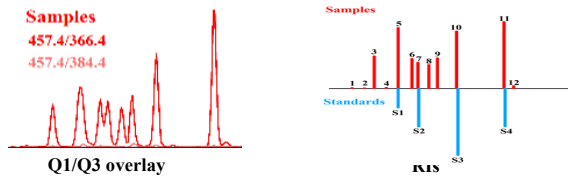
5112 folds

Oxylipins



RIs

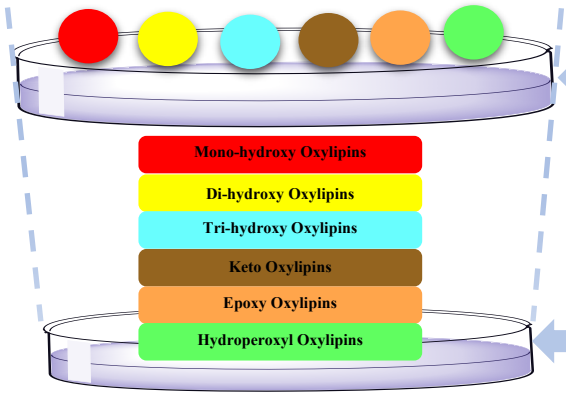
Q1/Q3 by NL fragments from DEPA



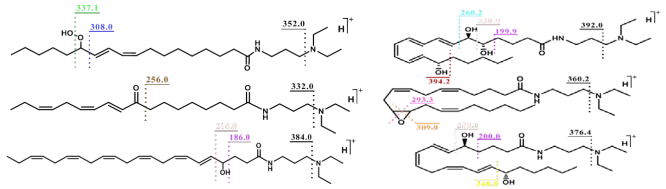

Significantly improve the qualitative effect

RSD < 1%

Six categories of oxylipins by different function groups




Diagnostic fragments by MS/MS



Identified by three levels

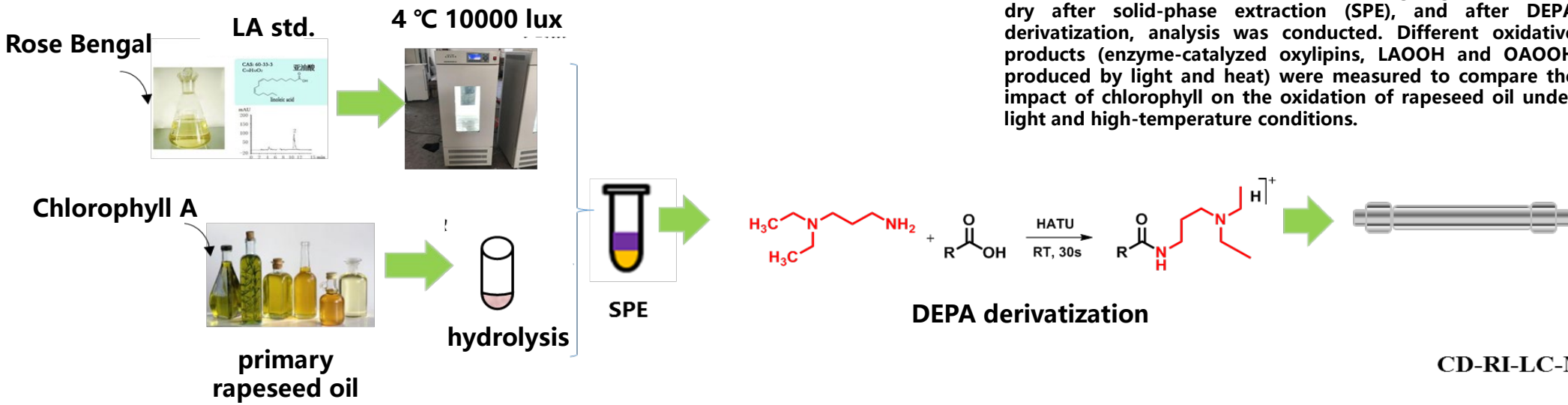

Another dimension to improve qualitative effect

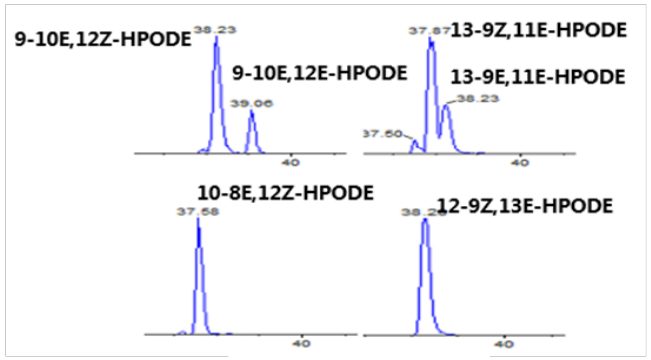
# Analysis of oxidative products

Unpublished data

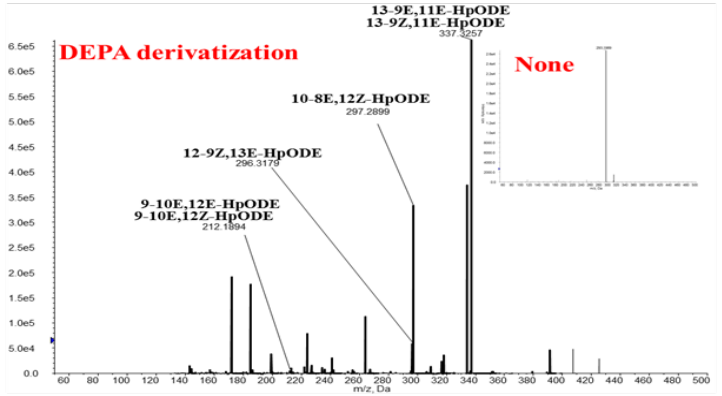
# Establishment of a targeted assay for linoleic acid and oleate hydroperoxides based on chemical derivatization

Addition and non-addition of chlorophyll a standard solution (10 ppm) to Grade 1 rapeseed oil, at a temperature of 17±1 ℃, in the absence of light, under light intensities of 1000 lux and 10000 lux, in the absence of light at 100 ℃ and 180 ℃ (with sampling every 10 minutes for a total of 90 minutes), the chlorophyll content was determined according to AOCS standards. After hydrolysis of the oil, nitrogen gas was blown dry after solid-phase extraction (SPE), and after DEPA derivatization, analysis was conducted. Different oxidative products (enzyme-catalyzed oxylipins, LAOOH and OAOOH produced by light and heat) were measured to compare the impact of chlorophyll on the oxidation of rapeseed oil under light and high-temperature conditions.

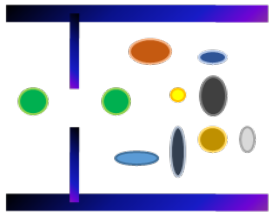



Efficient separation of cis-trans isomers




Significantly increased sensitivity



**●Method establishment**

# Experiment and methods

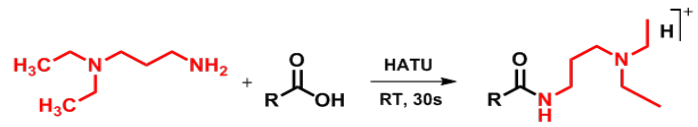
**HPLC conditions**:
Aglient C18 4.6×100mm, 1.8 μm; mobile phase:
A: acetonitrile/water/acetic acid 60/40/0.02 (v/v/v),
B: acetonitrile/isopropyl alcohol 50/50 (v/v) ; Flow rate 0.4 mL/min;
Gradient elution 0-4min 0.1-55% B; 4-15min 55-99% B; 15- 20min 99% B; 20.1-24.1min 55-0.1%

**Mass spectrometry conditions**:
ESI source, multiple reaction monitoring (MRM) scan, negative ion mode;
EPI scan range: m/z: 50-640; scan speed: 1000 Da/s; Curtain gas: 30psi; Ion Source Gas 1: 40psi; Ion Source Gas 2: 40psi; Temperature: 550℃.

## Sample preparation

Take 10mg of standard, add 2uL 20mM/L HATU, 4uL 20mM/L TEA, 2uL 20mM/L DEPA to each sample, add 92uL acetonitrile, vortex for 2 minutes, ultrasonic at room temperature for 5 minutes, the reaction is completed, for LC-MS/MS analysis.

SPE




DEPA derivatization


# Results

*Eur. J. Lipid Sci. Technol. 2019, 121, 1800078*

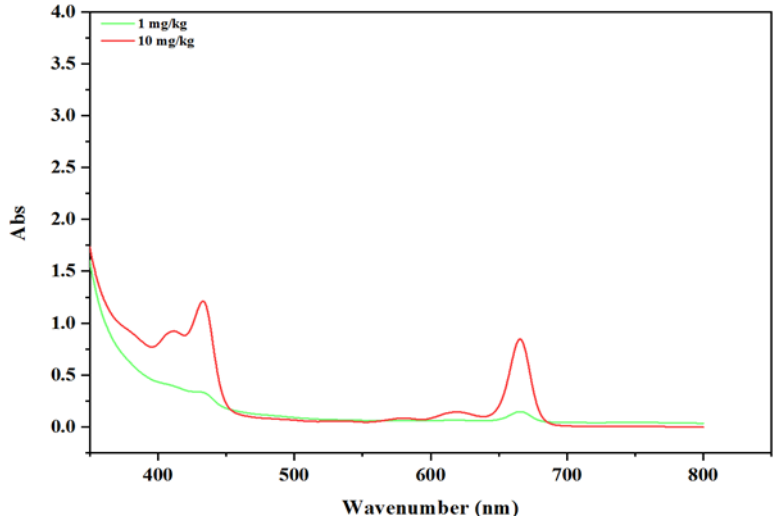
**CALCULATIONS**

$$\text{mg/kg C} = \frac{A_{670} - [(A_{630} + A_{710})/2]}{F \times L}$$

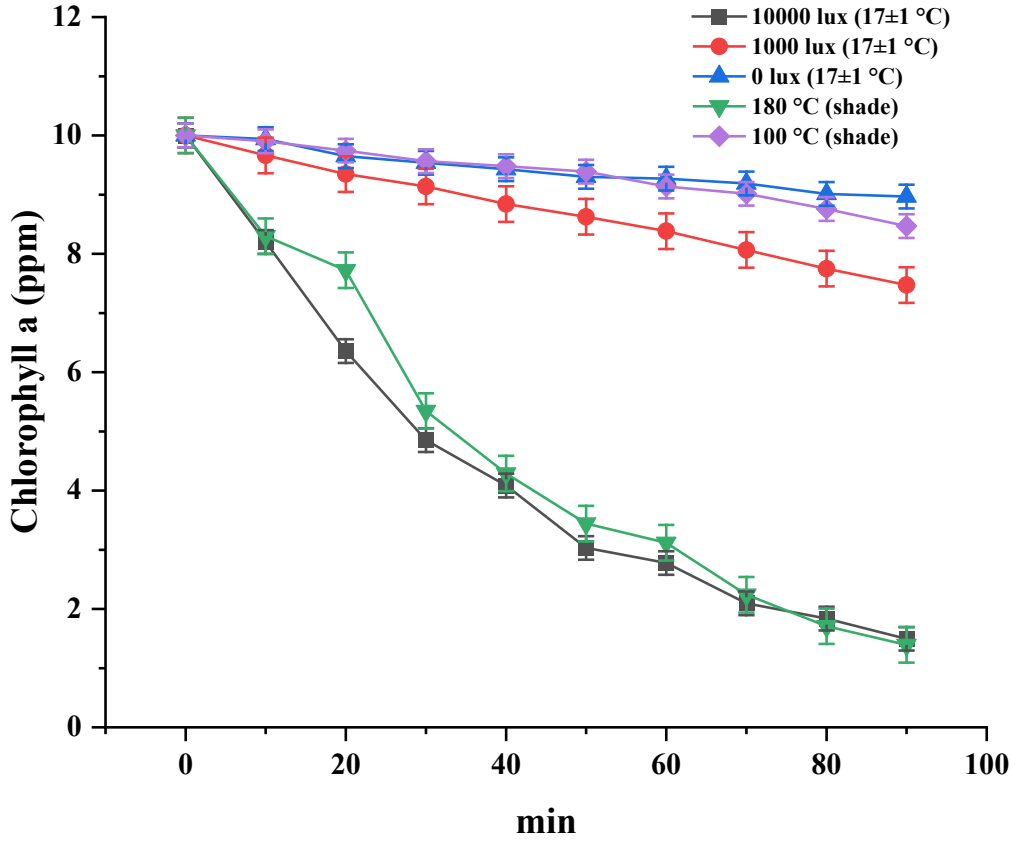
Where—

$C$ = chlorophyll pigments
$A$ = absorbance
$L$ = cuvette length, cm
$F$ = calibration factor (See Notes, 4)

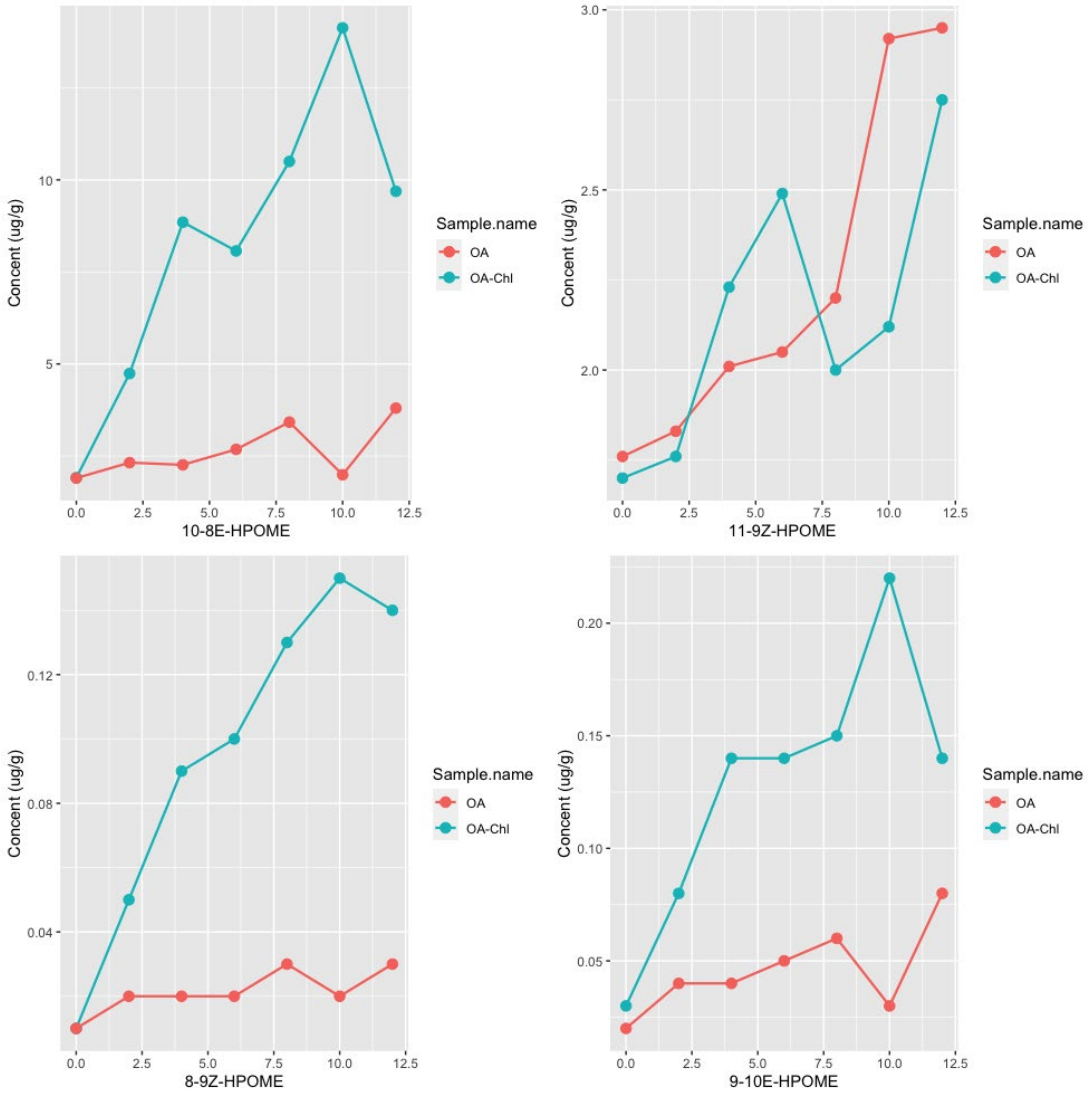
The chlorophyll content of rapeseed oil was determined according to the American Oil Chemists' Society (AOCS) official method Ch. 4–91 (reprinted in 2009), F was set to 0.1 in terms of chlorophyll a.



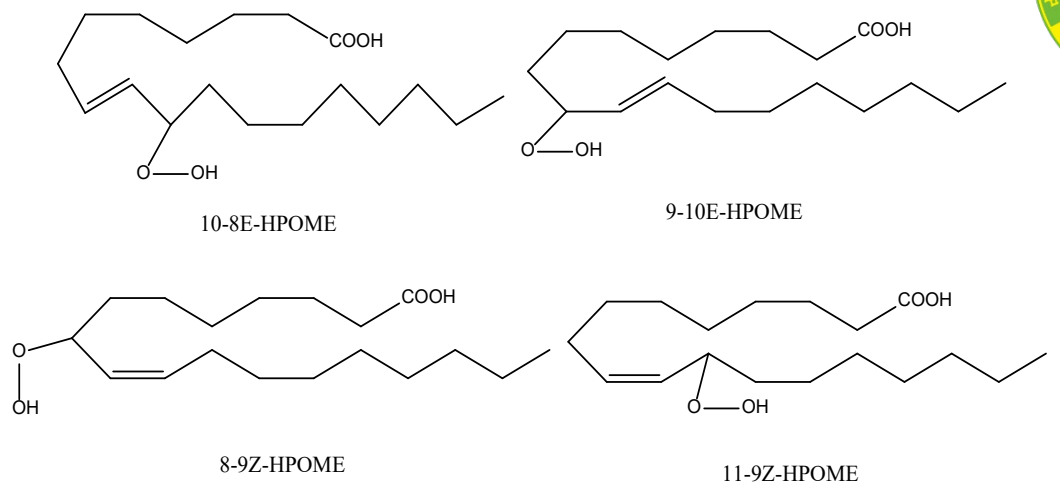

85% Chlorophyll a standard




$$C_c = C_{c0} \exp(-k_c t)$$

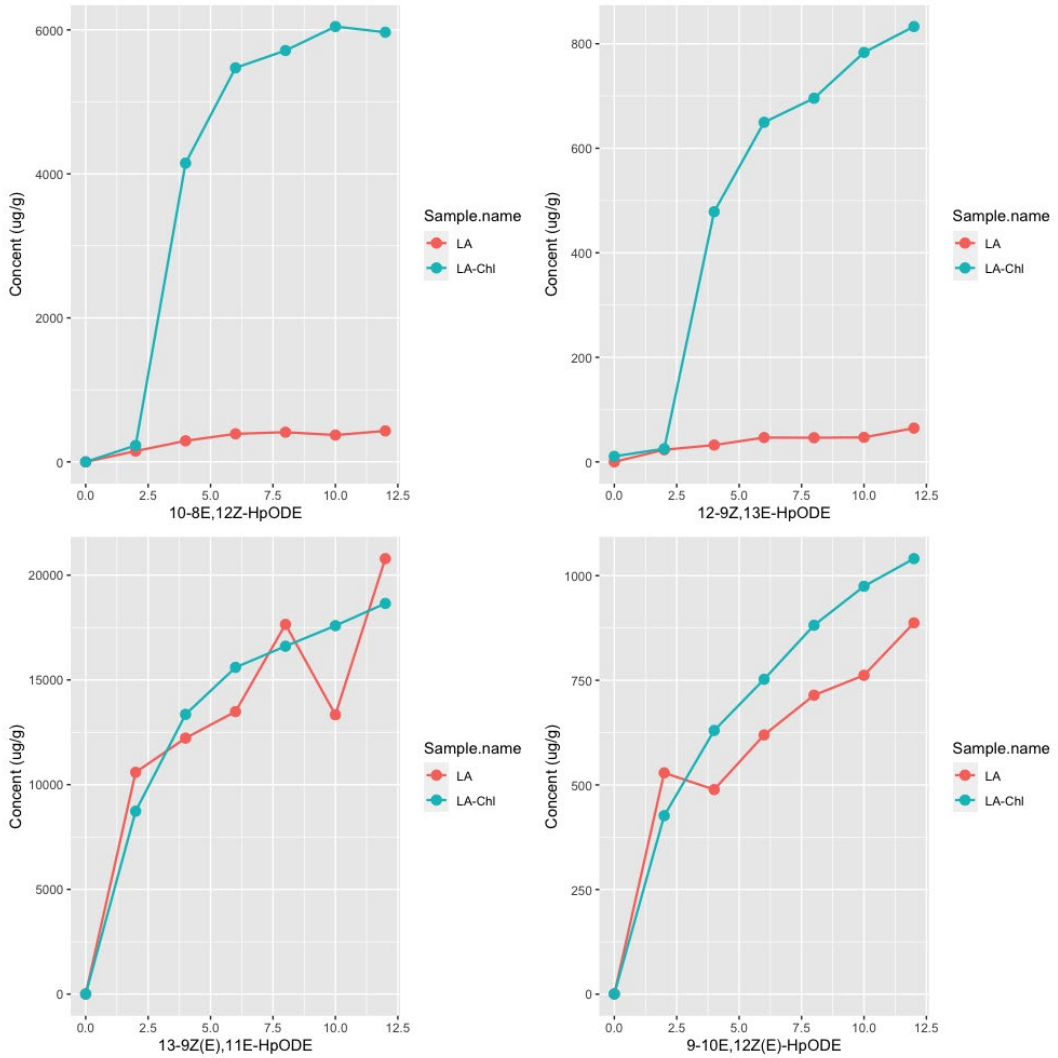



Products of oleic acid photo-oxidation

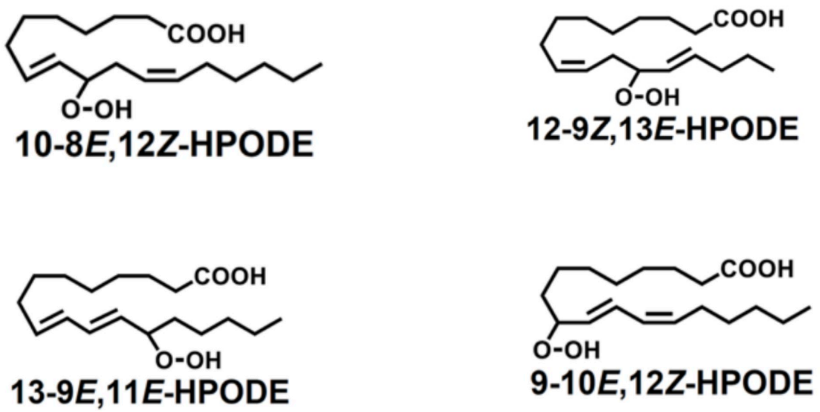



During the oxidation process, as the oxidation time accumulated, three out of the four hydroxyl oxidation products (8-9Z-HPOME, 9-10E-HPOME, 10-8E-HPOME) exhibited obvious changes compared to the control group. The trends for the two groups of 9Z-HPOME were similar, showing a slight increase.

Unpublished data




Products of Linoleic acid photo-oxidation




Aamong the four oxidation products of linoleic acid, two oxidation products (10-8E, 12Z-HpODE 12-9Z, 13E-HpODE) have exhibited obvious differences between the control and experiment group. The other two oxidation products (9-10E , 12Z(E)-HpODE 10-8E, 12Z-HpODE) showed a consistent slight increase, which may be causcould potentially be attributed byto auto-oxidation. There was no significant difference was found between the two groups.

Unpublished data


# Conclusion

- The more precise and detail molecular information of lipid oxidation of rapeseed oil could be provided by the novel approaches established by this research work
- Oxidation mechanism of rapeseed oil, especially photooxidation induced by chlorophyll, was studied based on these molecular information of lipid oxidation of rapeseed oil
- In the future， This research work could provide methodology, molecular structure database, and crucial theoretical support for studying the molecular mechanism of rapeseed oil oxidation deterioration during its processing and storage

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# Thank you!