What the analysis of triacylglycerol hydroperoxide isomers in foods can reveal

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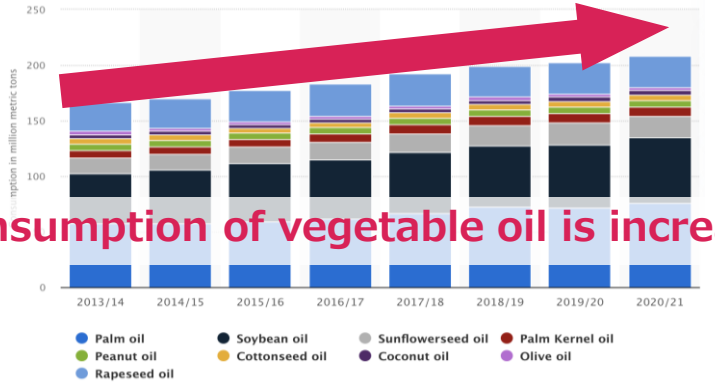
1. **Determination of oxidation mechanism in canola oil:**
   Analysis of triacylglycerol hydroperoxide (TGOOH) isomers

2. **Affect the different oxidation mechanism to quality of vegetable oil:**
   Analysis of secondary oxidation products of TGOOH

Vegetable Oils



Consumption of vegetable oil is increasing!

https://www.statista.com/statistics/263937/vegetable-oils-global-consumption/

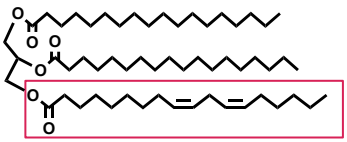
Safflower oil

Soybean oil

Canola oil

Sunflower oil

Triglyceriol,(TG)



Linoleic acid (LA)

αLinolenic acid (ALA)

Vegetable oil is vulnerable to oxidation which leads oil deterioration.

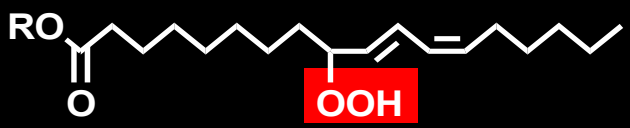
Why determination of oxidation cause is important?

Triglyceride (TG)

R=Dioleoylglycerol

O_2

Oxidation



Triglyceride hydroperoxide (TGOOH)

Aldehydes, Carboxylates etc..

Browning, Odor

To maintain oil quality,
prevention of TGOOH generation is important

Why determination of oxidation cause is important?

Triglyceride (TG)

R=Dioleoylglycerol

O_2

Radical

e.g. heat, auto-

① FA 18:2(9E,11E);13OOH

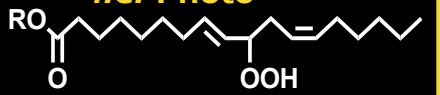
④ FA 18:2(10E,12E);9OOH

② FA 18:2(9Z,11E);13OOH

⑤ FA 18:2(10E,12Z);9OOH

Singlet Oxygen

i.e. Photo



③ FA 18:2(8E,12Z);10OOH

⑥ FA 18:2(8Z,13E);12OOH

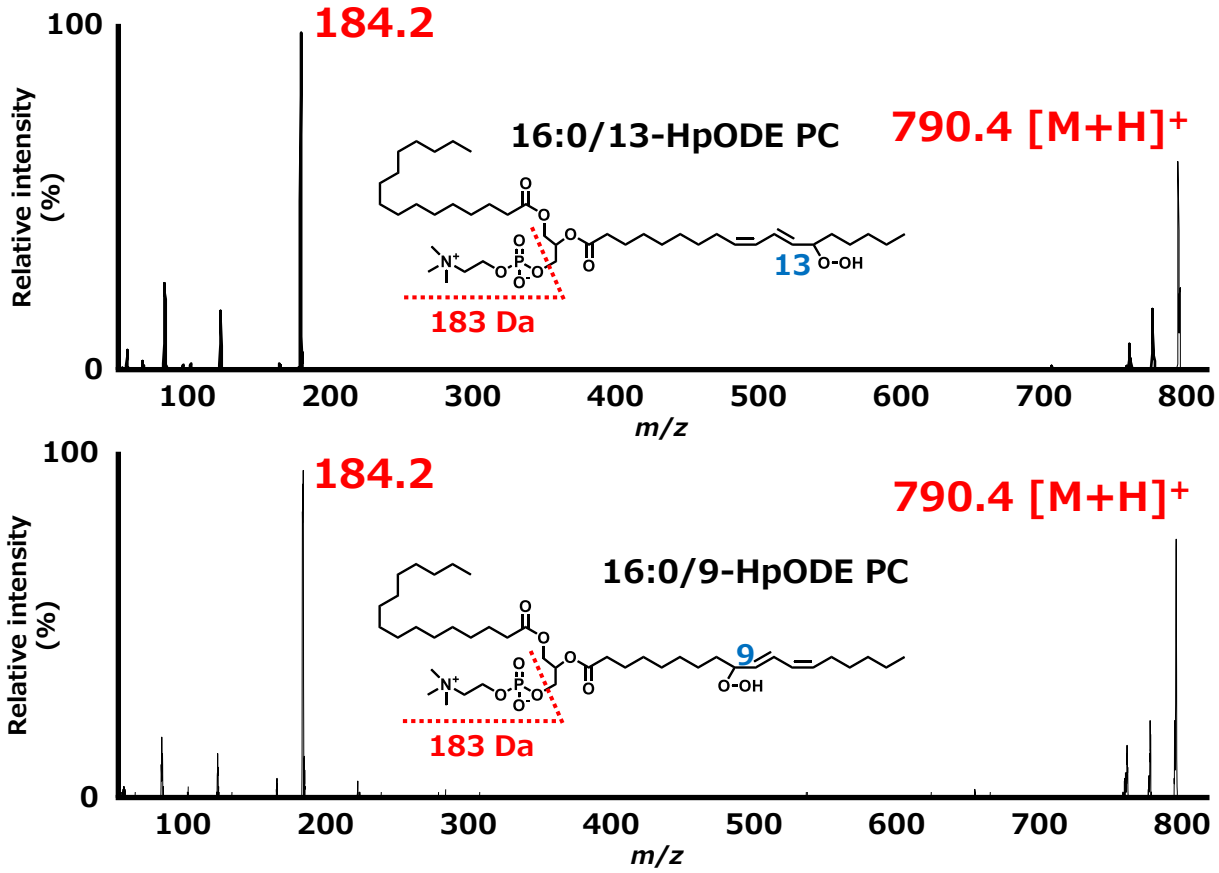
Frankel E.N. *Prog. Lipid Res.* 23 (1985)

Vitamin E, Polyphenol

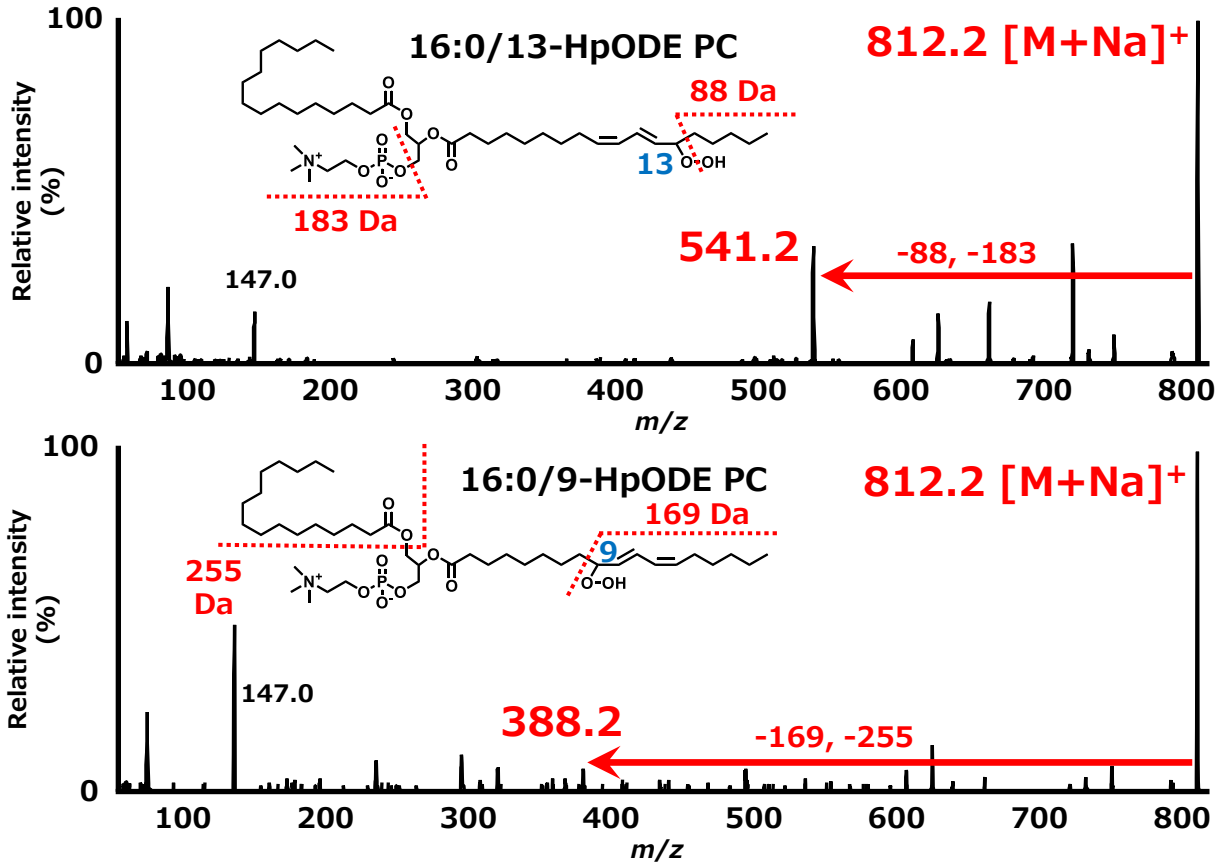
Carotenoid

Determination of oxidation mechanism is key

MS/MS analysis of PCOOH in the presence of proton

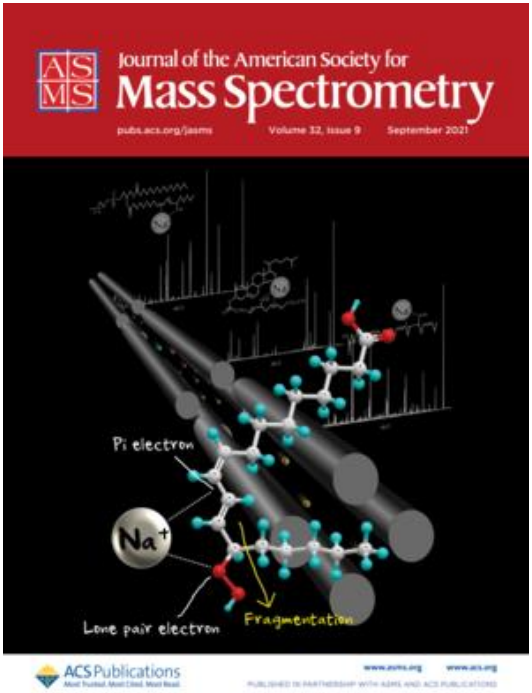


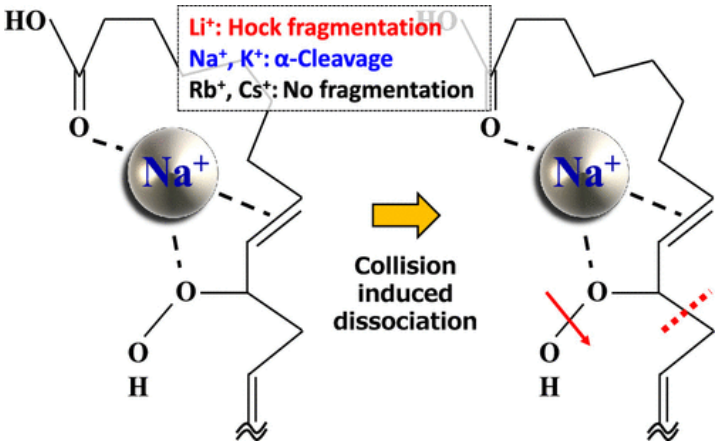
MS/MS analysis of PCOOH in the presence of sodium ion



S. Kato, et al., *Anal. Biochem.* 471:51-60 (2015)

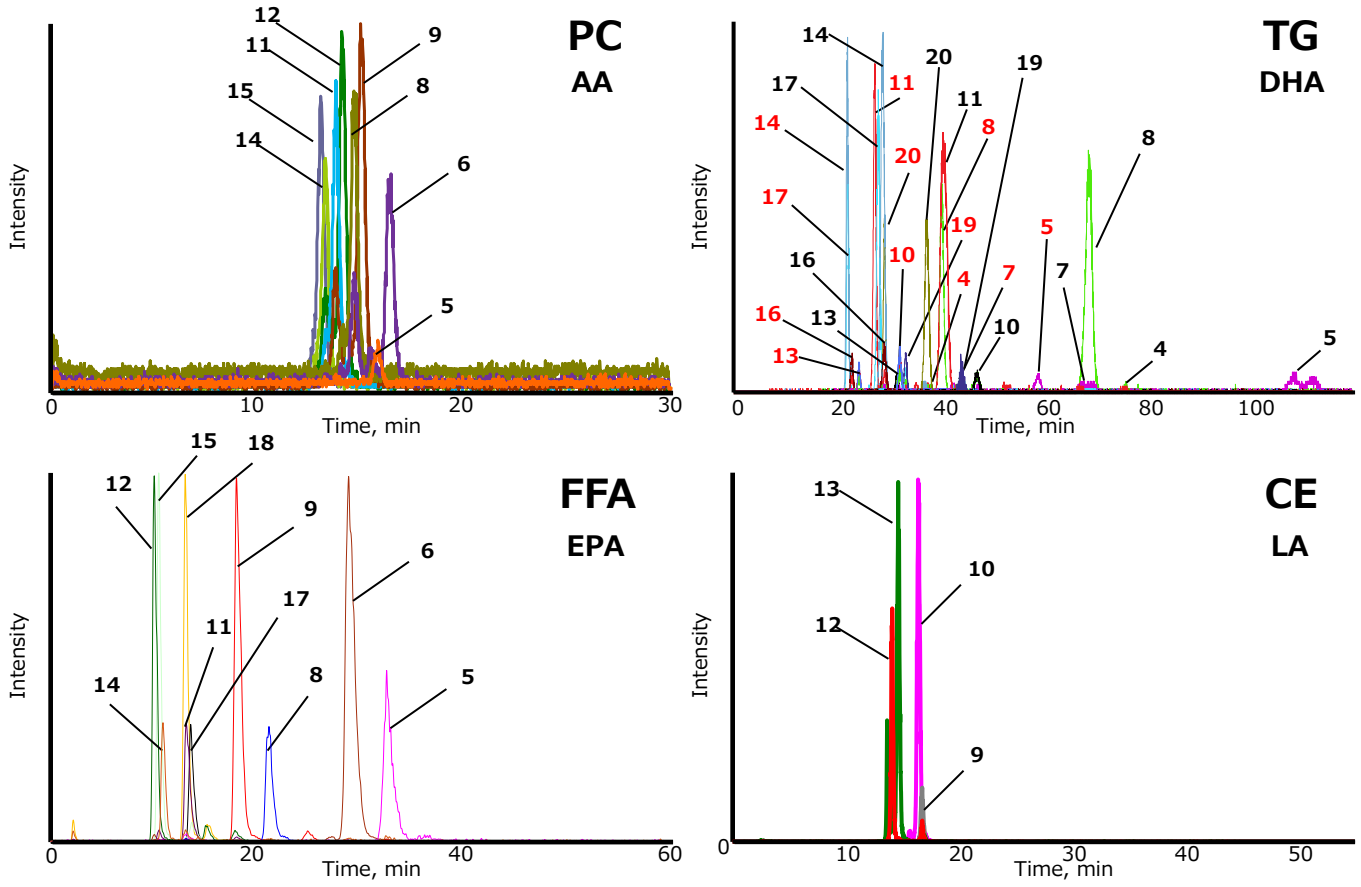
Analysis of isomeric structure by sodiated hydroperoxide





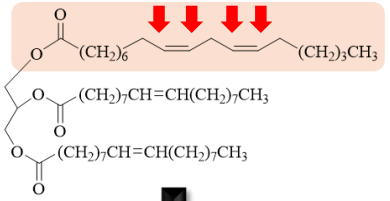
S. Kato et al., J. Am. Soc. Mass Spectrom. 32 (2021)

Analysis of various lipid hydroperoxides



S.Kato *et al., J. Am. Soc. Mass Spectrom.* 2021
J. Ito *et al. Anal. Chem.* 2015
S. Kato *et al. Anal. Biochem*. 2015

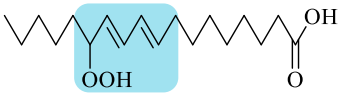
Lipid oxidation mechanisms and TGOOH isomers



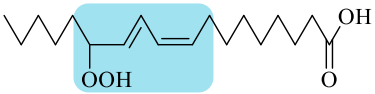
Dioleoyl-linoleoyl-TG

Radical
(e.g., Thermal, Auto-)

Singlet oxygen
(e.g., Photo-)



① FA 18:2(9E,11E);13OOH



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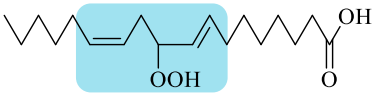
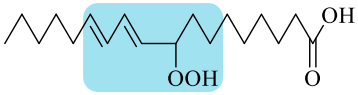
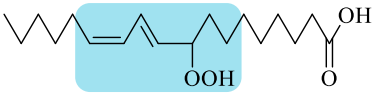


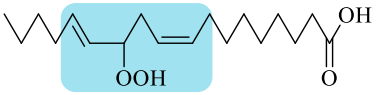
③ FA 18:2(8E,12Z);10OOH



④ FA 18:2(10E,12E);9OOH



⑤ FA 18:2(10E,12Z);9OOH



⑥ FA 18:2(8Z,13E);12OOH

Frankel E.N. *Prog. Lipid Res.* 23 (1985)

Synthesis of TGOOH standards

Linoleic acid (LA)

↓ **Oxidation** (50°C, 15,000 lux)

↓ **Purification** (semi-preparative HPLC)

FA 18:2;OOH isomers

↓ **Protection of -OOH group**
Pyridinium *p*-toluenesulfonate,
2-Methoxypropene

↓ **Purification** (semi-preparative HPLC)

FA 18:2;OOMxP

↓ **Esterification**
Dioleoyl glycerol
N,N'-Dicyclohexylcarbodiimide
N-Dimethyl-4-aminopyridine

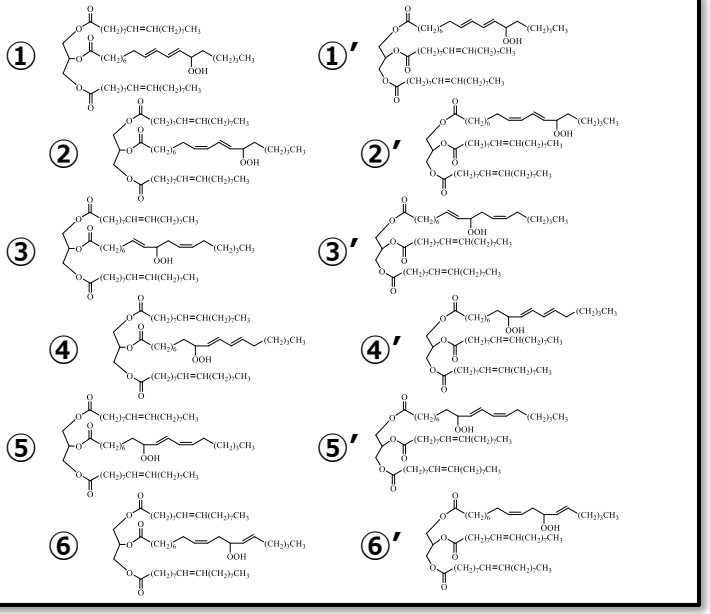
↓ **Purification** (semi-preparative HPLC)

↓ **Deprotection**

↓ **Purification**
(semi-preparative HPLC)

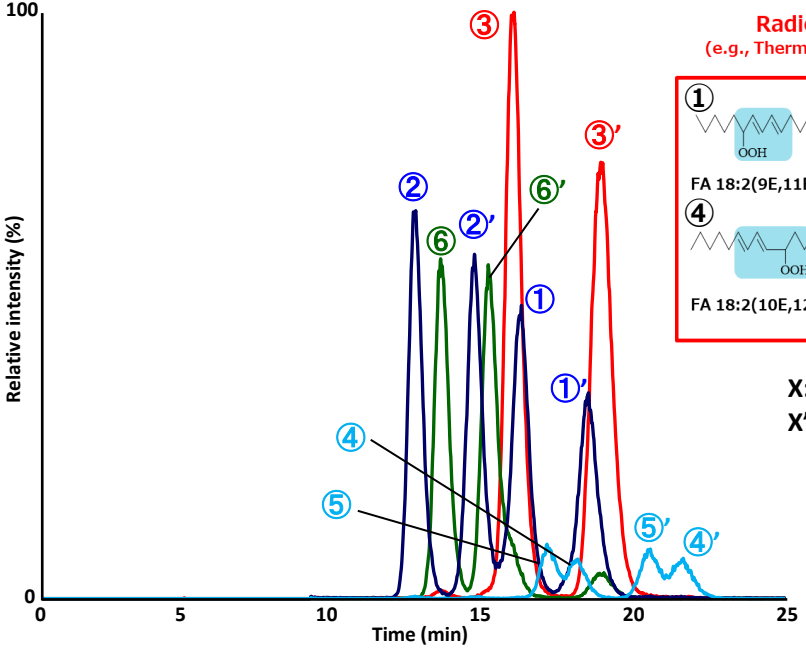
OOL/OLO type TGOOH isomers

① **FA 18:2(9E,11E);13OOH**
② **FA 18:2(9Z,11E);13OOH**
③ **FA 18:2(8E,12Z);10OOH**
④ **FA 18:2(10E,12E);9OOH**
⑤ **FA 18:2(10E,12Z);9OOH**
⑥ **FA 18:2(8Z,13E);12OOH**



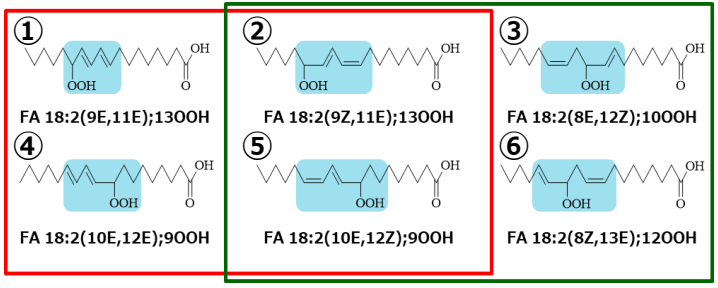
S. Kato et al., npj Science of Food, 2 (2018)

TGOOH Isomers (standards)



Radical
(e.g., Thermal, Auto-)

Singlet oxygen
(e.g., Photo-)

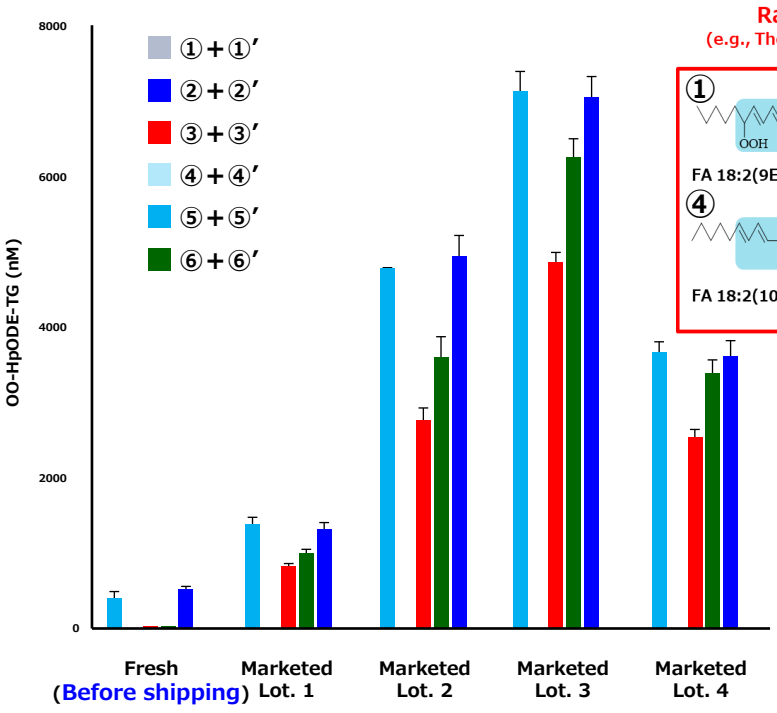


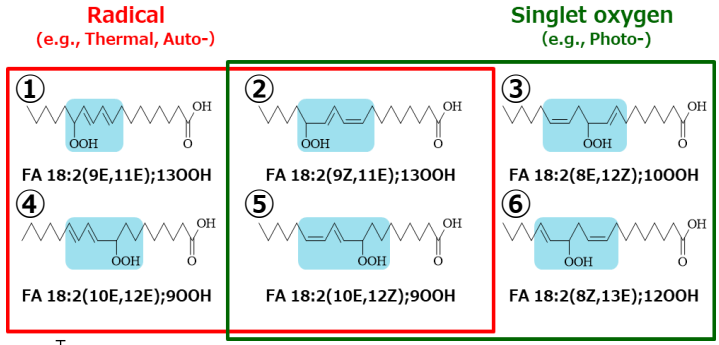
X: TGOOH possessing 18:2;OOH at β position
X': TGOOH possessing 18:2;OOH at α position

Mobile phase: Hexane : 2-propanol (100:0.5)
Column: InertSIL silica 2.1*250 mm
Post column: Methanol : 2-propanol (1:1, 0.2 mM CH_3COONa)
Mass spectrometry: Electrospray Ionization (ESI (+))

S. Kato et al., npj Science of Food, 2 (2018)

Is edible oil oxidized during transportation and selling?





S. Kato et al., npj Science of Food, 2 (2018)

The oxidation of marketed canola oil is mainly affected by photo irradiation

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e.g. heat, auto-

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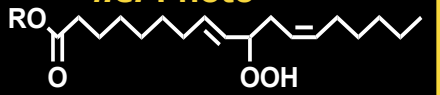
Singlet Oxygen

i.e. Photo



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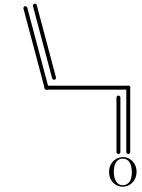
Frankel E.N. *Prog. Lipid Res.* 23 (1985)

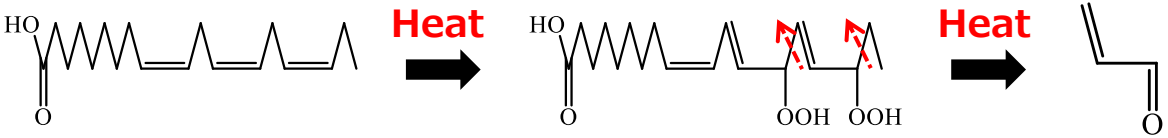
Carbonyl compounds such as aldehyde and ketone
Which is more volatile and affect flavor

Acrolein



Acrolein

- Acrolein reacts with proteins and DNA (Carcinogenic??)
- Acrolein is formed during the combustion of petroleum fuels and tobacco
- Thermal degradation of edible oils



α-Linolenic acid (ALA)

ALA hydroperoxide

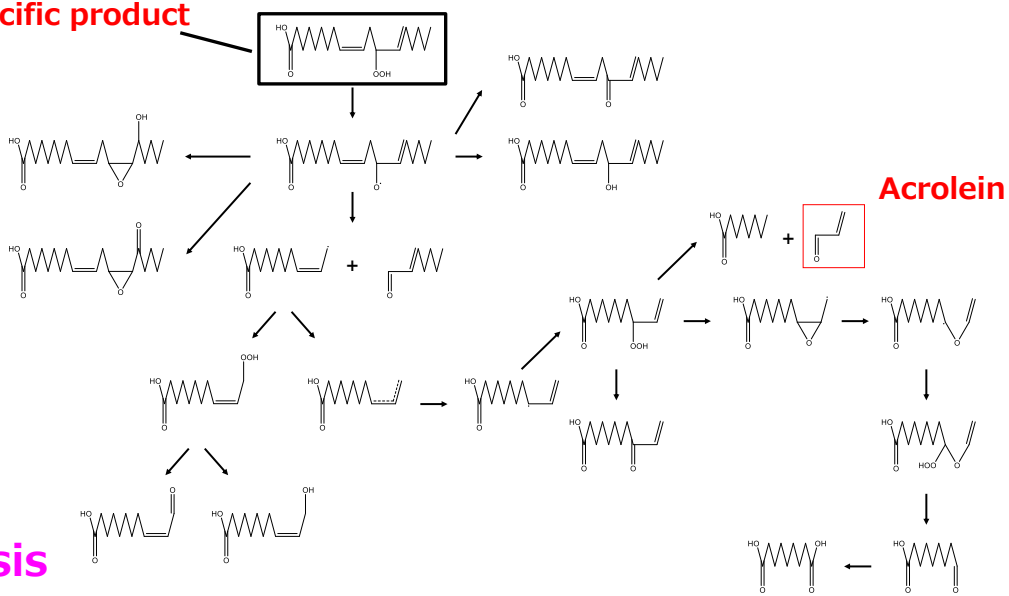
Endo, Y. et al., *J. Am. Oil Chem. Soc.* 90, 959-964 (2013).
Ewert, A. et al., *J. Agric. Food Chem.* 62, 8524-8529 (2014).

A lot of studies have persisted major ACR source is "radical oxidation of ALA".

Expectation of hydroperoxide decomposition pathways

Proposed decomposition pathway of LA-12-hydroperoxide

Linoleic acid-12-hydroperoxide
$^1O_2$ oxidation specific product



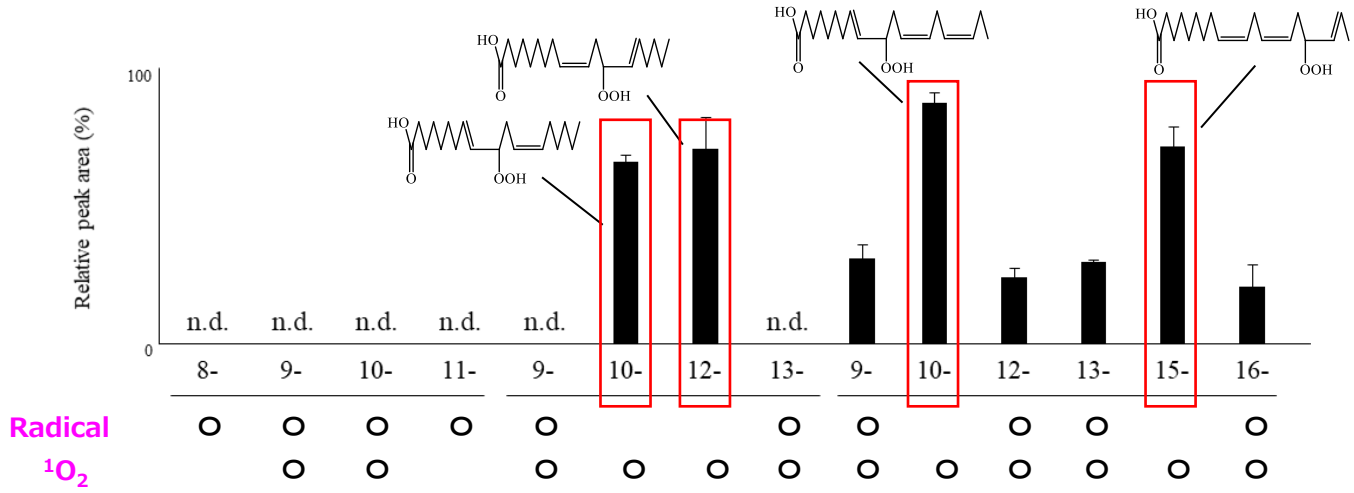
Hypothesis

- **$^1O_2$ oxidation of LA generates acrolein.**
- **$^1O_2$ oxidation of ALA increases acrolein generation.**

S. Kato et al., npj Science of Food, 6 (2022)

Acrolein generation

Equal mol of each hydroperoxide was heated (180℃, 30 sec)
Volatiles were collected by SPME and analyzed by GC-EI-MS



As we expected,

$^1O_2$ oxidation of LA generates acrolein.

$^1O_2$ oxidation of ALA increases acrolein generation.

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Acrolein derived from edible oils

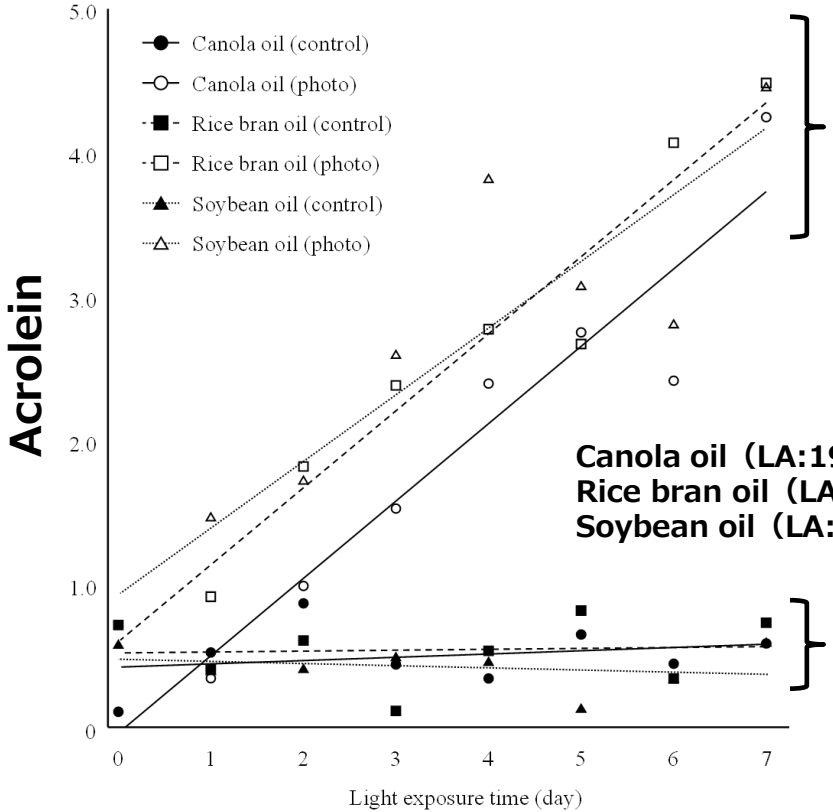


Photo-irradiated edible oils (5,000 lux, 20 ℃, 0~7 days)
After irradiation, oil was heated

Canola oil（LA:19.5%, ALA:7.7%）
Rice bran oil（LA:34.7%, ALA:1.0%）
Soybean oil（LA:54.9%, ALA:6.6%）

Edible oils placed at dark

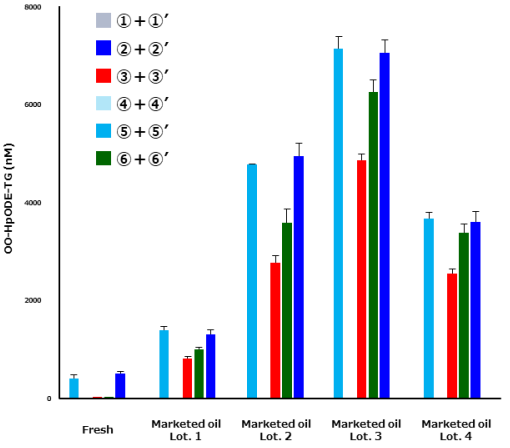
S. Kato et al., npj Science of Food, 6 (2022)

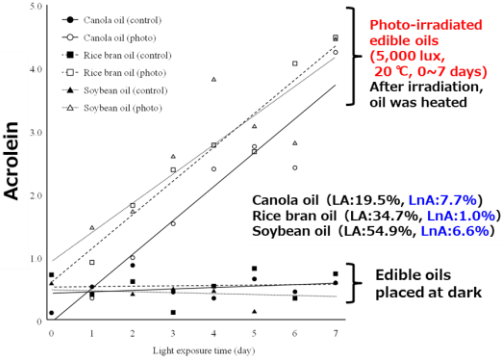
Summary

We completed the analytical method for TGOOH isomers.

This method allowed to determine the oxidation mechanisms of edible oils.

It was demonstrated that photo oxidation of linoleic acid (LA) also can be a source of acrolein.





Take home message

Please store edible oils

at dark place!!

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Thank you for your attention!