Fish Oil Oxidation: What is the Problem?

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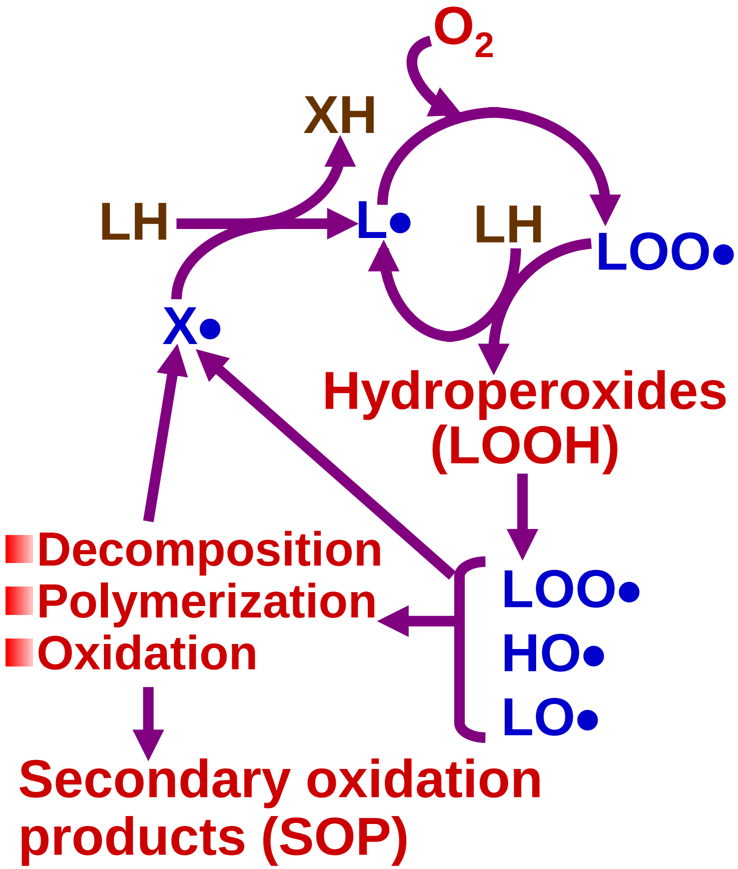
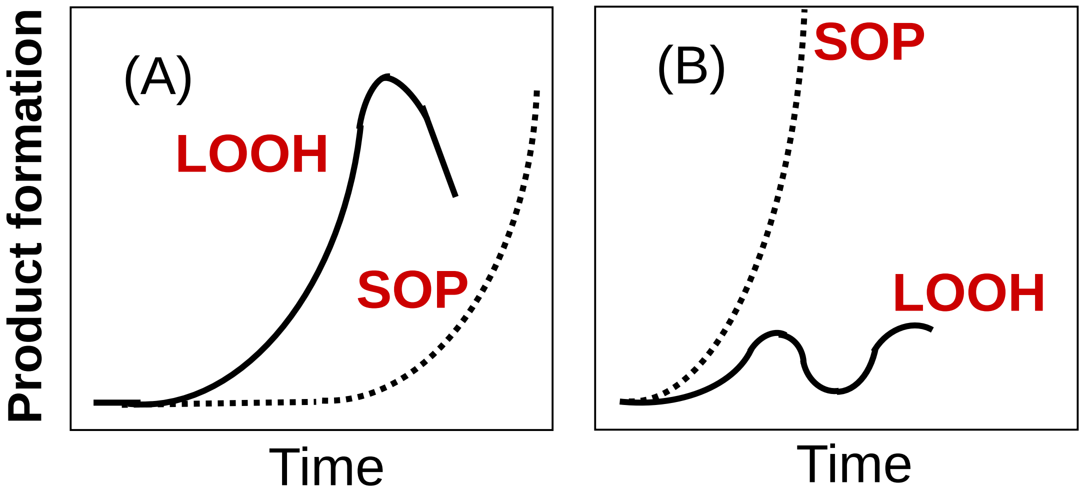
Why Fish Oil Is So Important?

- Much interest has been paid to fish oil intake, because of the high content of EPA and DHA.
- EPA (20:5n-3) and DHA (22:6n-3) are typical omega-3 polyunsaturated fatty acids (PUFA) mainly found in fish oils.
- The importance of both omega-3 PUFA on human health has been proven through research works across the globe.
- Epidemiological studies show that intake of EPA and DHA reduces the risk of cardiovascular diseases and of other kinds of non-communicable diseases.
- However, there is a problem in the application of fish oil to food products.

EPA and DHA as Important Nutrients, But Easily Oxidized

- EPA and DHA are susceptible to oxidation.
- The rapid formation of volatile aldehydes from the very early stage of fish oil oxidation is the most serious problem.





Conceptual Diagram of the Formation of LOOH and SOP in time course.

(A) Linoleate (18:2n-6)

(B) EPA (20:5n-3) and DHA (22:6n-3)

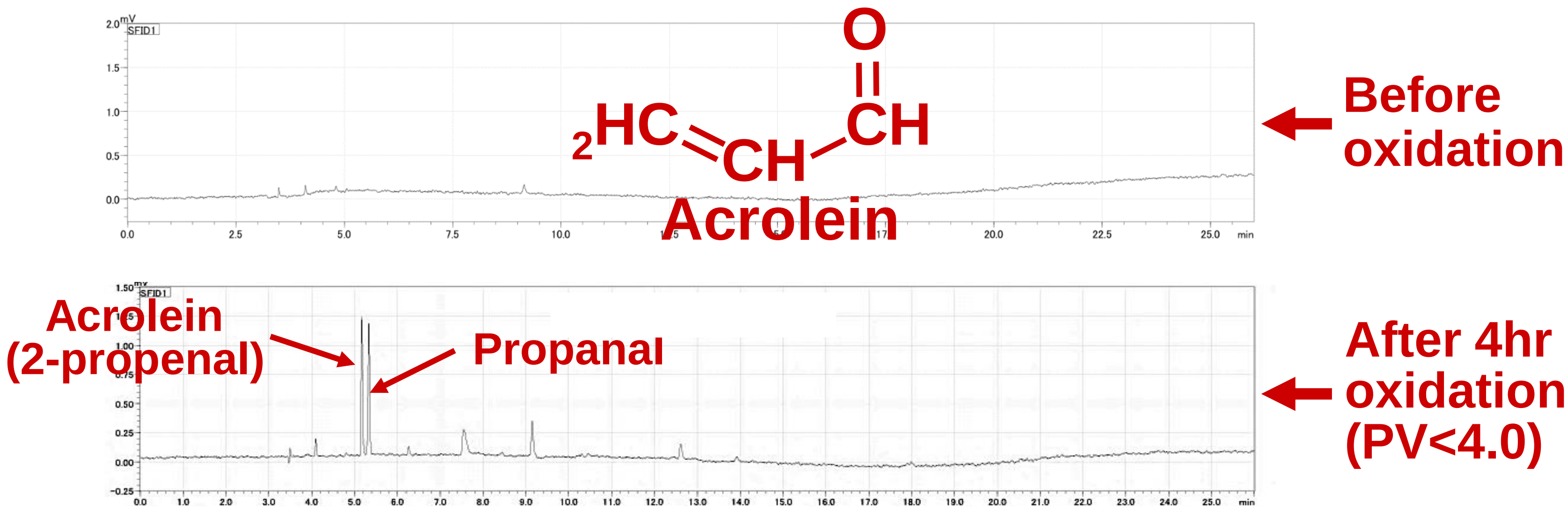
Volatile Analysis of Oxidized Fish Oil

- Most of the volatiles from oxidized fish oils were obtained after several days of oxidation and/or from the oxidation at high temperatures.
- However, the most notable and practical problem is the formation of volatiles found during early stage of the oxidation.
- The dynamic headspace (DHS) technique with solid-phase microextraction (SPME) method has been widely applied to measure the volatile compounds from oxidized lipids, including fish oils.
- However, during the SPME extraction, the lower-boiling compounds such as C3 aldehydes may not be concentrated or may be lost.

Volatile Analysis in Our Laboratory

- So, we analyzed volatiles formed during the very early stage of fish oil oxidation by using non-selective sample extraction method in the lower operating temperature.
- Another point for the analysis is to prepare the purified substrate.
- We have successfully obtained a highly purified fish oil triacylglecerol (TAG).
- The fish oil just after the chromatographic purification had little to no smell.
- However, the fish oil shows an unpleasant smell less than 1 h after leaving the chromatograph at room temperature.

Volatile Analysis of Fish Oil TAG Oxidation At Early Stage with Static Headspace Method



Concentrations (ppm) of Acrolein and Propanal

	Oxidation time (hr)		
	0	2	4
Acrolein	0	4.92 ± 0.09	14.45 ± 0.38
Propanal	0	6.02 ± 0.33	13.25 ± 0.33

Threshold value

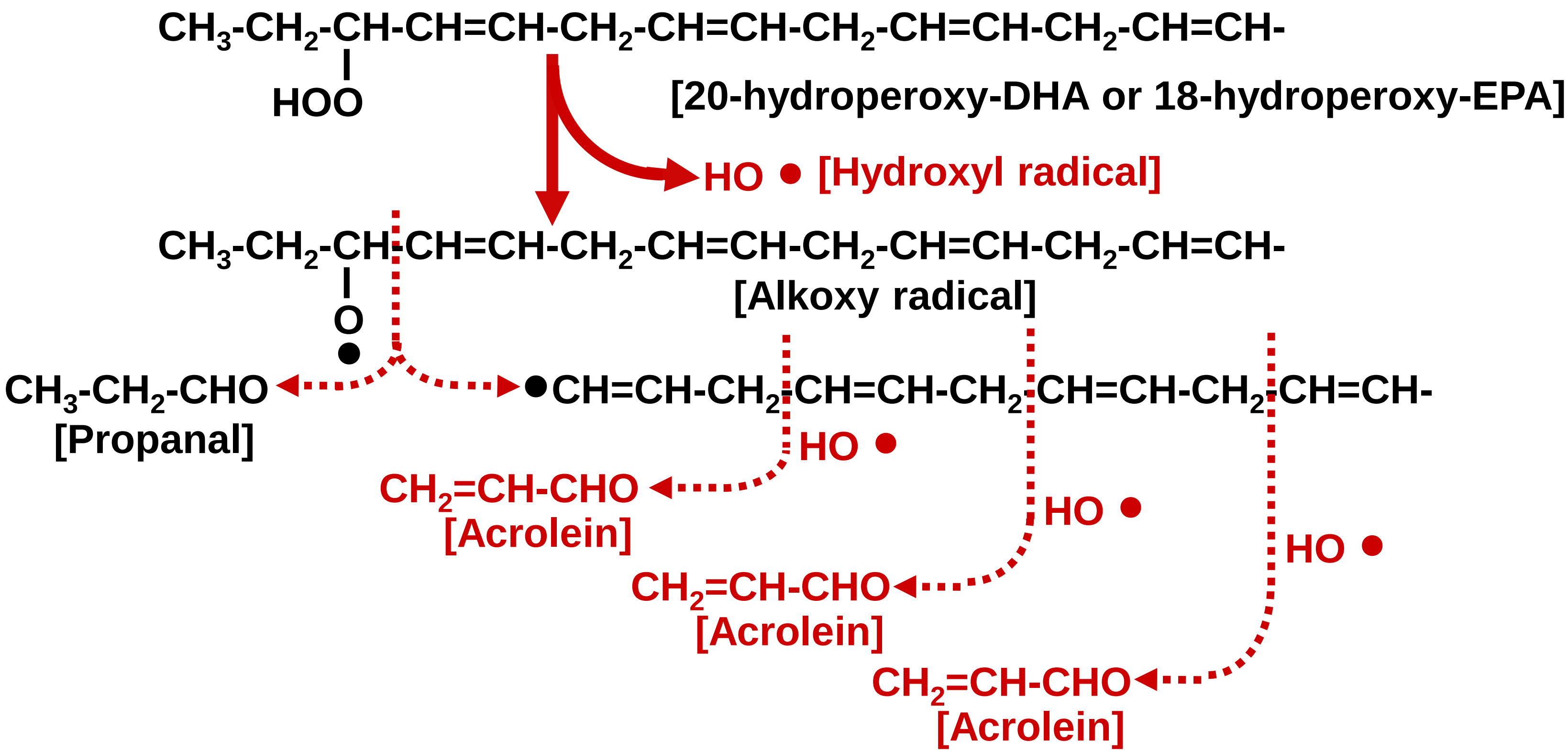
Acrolein: 3.6 ppb

Propanal: 60.0 ppb

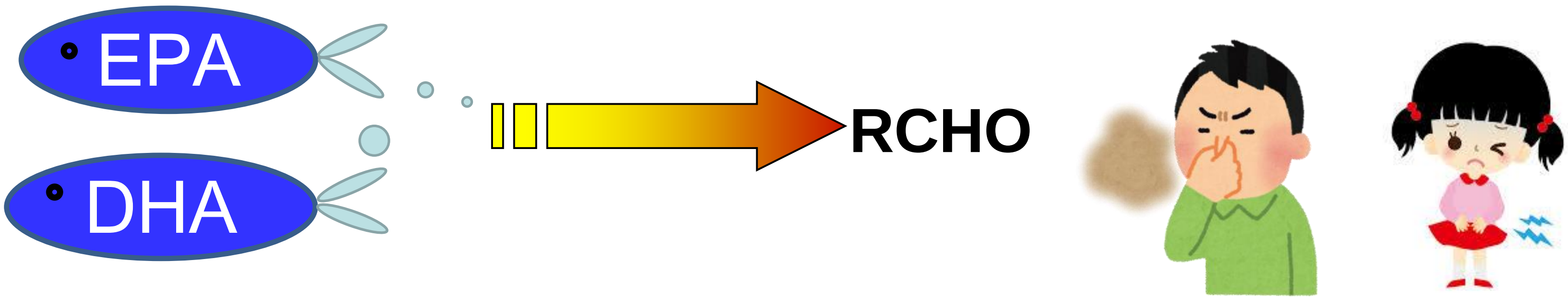
[Miyashita et al., Annu. Rev. Food Sci. Technol. 9:209-226, 2018]

Possible Acrolein Formation in Fish Oil Oxidation

- **Most probably, acrolein found in the early stage of fish oil oxidation is formed during the decomposition of the oxidation products from EPA and DHA as follows:**



How to Prevent Flavor Deterioration of Fish Oil?



- **Antioxidants? Of course, essential.**
  - **However, synthetic antioxidants are non-preferred.**
  - **Strong taste of antioxidants are not favorable.**
- **Micro-encapsulation? Maybe not bad, there are several problems.**
  - **Bioavailability of EPA and DHA are sometimes lower.**
  - **Taste, texture, and nutritional value can be influenced by the wall materials.**
  - **If the stability of encapsulated fish oil powder is high, it does not guarantee the stability of EPA and DHA in the final products.**
  - **The application of the powder products is limited.**

How Is the Stability of EPA and DHA in Natural Products

- EPA and DHA are sometimes very stable in natural products and traditional foods.
- It may give us a hint to create a stable and preferred EPA and DHA products.
- This is the concept for the research of our laboratory.

Traditional Foods in Japan

Salmon roe pickled in soy source

g/100g product		Wt% of total FA	
Lipid	Water	EPA	DHA
15.6	48.4	18.4	19.8

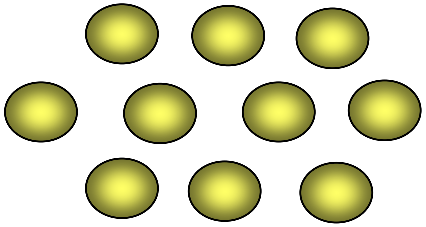
EPA and DHA are not oxidized more than 6 months, when the product is stored at 5ºC without not drying under a high humidity.

Fermented salted squid guts and muscle

g/100g product		Wt% of total FA	
Lipid	Water	EPA	DHA
3.4	67.4	12.4	35.8

EPA and DHA are not oxidized more than 1 year, when the product is stored in a bottle at 5ºC in the air with sealing to protect microbial spoilage.

Oxidation of Different Polyunsaturated Fatty Acids (PUFA) in Micelles



Purified PUFA or its ester
(With no peroxides and no antioxidants)

Tween 20 containing phosphate buffer (pH 7.4)

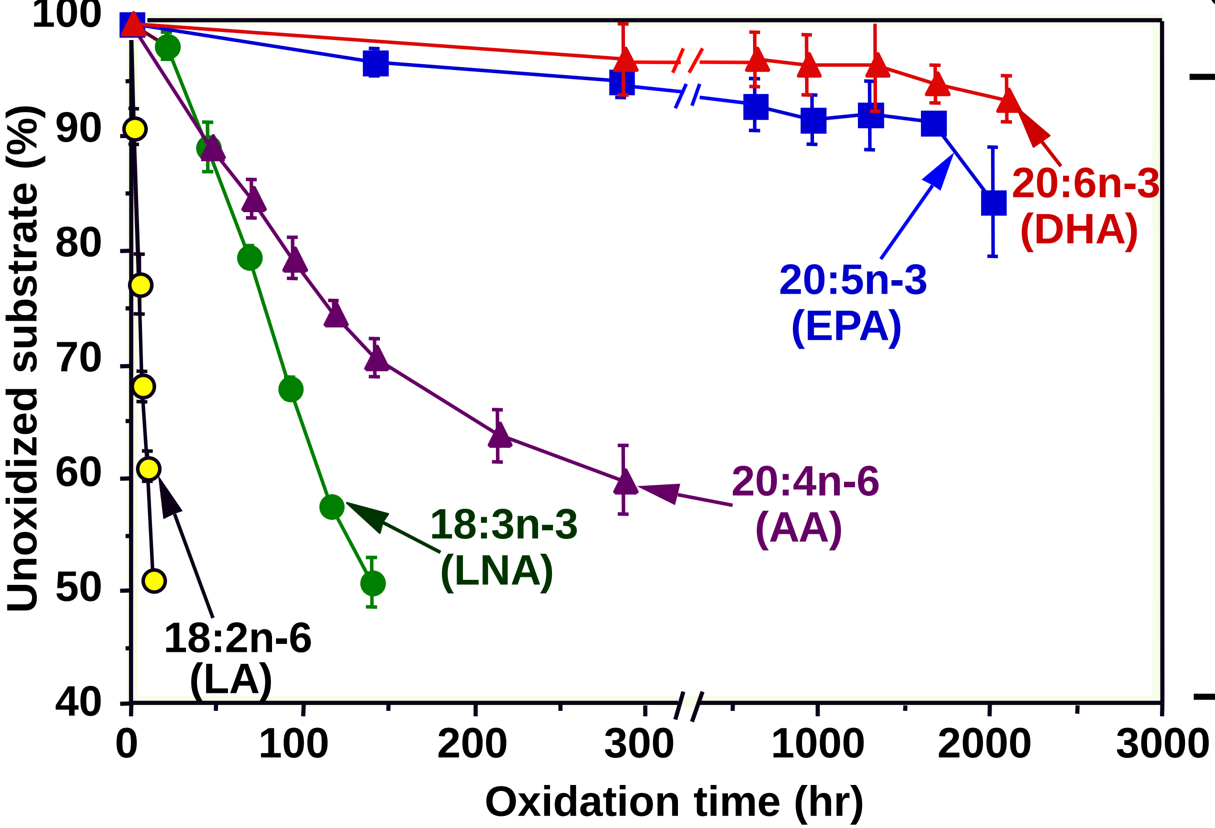
Dispersed as clear solution (PUFA:1.0 mM; Tween:1.0%)

+Fe(II) (1.0 μM) and ascorbic acid (20.0 μM)

Incubation (In the dark, 37^{o}C)

Oxidative stability of each lipid was evaluated by analyzing oxygen consumption, formation of peroxides.

Higher Oxidative Stability of EPA and DHA in Aqueous Micelles



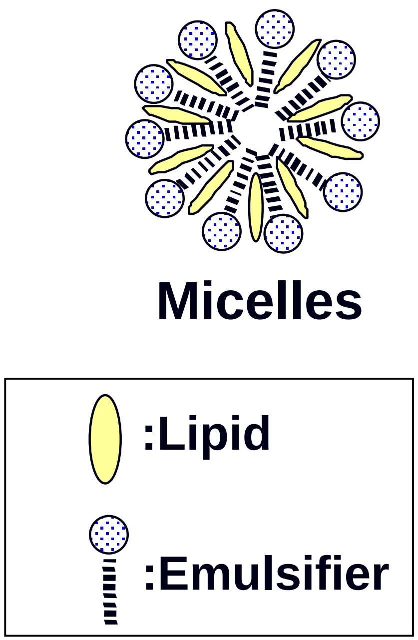
Oxidative stability	Aqueous micelles	Bulk phase
High	DHA	LA
	EPA	
		LNA
	AA	
		AA
	LNA	
		EPA
Low	LA	DHA

The order of the oxidative stability was reverse from those in the bulk phase and in organic solvent.

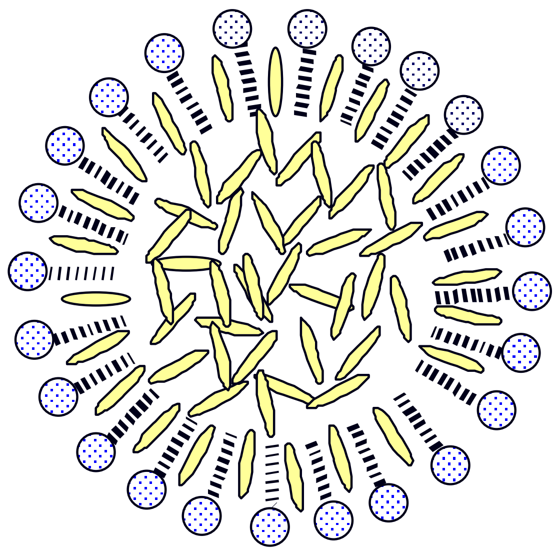
[Lipid Oxidation: Challenges in Food Systems, AOCS Press, pp. 155-176, 2013]

Reason for the Unusual High Oxidative Stability of EPA and DHA in Aqueous Micelles

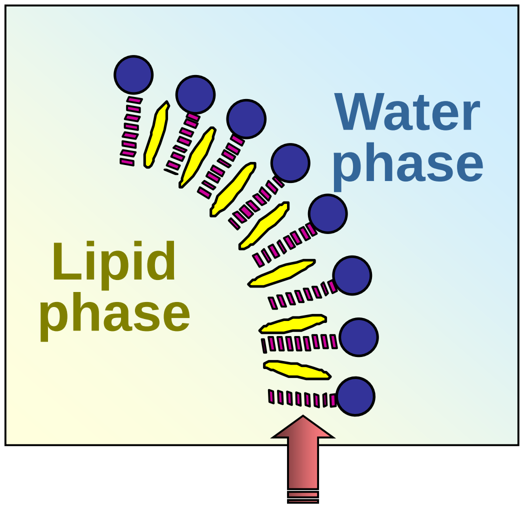
- Physical and stereochemical characteristics of EPA and DHA molecules.
- Interaction of PUFA molecule with other molecule such as emulsifier and other fatty acid chains.
- Tight packing conformation of EPA and DHA molecule.
- May result in specific inhibition of hydrogen abstraction from the bis-allylic positions of EPA and DHA.



Micelles



Emuslsion



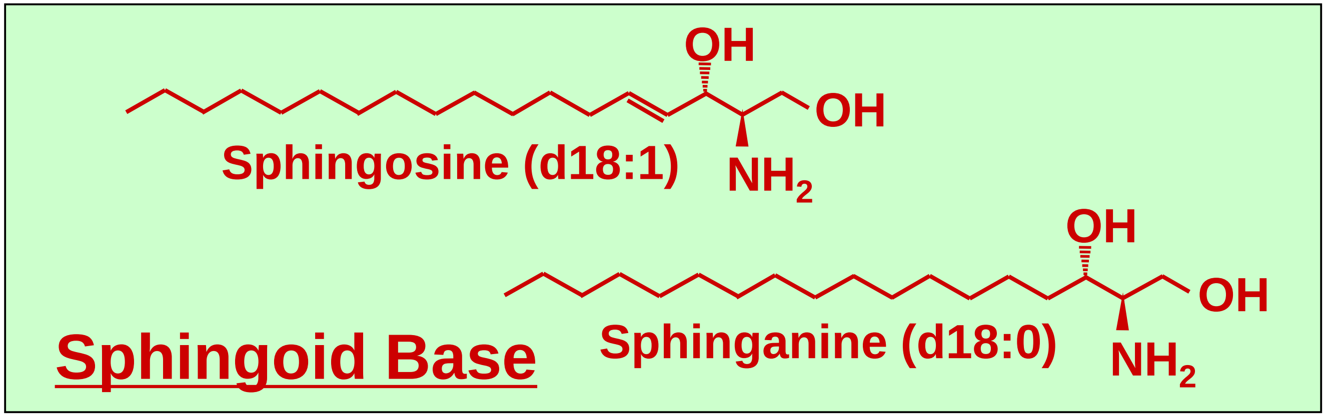
Interface (Lipids+Water+Emulsifier)

Presence of Sphingoid Base (SPG) in Fermented Salted Squid Guts and Muscle

Free Sphingoid Base Content (nmol/g dry weight) of Several Foods

Foods	PHS*1	SPO*2	SPA*3	Other SPG
Soybean	0.03±0.00	0.01±0.00	0.02±0.01	ND
Fermented soybean past (Miso)	3.02±0.26	0.20±0.02	0.73±0.07	ND
Non-fat dry milk	ND	0.87±0.14	0.45±0.08	ND
Powder cheese	ND	0.31±0.02	0.09±0.01	ND
Butter	ND	2.87±0.41	1.70±0.19	ND
Fermented salted squid *4	**ND**	**6.82±0.72**	**2.05±0.36**	**>90**

*1Phytosphingosine; *2Sphingosine (d18:1); *3Sphinganin (d18:0);
*4Considerable amount of other kinds of SPG (d16:1 and d19:3) were detected.

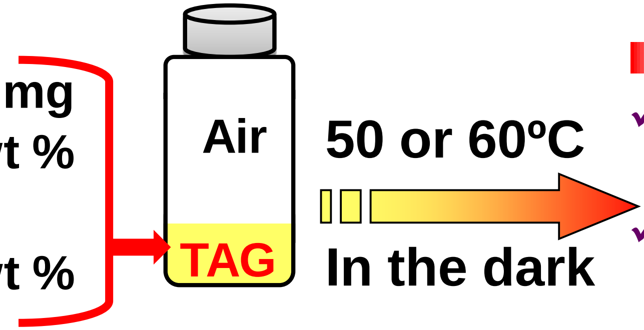


Effect of Sphingoid Base on the Oxidation of Different Types of Lipids

Oxidation and Analysis

- **Substrate: Fish, linseed, and soybean oil triacylglycerol (TAG)**
  - **Tocopherol free; Peroxide value: <1.0 meq/kg oil**

- **Oxidation:**
  - **TAG:300 mg**
  - **Sphingoid base:1.0 wt % (SPG)**
  - **α-Tocopherol:0.05 wt %**

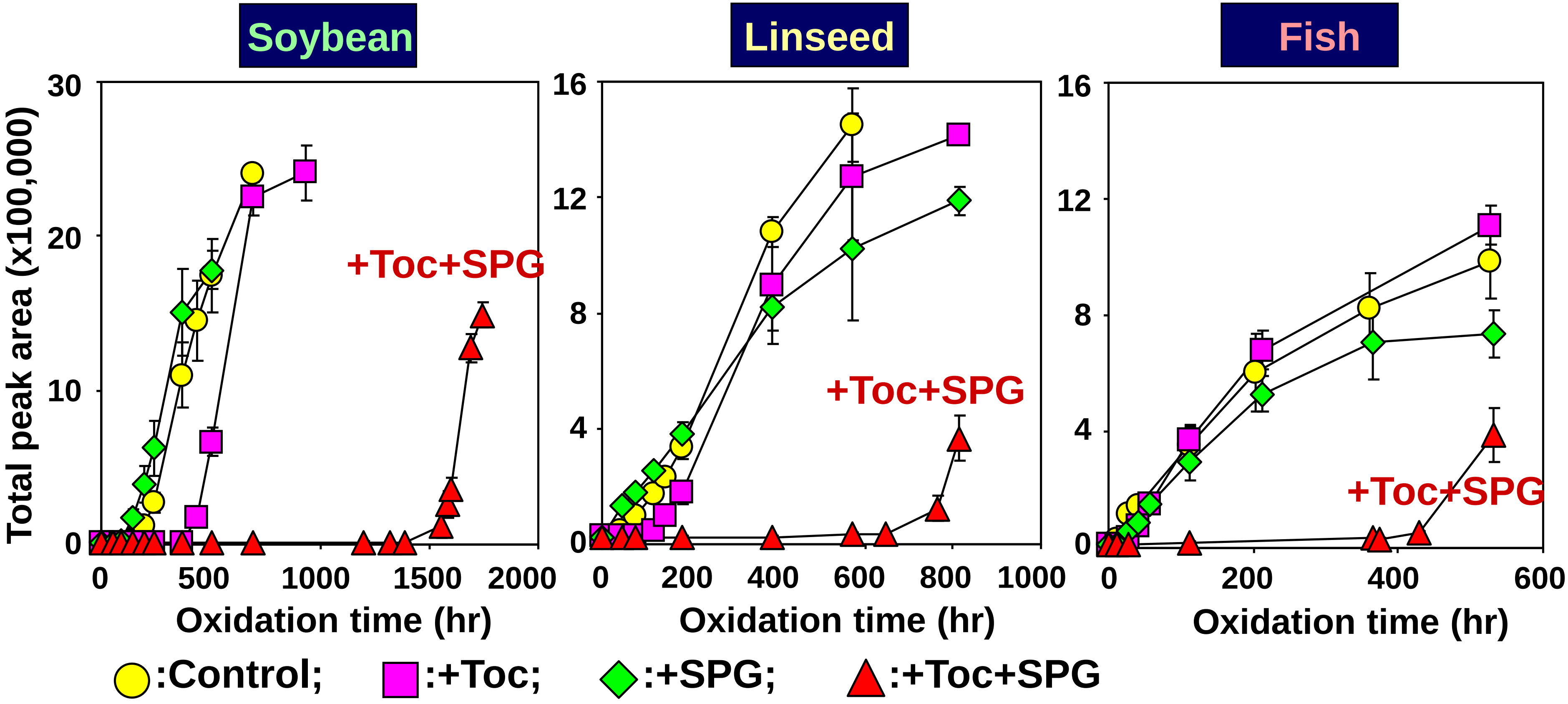


- **Analysis of lipid oxidation:**
  - ✓ **Oxygen concentration in headspace gas**
  - ✓ **Volatile compounds formation**

Fatty Acid Composition of TAG Used in This Study

Fatty acid (wt %)	Soybean	Linseed	Fish
14:0	-	-	5.41
16:0	11.64	5.89	12.96
18:0	4.09	4.08	3.65
16:1n-7	-	-	4.01
18:1n-9	24.82	25.32	6.92
18:2n-6	50.89	16.32	-
18:3n-3	4.71	45.31	-
18:4n-3	-	-	1.56
20:4n-6	-	-	2.70
20:5n-3	-	-	13.94
22:6n-3	-	-	25.44

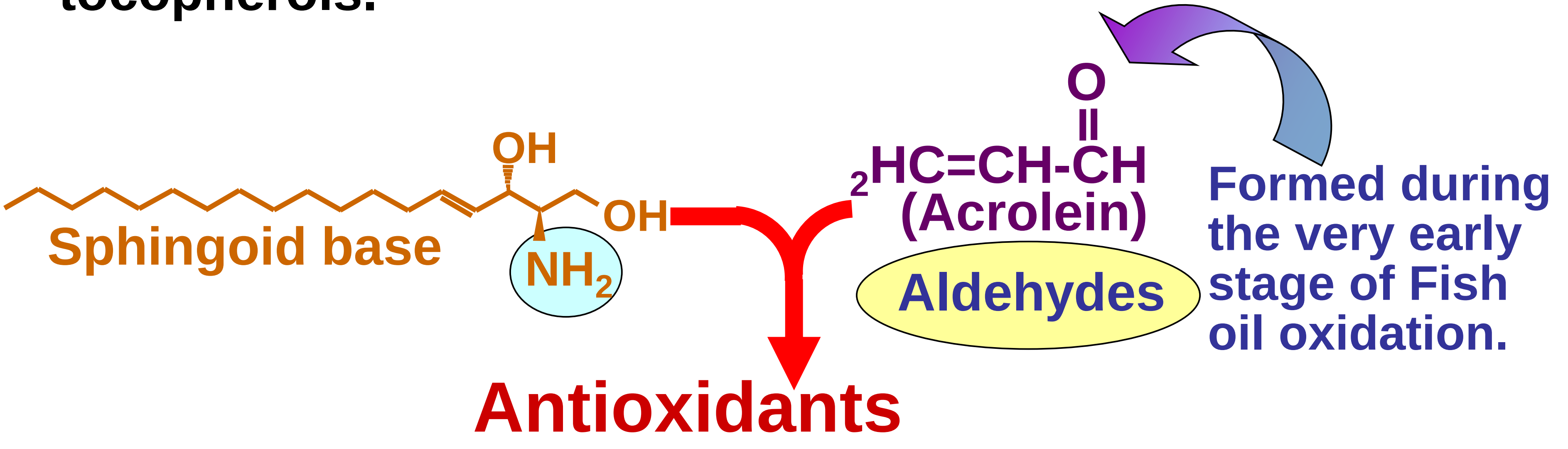
Effect of Sphingoid Base (SPG) and α-Tocopherol (Toc) on the Total Volatile Formation



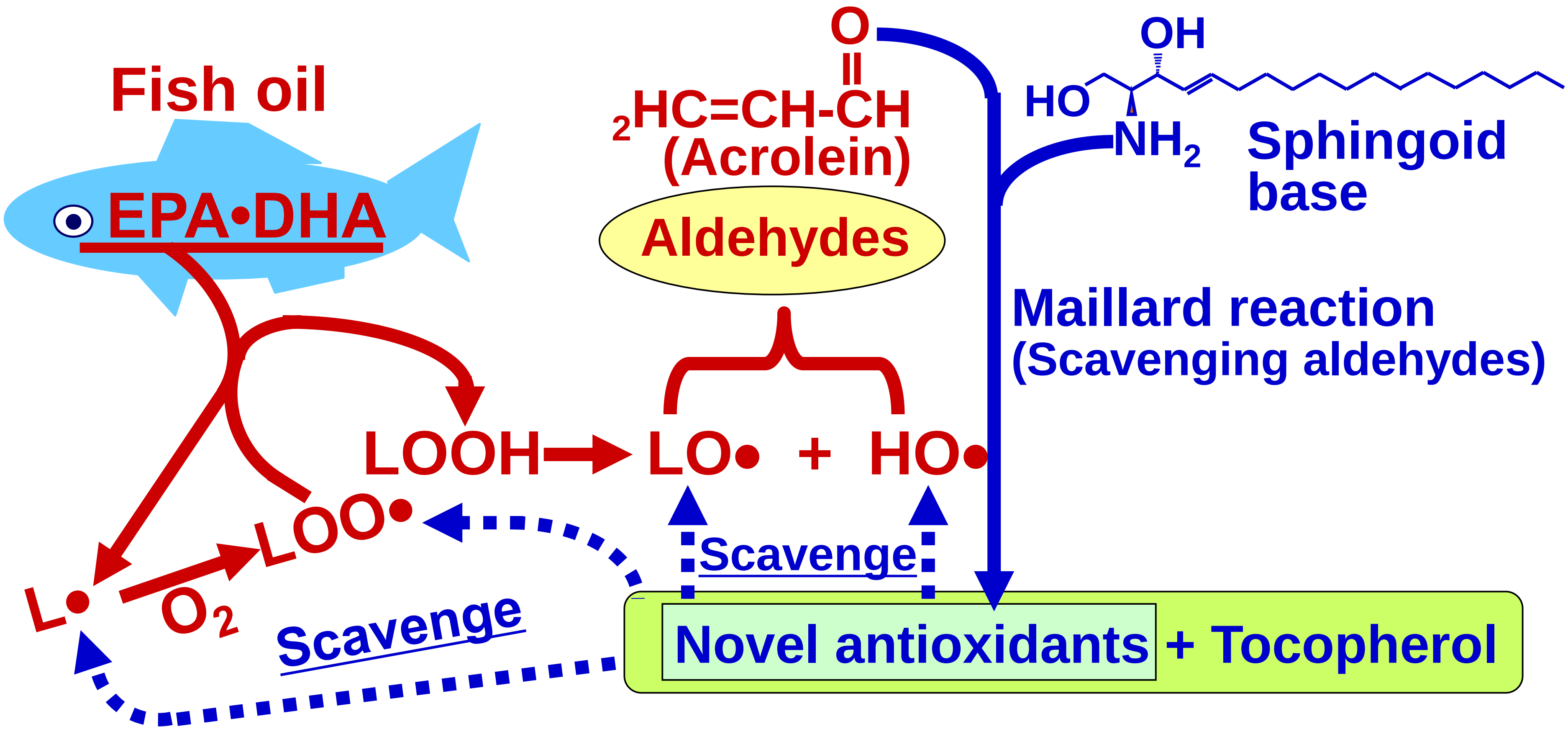
- **Various natural antioxidants have been used to prevent volatile formation in fish oil oxidation; however, a satisfactory effect has not yet been achieved.**
- **The combination of SPG and Toc could completely inhibit volatile formation during certain period of time even in fish oil TAG.**

Formation of Novel Antioxidants by the Reaction of Sphingoid Base and Aldehydes

- During the oxidation of fish oil in the presence of sphingoid base (SPG), novel antioxidants are formed by the Maillard reaction of SPG with aldehydes.
- The new antioxidants are not formed without oxidation products.
- The Maillard reaction products of SPG and acrolein showed strong antioxidant activity and synergistic effect on tocopherols.



Possible Mechanism for the Antioxidant Activity of Sphingoid Base and Tocopherol Combination



- The amine group of amine lipids such as sphingoid base can trap volatiles to form Maillard reaction products.
- The Maillard reaction products show strong synergistic antioxidant effects with conventional antioxidants.

Stability of EPA and DHA in Dried Seaweeds

Storage for more than 6 months at room temperature

Wakame (*Undaria pinnatifida*)

18:4n-3 : 37.3%
20:5n-3 : 17.6% → No decrease
20:4n-6 : 7.1%

Little formation of oxidation products

Konbu (*Laminariaceae bory*)

18:4n-3 : 27.9%
20:5n-3 : 18.4% → No decrease
20:4n-6 : 11.9%

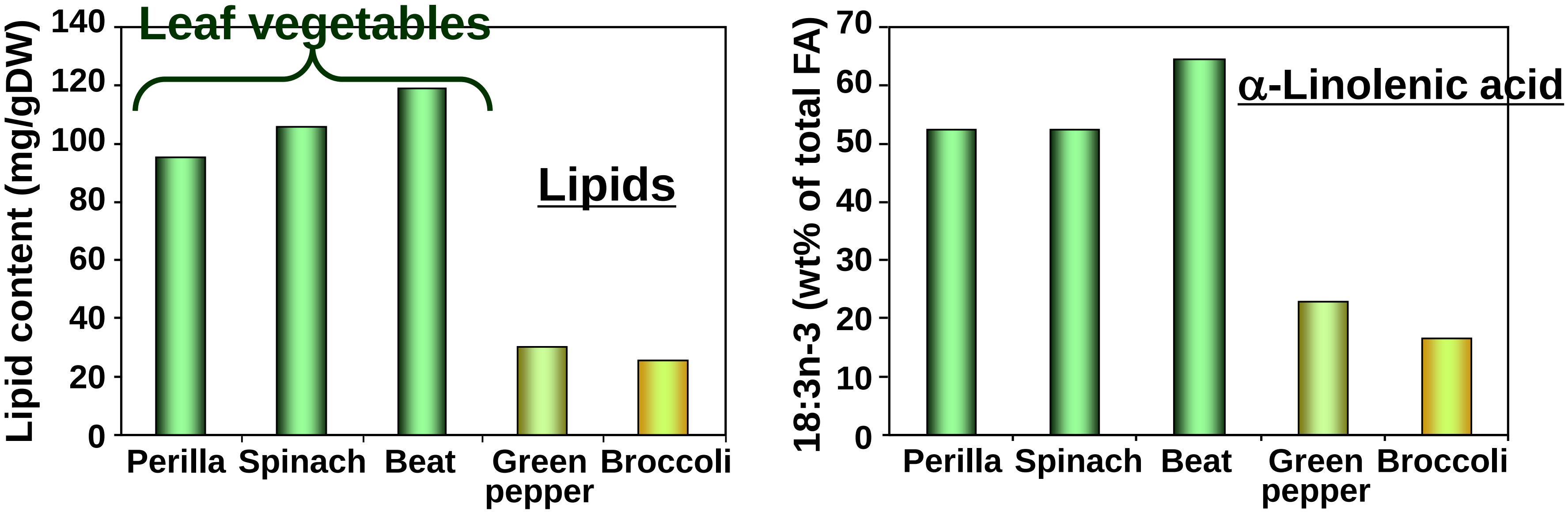
Little formation of oxidation products

Nori (*Pyropia yezoensis*)

18:4n-3 : 2.0%
20:5n-3 : 33.0% → No decrease
20:4n-6 : -

Little formation of oxidation products

Stability of α-Linolenic Acid in Dried Leaf Vegetables

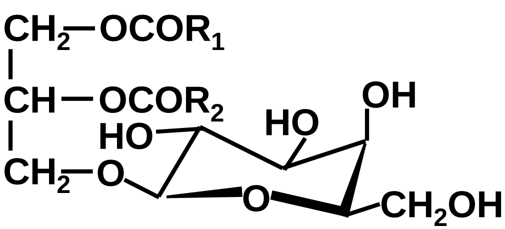


Contents of Lipids and α-Linolenic Acid (18:3n-3) in Leaf Vegetables and Others

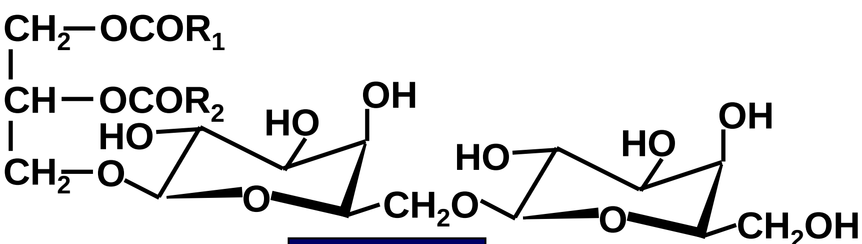
- **18:3n-3 (ALA) is main fatty acid in plant leave lipids, while 18:2n-6 is a main fatty acid in other parts.**
- **The oxidative stability of ALA is relatively low.**
- **On the other hand, we have found that there was little oxidation of ALA in the dried leaf vegetable powder during the storage more than half year at room temperature in the air.**

Why PUFA in Seaweeds and Leaves is So Oxidatively Stable?

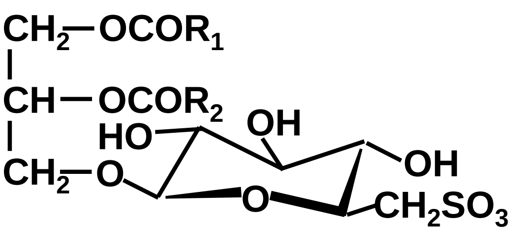
- The high oxidative stability of PUFA in dried seaweeds and dried leaf vegetables may be related to the presence of these PUFA as glycoglycerolipids (glycolipids; GL) form.
- GL is found in photosynthetic membranes of higher plant leaves and seaweeds as major lipid constituent.
- GL is rich in polyunsaturated fatty acids (PUFA).
- In higher plant leaves, the GL contain a high proportion of 18:3n-3 (α-linolenic acid; ALA), sometimes up to 95%.
- In seaweeds found in temperate and subarctic sea, GL contain high levels of 18:4n-3 (stearidonic acid) and 20:5n-3 (EPA) together with 20:4n-6 (arachidonic acid).



MGDG



DGDG



SQDG

Major GL Class Found in Plant Leave Lipids and Seaweed Lipids

Comparative Study on the Oxidative Stability of GL and Triacylglycerol (TAG)

Substrates:

- GL and TAG rich in ALA were obtained from spinach leave lipids and linseed oil, respectively, by using several column chromatographic separations.

Substrate purity:

- Tocopherol, carotenoid, chlorophyll, polyphenol free
- Peroxide value<1.0 meq/kg oil
- Metal free

Lipid class:

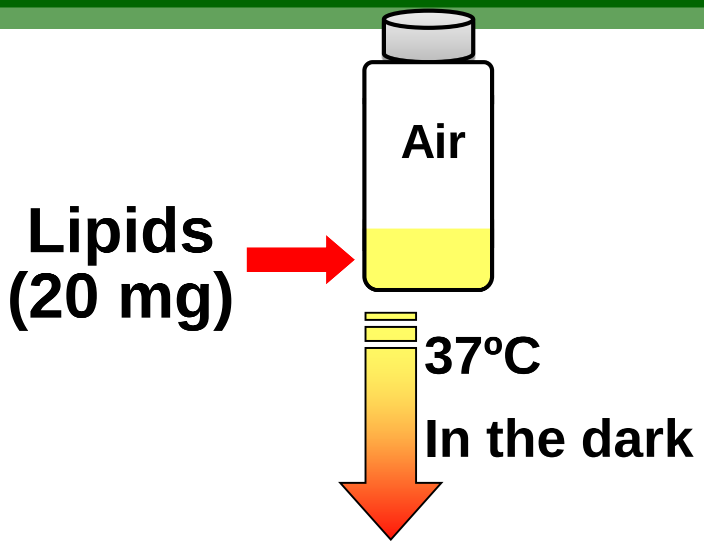
	MGDG	DGDG	SQDG	TAG
GL	75.3	19.0	5.7	0
TAG	0	0	0	100

Fatty acid profile

Fatty acid (weight%)	Linseed oil TAG	Spinach GL
16:0	5.3	2.2
16:1n-7	0.4	0.2
16:3n-3	0.2	18.2
18:1n-9	22.7	0.4
18:2n-6 (LA)	15.3	2.1
18:3n-3 (ALA)	52.7	74.0
Average number of bis-allylic positions per molecule	1.24	1.93

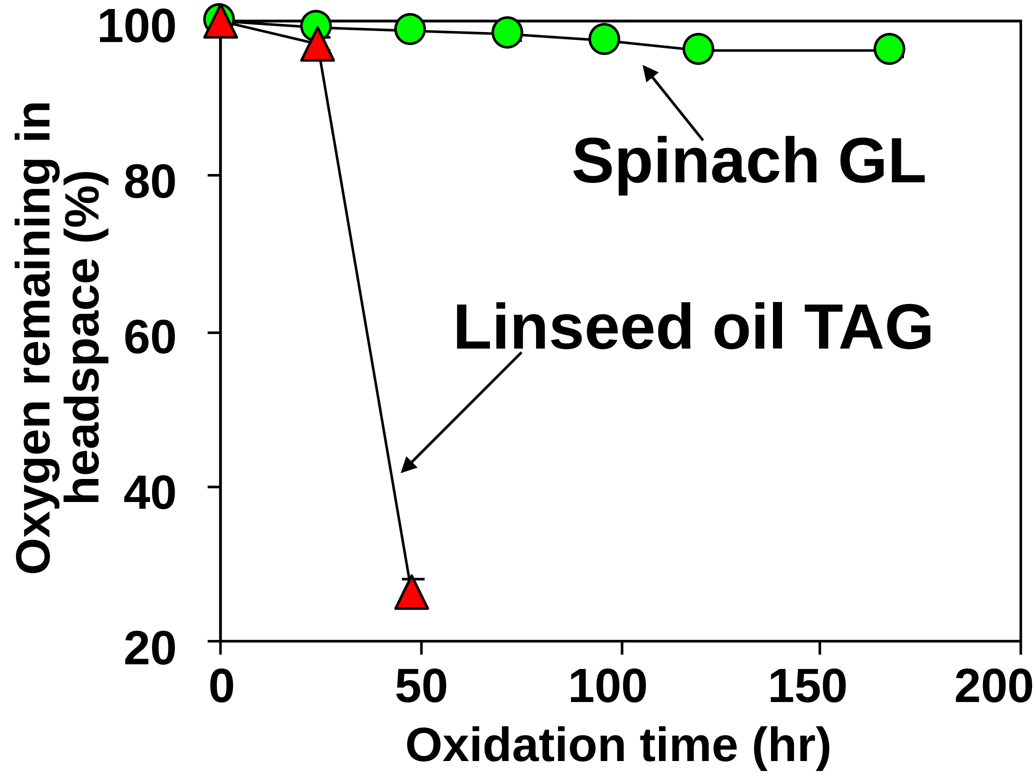
-CH=CH-CH2-CH=CH-
(Bis-allylic position)

Higher Oxidative Stability of ALA as GL Form



Analysis of lipid oxidation:

- ✓Oxygen concentration in headspace gas
- ✓Hydroperoxide formation
- ✓Propanal formation



Comparison of Oxidative Stability of Linseed oil TAG and Spinach GL

Although the number of bis-allylic positions of spinach GL (1.93) was higher than that (1.24) of linseed TAG, the GL was oxidatively more stable than the TAG.

Headspace analysis also showed a little formation of propanal after 168 hr incubation of spinach GL, while much propanal was detected in the oxidation of linseed oil TAG.

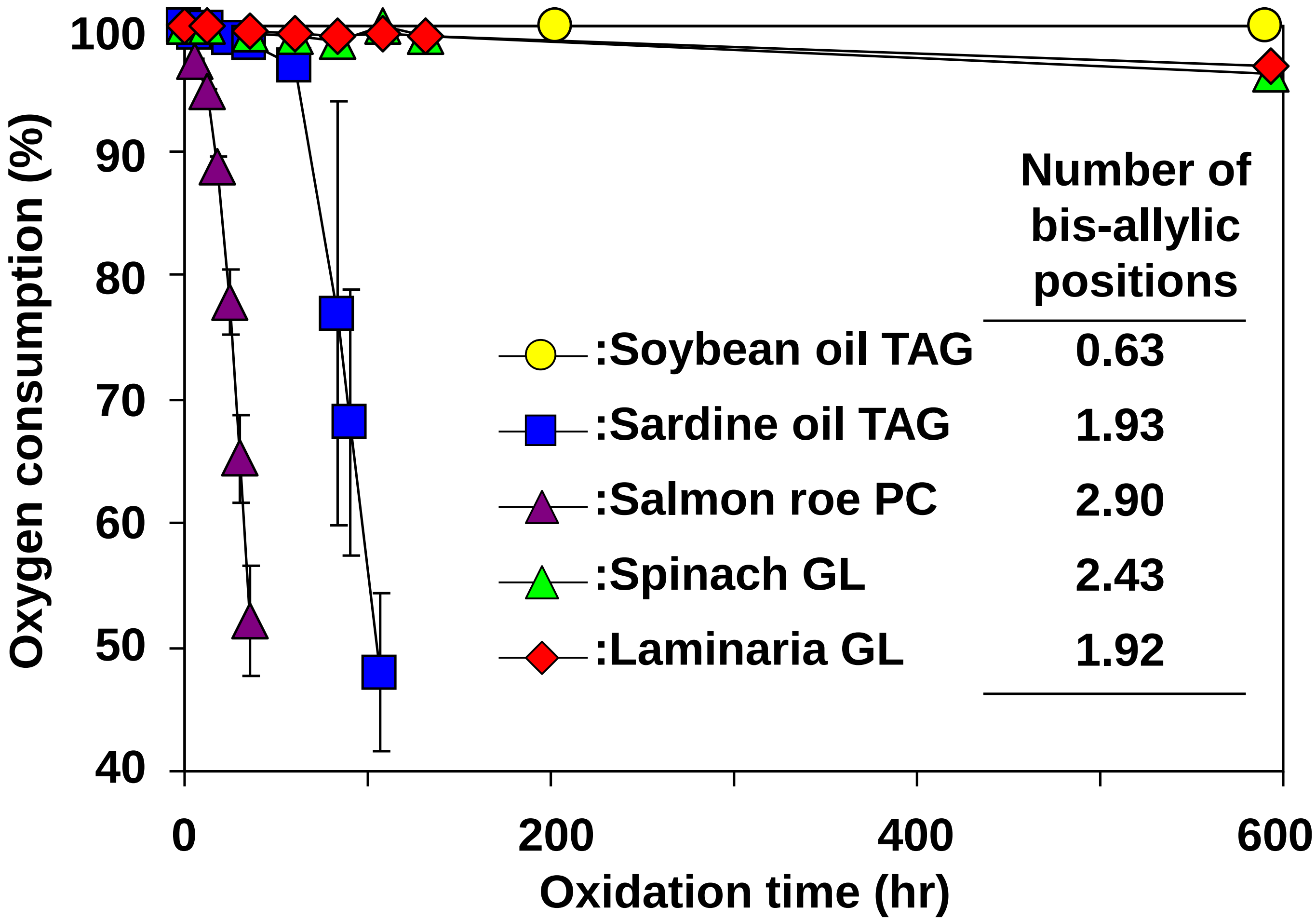
Another Comparison of Different Lipid Classes

Fatty acid (weight%)	Soybean oil TAG[b]	Sardine oil TAG[b]	Salmon roe PC[b]	Spinach GL[b,c]	Laminaria[a] GL[b,c]
16:0	11.3	8.4	16.7	2.4	12.1
16:1n-7	0.3	12.4	ND	ND	1.8
16:3n-3	ND	ND	1.3	18.2	ND
18:1n-9	27.7	14.1	7.8	0.4	16.5
18:2n-6	46.2	1.5	2.0	2.3	7.8
18:3n-3	3.2	0.7	ND	74.0	2.9
18:3n-6	ND	ND	ND	ND	8.1
18:4n-3	ND	2.0	0.1	ND	18.0
20:4n-6	ND	1.6	1.9	ND	7.8
20:5n-3	ND	19.5	10.3	ND	12.8
22:6n-3	ND	12.3	29.4	ND	ND
Average Number of bis-allylic positions					
per molecule:	0.55	1.71	2.11	1.93	1.60
per g lipid: ($x6.02x10^{20}$)	0.63	1.93	2.70	2.43	1.92

[a]Laminaria: edible brown seaweed.

[b]TAG, triacylglycerols; PC, phosphatidylcholine; GL, glycoglycerolipids.

GL Was Oxidatively Very Stable As Compared with Those of PC and TAG



Oxidative Stability of Soybean and Sardine Oil TAG, Salmon Roe PC, Spinach and Laminaria (Brown Seaweed) GL.

[Yamaguchi et al., J. Oleo Sci. 61:505-513, 2012]

Oxidative Stability of PUFA as GL Form

- Omega-3 PUFA as GL form was more stable to oxidation than those in other lipid classes.
- Plant leave lipids and seaweed lipids will be used as oxidatively stable omega-3 lipid sources.
- Galactosyl and sulphoquinovosyl moieties may protect the bisallylic positions of PUFA in GL by some kinds of interaction with the double bonds of the PUFA.
- Study on the oxidation of PUFA in natural products and traditional food products may give us novel approach to stabilize these PUFA.

It is always important to learn from nature.

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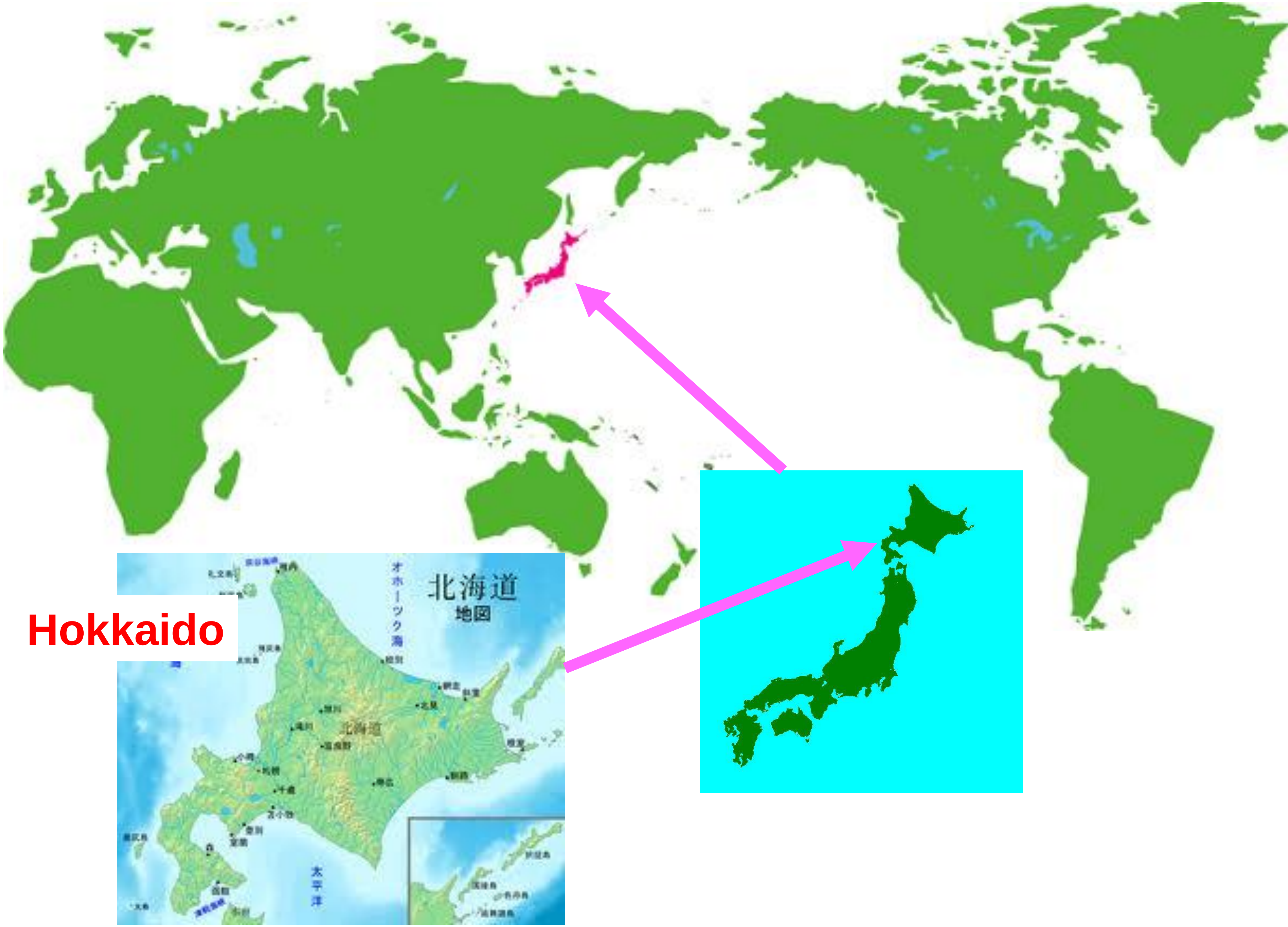
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