

Problems of Lipid Oxidation in Foods

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Thank you very much Mr. Chairman for your gracious introduction.

I am very honoured to have been invited to present a lecture at the regular meeting of the Korean Society of Food and Nutrition. This is all due the kindness of Prof. Park, the president of the Korean Society of Food and Nutrition, and Prof. Nam, the Secretary General of the Society.

Today I will talk about some problems of lipid oxidation in foods. Eating fresh foods in our daily life is an ideal condition. However, people living in the city have to be content with preserved or processed foods, more or less, in their daily life. Preserved materials or processed foods have usually undergone some deterioration related with lipid oxidation.

Now, I would like to start my lecture by talking about an incident which really occurred in Japan concerning so-called instant noodles. It happened 30 years ago when instant noodles began to appear on the market in Japan. Many people living in Osaka became ill after eating instant noodles. The physical symptoms were throwing up and loose bowels. The results from the investigation revealed that the noodles had been kept in a storehouse under unsuitable conditions. The roof of the storehouse was transparent made from plastic. When sunlight hit the roof, the inside became very bright, and the temperature rose. Therefore, lipid oxidation proceeded rapidly in that room. This incident was the first time that the problem of lipid oxidation was brought to light

in Japan. Since about that time, processed foods have increased rapidly in Japan, and care has been taken to prevent lipid oxidation.

As is well-known, lipid oxidation occurs with unsaturated fatty acids. The unsaturated fatty acids which are easily oxidized are linoleic, linolenic, and arachidonic acids, and these fatty acids are nutritionally "essential fatty acids" for humans.

1. Autoxidation

Autoxidation of unsaturated fatty acids is a slow reaction with molecular oxygen which occurs at an ordinary temperature. In the beginning, the reaction proceeds slowly but absorption of oxygen increases rapidly after a certain period. This period is called the "induction period." After this period, the reaction proceeds rapidly. This type of reaction is a radical chain reaction, where the products work in turn are the catalysts of further reactions.

In the case of linoleic acid, the hydrogen atom on the No. 11 carbon which is interposed between two double bonds is taken out easily. When this hydrogen atom is taken out, a radical is left behind. The oxygen molecule with 2 radicals binds to the carbon radical which has moved to both ends of the double bonds. The formed peroxy radical takes a hydrogen atom from another unsaturated fatty acid. Now hydroperoxide isomers 9- and 13-hydroperoxides, are formed. A new radical is left on the fatty acid molecule from which the

hydrogen atom was taken out. Then the same reactions are repeated.

The reactions are also shown schematically in this slide. Here, a double bond moves to the neighbouring position. So, conjugated double bonds are formed.

At the same time, *Cis-trans* transformation occurs. *Cis-trans* form also changes to *trans-trans* form with the passing of time. The reaction mechanism is explained by the release of a hydrogen atom and an oxygen molecule from a hydroperoxide group and the reforming of a hydroperoxy group by the coming together of another oxygen molecule and hydrogen atom. These processes have a free radical stage, and the free radical can move between the two double bonds. Thus, the position of the hydroperoxy group changes a half, 9- to 13-, and 13- to 9-.

Finally, four isomers are formed in linoleate hydroperoxides, positional and geometrical isomers.

The carbon atom interposed between the two double bonds is called an active methylene. Linolenic acid has 2 active methylenes and therefore 4 positional isomers are formed.

Arachidonic acid has 3 active methylenes and so 6 isomers are formed.

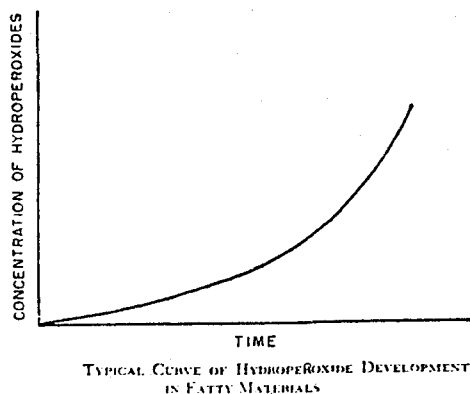
The rate of oxidation is parallel to the number of active methylenes.

Now I will show you how to detect the positional isomers of monohydroperoxides. Hydroperoxides are reduced to hydroxy compounds and next derivatized to TMS(trimethylsilyl) derivatives. These are applied on gas chromatograph-mass spectrometry(GC-MS). Both sides of the TMS position are split, and A and B and fragment ions are obtained.

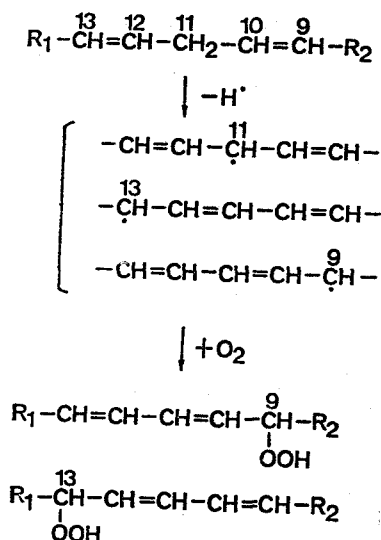
This is an example of a mass spectrogram of GC-MS. Fragment ions obtained from hydroperoxides of methyl oleate are shown.

This next slide shows the technique of "mass chromatography". The data shown here are

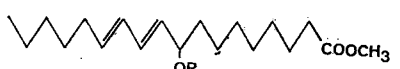
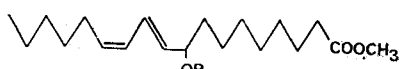
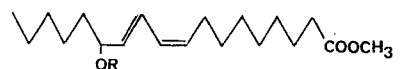
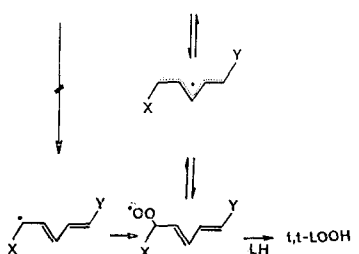
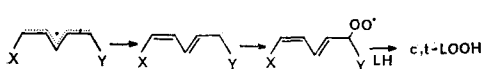
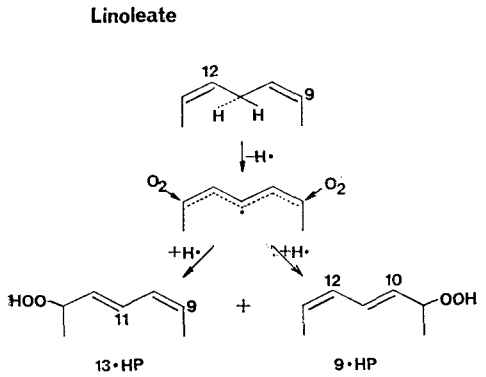
not those of autoxidation but of photosensitized oxidation, which will be presented later. Anyway, hydroperoxides of methyl oleate, methyl linoleate, and methyl linolenate were analyzed by GC-MS. The upper line shows the gas chromatograms of each derivatized fatty acid. In the case of mass chromatography, fragment ions are measured and memorized by a computer every 5 seconds. After the GC run is finished. The chromatograms are monitored by 2 fragment ions. A and B which are derived from a respective monohydroperoxide isomer (A and B are obtained by calculation), and



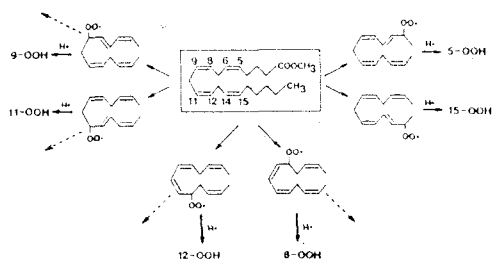
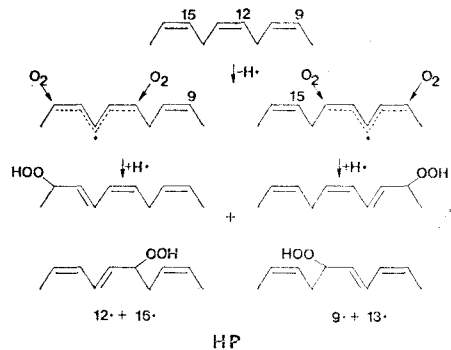
Linoleate Hydroperoxides



these peaks are obtained. A single peak obtained by GC contains 2 or 3 components by mass chromatography.

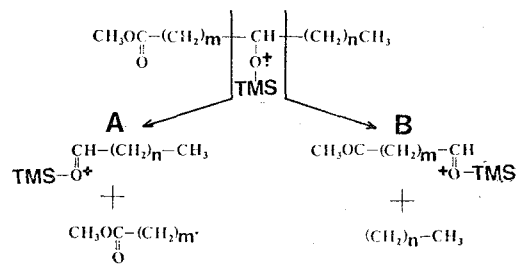


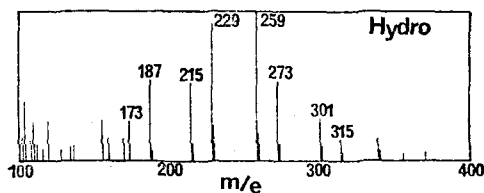
Linolenate



COMPARATIVE RATE OF OXIDATION

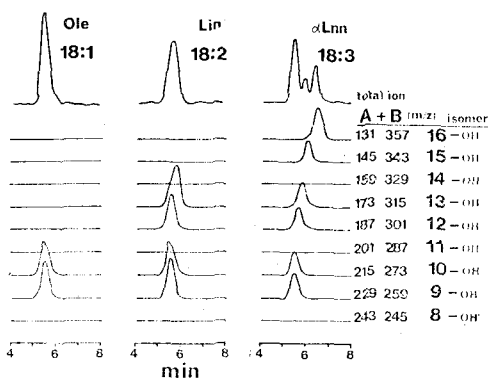
Fatty acids	Temperature		
	100 C	37 C	20 C
18 : 0	0.1		
18 : 1	6		
18 : 2	64	42	48
18 : 3	100	100	100
20 : 4		199	





TMS position	Fragment ion
9	259 229
10	273 215
12	301 187
13	315 173

Mass spectrum of TMS derivatives of methyl linoleate monohydroperoxides after hydrogenation



2. Photosensitized Oxidation

Another story I would like to tell you about concerned an incident in Tokyo about 10 years ago. Several ladies who had been taking chlorella tablets as a health food developed sores on their faces. The fact was that the chlorella products which they used contained pheophorbide which is a derived product from chlorophyll. Pheophorbide is formed when the phytol group of chlorophyll is split by an enzyme, chlorophyllase, which is contained in chloroplasts, and a magnesium atom is lost in an acidic medium. Pheophorbide can be absorbed into the body and flows through the veins. Under the skin of the face, hydroperoxides of lipids were formed from the absorption of sunlight

and the cells of the skin were broken.

When light hits a coloring matter which can be a photosensitizer, the coloring matter is excited. Sens means sensitizer. And the co-existing oxygen molecule (triplet oxygen takes the energy from the excited coloring matter and in turn becomes an excited state. This is called "singlet oxygen". Singlet oxygen attacks the substrate.

Electrons in the outer orbitals of the oxygen molecule are drawn simply in this slide. The arrows show the direction of electron spins. Oxygen at the ground state, is called "triplet oxygen". The life of singlet oxygen is extremely short but very reactive. If unsaturated fatty acids come in contact with singlet oxygen, the oxygen binds directly to the double bonds nucleophilically and hydroperoxides are formed.

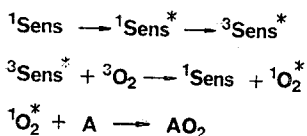
When singlet oxygen reacts with oleic acid, the oxygen molecule reacts directly with carbons on both sides of each double bond. Therefore, 9- and 10-hydroperoxide isomers are formed. When singlet oxygen attacks linoleic acid, 9-, 10-, 12-, and 13-hydroperoxide isomers are formed. 9- and 13-hydroperoxides have conjugated double bonds but 10- and 12-hydroperoxides are non-conjugated.

Here, the products of photosensitized oxidation are compared with those of autoxidation. Positional isomers formed by autoxidation and photosensitized oxidation are different.

The rate of autoxidation with triplet oxygen depends on the number of pentadienyl structures. On the other hand, the oxidation with singlet oxygen depends on double bonds and the reaction is very rapid. The reaction rate of singlet oxygen is 1500 times faster than that of triplet oxygen.

Photosensitizers which are contained in foods are chlorophyll and heme pigments and also their derived products. It is very difficult to eliminate chlorophyll derived products from

vegetable oils. Therefore, vegetable oils should be stored in dark places. Even though the amount of coloring matter is small and the formed hydroperoxides are a trace amount, hydroperoxides can initiate the radical chain reactions.



(Rawls and Van Santen, 1970)

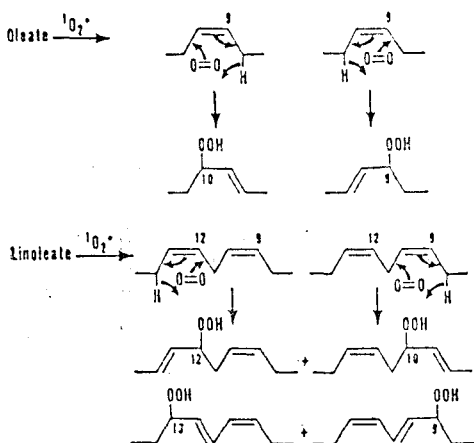
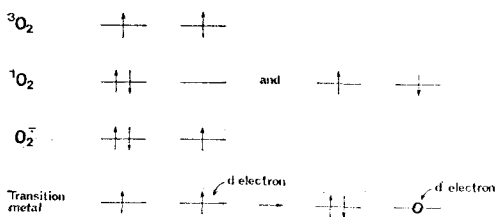
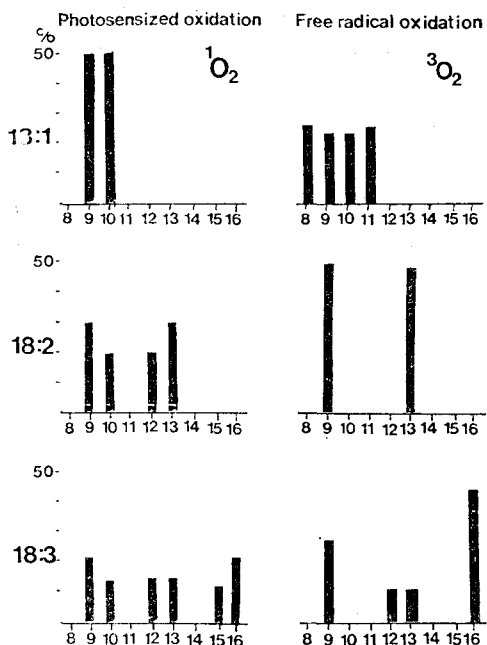


FIG. 4. Mechanism of photosensitized oxidation (13).



3. Lipoxygenase

Lipoxygenase is distributed widely in the plant kingdom. Soybean especially contains 3 isozymes and shows high activity. However, this enzyme can be easily inactivated by heating. I will not discuss this enzyme today.

4. Secondary Products

When hydroperoxides begin to accumulate, polymerized products and low molecular weight substances then begin to be formed. Monohydroperoxides can be caught temporarily as stable compounds, called primary stable compounds, and the compounds which are formed secondarily from monohydroperoxides are the so-called "secondary products". Secondary products consist of further oxygenated compounds and decomposition products from monohydroperoxides.

We see here the formation of endoperoxides in which the oxygen molecule is being cyclized.

As further oxygenated compounds, there are dihydroperoxide, epoxide, and others.

In this slide the monohydroperoxides seen to have decomposed. In the next 2 slides, the products from linoleate hydroperoxides and linolenate hydroperoxides are shown. Among the secondary products, volatile lower molecular weight substances are contained. These volatile compounds consist of many kinds of

aldehydes which have bad odors. Moreover, the radicals formed during the decomposition process of hydroperoxides bring about complex reactions.

Products from linolenate.

Here, hydroperoxy or alcohxy radicals react with each other and polymers are formed. There are more polymers than lower molecular weight substances.

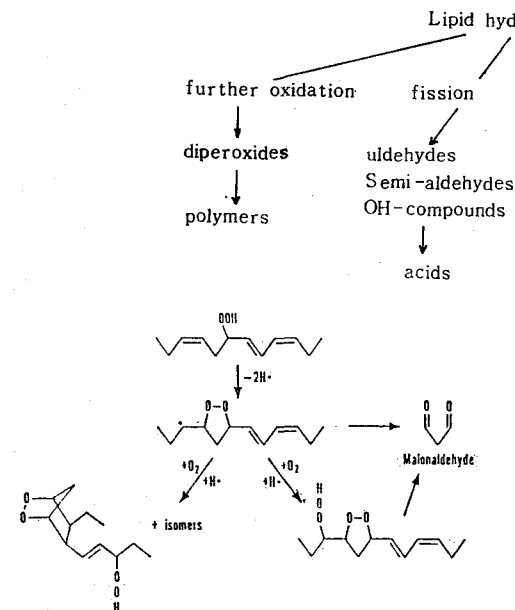
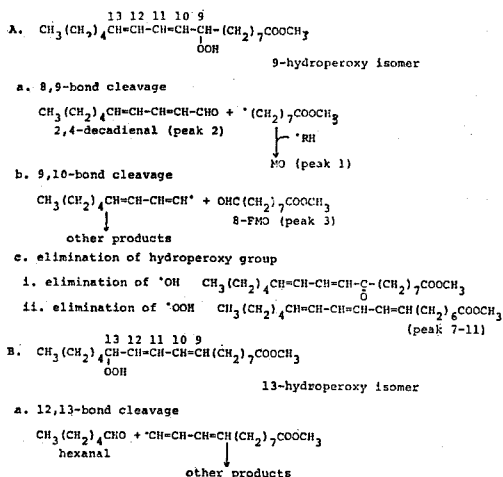


FIG. 5. Mechanism of 1,3-cyclization of 12- and 13-hydroperoxides of linolenate and formation of malonaldehyde (61).



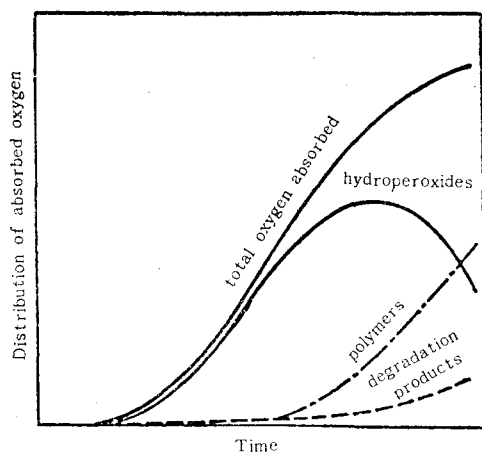
- b. 13,14-bond cleavage
 $\text{CH}_3(\text{CH}_2)_4 + \text{OHC}-\text{CH}=\text{CH}-\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOCH}_3$
 12-formyl methyl dodecadienoate (peak 5)
- c. elimination of hydroperoxy group
 1. elimination of $\cdot\text{OH}$ $\text{CH}_3(\text{CH}_2)_4\overset{13}{\text{C}}=\overset{12}{\text{C}}-\overset{11}{\text{C}}-\overset{10}{\text{C}}-\overset{9}{\text{C}}-\overset{8}{\text{C}}-\overset{7}{\text{C}}-\overset{6}{\text{C}}-\overset{5}{\text{C}}-\overset{4}{\text{C}}-\overset{3}{\text{C}}-\overset{2}{\text{C}}-\overset{1}{\text{C}}\text{COOCH}_3$
 11. elimination of $\cdot\text{OOH}$ $\text{CH}_3(\text{CH}_2)_4\overset{13}{\text{C}}=\overset{12}{\text{C}}-\overset{11}{\text{C}}-\overset{10}{\text{C}}-\overset{9}{\text{C}}-\overset{8}{\text{C}}-\overset{7}{\text{C}}-\overset{6}{\text{C}}-\overset{5}{\text{C}}-\overset{4}{\text{C}}-\overset{3}{\text{C}}-\overset{2}{\text{C}}-\overset{1}{\text{C}}\text{COOCH}_3$
 (peak 7-11)

GC-MS Analysis of Volatiles from Thermally Decomposed Methyl Linoleate Hydroperoxides

Compound	Elution temp (C)	Autooxidation (rel %)	Photooxidized oxidation (rel %)	Origin ^a
Acetaldehyde	70	0.3	0.4	?
Pentane	88	9.9	4.3	13-OOH
Hexane	117	0.8	0.3	13-OOH
1-Pentanol	129	1.3	0.3	13-OOH
Hexanal	136	15.2	1.7	12-/13-OOH
2-Pentanol ^b	145	7.2	9.9	12-OOH
1-Octen-3-ol ^b	163	Tr	1.9	10-OOH
2-Pentylpropanal ^b	165	2.8	0.6	?
Me heptanoate	170	1.0	0.3	?
2-Octenal	182	2.7	1.5	?
Me octanoate	189	1.5	7.6	9-OOH
2-Nonenal	195, 197 ^c	1.4	1.6	9-/10-OOH ^a
2,4-Nonadienal	200	0.3	0.3	?
7,8-Decadienal	219, 223 ^c	14	4.3	9-OOH
Me 8-Octadecanoate	220	1.3	0.9	?
Me 9-Octadecanoate	245	1.9	2.2	9-/10-OOH
Me 10-Octadecanoate ^d	256	0.7	0.7	?
Me 10-Octadecanoate ^d	265	4.9	14	10-OOH
Unidentified peaks		9.9	12	

GC-MS Analysis of Volatiles from Thermally Decomposed Methyl Linolenate Hydroperoxides

Compound	Elution temp (C)	Autooxidation (rel %)	Photooxidized oxidation (rel %)	Origin ^a
1-Hexene	65	10	3.2	14-OOH
Acetaldehyde	70	0.8	0.6	?
Propylaldehyde	80	7.7	9.3	15-/16-OOH
Diisoval	97	0.1	0.8	?
2-Butenal	109	0.5	11	15-OOH
2-Pentenal	131	1.6	1.1	13-OOH
2-/3-Pentenal	137	1.4	3.4	12-/13-OOH
2-Hexylpropanal	158	0.5	0.3	?
Me heptanoate	170	1.8	1.0	?
2,4-Hexadienal	174, 178 ^b	2.3	0.8	17-OOH
Me octanoate	189	1.2	12	9-OOH
4,5-Epoxyhept-2-enal	194	0.2	0.2	?
3,6-Nonadienal ^c	194, 199 ^b	0.5	1.1	9-/10-OOH
Me nonanoate	203	0.7	0.3	?
Decatrienal	219, 224 ^b	14	4.8	9-OOH
Me 8-Octadecanoate	220	0.8	0.9	?
Me 9-Octadecanoate	245	1.3	1.2	9-/10-OOH
Me 10-Octadecanoate	256	1.0	1.3	?
Me 10-Octadecanoate ^d	267	4.2	12	10-OOH
Unidentified peaks		11	12	



5. Deep Frying Oil

In the case of frying, oils are heated to a high temperature in the pan. At the surface of the oil in the pan oxidation occurs rapidly, and decomposed products or polymerized products are formed. Perhaps, hydroperoxides are formed as an intermediate but they do not accumulate because they decompose easily from heating. Hydrolysis occurs in the pan when steam evaporates from the frying materials, and free fatty acids are liberated. At the bottom of the pan, decomposition and polymerization of the oil occur due to over-heating. As a result, bubbling of the oil changes, the color becomes brown, and smoke is emitted.

6. Reactions with Food and Biological Components

Hydroperoxides and their secondary products can react with biological components. Chemical components of foods and animal tissues are the same, and therefore, if oxidized products react with food, the quality of foods decreases, and with animal tissues, toxicity is revealed.

When food proteins come in contact with lipid hydroperoxides, complexes are formed,

and then radicals are formed on the protein molecules. When 2 such radicals react with each other, a protein dimer is formed. If proteins are polymerized, solubility of the protein in water decreases and the texture of foods changes. Like amino acids, Met, Lys, His, and CySH, are easily damaged. In the case of the dry state, peptide bonds are easily splitted rather than forming polymers.

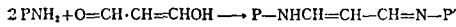
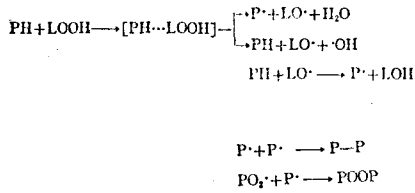
The secondary products, aldehydes, react with amino groups and a brawn pigment is formed. This reaction is called the "Maillard reaction".

Hydroperoxides are a kind of active oxygens, and they work as strong oxidants. Therefore, they can oxidize reducing substances such as ascorbic acid, tocopherol, glutathione and uric acid, and also decompose vitamin A or carotene which have many conjugated double bonds.

The reactions have described above lead to the deterioration of foods, and the shelf-life of processed foods becomes shorter.

It is still a question of whether hydroperoxides are absorbed into the body or not, when oxidized oils are eaten. But it is certain that the alimentary canal is upset. The symptoms are throwing up and loose bowels accompanied by stomach ache. This is from the acute toxicity of hydroperoxides due to the reaction with proteins. There are also some substances reacting with proteins among secondary products, namely, aldehydes. Aldehydes taken into the body are detoxicated in the liver but chronic toxicity occurs. This is called generally hypertrophy of the liver. Polymers formed by heating oils are also harmful to the body.

Apart from the extreme, some light symptoms such as feeling discomfort in the stomach or loose bowels may appear without any noticeable cause after a meal. The cause sometimes depends on the oxidation products from the meal.



Met., Lys., His., C₇H₇

7. Carcinogens

There is one more thing I would like to mention here. This is that some minor components contained in foods are oxidized and turn into dangerous carcinogens. Studies in this field have not yet been established. But I would like to show you some of our data.

Fe²⁺ and L-ascorbic acid induced oxidation of cholesterol was investigated in liposomes of egg yolk phosphatidyl choline (PC). Cholesterol 5,6-epoxide, 7-hydroxycholesterol, 7-oxycholesterol and cholestane-3, 5, 6-triol were detected as the oxidation products by gas chromatography or high performance liquid chromatography. These products indicated that epoxidation and hydroperoxidation took place dependently in cholesterol with the progress of lipid oxidation.

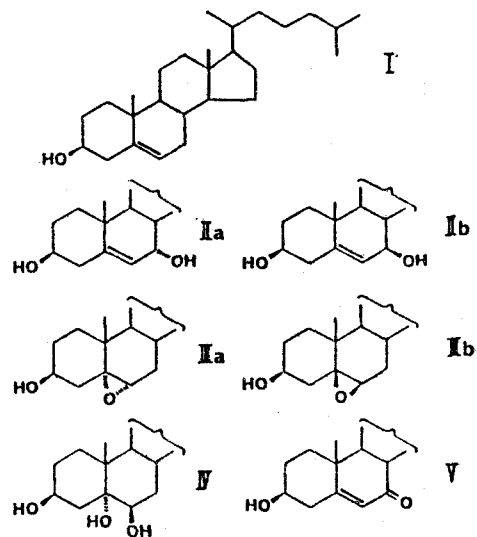
Oxidized cholesterols are considered to be toxic.

A shows the formation of cholesterol oxidation products in the liposomes made of cholesterol, egg yolk phosphatidylcholine, and oxidation catalists, Fe and AsA. 7-oxycholesterol, cholesteroltriol, 7-hydroxycholesterol, 7-hydroxycholesterol, and cholesterol epoxide are formed. B shows the [loss of cholesterol

and unsaturated fatty acid moiety of egg yolk phosphatidylcholine. That is, oxidation of cholesterol was accompanied by oxidative loss of unsaturated fatty acid moiety of phosphatidyl choline in liposomes made of egg yolk PC. When egg yolk PC was replaced with dimyristoyl-PC which is not oxidized, little oxidation was observed in the cholesterol fraction. These results indicate that cholesterol oxidation depends on the peroxidation of polyunsaturated fatty acids in liposomal phospholipids. Therefore, antioxidants must prevent the oxidation of unsaturated fatty acids and consequently oxidation of cholesterol. -Tocopherol incorporated into liposomes at a level of 1.0 mol% to egg yolk PC completely suppressed not only the loss of unsaturated fatty acid moieties but also oxidation of cholesterol.

Spray-dried egg contained cholesterol epoxides. The amounts were 30 μg/g or more.

In the case of benzopyrene, quinone of benyopyrene was formed in a system similar to cholesterol oxidation. Cholesterol and benzopyrene themselves do not show any carcinogenic activity, but their oxidized forms do.



I think these phenomena are significant problems in the matter of lipid oxidation. And I think the use of synthetic antioxidants should be considered again, though consumers these days do not like to use synthetic antioxidants.

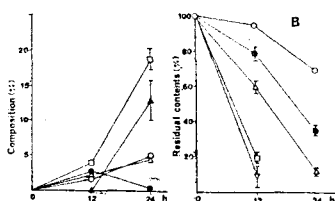
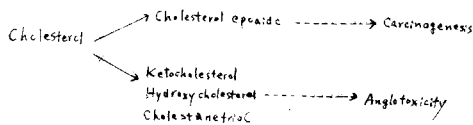
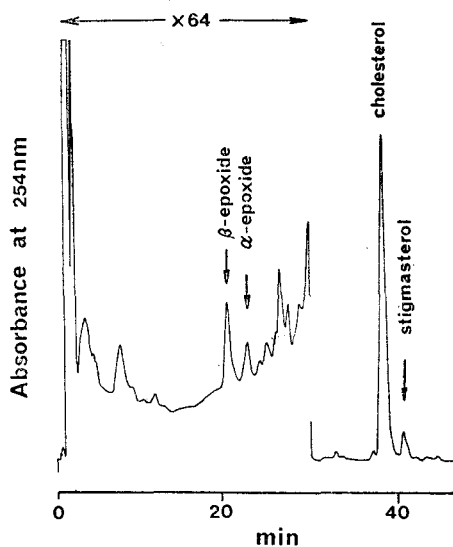


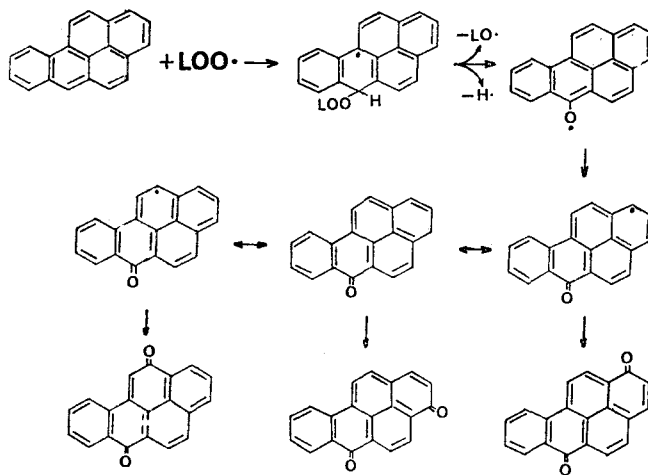
Fig. 3. Formation of cholesterol oxidation products (A), and the losses of cholesterol and unsaturated fatty acid moiety of egg yolk PC's during Fe^{2+} and ascorbic acid induced oxidation of liposomes. The composition of each oxidation product was determined by the ratio of its peak area to that of cholesterol before oxidation. (A) \circ , 7 α -hydroxycholesterol; Δ , 7 β -hydroxycholesterol; \bullet , cholesterol epoxide; \blacktriangle , cholestanetriol; \square , 7-oxocholesterol. (B) \bullet , cholesterol; \circ , 18:1; Δ , 18:2; \square , 20:4; ∇ , 22:6.



Column: YMC-PACKED ODS (6x150mm)

Solvent: Isopropanol / Acetonitrile

(Gradient: 10/90 → 50/50)



8. How to Control Oxidation

There is nothing to benefit from lipid oxidation in foods from the standpoint of both food quality and nutrition. Oxidation should be prevented as much as possible. Oxygen is

easily solubilized in oils, therefore, it is difficult to completely avoid oxidation.

The rate of oxidation can be affected by a number of things. Heat, light, trace metals, and biochemical catalysts including oxidative enzymes accelerate the oxidation rate consi-

derably. It may be possible to find ways to eliminate the physical factors. But there is no way to block the chemical catalyst except by using antioxidants.

Antioxidants cut the radical chain reactions and can delay the process of oxidation. Synthetic antioxidants, BHA and BHT, are very effective, but these days, their use is going down on account of reports which say that synthetic antioxidants have the possibility of being carcinogenic. So, tocopherols have been popular. But now, synthetic antioxidants are considered to be safe under the conditions required for food processing. I think the use of synthetic antioxidants should be allowed in some foods which are on the market for a long time.

To prevent photosensitized oxidation, we can get singlet oxygen quenchers. However, that can be used in foods are only β -carotene and tocopherols. There are two ways of quenching singlet oxygen. That is, one is to release the energy physically and the other is to eliminate singlet oxygen by reacting with it chemically.

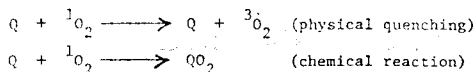
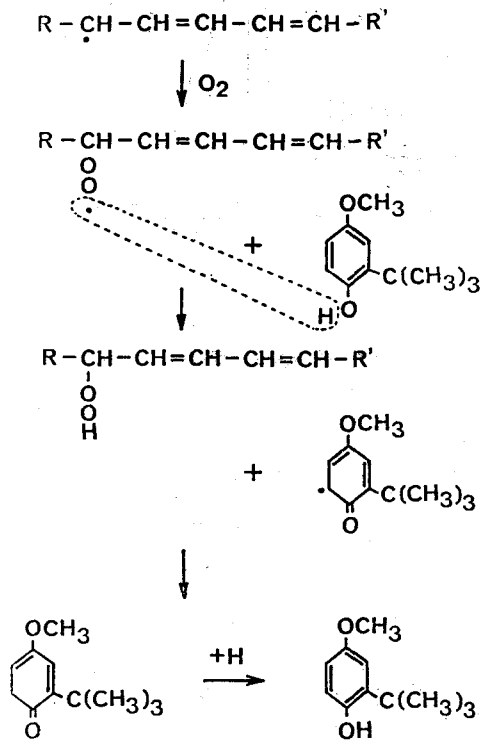
In the early stage, α -tocopherol showed the highest effects but they decreased with chemical reaction. Contrary to this, the effects of γ -tocopherol and δ -tocopherol can keep going longer. The quenching effect of β -carotene is superior, but radicals are formed as the reaction progresses and autoxidation is accelerated. Therefore, it is recommended that ρ -carotene be used together with δ -tocopherol.

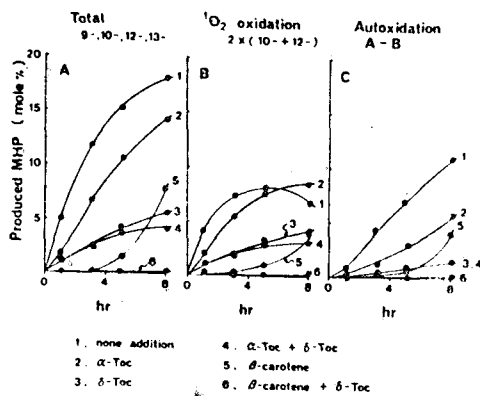
The ideal way is to eliminate oxygen. Some foods are sealed *in vacuo* or with nitrogen gas. Sometimes, a free-oxygen absorber is used. The most widely used absorber is made of iron.

The oxygen-removing mechanism is shown in this slide. One g of iron can bind with 0.4 g oxygen. This corresponds to about 1.5l of air. The use of this absorber is going to increase.

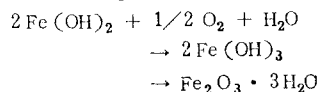
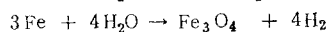
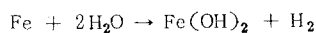
FACTORS AFFECTING LIPID OXIDATION

Accelerator	Inhibitor
High temperature	Cooling
Light (UV, blue color, photosensitizer)	Dark, Packing
Radiation	
Peroxide (oxidized lipid)	Antioxidant
Organometallic compounds (heme compounds)	Antioxidant
Trace metal catalyst (transition metal)	Chelating
Lipoxygenase	Heating





Free-Oxygen Absorber



9. Regulation

From several experiences, regulations were established in Japan in 1977 for oil-processed foods which contain more than 10% crude oils.

The foods with these values can not be sold.

1. AV over 3 and PV over 30
2. AV over 5 or PV over 50

This regulation applies to instant noodles, potato chips, fried rice cakes and so on. PV 30 corresponds to a condition where 1% of the oils becomes hydroperoxides.

Also, JAS, the Japanese Agricultural Standard, decided that the AV should be under

1.8 for instant noodles and under 3 for fried soybean curd.

Instant noodles are withdrawn from the market after 6 months.

As has already been pointed out, it is important to be careful not to form toxic substances in foods. In food processing, the proper choice of oils is important, and eliminating oxygen in the packing may be the ideal. We must use proper containers and packing methods to try to eliminate the several causes of oxidation. Storage conditions such as temperature and light should be controlled, and it is recommended that the period from processing to consumption be shortened. Not only in food manufacturing and distribution, but also in homes, the storage of foods should be carefully handled.

Regarding the use frying oils, generally, new oil is added to the pan to make up the amount that is used. But, when the turnover of the oil in the pan is slow, toxic substances accumulate. Therefore, It is necessary to replace all the oil in the pan with fresh oil sometimes.

FOOD SANITATION LAW

Processed foods which contain more than 10% lipids following can not be sold in the market.

1. AV over 3 and PV over 30
2. AV over 5 or PV over 50

JAPANESE AGRICULTURAL STANDARD

Instant noodle: AV should be under 1.8
Fried soybean curd: AV should be under 3.0