高度不飽和脂肪酸のケイ光燈照明下での自動酸化

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Autoxidation of Ethyl Eicosapentaenoate and Docosahexaenoate under Light Irradiation

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The oxidative stabilities of long chain polyunsaturated fatty acids (PUFA) such as eicosapentaenoate (EPA) and decosahexaenoate (DHA) were compared with those of linoleate (Lo) and linolenate (Ln) under light irradiation. Ethyl esters of these polyenoic acids were oxidized in separated sealed glass cylinders with constant agitation at 5°C under a fluorescent irradiation (90 Lux). Oxidation was followed by quantifying oxygen absorption, peroxide value, TLC and high pressure gel permeation chromatography. Both EPA and DHA were rapidly oxidized without a distinct induction period, while the induction periods of Ln and Lo were 1–2 and 15 days, respectively. The relative oxygen uptakes (Lo=1) during the first two days were as follows: Ln 99; EPA 743; DHA 948. We did not detect any significant differences in the oxidation rate during the propagation stage among the esters tested. The low values of OOH-oxygen/total oxygen uptake observed in polyenoic esters with more than 3 double bonds suggest the unstability of hydroperoxide in these esters. The major secondary products in EPA and DHA were polar materials consisting mainly of dimers. The intermolecular linkage distribution of dimers of PUFA commonly showed 80% C-O-O-C and 20% C-O-C.

Consumption of fish oils containing long-chain polyunsaturated fatty acids (PUFA) has gained special attention because these polyenoic acids may have benificial effects regarding ischemic heart disease and thrombosis.[1,2] However, polyenoic acids are known to be labile and easily oxidized to cause off-flavor. Oxidation mechanisms of linoleate (Lo) and linolenate (Ln) present in vegetable oils have been fully studied in the last decade,[3] while detailed information related to this matter on eicosapentaenoate (EPA) and docosahexaenoate (DHA) are not thoroughly understood. Monohydroperoxides and secondary products formed by auto and photooxidation of EPA were analyzed by Yamauchi *et al.*,[4,5] while those in autoxidized DHA were studied by Noble and Nawar.[6,7] As the assessment of oxidative stability should be required on handling these PUFA, we had previously compared the oxidation rate of EPA and DHA with Ln and Lo at 5°C in the dark.[8] This paper deals with the quantitative comparison of oxygen uptake and the patterns of oxidation products during autoxidation under irradiation with a fluorescent lamp. The intermolecular linkages of dimers were also characterized.

Materials and Methods

Materials

Ethyl esters of EPA (94.5%) and DHA (94.1%) were prepared from sardine oil by ethanolysis, urea adduction and fractional distillation.[9] Major contaminants in EPA were eicosatetraenoate and octadecatetraenoate, but were free from DHA. The contaminants in DHA were mainly composed of EPA and tetracosenoate. Ethyl Lo and Ln both >99% by GC were prepared from ethyl esters of safflower oil[10] and linseed oil[11], respectively. Each ester was further purified by silicic acid column chromatography prior to the autoxidation procedure.[14]

Autoxidation Procedure

Sample esters (1 g) were oxidized in a sealed glass cylinder (i.d. 4 cm) with constant agitation (ca. 500 rpm) by a teflon-coated magnetic bar (1 cm) at 5°C under irradiation with a fluorescent lamp (90 Lux).

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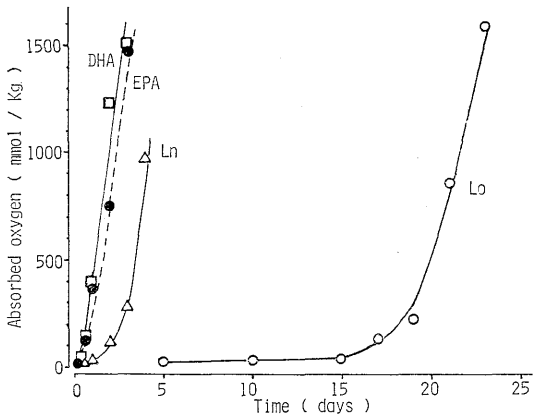

Fig. 1. Time-course of oxygen absorption during autoxidation of Lo, Ln, EPA and DHA at 5°C under a fluorescent lamp irradiation.

Analytical Methods

Oxygen absorption in the cylinder containing the sample ester was measured by gas chromatography with a thermoconductor detector as described previously.[8] Conjugated diene content was determined with UV absorbance at 233 nm (ε=26,000).[12] The peroxide values (POV) of the samples were determined iodometrically according to the AOCS method.[13] Patterns of oxidation products were analyzed by TLC-densitometer,[14] using Silica-Gel 60 plates (Merck) and hexane/ether (60: 40, v/v) as the mobile phase. Content of dimers and polymers was determined by high pressure gel permeation chromatography on a column of Ultrastyragel (500Å, Waters) using CH_2Cl_2 as the solvent with a refractive index detector and successive determination of molecular weight by the vapor pressure-equilibrium method as described previously.[14] The intermolecular linkages of dimers were characterized by selective splitting as follows: The cleavage of C-O-O-C was carried out treating with $SnCl_2$ according to the method of Mizuno and Chipault.[15] On the other hand, the cleavage of C-O-C was conducted by reducing the dimers with hydriodic acid following the method of Frankel *et al.*[16]

Results and Discussion

In Fig. 1 the time-course of oxygen absorption during autoxidation of ethyl Lo, Ln, EPA and DHA at 5°C under irradiation with a fluorescent lamp is shown. Both EPA and DHA were rapidly oxidized showing no induction period whatsoever, and Ln was also rapidly oxidized after an induction period of about 2 days. While it took about 18 days to Lo to start the oxygen uptake. The oxidation rate which was calculated from the oxygen absorption rate in the propagation stages of autoxidation was as follows: Lo, 15.4 mmol/Kg·h (relative rate 1); Ln, 25.7 (1.7); EPA, 28.5 (1.9); DHA, 35.0 (2.3). Comparing with the oxidation in the dark, the rate of oxygen uptake by EPA and DHA at the propagation stage was accelerated very slightly by light irradiation. However, the induction period of both EPA and DHA was shortened and not observed under light irradiation as shown Fig. 1, while their induction periods in the dark were 3 to 4 days. On the other hand, the oxidation of Ln was extremely accelerated and its rate reached almost 7 times of that in the dark. Thus, the oxidation stabilities of Ln, EPA and DHA were found to be extremely inferior to that of Lo judging from their induction period (Fig. 1, Table 1).

Table 1. Comparison of induction period and oxidation rate in the dark and under light irradiation

	Dark[8]		Light (90 Lux)	
	Induction period (days)	Rate (mmol·$Kg^{-1}\cdot h^{-1}$)	Induction period (days)	Rate (mmol·$Kg^{-1}\cdot h^{-1}$)
Lo	—	—	15.0	15.4 (1)
Ln	20.0	3.9 (1)	1.0	25.7 (1.7)
EPA	4.0	20.3 (5.2)	N.D.	28.5 (1.9)
DHA	3.6	33.3 (8.5)	N.D.	35.0 (2.3)

The formation of conjugated diene during autoxidation is shown in Fig. 2. The behavior of curve was very similar to that of oxygen uptake shown above. However, the content of conjugated diene in EPA and DHA on the 2nd day represented only about 70% of the total absorbed oxygen. The presence of secondary products not including conjugated diene was suggested in the early stage of oxidation.

Fig. 3 shows the ratio of OOH-oxygen determined by POV measurement to total oxygen absorbed during autoxidation of each ester under irradiation with a fluorescent lamp. The residual oxygen seems to be consumed for the formation of secondary products. The ratios in Ln, EPA and DHA found ranged between 50–70% in the early stage of oxidation, while for Lo in the initial stage was more than 90%. And a rapid decrease of those ratios was also suggested in both EPA and DHA with the progress of oxidation. These results indicate the unstability of hydroperoxides of fatty acids with more the three double bonds

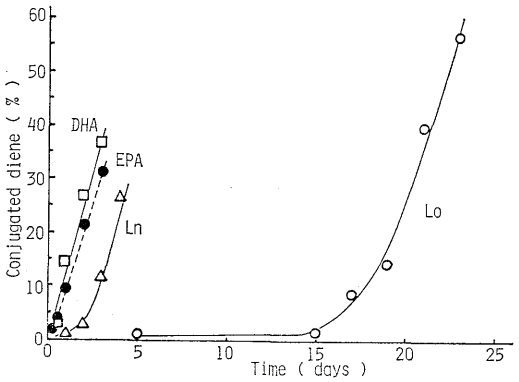

Fig. 2. Formation of conjugated diene during autoxidation of ethyl esters of Lo, Ln, EPA and DHA at 5°C under a fluorescent lamp irradiation.

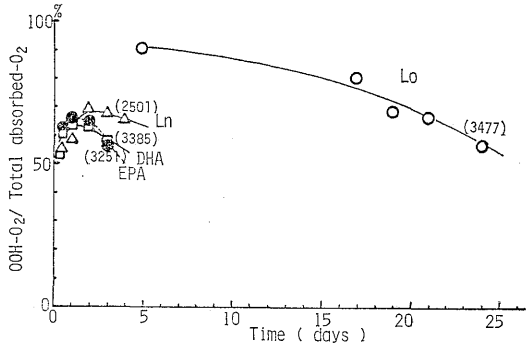

Fig. 3. Ratio of OOH-oxygen to total oxygen uptake during autoxidation of ethyl esters of Lo, Ln, EPA and DHA at 5°C under a fluorescent lamp irradiation. The values in parentheses indicate peroxide value.

comparing with that of Lo.

Fig. 4 shows TLC patterns of esters oxidized for 24 h. The first spot represents an unoxidized ester, while the 2nd spot indicates hydroperoxide (HPO) and the 3rd and 4th spots are presumed to be hydroperoxy cyclic peroxides as the major component.[17] The bottom polar spot is suggested to be composed of a mixture of polar materials including polymers as the major constituents.[14] The time-courses of distribution of oxidation products in Lo, Ln, EPA and DHA during autoxidation are summarized in Fig. 5. SP II and I in Fig. 5 represent secondary product corresponding to hydroperoxy cyclic peroxide, and the mixture of polymers and polar monomers, respectively. Both EPA and DHA showed a marked formation of highly polar secondary products from the initial stage of autoxidation. The amount of polar

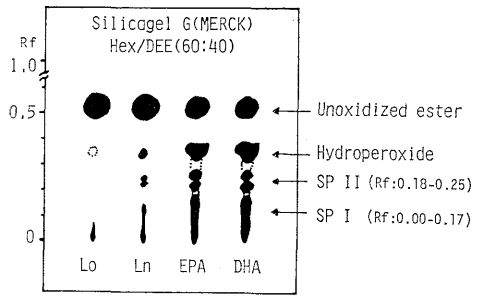

Fig. 4. TLC patterns of esters oxidized for 24 h.

materials was greater than that of HPO formed, while Ln preferentially formed hydroperoxy cyclic peroxides as the secondary products during autoxidation. On the other hand, the major oxidation products of Lo in propagation stage of autoxidation was HPO. Thus, the major oxidation products of EPA and DHA were highly polar secondary substances such as polymers and dihydroperoxides.

Fig. 6 shows the formation of dimers during autoxidation. Under this condition, in all of fatty acid esters, dimers increased linearly with the progress of oxidation. Especially, the increase in EPA and DHA was outstanding. Moreover, EPA and DHA produced trimers from the 1st day of autoxidation. The content of polymers determined by gel permeation chromatography was smaller than that estimated by TLC (Fig. 5). Since some of the polymers with high polarity are adsorbed by the column of Ultrastyragel, the polymer content might be underestimated in EPA and DHA. These combined results suggested that the polymerization play an important role in autoxidation of PUFA. The distribution of intermolecular linkages of dimers is shown in Table 2. In contrast to our previous report[18] presenting dimers formed in the initial stage of Lo autoxidation linked exclusively through C-O-O-C, the oxidized ethyl esters with peroxide value around 1,200 or 3,200 meq/Kg showed a common distribution of linkages, namely 80% C-O-O-C and 20% C-O-C. These distributions were similar to those found in dimers formed in aerated methyl Lo hydroperoxides.[18]

The present studies show that oxidation proceeds much faster in EPA and DHA, and a considerable amount of secondary products are formed from the early stage of oxidation. Polymers were found to be the major secondary products in PUFA. Therefore, the peroxide value is not

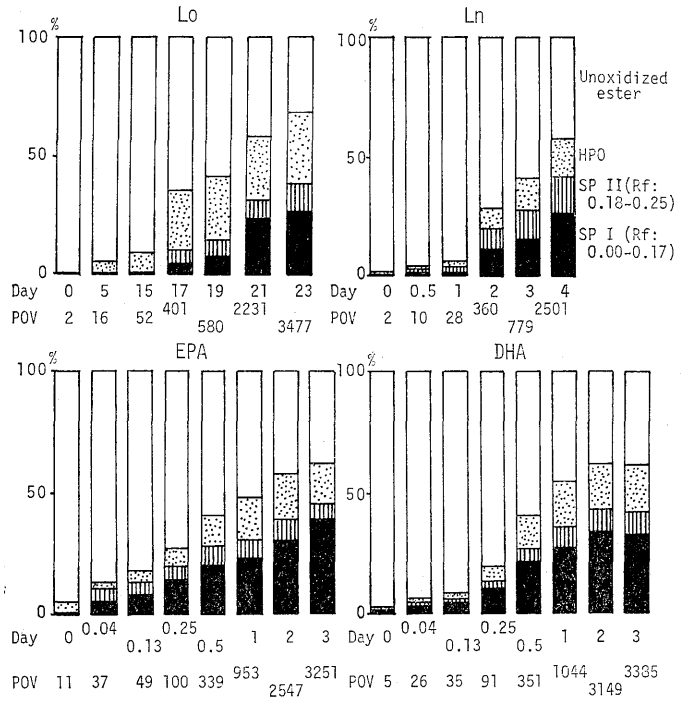

Fig. 5. Quantitative TLC analysis of autoxidized ethyl Lo, Ln, EPA and DHA at 5°C under a fluorescent lamp irradiation.

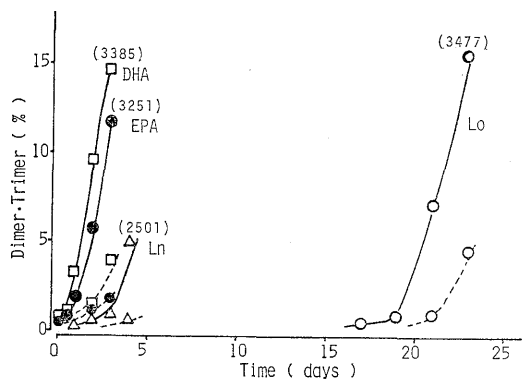

Fig. 6. Formation of dimer (—) and trimer (---) during autoxidation of ethyl esters of Lo, Ln, EPA and DHA at 5°C under a fluorescent lamp irradiation. The values in parentheses indicate peroxide value.

necessarily a good indication of oxidation in these PUFA because of the instability of their hydroperoxides. The determination of secondary products, such as polymers and carbonyl compounds, is suggested by this study to be important in evaluating the oxidative deterioration in PUFA. Light, of course, accelerated the oxidation of PUFA, and of all for EPA and DHA the most pronounced effect was found in shortening the induction period rather than in increasing the oxidation rate in the propagation stage. Together with refrigeration, shielding the light is confirmed to be very important to retard the oxidation of these PUFA.

Table 2. Intermolecular linkage distribution of dimers formed during autoxidation of ethyl esters of Lo, Ln, EPA and DHA at 5°C under irradiation with a fluorescent lamp

	POV (meq/Kg)	C-O-O-C (%)	C-O-C (%)
Lo	1170	73.7	26.3
	3285	69.8	30.2
Ln	1475	84.4	15.6
	3249	80.0	20.0
EPA	1130	79.6	20.4
	3185	80.0	20.0
DHA	1150	80.8	19.2
	3199	79.2	20.8

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