Autoxidation of Ethyl Eicosapentaenoate and Docosahexaenoate

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The extent of oxidation of ethyl esters of eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) was compared quantitatively with that of ethyl linoleate (Lo) and ethyl linolenate (Ln) by oxygen uptake and formation of conjugated diene, hydroperoxide and secondary oxidation products. EPA and DHA esters were oxidized rapidly even at 5 C in the dark after an induction period of 3-4 days, while the induction periods of Ln and Lo esters were 20 days and more than 60 days, respectively. Oxygen uptake of EPA and DHA esters after the induction period was 5.2 and 8.5 times faster than that of ethyl Ln, respectively. Hydroperoxides of EPA and DHA esters are much less stable than those of ethyl Lo. The peroxide value is not necessarily a good indication of oxidation in these polyenoic acids because a considerable amount of secondary products is formed at the early stage of oxidation. Polymers were found to be major secondary products in these polyenoic esters.

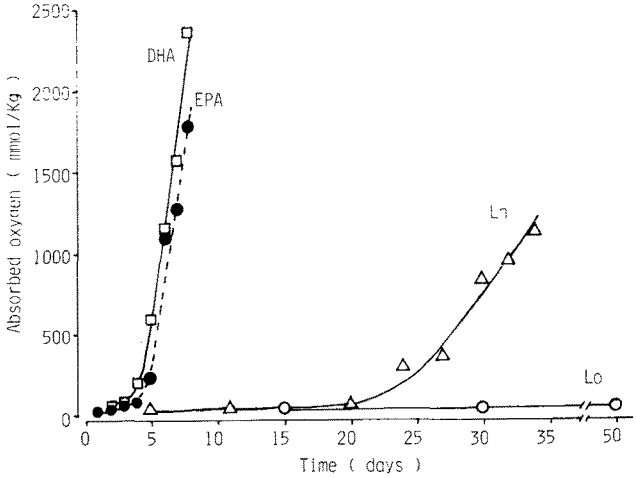

FIG. 1. Time course of oxygen absorption during autoxidation of ethyl esters of Lo, Ln, EPA and DHA at 5 C in the dark.

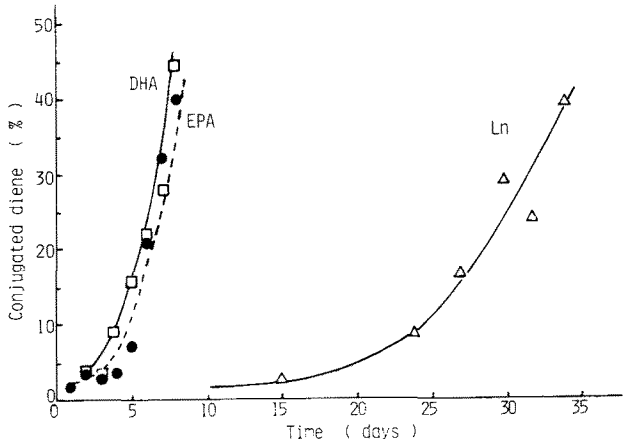

FIG. 2. Formation of conjugated diene during autoxidation of ethyl esters of Ln, EPA and DHA at 5 C in the dark.

Polyunsaturated fatty acids (PUFA) such as eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), particularly abundant in fish oils, currently are attracting much attention because epidemiologic studies in northern Greenland have shown that EPA, a fatty acid prevalent in the total plasma lipids in Eskimos, may account for the low incidence of coronary heart disease in the Eskimo population (1,2). Therefore, supplementation of western diets with PUFA has been recommended (3,4). However, because it has been suggested that PUFA are readily oxidized and the resultant oxidation products are toxic to animals (5,6), much care is required in their handling. The mechanisms of oxidation of linoleate (Lo) and linolenate (Ln) have been studied extensively in the last decade (7,8), and their oxidation rates have been determined (9). However, although hydroperoxides have been shown to form in these higher PUFA by free radical mechanisms similar to those in Lo and Ln (10-12), the details of the oxidation have not been resolved.

This paper compares the oxidation rate and the distribution of oxidation products from ethyl esters of EPA, DHA, Ln and Lo.

MATERIALS AND METHODS

Materials. Ethyl EPA (94.5%) and DHA (94.1%) were prepared from sardine oil by successive ethanolysis, urea adduction and fractional distillation (13). Major contaminants in the EPA ester were eicosatetraenoic and octadecatetraenoic acids, but it was free of ethyl DHA. The contaminants in the DHA esters were mainly ethyl EPA and tetracosenoate. Ethyl Lo (>99%) was prepared from ethyl ester of safflower oil by urea adduction (14). Ethyl Ln (>99%) was prepared from the mixture of ethyl esters of linseed oil according to the method of Mattews et al. (15). Each ester was used after purification by silicic acid column chromatography prior to autoxidation.

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 (4 × 10 mm) at 5 C in the dark.

Analytical methods. Oxygen absorption in the cylinder was measured by gas chromatography with a thermoconductivity detector. Conjugated diene content was measured by UV absorbance at 233 nm (ϵ=26,000) (16). The peroxide values of the samples were determined according to AOCS methods (17). Distribution of oxidation products was measured with a thin layer chromatography (TLC)-densitometer, using Silicagel

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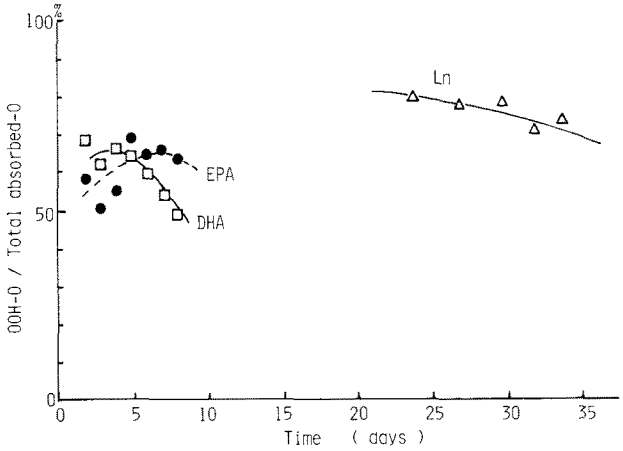

FIG. 3. Ratio of OOH-oxygen to total absorbed oxygen during autoxidation of ethyl esters of Ln, EPA and DHA at 5 C in the dark.

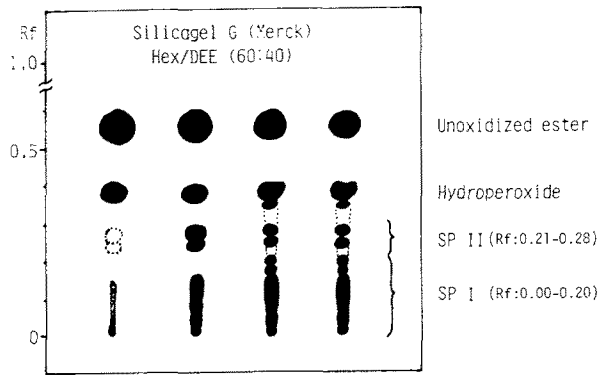

FIG. 4. Typical TLC patterns of oxidized esters.

60 (precoated, Merck, Darmstadt, Federal Republic of Germany) and hexane/ether (60:40, v/v) as the mobile phase. Oxidized ethyl EPA and DHA were fractionated on a silicic acid (Mallinckrodt, St. Louis, Missouri) column (2.7 × 30 cm) and eluted in steps with ether/hexane (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30 and 100:0) and methanol to separate polar materials. The polar materials obtained by silicic acid column chromatography were further fractionated by gel permeation chromatography on a Bio-Beads S-X3 (Bio-Rad Labs., Richmond, California) column (2.7 × 145 cm) with CH_2Cl_2 at a flow rate of 0.36 ml/min. Successive determination of mol wt with 6 ml fraction was done by vapor pressure equilibrium, as described previously for methyl linoleate (18). Dimer and polymer content were also determined by high performance gel permeation chromatography on a column of Ultrastyragel 500 Å (Waters, Milford, Massachusetts) using CH_2Cl_2 as the eluant with a refractive index detector.

RESULTS AND DISCUSSION

The time course of oxygen uptake during autoxidation of ethyl Lo, Ln, EPA and DHA at 5 C in the dark is shown in Figure 1. Both ethyl EPA and DHA were rapidly oxidized after an induction period of 3-4 days, while Ln was much more stable and oxidized after an induction period of three weeks. Under this condition, Lo was very stable and did not absorb any oxygen for more than 50 days. The oxidation rates, calculated from the oxygen absorption rate in the propagation stages while oxygen is absorbed linearly with time, were as follows: Ln, 3.9 mmol/kg/hr (relative rate 1); EPA, 20.3 (5.2); DHA, 33.3 (8.5). The relative rate of autoxidation of oleate:Lo:Ln has been reported to be on the order of 1:40-50:100 on the basis of oxygen uptake (19), and on

TABLE 1

Distribution of Polymers in Polar Materials of Ethyl EPA and DHA Autoxidized at 5 C in the Dark

SiO_2 column fractions (yield)	Bio-Beads S-X3 column fraction No.	Mean MW	Weight (%)	Distribution in polar materials (%) Tetra,trimer	Dimer	Monomer
EPA (POV:4734 meq/kg)						
DEE/Hex(40:60, 50:50)	35-47	548	10.5			
Rf: 0.05-0.19	49-52	418	54.0			
(9.8%)	55-80	308	35.5			
DEE/Hex(70:30), DEE(100) and EtOH(100)	30-43	1438	44.1			
Rf: 0.00-0.07	45-52	593	45.0			
(25.3%)	55-95	380	11.0			
Total polar materials				36.0	38.7	25.3
DHA (POV:5152 meq/kg)						
DEE/Hex(40:60, 50:50)	35-47	624	11.2			
Rf: 0.05-0.19	49-52	466	50.9			
(11.6%)	55-80	376	37.9			
DEE/Hex(70:30), DEE(100) and EtOH(100)	30-43	1576	46.4			
Rf: 0.00-0.07	45-52	734	46.7			
(31.9%)	55-95	362	7.1			
Total polar materials				38.1	40.5	21.4

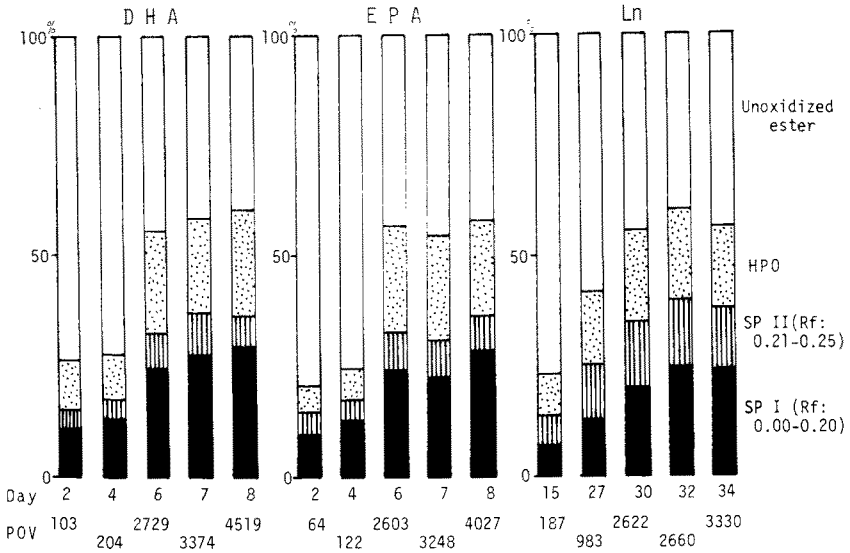

FIG. 5. Quantitative analysis of ethyl Ln, EPA and DHA autoxidized at 5 C in the dark.

the order of 1:12:25 on the basis of peroxide development (20). More recently, the initial oxidation rate of Ln was reported to be 2.0 to 2.5 faster than that of Lo, based on oxygen uptake (9). Moreover, Lo is known to be much less stable than monoenoic acids such as oleate. Consequently, these data strongly confirm the instability of EPA and DHA relative to Lo, Ln and other fatty acids found in edible oils.

The formation of conjugated diene during autoxidation is similar to that of oxygen absorption (Fig. 2). However, the conjugated diene content in EPA and DHA on the sixth day represented only about 70% of the total absorbed oxygen. This result suggests that the secondary oxidation products that do not contain conjugated diene had already formed in fairly large quantities in the early stages of autoxidation.

Figure 3 shows the ratio of OOH-oxygen to total absorbed oxygen during autoxidation of each ester in the dark. The residual oxygen, which comprises the total absorbed oxygen minus OOH-oxygen, is suggested to be consumed in the formation of secondary oxidation products. The ratios of OOH-oxygen in ethyl EPA and DHA were 50-70%, even in the early stages of oxidation, while that in Ln was slightly higher, 70-80%. A rapid decrease in the ratios in both ethyl EPA and DHA was also indicated. Lo monohydroperoxides were much more stable, and the ratio of OOH-oxygen in the early stages previously was reported to be more than 90% (18). Therefore, the instability of hydroperoxides of fatty acids with more than three double bonds compared with Lo was established. EPA and DHA are labile even at 5 C in the dark, and easily decomposed.

Figure 4 shows typical TLC patterns of oxidized esters. The first (top) spot is an unoxidized ester, while Rf of the second spot corresponds to the hydroperoxides of Lo and Ln. The third and fourth spots consist of secondary products which may include hydroperoxy cyclic peroxide as the major component (21). The bottom polar spot is composed of a mixture of polar materials, which were isolated by silicic acid column chromatography as shown in Table 1. Gel permeation

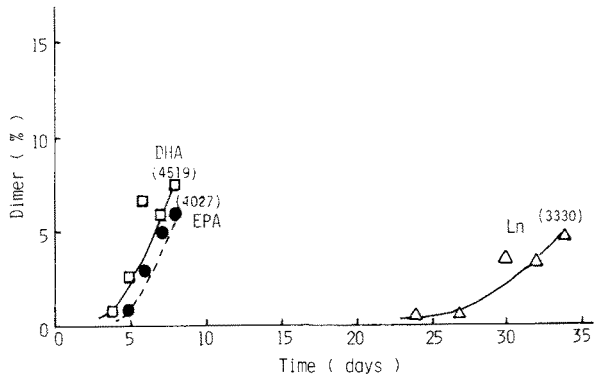

FIG. 6. Formation of dimer during autoxidation of ethyl esters of Ln, EPA and DHA at 5 C in the dark.

chromatography on Bio-Beads S-X3 established that more than 70% of polar materials from both ethyl EPA and DHA were dimers and polymers. Polymers and dimers by Bio-Beads S-X3 column chromatography gave isolated peaks by high performance gel permeation chromatography on Ultrastyragel with relative retention times (monomer:1) of 0.88 and 0.92, respectively. This result suggests the possibility of separation of polymers and dimers by Ultrastyragel. The time courses of distribution of oxidation products in Ln, EPA and DHA ethyl esters during autoxidation are shown in Figure 5. SP II and I in Figure 5 represent secondary products which may include hydroperoxy cyclic peroxide and mixtures of polymers and polar monomers, respectively. The ratios of monohydroperoxides/secondary products suggest the importance of the latter in the oxidation of these polyunsaturated esters.

The major secondary products in ethyl Ln were hydroperoxy cyclic peroxides (22), while those in ethyl EPA and DHA were polymers.

Figure 6 shows the formation of dimers during autoxidation at 5 C in the dark. In all fatty esters, dimers increased linearly along with the oxidation

process after the induction period. However, in the case of Ln esters, the formation rate was lower than that for EPA or DHA esters.

The present studies show that oxidation proceeds much faster in ethyl EPA and DHA than in ethyl Lo, and the peroxide value is not necessarily a good indication of oxidation in these PUFA because of the instability of their hydroperoxides. The determination of secondary products such as polymers is suggested by this study to be important in evaluating the oxidative deterioration in these fatty acid esters.

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