Oxidative Stability of Fish and Algae Oils Containing Long-Chain Polyunsaturated Fatty Acids in Bulk and in Oil-in-Water Emulsions

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The oxidative stability of long-chain polyunsaturated fatty acid (PUFA) and docosahexaenoic acid (DHA)-containing fish and algae oils varies widely according to their fatty acid composition, the physical and colloidal states of the lipids, the contents of tocopherols and other antioxidants, and the presence and activity of transition metals. Fish and algal oils were initially much more stable to oxidation in bulk systems than in the corresponding oil-in-water emulsions. The oxidative stability of emulsions cannot, therefore, be predicted on the basis of stability data obtained with bulk long-chain PUFA-containing fish oils and DHA-containing algal oils. The relatively high oxidative stability of an algal oil containing 42% DHA was completely lost after chromatographic purification to remove tocopherols and other antioxidants. Therefore, this evidence does not support the claim that DHA-rich oils from algae are unusually stable to oxidation. Addition of ethylenediaminetetraacetic acid (EDTA) prevented oxidation of both fish and algal oil emulsions without added iron and at low iron:EDTA molar concentrations. EDTA, however, promoted the oxidation of the corresponding emulsions that contained high iron:EDTA ratios. Therefore, to be effective as a metal chelator, EDTA must be added at molar concentrations higher than that of iron to inhibit oxidation of foods containing long-chain PUFA from either fish or algae and fortified with iron.

KEYWORDS: Docosahexaenoic acid (DHA)-containing oils; fish oils; algae oils; oxidative stability; bulk systems; emulsions; antioxidants; ethylenediaminetetraacetic acid (EDTA); iron

INTRODUCTION

Oils rich in long-chain *n*-3 polyunsaturated fatty acids (PUFA) have long been recognized for their nutritional importance (*1*–*7*). Lipid oxidation, however, limits the utilization of these oils in processed foods and as nutritional supplements in fortified food. The literature on the oxidative stability of long-chain *n*-3 PUFA is controversial. This problem may be attributed to the wide variation in fatty acid and triglyceride composition of fish oils (*6*) and to the wide range of methods and lipid systems used in oxidative stability tests. Many studies on the stability of fish oil PUFA are based on unreliable and unspecific methods for measurement of lipid oxidation and oxidative stability, including the test for thiobarbituric acid reactive substances (*8*–*11*), weight-gain (*11*), oxygen absorption (*12*–*14*), and fatty acid analyses by gas chromatography (*15*, *16*). The many problems encountered with the applications of these methods to evaluate relatively unstable polyunsaturated oils were previously reviewed (*17*, *18*). A fish taste model based on 2,6-nonadienal, 4-heptenal, and 3,6-nonadienal as key volatiles was proposed recently by using gas chromatography and mass spectral detection with ammonia negative chemical ionization in the parts per billion range (*19*). The effect of long-chain *n*-3 PUFA on the oxidative and flavor stability of triglycerides remains however an open and important question.

The results of studies of the effectiveness of antioxidants in protecting oils with long-chain PUFA have been particularly difficult to interpret because of the unreliable methods used to measure lipid oxidation and stability and the use of model systems in which the lipids are dispersed in different colloidal states known to affect their oxidative stability (*20*–*23*). Wide variations are also reported on the oxidative stability of emulsified food systems containing long-chain PUFA. In one report, *tert*-butylhydroquinone was effective but ascorbate was not effective in stabilizing mayonnaise prepared with 70% menhaden oil (*24*). In that study, calcium disodium ethylenediaminetetraacetic acid (EDTA), sorbic acid, and lecithin were included in the fish oil emulsion formulation. In another report, the shelf life of mayonnaise prepared with menhaden oil was significantly increased by adding citric acid or sodium citrate and propyl gallate in the oil phase and EDTA and ascorbic acid

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Table 1. Composition of Long-Chain Polyunsaturated Fatty Acid-Containing Oils[a]

fatty acid (%)	fish 1	fish 2	fish 3	tuna	mackerel	salmon	sand eel	algae 1	algae 2	algae 3
C12:0								5.0	5.1	4.4
C14:0	4.2	4.1	7.7	3.5	8.2	5.9	7.0	17.8	18.4	15.6
C16:0	21.9	22.0	18.4	20.4	13.6	15.2	19.9	15.0	15.6	12.8
C16:1, *n*-7	5.2	4.8	7.4	4.2	4.7	7.1	7.9	1.4	1.5	1.4
C18:0	6.2	6.3	3.8	5.5	2.4	3.2	2.3			0.9
C18:1, *n*-9	12.0	11.4	10.9	15.7	12.0	15.8	9.8	8.4	8.7	21.6
C18:1, *n*-7	2.4	2.3	3.2	2.4	2.2	3.3	2.2			
C18:2, *n*-6	1.3	1.4	2.0	1.8	2.3	3.3	2.9			0.8
C18:3, *n*-6	0.5	0.6	0.4	1.0	0.4	0.5	0.4			
C18:3, *n*-3	0.5	0.5	1.2	0.6	2.2	1.0	2.2			
C18:4, *n*-3				1.3	7.0	2.2	6.1			
C20:1, *n*-9	0.7	0.8	1.8	1.7	11.8	14.5	7.6			
C20:4, *n*-3				0.6	1.7	1.8	0.9			
C20:4, *n*-6 (AA)[b]	2.3	2.4	1.4	1.7	0.5	0.4	0.4			
C20:5, *n*-3 (EPA)[b]	5.1	5.5	11.8	7.1	9.7	7.9	12.7			
C22:1, *n*-9				0.4	1.2	1.6	1.1			
C22:5, *n*-3	1.1	1.1	1.7	1.5	1.9	3.3	0.9			
C22:6, *n*-3 (DHA)[b]	26.6	27.7	16.1	26.3	14.5	10.5	12.7	52.4	50.8	42.4
C24:1, *n*-9	0.7	0.7	0.7	0.7	1.2	0.8	1.1			
other fatty acids	9.3	8.5	11.4	3.6	2.5	1.7	2.0			0.2
total PUFA[b]	37.5	39.0	34.6	41.9	40.2	30.9	39.2	52.4	50.8	42.4
minor components (ppm)										
α-tocopherol	556	451	55	171	11	82	76	836	420	1729
β- + γ-tocopherols	413	0	24	8	0	0	0	0	0	208
δ-tocopherol	160	0	12	0	0	0	0	0	0	0
total tocopherols	1129	451	91	179	11	82	76	836	420	1937
carotenoids	ND	ND	ND	ND	ND	ND	ND	2823	577	1687

[a] Sources: fish-1 oil from Nestlé R&D, Lausanne, Switzerland; fish-2 and fish-3 oils from Roche Vitamins, Inc., Nutley, NJ; tuna oil from Maruha Corp., Japan; mackerel, salmon, sand eel, algae-1, and algae-2 oils experimentally bleached and deodorized at Technical University of Denmark, Lyngby, Denmark; algae-3 oil contained 0.025% ascorbyl palmitate according to specifications from Martek Bioscience Corp., Columbia, MD. Fatty acid compositions were analyzed by capillary gas chromatography (*31*), tocopherols were analyzed by high performance liquid chromatography (*32*), and carotenoids in the algae oils were analyzed by spectrophotometry (*33*). [b] ND, not determined.

in the aqueous phase (*25*). Wide variation in quality was observed between batches of menhaden oil. More recent studies showed EDTA to be a very effective inhibitor of oxidative deterioration in mayonnaise containing 16% fish oil, but gallic acid and propyl gallate were weak prooxidants (*26, 27*). Although the effect of tocopherol was insignificant, a mixture of ascorbic acid, lecithin, and tocopherol was a strong prooxidant in this fish oil mayonnaise (*28*).

To better understand the relation of composition to the oxidative stability of polyunsaturated fats, the present study used sensitive quantitative methods to determine oxidation and stability (*21, 29*) of commercial sources of long-chain polyunsaturated oils derived either from algae or from fish. Because of current interest in supplementing infant formulas and clinical nutrition products with long-chain PUFA-containing oils, including those containing docosahexaenoic acid (DHA) (*30*), the oxidative stability of these oils was compared with that of corresponding oil-in-water emulsions. Because commercial infant formulas are supplemented with iron, the effect of iron and EDTA, as a metal chelator, was tested in model emulsions of polyunsaturated fish oils and algal oils.

MATERIALS AND METHODS

Materials. The fish and algal oils used in this study are listed in **Table 1** with their fatty acid, tocopherol, and carotenoid composition. Three commercial fish oils (fish-1, Nestlé R&D, Lausanne, Switzerland; fish-2 and fish-3, Roche Vitamins, Inc., Nutley, NJ) were similar in fatty acid composition to that of a sample of tuna oil obtained from Japan (Maruha Corp., Japan); fish-1 and fish-2 oils appeared to be supplemented with mixed and α-tocopherols (**Table 1**). The raw mackerel oil (Scomber scombrus, Saeby Fish Industries, Saeby, Denmark), salmon oil ("sea farmed", Vikholmen Bioprocess A/S, Vikholmen, Norway), and raw sand eel oil (Esbjerg Fiskeindustri, Esbjerg, Denmark) were also obtained commercially and refined at the

Technical University of Denmark (BioCentrum-DTU, Lyngby, Denmark). The raw fish oils were bleached (1.5 wt % bleaching earth) and subjected to thin-film deodorization for 3 h at 190 (mackerel) and 180 °C (salmon and sand eel). One commercial algal oil was formulated with tocopherols and ascorbyl palmitate and blended with high-oleic sunflower oil to standardize the DHA content to 42% (algae-3, Martek Bioscience Corp., Columbia, MD); algae-1 and algae-2 oils were obtained from the Martek Bioscience Corp. without antioxidant supplementation and subjected to thin-film deodorization at 200 and 230 °C, respectively, at the Technical University of Denmark (Bio-Centrum-DTU, Lyngby, Denmark).

Chromatographic Purification. A sample of algae oil-3 (**Table 1**) was purified chromatographically to remove tocopherols and other antioxidants and carotenoids by the following procedure. A glass column was packed with a hexane slurry of 14 g of high-purity Merck silica gel (grade 60, 230−400 mesh, 60 Å, Aldrich Co., Milwaukee, WI, activated overnight at 110 °C), followed by a hexane slurry of 4 g of Fuller's earth (100−200 mesh, Sigma Chemical Co., St. Louis, MO), and then was washed with hexane. A 3.0 g sample of oil was passed through the column and eluted with 200 mL of hexane, followed by 100 mL of 10% ethyl ether in hexane (v/v). The chromatographed oil recovered in 98% yield was not significantly changed in fatty acid composition, with a DHA concentration of 41.7%, and contained 258 ppm total tocopherols (29 ppm α-, 171 ppm γ-, and 58 ppm δ-tocopherol) and no measurable carotenoids.

Preparations of Emulsions. Emulsions containing 5% oil and 1% lecithin were prepared by a previously described procedure (*34*) using a phosphate−citrate buffer (25 mM, pH 6.6).

Oxidation. Oxidation of oils (5 g) and emulsions (30 mL) was carried out in stoppered 25 mL Erlenmeyer flasks at 40 °C in a shaker oven (Lab-Line Instrument, Inc., Melrose Park, IL). Two oil samples (algae-3 and fish-1) were also oxidized at 50 and 60 °C. Oxidative stability of the oils and emulsions was determined by measuring peroxide values colorimetrically, and propanal was determined by static headspace gas chromatography. Peroxide values were determined by the ferric thiocyanate method (*35*) modified for safety reasons by using chloroform:methanol (3:1, v/v) instead of benzene:methanol as solvent.

Table 2. Increase in Peroxide Values and Propanal Content in Long-Chain Polyunsaturated Oils Oxidized at 40 °C

|	peroxide value (mequiv/kg)			propanal (mmol/kg)		
	hours					
sample	48	72	96	48	72	96
algae-3	1.0	1.3	1.6	0	0.007	0.028
fish-3	3.0	11.3	22.9	0	0.022	0.041
sand eel	19.7	55.4		0.040	0.097	0.180
salmon	27.3	62.2		0.013	0.051	0.080
mackerel	34.9	148.1		0.068	0.741	1.804
algae-2	38.7	137.9		0.031	0.139	0.622
tuna	53.9			0.100	0.185	0.561
fish-2	58.8	163.2	287.9	0.010	0.063	0.131
fish-1	98.3	242.9	343.6	0.015	0.060	0.159
algae-1	117.9	270.4		0.082	0.285	1.584
algae-3[a]	151.4			0.031	0.112	

[a] Chromatographically purified to remove tocopherols (87%) and other antioxidants (see Materials and Methods).

Measurements of propanal by static headspace gas chromatography (*29*) were carried out by weighing aliquots of either oils (0.10 g) or emulsions (1.0 mL) into 22 mL headspace vials, sealing the vials, and equilibrating the samples at 60 °C for 15 min in an HS-40 headspace autosampler. An aliquot of the headspace was then injected in an autosystem gas chromatograph (Perkin-Elmer, Norwalk, CT) equipped with a DB-1701 column, 30 m long, 0.32 mm i.d., and 1 μm film thickness (J&W Scientific, Folsom, CA). The injector and detector temperatures were 180 and 200 °C, respectively. The oven temperature was programmed at 30 °C for 4 min, followed by an increase to 80 °C at a rate of 10 °C/min. Propanal was quantified by using standard propanal solutions of known concentrations. All experiments and analyses were carried out in duplicate. Standard deviations averaged 3.5 mequiv/kg for peroxide values in oils, 2.7 mequiv/kg in emulsions, and 0.015 mmol/kg for propanal in oils and emulsion.

RESULTS

Oxidative Stability of Bulk Oils. The fatty acid compositions of the fish- and algae-derived oils varied in total PUFA content from 30.9 to 64.4%, eicosapentaenoic acid (EPA) from 5.1 to 12.7%, and DHA from 10.5 to 52.4%; total tocopherols varied from 11 to 1937 ppm (**Table 1**). The carotenoids in the algal oils varied from 577 to 2823 ppm. As expected from composition differences alone, the oxidative stability of these oils varied widely. The relative stability of DHA oils, measured as peroxide value after oxidation for 48 h at 40 °C, decreased in the order algae-3 > fish-3 > sand eel > salmon > mackerel > algae-2 > tuna > fish-2 > fish-1 > algae-1 ≫ chromatographed algae-3 (**Table 2**). When the algae-3 oil was purified chromatographically with silicic acid and Fuller's earth to remove tocopherols (87%) and other antioxidants, its stability was drastically reduced and developed a peroxide value of 151 after oxidation for 48 h as compared with 1.0 for the original oil. The relatively high oxidative stability of algae oil-3 can be attributed therefore to its high tocopherol content of 1937 ppm and the presence of 0.025% ascorbyl palmitate, which may behave synergistically in reinforcing the antioxidant activity of tocopherols. On the other hand, the larger amount of total tocopherols in algae-1 than in algae-2 did not effectively stabilize these oils in the presence of high concentrations of carotenoids. The higher concentration of carotenoids in algae-1 than algae-2 may have a prooxidant effect by lowering the relative stability of algae-1.

A different order of stability was observed on the basis of propanal analyses after 72 h of oxidation, decreasing in the order algae-3 > fish-3 > salmon > fish-1 ~ fish-2 > sand eel > chromatographed algae-3 > algae-2 > tuna > algae-1 >
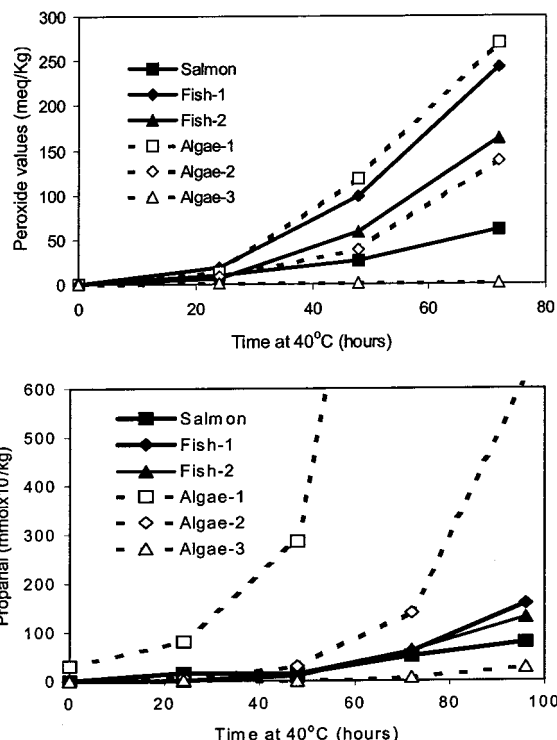
Figure 1. Oxidative stability of long-chain polyunsaturated fish and algal oils. Top panel, peroxide values; bottom panel, propanal values.

Table 3. Increase in Peroxide Values and Propanal Content in Long-Chain Polyunsaturated Oils Oxidized at Different Temperatures

		peroxide value (mequiv/kg)			propanal (mmol/kg)		
	temp	hours					
sample	(°C)	48	72	96	48	72	96
algae-3	40	1.0	1.3	1.6	0.003	0.000	0.007
	50	1.2	2.2	3.8	0.000	0.073	0.145
	60	2.6	6.0		0.000	0.239	0.412
fish-1	40	3.0	11.3	22.9	0.000	0.000	0.022
	50	6.3	26.1	80.0	0.000	0.086	0.516
	60	131.3	196.4		0.000	1.517	2.258

mackerel (**Table 2**). Propanal formation was significantly higher in algae-1 and algae-2 oils than in the salmon and fish oil-1 and fish oil-2 (**Figure 1**). The low propanal values in algae-3 oil agreed with the corresponding peroxide values obtained for this oil. These differences in relative stability may be due not only to differences in fatty acid composition but also to the relative amounts and types of tocopherols and/or the presence of trace metals. Algae-1 oil was much less stable than algae-2 and algae-3 oils. This difference in oxidative stability may be due to its much higher content of total carotenoids that may have prooxidant activity. Thus, β-carotene acted as a prooxidant by promoting oxidation of soybean oil chromatographed to remove the natural tocopherols (*36*).

The increase in oxidation temperature between 40 and 60 °C had a significantly greater effect on the oxidative stability of the fish-1 oil than the algae-3 oil (**Table 3**). Between 40 and 50 °C, after oxidation for 48 h, the peroxide values increased 2.1-fold in the fish oil and 1.2-fold in the algal oil. Between 50 and 60 °C, after oxidation for 48 h, the peroxide value increased 20.8-fold in the fish oil and 2.2-fold in the algal oil. This difference in effect of temperature may be attributed to the increase in activation energy caused by the higher concentration of antioxidants in algae-3 oil (*18*).
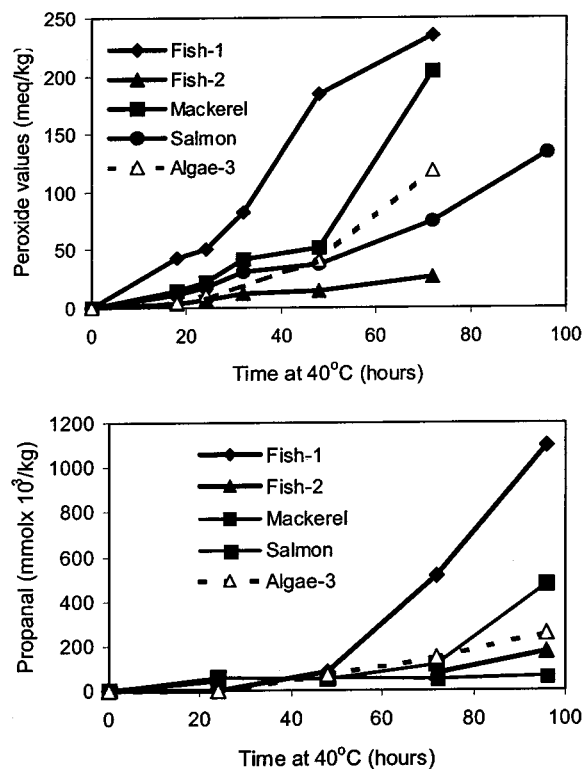
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Figure 2. Oxidative stability of long-chain polyunsaturated fish and algal oil emulsions. Top panel, peroxide values; bottom panel, propanal values.
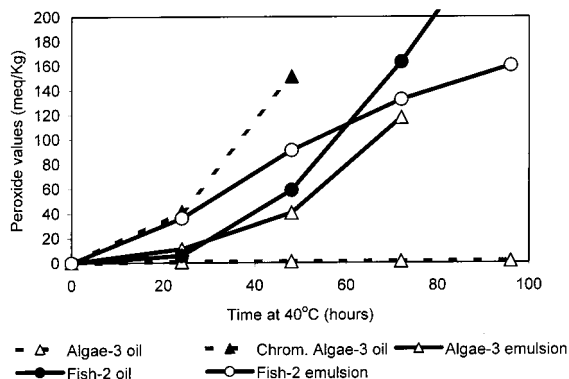

Figure 3. Oxidative stability of algae-3 oil, chromatographed algae-3 oil, algae-3 emulsion, fish-2 oil, and fish-2 emulsion at 40 °C.

Table 4. Increase in Peroxide Values in Long-Chain Polyunsaturated Oil-in-Water Emulsions in Lecithin at 40 °C

| | peroxide value (mequiv/kg) | | | |
| | hours | | | |
emulsion	24	48	72	96
fish-3	6.6	14.6	26.5	
algae-3	11.2	40.5		
salmon	17.4	37.4	74.5	134.0
sand eel	16.6	41.2	51.0	
mackerel	21.6	52.1	203.6	
fish-2	36.1	91.3	132.4	160.2
fish-1	50.1	183.7	234.4	

Table 5. Effect of Iron and EDTA on Increase in Peroxide Values of Oil-in-Water Emulsions Containing Long-Chain Polyunsaturated Oils[a]

| | peroxide value (mequiv/kg) | | | | |
| | hours | | | | |
emulsified oil	18	24	42	48	72
algae-3	3.9	11.2	36.6	40.5	90.9
+ 100 μM Fe	6.4	13.0	37.2	46.9	89.4
+ 200 μM Fe	19.1	20.1	44.6	55.8	104.1
+ 100 μM EDTA	0.0	0.0	0.0	0.2	1.6
+ 100 μM Fe + 100 μM EDTA	18.7	37.0	72.1	152.9	212.9
+ 200 μM Fe + 100 μM EDTA	25.3	46.0	87.7	157.6	226.4
fish-2	22.9	36.1	45.6	91.3	132.4
+ 100 μM Fe	37.4	49.8	59.6	108.5	157.1
+ 200 μM Fe	61.3	60.8	64.2	126.6	181.2
+ 100 μM EDTA	0.5	0.0	0.0	0.9	5.3
+ 100 μM Fe + 100 μM EDTA	27.5	54.6	70.0	168.9	246.3
+ 200 μM Fe + 100 μM EDTA	74.7	86.8	76.9	247.2	296.4
mackerel	14.7	21.6	41.7	52.1	203.6
+ 100 μM Fe	32.1	32.5	61.2	62.8	234.3
+ 100 μM EDTA	0.0	0.2	1.0	1.0	2.9
+ 100 μM Fe + 100 μM EDTA	39.2	61.3	84.5	253.0	327.7
+ 50 μM Fe + 100 μM EDTA	1.9	2.9	5.9	6.2	13.2
+ 25 μM Fe + 100 μM EDTA	0.1	1.0	2.9	2.9	7.0

[a] Oil-in-water emulsions prepared with lecithin as emulsifier (see Materials and Methods).

the corresponding oil. The emulsion of fish-2 oil was less stable during the first 48 h than was the corresponding fish oil. After 48 h, the fish oil emulsion had a smaller increase in peroxide value than the corresponding fish oil.

The effects of iron and EDTA were tested in several emulsions prepared with algae-3 and two fish oils (**Table 5**). Added iron decreased the stability of the fish oil emulsions much more significantly than that of the algal oil emulsion. This difference may be due to a synergistic effect of the mixture of tocopherols and ascorbyl palmitate present in the algal oil. In the absence of added iron, 100 μM EDTA almost completely inhibited the oxidation of all three emulsions. However, in the presence of 100 or 200 μM of Fe^{2+}, EDTA significantly promoted the oxidation of these emulsions as compared with the emulsions containing the same concentrations of iron without EDTA. On the other hand, EDTA effectively inhibited oxidation of these emulsions at higher molar ratios of EDTA to iron (2:1 and 4:1). Therefore, EDTA is an effective iron chelator in these emulsions when added at equivalent or higher molar concentrations than those of iron.

DISCUSSION

The extensive literature on oxidative stability evaluations of different polyunsaturated fats shows a wide variation of results that are difficult to interpret. Such inconsistent results may be due to changes in oxidation conditions, to the application of

Stability of Oil-in-Water Emulsions.
Oil-in-water emulsions were prepared with 5 wt % of the same long-chain PUFA-containing fish oils and DHA-containing algal oils described above and 1% soybean lecithin as emulsifier in phosphate−citrate buffer at pH 6.6. The oxidative stability of these emulsions was significantly lower during the first 48 h than that of the corresponding oils (**Table 4** and **Figure 2**). After oxidation for 48 h at 40 °C, the peroxide values decreased in the order fish-3 > algae-3 > salmon > sand eel > mackerel > fish-2 > fish-1. The emulsion of algae-3 oil increased 40-fold in peroxide value after 48 h oxidation as compared with the corresponding oil. The relative effect of removing added antioxidants and altering the lipid system used is shown by comparing the oxidative stability of algae-3 oil before and after chromatography and comparing the stability of the algal and fish oils with that of the corresponding oil-in-water emulsions (**Figure 3**). The greater stability of algae-3 oil was completely lost after chromatographic removal of antioxidants and carotenoids. The emulsion of algae-3 oil was much less stable than

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analytical methods that measure different endpoints of lipid oxidation, and to the unreliable methodology used to determine stability. Many stability methods accelerate oxidation under conditions that are too drastic, and many methods employed to determine the extent of oxidation are unspecific and not sufficiently sensitive to be relevant to flavor deterioration. The current interest in using long-chain polyunsaturated oils, including DHA-containing oils, in infant foods (*30*) demands improved evaluations by employing more valid stability tests and endpoints of quality deterioration (*17, 18*). Natural antioxidants have been particularly difficult to evaluate in oils and food emulsions due in part to the complex interfacial phenomena involved (*18*). In multiphase food systems, physical properties and partitioning between the aqueous and the lipid phases and the interface are important factors that affect antioxidant activity (*37*). The application of several complementary methods was recommended to determine different lipid oxidation products, including methods to measure the primary hydroperoxides and their decomposition aldehyde products (*18*).

In the present study, a standard peroxide value was used as a convenient assay of hydroperoxides formed under mild oxidation temperatures (40–60 °C). Static headspace gas chromatography was used to analyze propanal, as a specific marker for the oxidation of *n*-3 PUFA (*29*). Oxidized fish oils, rich in *n*-3 PUFA, were previously shown to produce volatile compounds more readily than oxidized vegetable oils, rich in linoleic acid (*29*). Thus, the activation energy for the formation of propanal from fish oils was lower than for the formation of hexanal from vegetable oils. Different trends observed in oxidative stability based on measurement of peroxide values as compared with analysis of headspace propanal (**Table 2**) may be attributed to the wide range in fatty acid composition and in the concentrations of tocopherols and carotenoids (**Table 1**).

DHA-rich oils from algae were claimed to have an unusually high oxidative stability (*38*), but the evidence for the basis of this stability may be questionable. Claims for high stability of DHA-rich oils based on the Rancimat are subject to serious pitfalls (*17, 18*). The use of conductivity detection in the Rancimat method is neither sufficiently sensitive nor specific for measurement of oxidative decomposition products of *n*-3 PUFA responsible for undesirable fishy flavor development (*29*). The claim that triglycerides containing DHA are unusually stable (*38*) is invalidated in the present study by evidence that the high oxidative stability of the commercial algae-3 oil is lost when the triglycerides are purified to remove tocopherols (87%) and other antioxidants (**Table 2**, **Figure 3**). The oil-in-water emulsion prepared from the same commercial DHA-containing algal oil had a much lower oxidative stability than the corresponding bulk oils (**Figure 3**, **Table 4**). The significant decrease in oxidative stability in corresponding emulsions is in marked contrast to the evaluation in bulk oil systems and further invalidates the claim that DHA triglycerides are unusually stable to oxidation (*38*). This decrease in stability may be explained by the partitioning of ascorbyl palmitate into the water phase. A related decrease in oxidative stability by the addition of ascorbic acid to fish oil-containing mayonnaise was reported (*39*). The emulsion prepared from fish-2 oil was less stable than the corresponding fish oil only during the first 48 h of oxidation (**Figure 3**).

A mechanism was suggested for the oxidative deterioration of fish oil mayonnaise that involved the disruption of iron bridges between egg yolk proteins into the aqueous phase (*39*). The iron released from this break catalyzes the decomposition of lipid hydroperoxides located either at the oil–water interface or in the aqueous phase where oxidation is initiated with the formation of potent flavor volatiles with low threshold values (*27*). In this fish oil mayonnaise, metal chelation is evidently a more effective means of preventing oxidation of long-chain PUFA than free radical scavenging phenolic antioxidants.

In the present study, EDTA effectively stabilized emulsified fish and algal oils by chelation of iron either initially present in traces or if added at lower than equimolar concentrations (**Table 5**). At higher iron:EDTA molar ratios, however, EDTA actively promoted the oxidation of these long-chain PUFA-containing emulsions. The concentration effect is thus a particularly important factor in the application of EDTA and perhaps other metal chelating agents in stabilizing food emulsions. The study of other metal chelators is needed to develop natural additives to stabilize emulsions and foods containing long-chain PUFA including DHA oils, especially if they are fortified with iron to meet dietary nutritional requirements.

ABBREVIATIONS USED

Docosahexaenoic acid, DHA; ethylenediaminetetraacetic acid, EDTA; eicosapentaenioc acid, EPA; polyunsaturated fatty acids, PUFA.

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