Photo-oxidation of Lipids Impregnated on the Surface of Dried Seaweed (*Porphyra yezoensis* Ueda). Hydroperoxide Distribution

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ABSTRACT: The distribution of hydroperoxide isomers generated by photo-oxidation of natural lipids impregnated on the surface of dried seaweed previously exposed to visible light and without added photosensitizer were studied. The surface of dried seaweed was impregnated with linoleic acid methyl ester, and the sample was divided into two parts. One part was exposed to light from a 100-W tungsten bulb (4500 lux) in a low-temperature room (5°C). The other part was kept in the dark as a control. Positional isomers of the hydroperoxides generated from the impregnated linoleic acid methyl ester were separated individually by HPLC and further identified by MS. The dried seaweed kept in the dark contained four hydroperoxide isomers, namely, 13-hydroperoxy-*cis*-9,*trans*-11-octadecadienoate, 13-hydroperoxy-*trans*-9,*trans*-11-octadecadienoate, 9-hydroperoxy-*trans*-10,*cis*-12-octadecadienoate, and 9-hydroperoxy-*trans*-10,*trans*-12-octadecadienoate. For the dried seaweed exposed to light, the oxidized lipids contained not only the same four isomers, but also 12-hydroperoxy-*cis*-9,*trans*-13-octadecadienoate and 10-hydroperoxy-*trans*-8,*cis*-12-octadecadienoate. When fresh seaweed was dried in the sunlight, the formation of 12-*cis,trans*- and 10-*cis,trans*-hydroperoxides of naturally occurring methyl linoleate was verified. Dried seaweed was then impregnated with eicosapentaenoic acid ethyl ester and exposed to light. Light exposure also generated certain hydroperoxide isomers attributable to singlet oxygen oxidation, namely, 6-hydroperoxy-*trans*-4,*cis*-8,*cis*-11,*cis*-14,*cis*-17-ethyl and 17-hydroperoxy-*cis*-5,*cis*-8,*cis*-11,*cis*-14,*trans*-18-ethyl eicosapentaenoate. When dried seaweed without any impregnated lipids was exposed to the light for 24 h in a cold room (5°C), characteristic isomers, including both the 20-carbon FA isomers 6-OOH and 17-OOH as well as the 18-carbon FA isomers 10-OOH and 12-OOH, were detected in the light-exposed sample but were not found in the control. These results clearly show that singlet oxygen oxidation of lipids occurred in the seaweed exposed to light. We concluded that this lipid oxidation was catalyzed by chlorophyll as a photosensitizer in seaweed.

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KEY WORDS: Autoxidation, eicosapentaenoic acid ethyl ester, hydroperoxide, linoleic acid methyl ester, lipids, photosensitized oxidation, singlet oxygen.

Lipid oxidation affects many properties of foods, including flavor, taste, color, texture, and nutrients, and it is a decisive factor in the useful processing or storage of food products (1–3). Photosensitized oxidation of lipids generates allylic hydroperoxides from unsaturated lipids that coexist with oxygen and a photosensitizer (4). Studies on photosensitized oxidation usually have been performed on unrealistic model systems consisting of authentic compounds such as methylene blue and purified chlorophyll as photosensitizers and purified unsaturated lipids in a bulk oil system (4-7). Photosensitized oxidation during food processing and preservation has been verified in terms of the accelerated reaction rate of lipid oxidation in photoirradiated foods and oils compared with those kept in the dark (8). It is usually difficult to distinguish singlet oxygen oxidation from free radical oxidation in complex food systems since hydroperoxides decompose easily in the presence of metals, metmyoglobin, or hematin (9). Therefore, detailed information on the distribution of positional isomers of hydroperoxides that could distinguish photosensitized oxidation from traditional autoxidation in food systems could be very meaningful. Since seafood is rich in PUFA that are labile to oxidation, lipid oxidation is an important form of deterioration of seafood quality even when the lipid content is very low. The reaction rate of singlet oxygen oxidation of linoleic acid is at least 1500 times faster than that of common triplet oxygen (10). It is therefore very important to evaluate the effects of photosensitized oxidation of lipids on food quality. The aim of this study was to obtain exact evidence of photosensitized oxidation of unsaturated lipids in a complex food system. Certain species of edible seaweed rich in chlorophyll were used in the present study as a model food to be photosensitized. Detailed distributions of positional isomers of the hydroperoxides generated in the photosensitized dried seaweed were compared with those formed by free radical oxidation.

MATERIALS AND METHODS

Materials. *cis*-9,*cis*-12-Octadecadienoic acid methyl ester [linoleic acid (LA) methyl ester] of >99% purity was purchased from Sigma (St. Louis, MO). All-*cis*-5,8,11,14,17-eicosapentaenoic acid ethyl ester (EPA ethyl ester) of >92% purity was obtained from Mochida Pharmaceutical Co. Ltd. (Tokyo, Japan) in the form of Epadels® edible gelatin capsules of EPA ethyl ester, and purified with Sep-pak® silica cartridges (Waters Co., Milford, MA) to eliminate antioxidants before use. Fresh seaweed (*Porphyra yezoensis* Ueda) was harvested

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at Kisarazu in Chiba Prefecture. Mechanically dried *P. yezoensis* seaweed was purchased from a local retailer.

Analysis of lipid classes. Total lipids (TL) of ground, dried seaweed were extracted and purified according to the method of Bligh and Dyer (11), and the lipid content was determined gravimetrically. Lipid classes were determined by Iatroscan TLC-FID, using a model MK-5 Iatroscan thin-layer chromatograph with an FID (Iatron Lab. Inc., Tokyo, Japan). Briefly, a 1-μL aliquot of chloroform solution of TL was spotted on a Chromarod-S III (Iatron Lab. Inc.) and developed in two different solvent systems, i.e., *n*-hexane/diethyl ether/formic acid (97:3:1, by vol) for the separation of neutral lipids (12) and chloroform/methanol/water/acetic acid (70:35:3.5:0.5, by vol) for polar lipids (13). The hydrogen pressure was 0.8 kg/cm^2, and the scan speed was set at 30 s/scan. The peak areas were integrated with an Iatrocorder TC-II (Iatron Lab. Inc.).

Analysis of FA compositions. An aliquot (*ca.* 50 mg) of TL was saponified with 1 M KOH in 95% ethanol and subsequently methylated with 14% BF_3 in methanol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) to obtain the corresponding FAME. Quantitative analysis of the FAME was carried out by GLC using a Shimadzu model 15A PF GC instrument (Kyoto, Japan) equipped with a SUPELCOWAX-10™ fused-silica open tubular capillary column (0.25 mm i.d. × 30 m, 0.25 μm film thickness; Supelco Japan, Tokyo, Japan) and an FID. Helium was used as a carrier gas with a column inlet pressure of 2 kg/cm^2. The column temperature was held at 150°C for 1 min and then programmed to 240°C at a rate of 1°C/min. Injector and detector temperatures were set isothermally at 250°C.

Photosensitized oxidation of lipids impregnated on the surface of seaweed. A 200-mg portion of FA esters was impregnated on the surface of 40 g of dried seaweed by dissolving the lipids in 300 mL of 95% ethanol. The ethanol was then evaporated at 30°C in the dark. The seaweed treated with FA esters was divided evenly into two parts. One part was exposed to a 100-W tungsten light source (4500 lux) in a cold room (5°C) through a 3-cm layer of water to filter out IR radiation (5–7). The other part was kept in the dark at the same temperature as a control. At appropriate intervals, a 4-g portion of seaweed was sampled and subjected to lipid extraction with 75 mL of *n*-hexane. The *n*-hexane solution of lipids was filtered with a membrane filter (0.2 μm; Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and subsequently concentrated to *ca.* 5 mL. A 20-μL portion of the solution was injected into a high-performance liquid chromatograph to analyze the hydroperoxide isomers (5).

Determination of PV. The PV of the oxidized lipids was determined photometrically by the ferric thiocyanate method (14).

Determination of hydroperoxide isomers. Positional isomers of the hydroperoxides were analyzed by a high-performance liquid chromatograph equipped with a postcolumn diphenyl-1-pyrenylphosphine (DPPP) fluorescence detection system as described previously (5,15). Briefly, the hydroperoxide isomers were separated through a Supelcosil LC-Si silica column (2.1 mm i.d. × 250 mm, 5 μm; Supelco, Bellefonte, PA) using a mixture of 500 mL of *n*-hexane and 30 mL of diethyl ether as a mobile phase, with a flow rate of 0.6 mL/min. The eluate from the column was passed through a Shimadzu model SPD-10A UV spectrophotometric detector to monitor conjugated dienes at 234 nm and then mixed with a DPPP solution (3 mg in a mixture of 200 mL of 1-butanol and 200 mL of methanol) pumped by a Shimadzu model LC-9A HPLC pump with a flow rate of 0.3 mL/min to form DPPP oxide in a postcolumn reaction coil held at 89°C. The fluorescence intensity of the DPPP oxide was monitored at an emission wavelength of 380 nm and an excitation wavelength of 352 nm using a Shimadzu model RF 535 fluorescence spectrophotometer.

The hydroperoxide isomers were identified by GC–MS as described previously (5,15). Briefly, the fractionated hydroperoxide was reduced to the corresponding hydroxy ester by the addition of $NaBH_4$, hydrogenated, and subsequently converted to the trimethylsilyl (TMS) ether derivative. The TMS derivative was separated by GC using a Shimadzu model 17A GC instrument equipped with a SUPELCOWAX-10 fused-silica capillary column (0.25 mm i.d. × 25 m, 0.25 μm), and the outlet of the column was directly connected to a Shimadzu model QP 5000 mass spectrometer. The column temperature was programmed from 150 to 180°C at a rate of 5°C/min, and then from 180 to 240°C at a rate of 2°C/min. The sample injection port temperature was 250°C, and helium was used as a carrier gas.

Photo-oxidation of lipids of seaweed without impregnation. A 20-g portion of the commercially available dried seaweed without added lipids was exposed to light for 24 h as already described, and TL of the dried seaweed were extracted and purified in a cold room (5°C) according to the method of Bligh and Dyer (11). The control sample was kept in the dark at the same temperature. Hydroperoxides in TG of the TL were reduced to the corresponding hydroxy lipids by the addition of $NaBH_4$ and were then hydrogenated to saturated lipids. The hydroxy saturated lipids were saponified with 1 M KOH in 95% ethanol and subsequently methylated with 14% BF_3 in methanol to obtain the corresponding hydroxy FA methyl ester, which was separated with silica gel 60 thin-layer plates (20 cm × 20 cm; Merck, Darmstadt, Germany). The separated hydroxy FA methyl ester was converted to the TMS ether derivative and identified by GC–MS.

RESULTS AND DISCUSSION

Lipid classes and FA composition of the dried seaweed sample without impregnation. The contents of TL were 850 mg/100 g dried seaweed. Phospholipids were predominant, accounting for 75% of TL. PE (59% of TL), sphingomyelin (9.1% of TL), and PC (7.3% of TL) were the dominant lipid classes. The neutral lipids (24.6% of TL) consisted mainly of FFA (8.9% of TL), TG (2.2% of TL), and sterol esters (13.6% of TL).

FA compositions of the dried seaweed are shown in Table 1. The predominant FA were 20:5n-3 (55.5%) and 16:0 (25.1%). The amounts of unsaturated FA and saturated FA were 70.8 and 29.2%, respectively. Although the EPA content in the seaweed was relatively high, it seemed necessary to impregnate some authentic lipids on the surface of the seaweed to eliminate contamination of the complicated hydroperoxide isomers generated from the original seaweed lipids. Based on the FA compositions of the dried seaweed, the EPA ethyl ester and the LA methyl ester were chosen as authentic lipids to be impregnated on the dried seaweed.

Changes in PV. PV of the added LA methyl ester recovered from the dried seaweed increased with an increase in the duration of light exposure, as shown in Figure 1. In contrast, the PV of the control sample kept in the dark at the same temperature remained almost unchanged. A similar tendency was observed in the case of the dried seaweed impregnated with the EPA ethyl ester and exposed to light; the PV of the light-exposed samples showed markedly higher values compared with those of the control samples, as shown in Figure 2. These results strongly suggest that photosensitized oxidation of lipids proceeded rapidly in the light-exposed seaweed.

Isomeric hydroperoxides of the LA methyl ester. After exposure to light for 1.5 h, five peaks in the recovered lipid FA attributable to hydroperoxide isomers were partially separated from each other by fluorescence detection, as shown in Figure 3A. With an increase in the duration of light exposure, the relative ratios of peaks 2 and 5 became larger, as shown on the chromatogram of the sample irradiated with light for 4.5 h (Fig. 3C). The components of peaks 2 and 5 had no UV absorption at 234 nm attributable to conjugated dienes, as shown in Figures 3B and 3D.

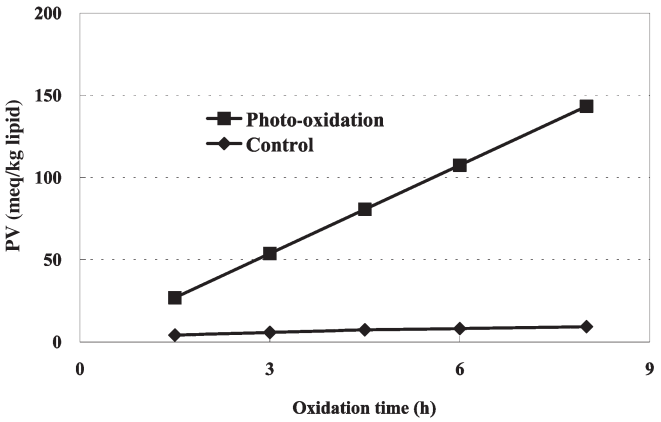

FIG. 1. Changes in PV of impregnated linoleic acid methyl ester recovered from dried seaweed. Oxidation was carried out at 5°C. (■) Photo-irradiated dried seaweed; (◆) control dried seaweed kept in the dark.

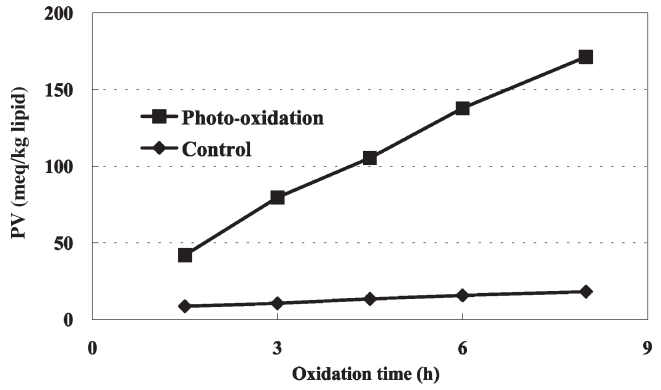

FIG. 2. Changes in PV of impregnated eicosapentaenoic acid ethyl ester recovered from dried seaweed. Oxidation was carried out at 5°C. (■) Photo-irradiated dried seaweed; (◆) control dried seaweed kept in the dark.

TABLE 1
FA Compositions of Dried Seaweed Lipids (mg/100 g)[a]

FA	Amount (mg)	Percent (%)
14:0	1.38	0.24
15:0	1.64	0.29
16:0	143.37	25.08
7-Me 16:0	16.00	2.80
Phytanic + ?	1.26	0.22
18:0	3.13	0.55
18:1n-9	7.71	1.35
18:1n-5	3.35	0.59
18:2n-6	9.17	1.60
18:4n-3	1.41	0.25
20:1n-9	16.64	2.91
20:2n-6	5.20	0.91
20:3n-9	11.77	2.06
20:4n-6	21.26	3.72
20:4n-3	5.04	0.88
20:5n-3	317.08	55.47
22:1n-9	2.36	0.41
22:2n-6	3.84	0.67
TFA	571.60	
TSFA	166.91	29.20
TUFA	404.69	70.80
Total lipids	850.00	

[a]TUFA, total unsaturated FA; TSFA, total saturated FA; TFA, total FA.

MS of the fractionated isomers confirmed the following identification of the positional isomers by comparison with the previous results (5): peak 1, 13-hydroperoxy-*cis*-9,*trans*-11-octadecadienoate (13-*cis,trans*-18:2-OOH); peak 2, 12-hydroperoxy-*cis*-9,*trans*-13-octadecadienoate (12-*cis,trans*-18:2-OOH); peak 3, 13-hydroperoxy-*trans*-9,*trans*-11-octadecadienoate (13-*trans,trans*-18:2-OOH); peak 4, 9-hydroperoxy-*trans*-10,*cis*-12-octadecadienoate (9-*cis,trans*-18:2-OOH); peak 5, a mixture of 10-hydroperoxy-*trans*-8,*cis*-12-octadecadienoate (10-*cis,trans*-18:2-OOH) and 9-hydroperoxy-*trans*-10,*trans*-12-octadecadienoate (9-*trans,trans*-18:2-OOH) (data not shown).

Because it was difficult to find an appropriate hydroperoxide isomer as an internal standard compound, changes in the amounts of isomers were expressed as percentages of composition in this study. For up to 8 h of light exposure, the area ratios of peaks 2 and 5, containing nonconjugated hydroperoxide isomers, i.e., 12-*cis,trans*-18:2-OOH and 10-*cis,trans*-18:2-OOH, in the photo-irradiated sample increased gradually, as shown in Figure 4. In contrast, the control samples, which were kept in the dark, remained almost unchanged in the peak area ratios of hydroperoxide isomers.

These results revealed that 12-*cis,trans*-18:2-OOH and 10-*cis,trans*-18:2-OOH, which are both characteristic hydroper-

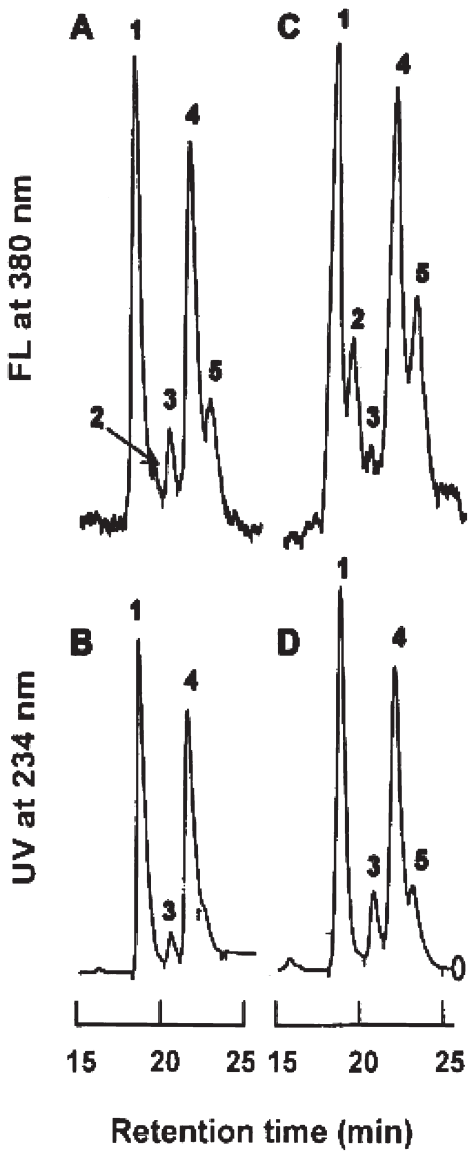

FIG. 3. Typical HPLC chromatograms of isomeric hydroperoxides of impregnated linoleic acid methyl ester recovered from dried seaweed. Detected by fluorescence (FL) after (A) 1.5 and (C) 4.5 h of oxidation time, respectively; detected by UV after (B) 1.5 and (D) 4.5 h of oxidation time, respectively. Peak 1, 13-*cis,trans*-18:2-OOH; peak 2, 12-*cis,trans*-18:2-OOH; peak 3, 13-*trans,trans*-18:2-OOH; peak 4, 9-*cis,trans*-18:2-OOH; peak 5, a mixture of 10-*cis,trans*-18:2-OOH and 9-*trans,trans*-18:2-OOH.

oxide isomers generated only by $^1O_2$-mediated oxidation of the LA methyl ester, were produced and increased in the amounts accumulated during exposure to light for up to 8 h.

Isomeric hydroperoxides of EPA ethyl ester. A typical HPLC chromatogram of hydroperoxide isomers recovered from the light-exposed dried seaweed treated with the EPA ethyl ester (Fig. 5A) showed eight partially separated peaks when the peak components were monitored with the fluorescence detection system. The results of UV light detection of the eluate (Fig. 5B) revealed that the components of peaks 5 and 8 did not show UV absorption at 234 nm, again suggesting that these hydroperoxides were

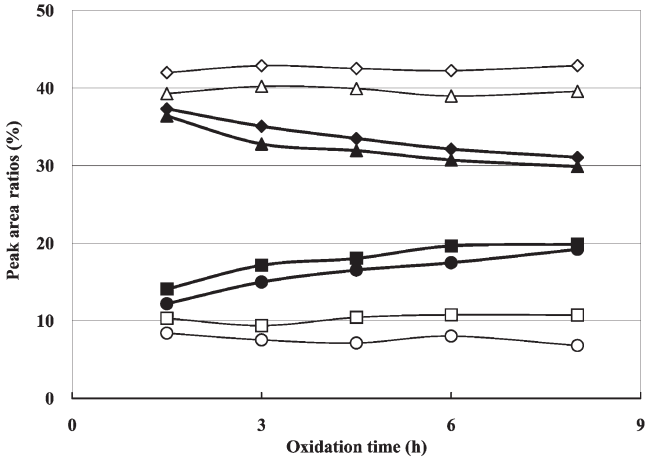

FIG. 4. Changes in isomeric hydroperoxide compositions of impregnated linoleic acid methyl ester recovered from dried seaweed. Oxidation was carried out at 5°C. (◇) 13-*cis,trans*-18:2-OOH from the control; (○) 13-*trans,trans*-18:2-OOH from the control; (△) 9-*cis,trans*-18:2-OOH from the control; (□) 9-*trans,trans*-18:2-OOH from the control; (◆) 13-*cis,trans*-18:2-OOH from the light-exposed sample; (●) 13-*trans,trans*-/12-*cis,trans*-18:2-OOH from the light-exposed sample; (▲) 9-*cis,trans*-18:2-OOH from the light-exposed sample; (■) 9-*trans,trans*-/10-*cis,trans*-18:2-OOH from the light-exposed sample.

nonconjugated dienes. MS confirmed the identification of the positional isomers as follows: peak 1, 15-hydroperoxy-*cis*-5,*cis*-8,*cis*-11,*trans*-13,*cis*-17-eicosapentaenoate (15-*cis,trans*-20:5-OOH); peak 2, 18-hydroperoxy-*cis*-5,*cis*-8,*cis*-11,*cis*-14,*trans*-16-eicosapentaenoate (18-*cis,trans*-20:5-OOH); peak 3, a mixture of 12-hydroperoxy-*cis*-5,*cis*-8,*trans*-10,*cis*-14,*cis*-17-eicosapentaenoate (12-*cis,trans*-20:5-OOH) and 14-hydroperoxy-*cis*-5,*cis*-8,*cis*-11,*trans*-15,*cis*-17-eicosapentaenoate (14-*cis,trans*-20:5-OOH); peak 4, 11-hydroperoxy-*cis*-5,*cis*-8,*trans*-12,*cis*-14,*cis*-17-eicosapentaenoate (11-*cis,trans*-20:5-OOH); peak 5, 17-hydroperoxy-*cis*-5,*cis*-8,*cis*-11,*cis*-14,*trans*-18-eicosapentaenoate (17-*cis,trans*-20:5-OOH); peak 6, a mixture of 8-hydroperoxy-*cis*-5,*trans*-9,*cis*-11,*cis*-14,*cis*-17-eicosapentaenoate (8-*cis,trans*-20:5-OOH) and 9-hydroperoxy-*cis*-5, *trans*-7,*cis*-11,*cis*-14,*cis*-17-eicosapentaenoate (9-*cis,trans*-20:5-OOH); peak 7, 5-hydroperoxy-*trans*-6,*cis*-8,*cis*-11,*cis*-14,*cis*-17-eicosapentaenoate (5-*cis,trans*-20:5-OOH); peak 8, 6-hydroperoxy-*trans*-4,*cis*-8,*cis*-11,*cis*-14,*cis*-17-eicosapentaenoate (6-*cis,trans*-20:5-OOH). Thus, the two missing peaks in UV detection (Fig. 5B), peaks 5 and 8, belonged to 17-*cis,trans*-20:5-OOH and 6-*cis,trans*-20:5-OOH, respectively (data not shown).

No hydroperoxide isomers in the sample kept in the dark were detected by either fluorescence or UV detection. The extremely low PV of this sample (Fig. 2) suggests that hydroperoxide formation was very slow in the control kept in the dark.

When the fresh seaweed was dried in the sunlight, the same distributions of hydroperoxide isomers were obtained as when the tungsten lightbulb was used as a source of light (data not shown).

Distributions of hydroperoxide isomers in the sun-dried seaweed without impregnation. A series of methyl trimethyl-

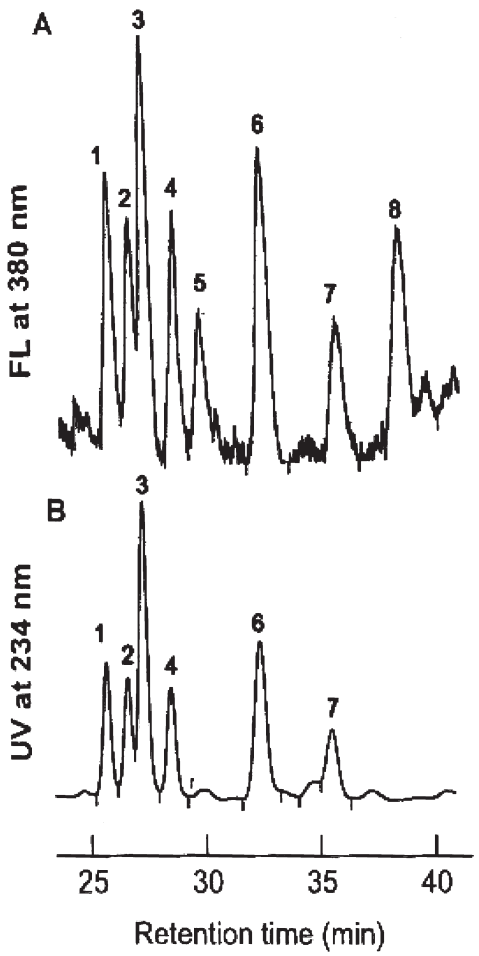

FIG. 5. Typical HPLC chromatograms of isomeric hydroperoxides from eicosapentaenoic acid ethyl ester impregnated on dried seaweed. (A) Detected by fluorescence (FL) after 1.5 h of light exposure; (B) detected by UV after 1.5 h of light exposure. Peak 1, 15-*cis,trans*-20:5-OOH; peak 2, 18-*cis,trans*-20:5-OOH; peak 3, a mixture of 12-*cis,trans*-20:5-OOH and 14-*cis,trans*-20:5-OOH; peak 4, 11-*cis,trans*-20:5-OOH; peak 5, 17-*cis,trans*-20:5-OOH; peak 6, a mixture of 8-*cis,trans*-20:5-OOH and 9-*cis,trans*-20:5-OOH; peak 7, 5-*cis,trans*-20:5-OOH; peak 8, 6-*cis,trans*-20:5-OOH.

siloxy eicosanoates corresponding to 20-carbon FA were identified in the light-exposed dried seaweed, i.e., methyl 5-trimethyl-siloxy eicosanoate (*m/z* 203 and 313), methyl 8-trimethylsiloxy eicosanoate (*m/z* 245 and 271), methyl 9-trimethylsiloxy eicosanoate (*m/z* 257 and 259), methyl 11-trimethylsiloxy eicosanoate (*m/z* 229 and 287), methyl 12-trimethylsiloxy eicosanoate (*m/z* 215 and 301), methyl 14-trimethylsiloxy eicosanoate (*m/z* 187 and 329), methyl 15-trimethylsiloxy eicosanoate (*m/z* 173 and 343), methyl 18-trimethylsiloxy eicosanoate (*m/z* 131 and 385), methyl 6-trimethylsiloxy eicosanoate (*m/z* 217 and 299), and methyl 17-trimethylsiloxy eicosanoate (*m/z* 145 and 371). The latter two isomers did not exist in the sample kept in the dark. Based on the FA compositions of the seaweed lipids, the 20-carbon unsaturated FA consisted mainly of 20:1n-9, 20:2n-6, 20:3n-9, 20:4n-6, 20:4n-3, and 20:5n-3. The theoretically produced isomeric hydroperoxides attributable to photo-oxidation and autoxidation of these 20-carbon unsaturated FA are summarized in Table 2. 6-OOH (*m/z* 217 and 299) and 17-OOH (*m/z* 145 and 371) of the 20-carbon unsaturated FA were specific hydroperoxide isomers generated by photosensitized oxidation. The 6-OOH isomer could have been generated only from photosensitized oxidation of 20:3n-9, 20:4n-6, and 20:5n-3, and the 17-OOH isomer could have been produced only by photosensitized oxidation of 20:4n-3 and 20:5n-3.

Another important group of FA in the lipids of seaweed were the 18-carbon FA. A series of methyl trimethylsiloxy octadecanoate, namely, methyl 9-trimethylsiloxy octadecanoate (*m/z* 259 and 229), methyl 13-trimethylsiloxy octadecanoate (*m/z* 315 and 173), methyl 10-trimethylsiloxy octadecanoate (*m/z* 273 and 215), and methyl 12-trimethylsiloxy octadecanoate (*m/z* 301 and 187) existed in the light-exposed dried seaweed (data not shown). The latter two were not found in the sample kept in the dark.

The 18-carbon unsaturated FA of sun-dried seaweed consisted mainly of 18:1n-9, 18:1n-5, 18:2n-6, and 18:4n-3. The theoretically produced isomeric hydroperoxides from these 18-carbon unsaturated FA by photo-oxidation and autoxidation are summarized in Table 2. The isomeric 10-OOH of 18-carbon unsaturated FA could include 18:2n-6, 18:1n-9, and 18:4n-3 generated by photosensitized oxidation as well as 18:1n-9 by autoxidation. The 10-OOH and 8-OOH should be generated from autoxidation of 18:1n-9 (27). However, no fragment ions of *m/z* 245 and 243 attributable to 8-OOH were detected, showing that 8-OOH could not have been generated by autoxidation but by photosensitized oxidation. Thus, 10-OOH would have been generated from autoxidation of 18:1n-9. In addition to these, since the amount of 18:4n-3 was small in the TL, the amounts of 10-OOH produced from photosensitized oxidation of 18:4n-3 should be negligible. A negligible amount of 10-OOH was detected in the control sample. These results strongly suggest that the main precursors of 10-OOH attributable to photosensitized oxidation were 18:2n-6 and 18:1n-9.

The 12-OOH of 18-carbon unsaturated FA could have been generated by photosensitized oxidation of 18:2n-6 and 18:4n-3 as well as by autoxidation of 18:1n-5 and 18:4n-3. One possible precursor of 12-OOH attributable to autoxidation was 18:1n-5. However, the amount of 18:1n-5 was negligible, and even 14-OOH (generated from 18:1n-5 by both photosensitized oxidation and autoxidation) could not be detected. Thus, the generation of 12-OOH from 18:1n-5 would be negligible. Another possible precursor of 12-OOH was 18:4n-3. For the same reason, the amount of 18:4n-3 would be very small, and even 16-OOH (generated from 18:4n-3 by both photosensitized oxidation and autoxidation) could not be detected. Therefore, the 12-OOH produced from 18:4n-3 also could have been neglected. Based on the above analysis, we concluded that 10-OOH and 12-OOH of 18-carbon unsaturated FA were generated mainly by the singlet oxygen oxidation of lipids in the seaweeds.

The isomeric hydroperoxides of 18- and 20-carbon FA analysis clearly showed that the singlet oxygen oxidation of

TABLE 2
Monohydroperoxide Isomers Theoretically Generated by Oxidation of FA with 20 and 18 Carbons in Seaweed and the Fragment Ions of Hydrogenated Trimethylsilyl Derivatives[a]

FA	Hydroperoxide Characteristic fragment (*m/z*)	5-OOH 203, 313	6-OOH 217, 299	8-OOH 245, 271	9-OOH 257, 259	10-OOH 243, 273	11-OOH 229, 289	12-OOH 215, 301	13-OOH 201, 315	14-OOH 187, 329	15-OOH 173, 343	17-OOH 145, 371	18-OOH 131, 385
20:1n-9						#	*,#	*,#	#				
20:2n-6							*,#	*		*	*,#		
20:3n-9		*,#	*	*,#	*,#		*	*,#					
20:4n-6		*,#	*	*,#	*,#		*,#	*,#		*	*,#		
20:4n-3							*,#	*		*,#	*,#	*	*,#
20:5n-3		*,#	*	*,#	*,#		*,#	*,#		*,#	*,#	*	*,#
FA	Hydroperoxide Characteristic fragment (*m/z*)			(8-OOH) 245, 243	9-OOH 259, 229	10-OOH 253, 215	(11-OOH) 287, 201	12-OOH 301, 187	13-OOH 315, 173	(14-OOH) 329, 159	(15-OOH) 343, 145	(16-OOH) 357, 131	
18:1n-9				#	*,#	*,#	#						
18:1n-5								#	*,#	*,#	#		
18:2n-6					*,#	*		*	*,#				
18:4n-3					*,#	*		*,#	*,#		*	*,#	

[a]Symbols: *, theoretically produced hydroperoxides by singlet oxygen oxidation; #, theoretically produced hydroperoxides by free radical oxidation; isomers in parentheses were not detected in this study.

lipids in the cells of seaweed occurred only upon exposure to light; thus, we concluded that this lipid oxidation was catalyzed by chlorophyll, which served as a photosensitizer in the seaweed. Indeed, chlorophyll serves as a photosensitizer in certain phytoplankton (16).

The isomers produced from oxidized FA are extremely unstable and easily decompose into small-M.W. volatile compounds when lipid oxidation proceeds to certain levels. Since the distribution of hydroperoxide isomers generated by photosensitized oxidation is different from that generated by free radical oxidation of the same lipids, this means the decomposition products of the hydroperoxide isomers should be different, which may lead to the special aroma of sun-dried seafood generally favored by some consumers.

The distributions of LA hydroperoxides generated by photosensitized oxidation and free radical oxidation have been compared (5). The formation of 10-OOH and 12-OOH in the photosensitized oxidation of LA methyl ester caused decreased ratios of other hydroperoxide isomers, which could also have been formed by free radical oxidation. The results of the present study clearly showed that 6-*trans,cis,cis,cis,cis*-20:5-OOH and 17-*cis,cis,cis,cis,trans*-20:5-OOH were formed only by photosensitized oxidation specifically associated with the seaweed. The distribution of hydroperoxide isomers formed by photosensitized oxidation of EPA has been reported previously (15), but the exact ratio of formation is still obscure since hydroperoxides formed from PUFA easily undergo cyclization and decomposition (17). However, differences between photosensitized oxidation and free radical oxidation in the distribution of hydroperoxides could affect the distribution of volatile compounds formed as secondary oxidation products of the hydroperoxides. That sun-dried seafood has a different odor from the mechanically dried product under dark conditions is well known. The present study suggests that photosensitized oxidation proceeds rapidly in sun-dried products and probably affords the characteristic aromas that consumers desire.

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