TABLE IV

Tocopherol Contents of Deep-Frozen Fillets and Preserved Fish Products

Sample	Tocopherols mg/100 g				Fat g/100 g
	α-T	β-T	γ-T	δ-T	
Pollack, deep-frozen	0.78	–	–	–	0.84
Red fish, deep-frozen	0.90	–	–	–	4.56
Baltic herring, deep-frozen	2.89	–	–	–	8.11
Baltic herring fish fingers	2.55	–	3.54	–	14.10
Herring, marinated	1.55	tr	–	–	9.16
Sprat, in oil, preserved	2.31	0.04	3.80	1.18	19.00
Sprat, preserved	2.51	–	–	–	9.91

vitamin E measured with Finnish men (17) and also about 10% of the 1980 Recommended Daily Allowance (18).

Fish consumption varies widely over the different parts of Finland and seasons of the year. It is higher along the coasts and in the lake areas than in other parts of the interior, and higher in the east than in the west. Certain Lapps may eat as much as 300 g of fish daily (19), which will provide no less than one half of the total recommended allowance of vitamin E.

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Study of Oxidation by Chemiluminescence. IV. Detection of Low Levels of Lipid Hydroperoxides by Chemiluminescence

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ABSTRACT

Sodium hypochlorite (NaOCl) induced decomposition of organic hydroperoxides gave strong chemiluminescence. Chemiluminescence intensity reached its maximum a few seconds after the addition of sodium hypochlorite and decreased to the background level in three min. Good linear relationships were observed between total chemiluminescence counts in three min and the amounts of hydroperoxides. This chemiluminescence method can be applied to the detection of low levels of lipid hydroperoxides.

INTRODUCTION

The oxidation of lipids has received renewed attention recently in connection with the deterioration of foods and oils and with peroxidation in biological systems (1,2). Lipid hydroperoxide is the primary product of lipid oxidation. Therefore, the detection of low levels of lipid hydroperoxides is very important to estimate the progress of oxidation. However, a convenient method has not been established that is sensitive enough for the analysis of trace amounts of lipid hydroperoxide.

It has been observed that weak chemiluminescence arises during the autoxidation of many organic compounds (3) and biological molecules (4). We have reported recently (5-8) that the chemiluminescence arises from the radical-induced decomposition of hydroperoxides and that this method can be applied to estimate antioxidant activity and extent of oxidation. The chemiluminescence method is a very sensitive method, and its application has been studied (4,7,9,10). In the course of our study on chemiluminescence, we extended this method to the detection of low levels of lipid hydroperoxides.

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EXPERIMENTAL PROCEDURES

Materials

Tetralyl hydroperoxide (TOOH) was prepared by the autoxidation of tetraline and recrystallized from pentane *tert*-Butyl hydroperoxide (BOOH) and di-*tert*-butyl peroxide (BOOB) were distilled under reduced pressure. Methyl linoleate hydroperoxide (18:2 LOOH) and methyl linolenate hydroperoxide (18:3 LOOH) were prepared by the oxidation of methyl linoleate and methyl linolenate, respectively, with soybean lipoxygenase and purified with silica gel column chromatography, using hexane and ether as eluents. The purity of hydroperoxides was determined by TLC and HPLC (11). 18:2 LOOH and 18:3 LOOH gave only one spot on TLC, indicating that they were free from methyl linoleate and linolenate. It was found by HPLC analysis that methyl 13-hydroperoxy-9-*cis*,11-*trans*-octadecadienoate (12) and methyl 13-hydroperoxy-9-*cis*,11-*trans*,15-*cis*-octa-

decatrienoate (13) were predominant in 18:2 LOOH and 18:3 LOOH, respectively. Dilinoleoylphosphatidylcholine was prepared as described in the literature (14). Dilinoleoylphosphatidylcholine hydroperoxide (18:2 PC-OOH) was prepared by the autoxidation of dilinoleoylphosphatidylcholine at room temperature. The amount of hydroperoxide was determined as methyl linoleate hydroperoxide by HPLC after methanolysis with tetramethylammonium hydroxide in methanol, followed by extraction with hexane. Hydrogen peroxide and aqueous sodium hypochlorite (NaOCl) were used as received. The concentration of NaOCl was determined by iodometric titration. 2,6-Di-*tert*-butyl-4-methylphenol (BMP) was recrystallized from methanol. Phenyl-*tert*-butyl nitrone (PBN) was used as received from Aldrich Chemical Co., Milwaukee, Wisconsin. Electron spin resonance (ESR) spectra were recorded on an X-band JEOL FE1X spectrometer.

Procedure

Two ml of sample solution containing hydroperoxide (solvent; *tert*-butyl alcohol/water, 1/1 by volume) was taken into a stainless steel cell. The cell, sealed with a stainless steel cover, was equipped with quartz window and Teflon capillary as shown in Figure 1. 50 µl of 0.44M aqueous NaOCl was introduced into the capillary and allowed to stand for five min to minimize the effect of laboratory light and to get a stable background level. Air was then blown into the capillary with the injector to mix NaOCl with sample solution. Chemiluminescence was detected through the quartz window by a photomultiplier (Hamamatsu TV Ind., Model R 878) with the chemiluminescence analyzer Model OX-7 (Tohoku Denshi Co., Sendai, Japan).

RESULTS AND DISCUSSION

Figure 2 shows the time course of chemiluminescence observed when 50 µl of aqueous NaOCl was introduced to 0.47 µmol methyl linoleate hydroperoxide in 2 ml of *tert*-butyl alcohol/water solvent. As shown in Figure 2, chemiluminescence intensity reached its maximum in a few seconds and decreased to the background level in three min. Similar patterns of chemiluminescence intensity were observed with other hydroperoxides. The iodometric titration shows that few hydroperoxides remained after three min. The background level was 72 counts/s (1.3×10^4 counts/3 min) on the average. The blank chemiluminescence counts observed three min after 50 µl of aqueous NaOCl was added to 2 ml of *tert*-butyl alcohol and water solvent were 1.5×10^4 counts. Unoxidized methyl linoleate did not give any appreciable chemiluminescence.

Figure 3 shows the plot of the total chemiluminescence counts three min after introduction of NaOCl as a function

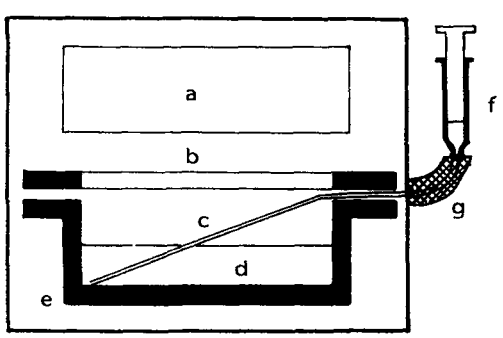

FIG. 1. Schematic diagram of sample cell and NaOCl introducing capillary. a, Photomultiplier; b, quartz window; c, capillary; d, sample solution; e, cell; f, injector; g, sealed cover.

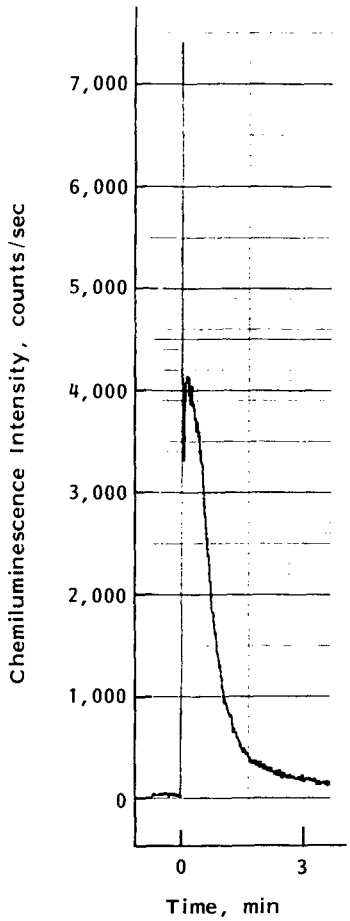

FIG. 2. Chemiluminescence observed when 50 µl of 0.44M aqueous NaOCl was added to 0.47 µmol of methyl linoleate hydroperoxide in 2 ml of tert-butyl alcohol/water solvent (1/1 by volume) at 30 C.

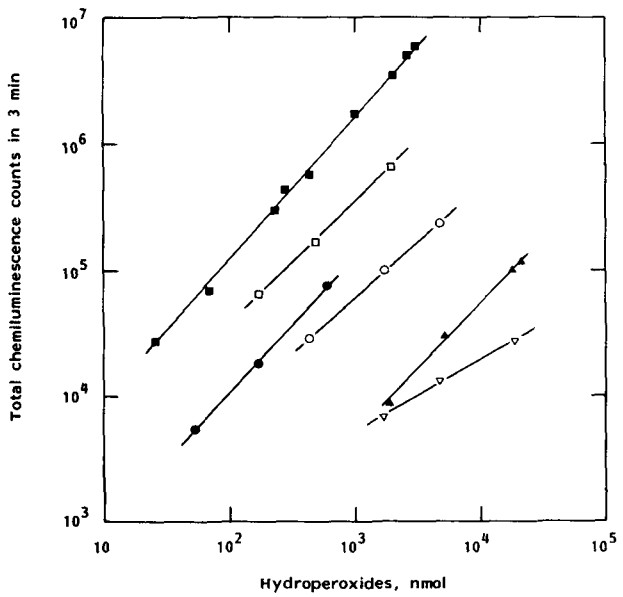

FIG. 3. Plot of total chemiluminescence counts 3 min after introducing NaOCl versus the amount of hydroperoxides. ■, TOOH; □, 18:3 LOOH; ●, 18:2 PC-OOH; ○, 18:2 LOOH; ▲, BOOH; ▽, H_2O_2.

of the amounts of hydroperoxide. The blank chemiluminescence counts were subtracted from the total counts. Good linear relationships were obtained over a wide concentration range. The slope was close to 1. Chemiluminescence intensity was dependent on the type of hydroperoxide and decreased in the order of TOOH > 18:3 LOOH > 18:2 PC-OOH > 18:2 LOOH > BOOH for any specific concentration. BOOB gave little chemiluminescence when NaOCl was added. Figure 3 shows that this chemiluminescence method is sensitive enough to determine hydroperoxides at peroxide values below 1.

Figure 4 shows the effect of BMP, an oxygen radical scavenger, on the chemiluminescence intensity during the NaOCl-induced decomposition of TOOH, BOOH and hydrogen peroxide. As shown, chemiluminescence intensity decreased with increasing BMP. However, chemiluminescence intensity from the decomposition of hydrogen peroxide by NaOCl was not changed by the addition of BMP.

The technique of spin trapping has been developed and applied in the studies of free radical chemistry and biology

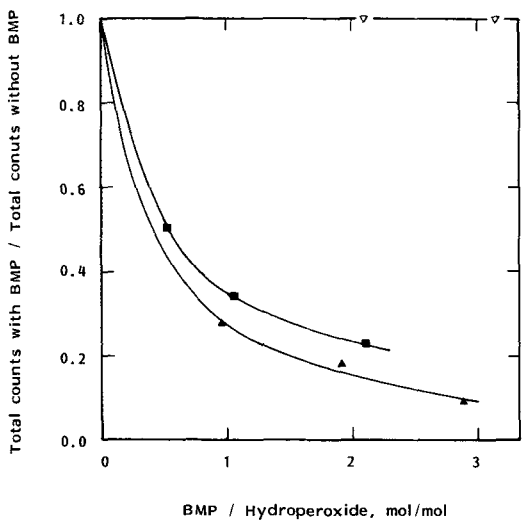

FIG. 4. Effect of BMP on the chemiluminescence intensity from 2 μmol of TOOH (■), 20 μmol of BOOH (▲) and 20 μmol of H_2O_2 (▽).

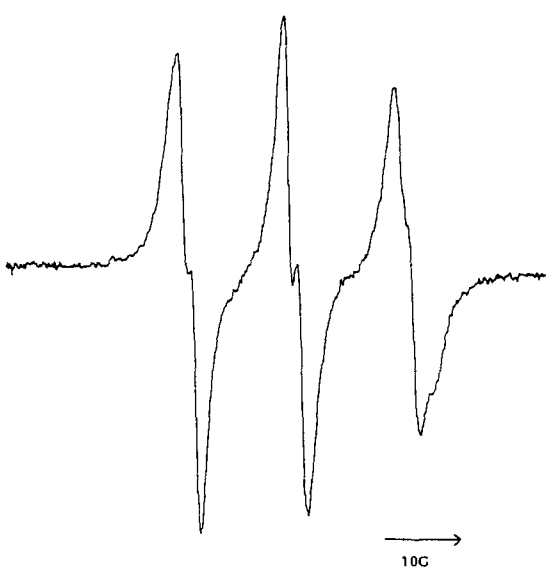

FIG. 5. ESR spectrum observed in the NaOCl-induced decomposition of BOOH in the presence of PBN at room temperature under air. 56 μmol of PBN was dissolved in 1 ml of neat BOOH, and 0.2 ml of 0.44 M aqueous NaOCl was added.

(15). It gives us valuable information concerning the structure of the intermediate radicals and the mechanism of the reactions in which they are involved. Figure 5 shows an ESR spectrum observed in the NaOCl-induced decomposition of BOOH in the presence of a spin trap, PBN. The hyperfine splitting was not good, probably due to the polar protic solvent used and the presence of oxygen, but the coupling constant a_N = 13.7 G indicates the spin adduct of *tert*-butylperoxy radicals (16,17).

This ESR study and the effect of BMP described above strongly suggest the involvement of peroxy radicals in the emission of chemiluminescence from a hydroperoxide-NaOCl system. It is known that the peroxy radicals generate chemiluminescence (3,5), probably by their bimolecular interactions to give singlet oxygen and triplet carbonyl compounds (18-21).

It is well known that the NaOCl-induced decomposition of hydrogen peroxide gives singlet oxygen (22,23). The finding that BMP had little effect on the chemiluminescence intensity from the NaOCl-hydrogen peroxide system may imply that peroxy radicals are not involved in this system.

Figure 3 shows rather big differences in chemiluminescence intensities with different hydroperoxides. This may be ascribed to different yields of excited carbonyl compound and singlet oxygen and their different quantum yields. However, we hesitate to speculate further at the present, because the exact mechanism is not really established. In any event, this chemiluminescence method is very sensitive and convenient, and it can be applied to the detection of low levels of lipid hydroperoxides.

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