Effects of High-Energy Radiation on the Lipids of Fish

M. F. Dubravcic[1] and W. W. Nawar

The volatile compounds formed in the lipid fraction of mackerel by gamma irradiation under vacuum at 0.3, 2, and 6 Mrads and at 0° and 25° C. were investigated. Using gas chromatography and mass spectrometry, 56 compounds, most of which were not reported previously, were identified. Radiolytic products included the normal alkanes C_1 to C_{17}, the 1-alkenes C_2 to C_{17}, the alkadienes C_{12} to C_{21}, the internally unsaturated alkenes C_{14} to C_{21}, the C_{17} triene, the C_{11} alkyne, and the C_{16}, $C_{16:1}$, and $C_{18:1}$ normal aldehydes. Quantitative analysis demonstrated that the major products of irradiation were the longer-chain compounds, probably originating from radiolytic cleavage of the fatty acids near the carbonyl groups.

The potential for the application of radiation energy in the preservation of fresh and precooked seafoods has been generally recognized. Irradiation at sterilization levels, however, usually produces changes in flavor, texture, and color which render these products unacceptable to the consumer. Recent efforts by a number of investigators to study the treatment of marine foods with low-dose radiation for the purpose of extending their refrigerated shelf life have shown considerable promise (AEC, 1967; Emerson *et al.*, 1966; Novak *et al.*, 1966; Ronsivalli *et al.*, 1965).

Although a great deal of research has been done on the microbiological and technological aspects of irradiation, little information is available concerning the chemical changes induced in fish by this process. The lipids were implicated in the development of off-odors and the increased susceptibility of irradiated fish to oxidative rancidity (Lerke *et al.*, 1961; Stansby and Kudo, 1964), particularly with species containing a high percentage of fat. Changes in certain general characteristics, such as peroxide value, acid number, etc., have been followed in some oils (Astrack *et al.*, 1952; Luck and Kohn, 1959), but no detailed research has been reported on the chemical compounds formed by irradiation of fish or fish oil.

Merritt and coworkers (1965, 1966, 1967) identified a homologous series of normal alkanes and 1-alkenes in irradiated fats, but no hydrocarbons higher than C_{13} were detected in irradiated milk fat, tristearin, or methyl oleate. More recently, Champagne and Nawar (1968) irradiated beef and pork fats under vacuum and were able to identify additional longer chain hydrocarbons up to C_{17}. These included a series of alkadienes and internally unsaturated alkenes. A number of hydrocarbons were also found in irradiated milk fat by Khatri *et al.* (1966). In addition, these authors identified aldehydes up to C_{12}, CO, CO_2, and H_2.

On the basis of qualitative and quantitative analysis of the radiolytic products formed in simple triglycerides, Dubravcic and Nawar (1968) proposed a mechanism applicable to the radiolysis of natural fats. They speculated that the principal cleavages of glyceride molecules occur in the vicinity of the carbonyl group, giving rise to relatively large amounts of the hydrocarbons containing one and two carbon atoms less than the glyceride fatty acid and, in addition, some oxygen-containing compounds of the same carbon numbers as the fatty acid.

Fish oils are unique among food lipids in that they contain the characteristic long chain highly unsaturated fatty acids. The present study was undertaken to obtain fundamental information on the primary effects of ionizing radiation under nonoxidative conditions on the lipid fraction of fish. Mackerel was selected because it is high in fat content, it lends itself to mild techniques of lipid extraction, and it can be easily obtained on the east coast in a fresh condition.

EXPERIMENTAL

Preparation of Sample. Fresh mackerel, caught off the Massachusetts shore, was covered with ice and brought to the laboratory within 3 hours after catching. The fish were cleaned and the fillets stored under nitrogen in plastic containers at −29° C. until used. The oil was obtained from the fillets by pressing at room temperature. The resulting emulsion was then centrifuged under nitrogen, the clear oil transferred to glass ampoules, degassed, and sealed at a pressure of 10^{-2} torr. The ampoules of oil were stored in the dark at −29° C. until irradiation, which occurred within 6 days after the fish had been caught.

Irradiation. Fish oil samples were irradiated with gamma rays from a Co^{60} source at the Marine Products Development Irradiator, Gloucester, Mass., and those of the U. S. Army Natick Laboratories, Natick, Mass.

Irradiation was carried out under vacuum at two temperatures, 0° and 25° C., and three doses, 0.3, 2.0, and 6.0 Mrads. Immediately after the treatment, the samples were packed in dry ice, returned by automobile to the laboratory, and stored at −29° C. until analyzed in the shortest possible time.

Department of Food Science and Technology, University of Massachusetts, Amherst, Mass. 01002

[1] Present address, University of Akron, Akron, Ohio

Table I. Fatty Acid Composition of Mackerel Oil

Acid	%	Acid	%	Acid	%
14:0	6.7	18:0	2.5	20:5	7.4
14:1	0.5	18:1	11.7	22:1	16.5
15:0	0.6	18:2	1.4	22:4	1.6
16:0	16.7	18:3	2.1	22:5	0.9
16:1	5.5	18:4	5.7	22:6	8.3
17:0	0.5	20:1	9.3		
17:1	0.7	20:4	1.4		

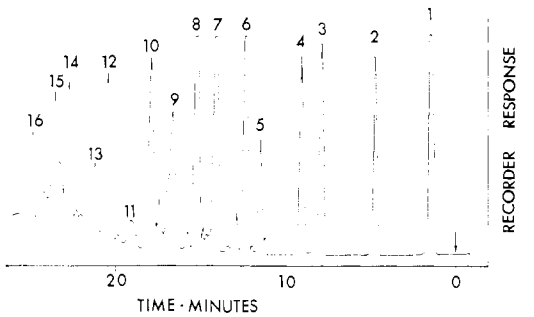

Figure 1. Gas chromatographic analysis of the lower-boiling compounds in irradiated mackerel oil (12-foot × 1/8-inch alumina column, temp. prog. 30° to 350° C. at 15° C. per min.). Designations of peak numbers are given in Table II

Analytical Methods. The fatty acid composition of the oil used in this study (Table I) was determined by refluxing the fat with 10% HCl–methanol as described by Stoffel *et al.* (1959), and analyzing the methyl esters by gas chromatography on a 12-foot × ⅛-inch diethylene glycol succinate column. Identification of the various methyl esters was further verified by mass spectrometry.

The techniques for the collection of radiolytic products and their qualitative and quantitative analysis have been described (Nawar *et al.*, 1969). Only a brief account of the methodology is given here.

The higher-boiling compounds were collected by high-vacuum distillation in an apparatus similar to that of de Bruyn and Schogt (1961). The cold finger was rinsed with ethyl ether and the ether extract analyzed by gas chromatography. The lower-boiling compounds were trapped in a precolumn, which was subsequently fitted to the gas chromatographic system. The volatiles were separated on three different columns: a 12-foot × ⅛-inch Carbowax 20M, a 6-foot × ⅛-inch silicone rubber SE-30, and a 12-foot × ⅛-inch alumina F-1. For quantitative analyses, two internal standards, 6-dodecyne and eicosane, were used. Appropriate amounts of each were accurately weighed and added to the oil samples immediately before distillation. Conversion factors relating peak areas of the identified components to those of the internal standards were established by previous tests.

Mass spectrometric analysis was conducted in a Perkin-Elmer-Hitachi gas chromatograph–mass spectrometer system, in which the effluent from the GC column was admitted directly into the ion source.

RESULTS AND DISCUSSION

Unirradiated mackerel oil contained small amounts of straight chain hydrocarbons, including the alkanes C_{10} to C_{15} and the 1-alkenes C_{10}, C_{11}, and C_{14}. The major component, however, was pristane (2,6,10,14-tetramethyl pentadecane), which was present in much greater quantity than all other volatile compounds (500 μmoles per 100 grams of fat). Five additional branched hydrocarbons were also present, but in much smaller quantities (less than 2 μmoles per 100 grams). These were nonadecene (two isomers), nonadecadiene, nonadecatriene and eicosadiene. The presence of pristane in shark and herring oils has been previously observed (Blumer, 1967; Lambertsen and Holman, 1963). However, the present study is the first to report on the presence of pristane in mackerel.

Irradiation of mackerel oil results in the production of a wide range of volatile compounds (Figures 1 and 2). Evidence for the identification of these compounds is given in Table II. The normal alkanes and 1-alkenes in the irradiated oil range from the lowest members of the homologous series up to C_{17}. General observations which can be made from this and other work (Champagne and Nawar, 1968; Khatri *et al.*, 1966) regarding the normal alkanes and 1-alkenes are that they are present in different irradiated fats and that both series of hydrocarbons go up to C_{17} only. This is explained by the formation of the normal alkanes and 1-alkenes from saturated fatty acids predominantly by cleavages of carbon-carbon bonds alpha and beta to the carbonyl group (Dubravcic and Nawar, 1968) and by the fact that the highest major saturated fatty acid present in these irradiated fats was octadecanoic acid.

The production of 1-alkenes upon irradiation of fish oil is important from the standpoint of off-flavor production, since the lower members of this series are the most unpleasant among the hydrocarbons. Champagne and Nawar (1968) determined odor threshold values

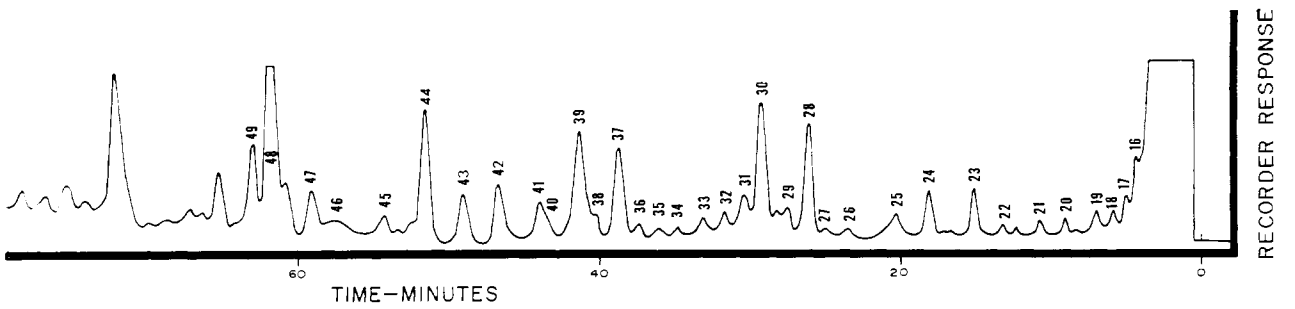

Figure 2. Gas chromatographic analysis of the higher-boiling compounds formed by irradiation of mackerel oil (12-foot × 1/8-inch Carbowax 20M, temp. prog. 60° to 220° C. at 2° C. per min.). Designations of peaks are given in Table II

Table II. Identification of Hydrocarbons in Irradiated Mackerel Oil

Straight Chain Hydrocarbons, C No.	GLC Retention: Agrees with Ref. Compds. on Al_2O_3	GLC Retention: Agrees with Ref. Compds. on SE-30	GLC Retention: Agrees with Ref. Compds. on CW	GLC Retention: Extrapolation	Mass Spectrometry: Agrees with ref. compds.	Mass Spectrometry: Interpretation	Corresp. Peaks in Figures 1 & 2
Alkanes							
1	x				x		1
2	x				x		2
3	x				x		4
4	x				x		6
5	x				x		8
6	x				x		10
7	x	x			x		12
8	x	x	x		x		14
9	x	x	x		x		16
10	x	x	x		x		18
11		x	x		x		20
12		x	x		x		22
13		x	x		x		24
14		x	x		x		26
15		x	x		x		30
16		x	x		x		34
17		x	x		x		38
1-Alkenes							
2				x	x		2
3				x	x		5
4	x				x		7
5	x				x		9
6	x				x		11
7	x	x			x		13
8	x	x	x		x		15
9	x	x	x		x		17
10	x	x	x		x		19
11		x	x		x		21
12		x	x		x		23
13		x	x		x		25
14		x	x		x		28
15		x	x		x		32
16		x	x		x		36
17		x	x		x		40
Internal alkenes							
14				x		x	27
15				x		x	31
16				x		x	35
17				x		x	39
18				x		x	42
19				x		x	44
20				x		x	46
21				x		x	48
Alkadienes							
12				x		x	
13				x		x	
14				x		x	29
15				x		x	33
16				x		x	37
17		x	x		x		41
18				x		x	43
19				x		x	45
20				x		x	47
21				x		x	49
Alkynes							
11		x	x		x		

Table III. Quantitative Analysis of the Volatiles in Irradiated Mackerel Oil (Micromoles per 100 Grams)

Product	C No.	Control (Not Irrad.)	0.3 Mrad 0° C.	0.3 Mrad 25° C.	2 Mrads 0° C.	2 Mrads 25° C.	6 Mrads 0° C.	6 Mrads 25° C.
Straight-Chain H.C.								
Alkanes								
	1	0	0	0.1	0.1	0.2	0.1	1.0
	2	0	0	0.1	0.1	0.7	0.1	0.7
	3	0	0	0	0.2	1.0	0.1	1.4
	4	0	0	0	0.2	1.0	0.2	1.9
	5	0	0	0.1	0.1	0.8	0.3	1.5
	6	0	0	0.1	0.1	0.6	0.3	1.4
	7	0	0	0.1	0.1	0.5	0.4	1.2
	8	0	0	0	0.1	0.6	0.4	1.5
	9	0	0	0	0	0.3	0.4	1.4
	10	0.2	0.2	0.2	0.5	1.1	0.6	2.2
	11	1.0	1.0	1.0	1.0	1.2	1.1	2.1
	12	1.9	1.9	2.0	1.9	2.0	2.0	2.7
	13	2.0	2.2	2.4	2.3	3.1	2.8	6.0
	14	0.1	0.1	0.2	0.2	0.3	0.2	0.6
	15	1.1	1.3	1.8	1.8	3.4	2.8	9.1
	16	0	[a]	[a]	[a]	[a]	[a]	0.5
	17	0	[a]	[a]	[a]	[a]	[a]	1.7
1-Alkenes								
	2	0	0	0	0.1	3.4	0.1	5.6
	3	0	0	0	0.2	1.2	0.2	1.5
	4	0	0	0	0.2	1.6	0.2	3.2
	5	0	0	0	0.1	0.5	0.1	1.3
	6	0	0	0	0	0.3	0.1	0.7
	7	0	0	0	0.1	0.6	0.5	1.6
	8	0	0	0.1	0.2	0.8	0.6	1.9
	9	0	0	0.2	0	0.3	0.3	0.9
	10	0.2	0.2	0.2	0.4	0.8	0.9	1.2
	11	0.9	0.9	1.0	1.0	1.2	1.2	2.0
	12	0	0	0.3	0.3	1.4	1.0	4.6
	13	0	0	0.1	0.1	0.2	0.3	2.2
	14	0.5	0.5	0.7	1.0	1.8	1.1	7.0
	15	0	0	0.1	0.1	0.3	0.2	0.7
	16	0	[a]	[a]	[a]	[a]	[a]	1.2
	17	0	[a]	[a]	[a]	[a]	[a]	0.4
Internal alkenes								
	14	0	[a]	[a]	[a]	[a]	[a]	0.3
	15	0	[a]	[a]	[a]	[a]	[a]	3.4
	16	0	[a]	[a]	[a]	[a]	[a]	0.7
	17	0	[a]	[a]	[a]	[a]	[a]	11.1
	18	0	[a]	[a]	[a]	[a]	[a]	[b]
	19	0	[a]	0.6	1.0	3.3	3.0	10.3
	20	0	[a]	[a]	[a]	[a]	[a]	1.0
	21	0	0.3	1.6	2.2	7.9	6.2	21.3
Alkadienes								
	14	0	0.7	1.1	2.8	6.6	5.1	20.2
	15	0.1	0.1	0.1	0.1	0.4	0.4	1.0
	16	0	[a]	[a]	[a]	[a]	[a]	9.4
	17	0	[a]	[a]	[a]	[a]	[a]	4.3
	18	0	0	0.4	0.5	1.6	1.6	5.1
	19	0	[a]	[a]	[a]	[a]	[a]	2.5
	20	0	0	0.2	0.5	1.4	1.7	4.8
	21	0	0.4	0.8	1.4	3.2	3.1	7.8

[a] The compound could be measured only in stripped samples free from interfering natural volatiles.
[b] Present but overlapping with other peaks. See text.

of a series of normal alkanes, alkenes, alkynes, and alkadienes and found that the C_6, C_7, and C_8 1-alkenes were the most odorous. These were detectable in mineral oil at concentrations of 3, 1, and 2 p.p.m., respectively, and exhibited odors described as "chemical," "paint-like," and "sharp." In the present work on mackerel oil, no formal evaluation of flavor was attempted. However, samples irradiated at or above 2 Mrads were easily distinguishable from the unirradiated controls, as they possessed a characteristic off-odor which may be described as "musty" or "stale."

In addition to the above, irradiation of fish oil produced internally unsaturated alkenes from C_{14} to C_{21} and alkadienes from C_{12} to C_{21} (Table II). The presence of the internal alkenes was reported in irradiated beef and pork fats only by Champagne and Nawar (1968), who found the C_{15}, C_{16}, and C_{17} members of this series. The formation of the C_{10}-C_{12}, C_{16}, and C_{17} alkadienes was suspected by Khatri *et al.* (1966) in irradiated milk fat.

The C_{17} alkadienes and the C_{17} internally unsaturated alkenes were the highest members of these two series in the radiolytic products of those animal fats where the highest monounsaturated acid was oleic. On the other hand, in fish oil, which contains also eicosenoic and docosenoic acids, irradiation produces members of the two series up to C_{21}.

Hendecyne was the only alkyne found in irradiated fish oil (Table II). The same compound was previously detected by Dubravcic and Nawar (1968) among the radiolytic products of triolein. Decyne and dodecyne were reported in irradiated beef (Merritt *et al.,* 1966) and all the lower members of the normal 1-alkyne series in irradiated milk fat (Merritt *et al.,* 1967).

The presence of heptadecatriene in irradiated fish oil was indicated by mass spectral analysis, but the compound could not be separated from the internal octadecene. Its GLC retention time agreed with the major peak from irradiated trilinolenin, which was also identified as heptadecatriene.

Evidence for the presence of hexadecanal, hexadecenal, and octadecenal in irradiated fish oil was also obtained. Only aldehydes of shorter chain length have been previously reported in irradiated fats. Merritt *et al.* (1966, 1967) identified the saturated aldehydes C_2 to C_8 in irradiated butterfat and the C_5 to C_8 aldehydes in irradiated meats. Khatri *et al.* (1966) identified C_5 to C_{12} saturated aldehydes in irradiated milk fat.

The compounds identified in irradiated mackerel oil, as listed in Table II, were essentially the same as those found when mackerel fillets were irradiated first, in glass containers under vacuum, and then the oil extracted and analyzed. This observation was confirmed more recently by Kavalam (1968).

Table III shows the amounts of hydrocarbons formed by irradiation of mackerel oil at different doses and temperatures. With the exception of ethene, the quantities of the lower-boiling compounds produced by radiation are, in general, small compared with the quantities of higher-boilers. Of the normal alkanes, the C_{15} compound was produced in greater quantity than all others, and the most abundant in the 1-alkene series was the C_{14} compound. The conclusion that pentadecane and 1-tetradecene were the two major radiolytic products of palmitic acid (16.7% in mackerel oil) was reached in the earlier work on irradiation of tripalmitin (Dubravcic and Nawar, 1968). Similarly, the origin of the other major radiolytic products can be established. The presence of the 14:0, 18:1, 20:1 and 22:1 fatty acids is thus reflected in the formation by irradiation of relatively large amounts of tridecane and 1-dodecene, internal heptadecene and hexadecadiene, internal nonadecene and octadecadiene, and internal heneicosene and eicosadiene, respectively.

Contrary to expectation, however, no significant quantities of the corresponding radiolysis products from the major polyunsaturated fatty acids—18:4, 20:5, and 22:6—were found. A similar apparent stability was shown by methyl docosahexaenoate, irradiated under vacuum at 6 Mrads, and later also at 10 Mrads (Lien, 1968). Two suggestions can be offered at present to explain these observations. Since the unsaturated fatty acids of fish oils are predominantly in the cis-configuration, it is possible that the compactness of the molecule (so shaped by repetition of cis-double bonds) is contributing to the stability to radiation, as compared with the extended chain molecules. This was shown to be the case in thermal degradation (Patai, 1964). Also, our earlier work on radiolysis of triolein and trilinolenin indicated that with the increased number of cis-double bonds, significantly lower quantities of the major hydrocarbons were produced. The second suggestion refers to the reduced possibility of radiolytic homolysis of carbon-carbon bonds along the chains of the polyunsaturated fatty acids. As shown by NMR studies (Hashimoto, 1963) in mackerel and other fish oils, the double bond arrangement is that of the methylene interrupted type. Accordingly, most of the single carbon-carbon bonds present are alpha to a double bond, and this position usually increases the strength of the bond well above that of the normal carbon-carbon bond (Morrison and Boyd, 1964).

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