Autoxidation of Polyunsaturated Fatty Compounds in Mackerel Oil: Formation of 2,4,7-Decatrienals[1]

P.J. KE, R.G. ACKMAN, and B.A. LINKE, Environment Canada, Fisheries and Marine Service, Halifax Laboratory, Halifax, Nova Scotia B3J 2R3, Canada

ABSTRACT

The formation of potentially "fishy" off flavor components, especially 2,4,7-decatrienals, in various rancid mackerel oils has been semiquantitatively investigated using preparative thin layer chromatography (TLC) and gas liquid chromatography (GLC) methods. A combination of 2 GLC analyses can be directly employed for free aldehyde analysis. This GLC method is faster and gives a better recovery than the alternative TLC proceeding through the dinitrophenylhydrazone derivatives of the carbonyl compounds. Kinetic relations between decatrienal formation and the degree of autoxidation of polyenoic fatty compounds present in mackerel oil are discussed. The decreases in major polyenoic fatty acids rancid oils, measured by the ratios

$$\frac{18{:}4\omega3 + 20{:}5\omega3 + 22{:}6\omega3}{14{:}0 + 16{:}0 + 18{:}0}$$

or

$$\frac{\text{total polyenoic acids}}{14{:}0 + 16{:}0 + 18{:}0}$$

can be related to the formation of 2,4,7-decatrienals and other unsaturated aldehydes.

INTRODUCTION

Studies on the occurrence of a "fishy" off flavor in autoxidized butter have been reviewed by Meijboom and Stroink (1). The suggestion that unsaturated aldehydes with a double bond not conjugated to the carbonyl group were responsible for this particular off flavor in strongly oxidized fats held sway for some years. Later, Badings (2,3) reported that 4-*cis*-heptenal, if present at higher than 15 parts per billion, was characterized as having a fishy or whale oil flavor. Meijboom and Stroink (1) and Badings (3) have stated recently that 2-*trans*,4-*cis*,7-*cis*-decatrienal should be considered as a leading element of the fishy, trainy, or whale oil flavors in the complex mixture of volatiles from a strongly autoxidized oil containing linolenic acid. This decatrienal also has been found (1) among the decomposition products of strongly autoxidized oils having longer chain polyenoic fatty acids with the basic linolenic acid double bond pattern of $\omega9$, $\omega6$, and $\omega3$, combined with 1 or more other double bonds. Marine fish oils and lipids contain little (1%) linolenic acid (18:3$\omega3$); but substantial amounts (total usually $>20\%$) of 6,9,12,15-octadecatetraenoic acid (18:4$\omega3$), 5,8,11,14,17-eicosapentaenoic acid (20:5$\omega3$), and 4,7,10,13,16,19-docosahexaenoic acid (22:6$\omega3$) are usually found along with smaller amounts ($<5\%$) of all other polyenoic acids (4,5,6).

In this report a combination gas liquid chromatographic (GLC) technique has been used directly to estimate the aldehyde content of volatiles from autoxidized oils. This gives a much better result than the thin layer chromatographic (TLC) method applied to derivatives of aldehydes (1). Preliminary evaluation of data obtained by this method for the formation of 2,4,7-decatrienals in oxidized mackerel oil is used to demonstrate that the relationship to the 3 major polyenoic fatty acids (18:4$\omega3$, 20:5$\omega3$, and 22:6$\omega3$) is adequate for study, and that evaluation of all polyenoic acids is not necessary.

EXPERIMENTAL PROCEDURES

Chemicals

Alkanals, alkenals, and alkadienals were ACS grade reagent obtained from Aldrich Chem. Co. (Montreal, Canada) and Pfaltz & Bauer, Inc. (New York, N.Y.), and were used as calibration standards without purification. Handling precautions included a nitrogen atmosphere wherever practical. 2-*trans*-, 4-*cis*-, 7-*cis*-, and 2-*trans*-, 4-*trans*-, 7-*cis*-Decatrienals were prepared from 2-*trans*-decen-4,7-diynol (7) and 4-*trans*-decen-2,7-diynol (8), respectively, as in a previous study (1). n-Hexane, GC-Spectrophotometric Grade (Baker Chem. Co., Phillipsburg, N.J.) was used satisfactorily as a carbonyl free solvent. This was comparable in quality to the n-hexane specially purified with the modified 2,4-dinitrophenylhydrazine reaction column originally described by Schwartz and Parks (1,9).

Preparation of Rancid Mackerel Oils

Oil was extracted by the method of Bligh and Dyer (10) from whole mackerel (*Scomber scombrus*) landed in Nova Scotia, June, 1973, and held frozen in plastic bags (ca. 20 round fish per bag) at -40 C for 5 months. The average oil yield from 19 extractions was 11.5%. Each extract was subjected to prolonged high vacuum treatment to remove all traces of solvent and existing volatile components. A total of 1.1 kg oil was obtained from pooled extracts. Mackerel oil, 150 g, with 15 mg Fe(III) in the form of ferric chloride (Nuodex) added, was placed in a specially designed glass vessel (11) for oxidization at 40 C. In addition to constant stirring, compressed air was bubbled through the oil at a flow rate of 3 ml/min. After oxidation for 0,5,10, and 23 days, oils with final peroxide values of 1 (control), 15, 70, and 151, respectively, were produced for detailed investigations. The total content of volatile carbonyls from the 4 oxidized mackerel oils was estimated spectrophotometrically by Wyatt and Day's procedure (12), using a molar absorptivity index of 25,000 at 358 nm. The peroxide value (POV), free fatty acid content, and iodine value (IV) were determined by the AOCS Official Methods, Cd 8-53, Ca 5a-40, and Tg 1-64, respectively. The analytical data for these oxidized oils are listed in Table I.

TABLE I

Analytical Data for 4 Oxidized Mackerel Oils

Sample	1	2	3	4
Iodine Value (calculated from GLC[a])	145 (142[b])	138	128	104
Peroxide Value (MEq/Kg)	1	15	70	151
Free fatty acid, %	1.8	2.1	6.9	9.2
Volatile carbonyls (Mole/G Oil x 10^{-9})	25.1	107	224	467
Total aldehydes (Mole/G Oil x 10^{-9})	17.3	75.6	187	340
2,4,7-Decatrienals (Mole/G Oil x 10^{-9})	None	Trace	0.31	0.71

[a]GLC = Gas liquid chromatography.

[b]Iodine value was determined by WIJS method.

[1]Presented in part at the Spring AOCS Meeting, Mexico City, 1974.

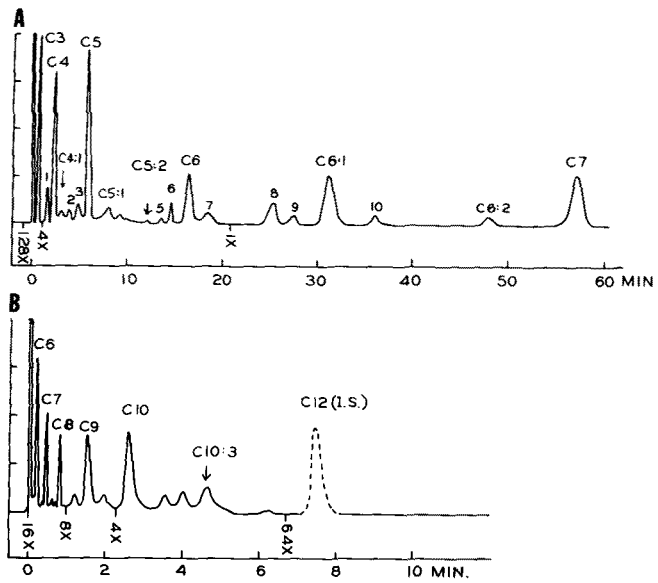

FIG. 1. Chromatograms for the analyses of monocarbonyl aldehydes from oxidized mackerel oils (peroxide value [POV] = 70) by: (A) a glass column (10 ft x 3 mm) packaged with 0.4% Carbowax 1500 on Carbopack A and operated at 130 C and 20 ml/min He; and (B) a glass column (5 ft x 3 mm) with Ap-M on Chromosorb W operated at 150 C and 30 ml/min He. The C_{12} was decanal added as in internal standard.

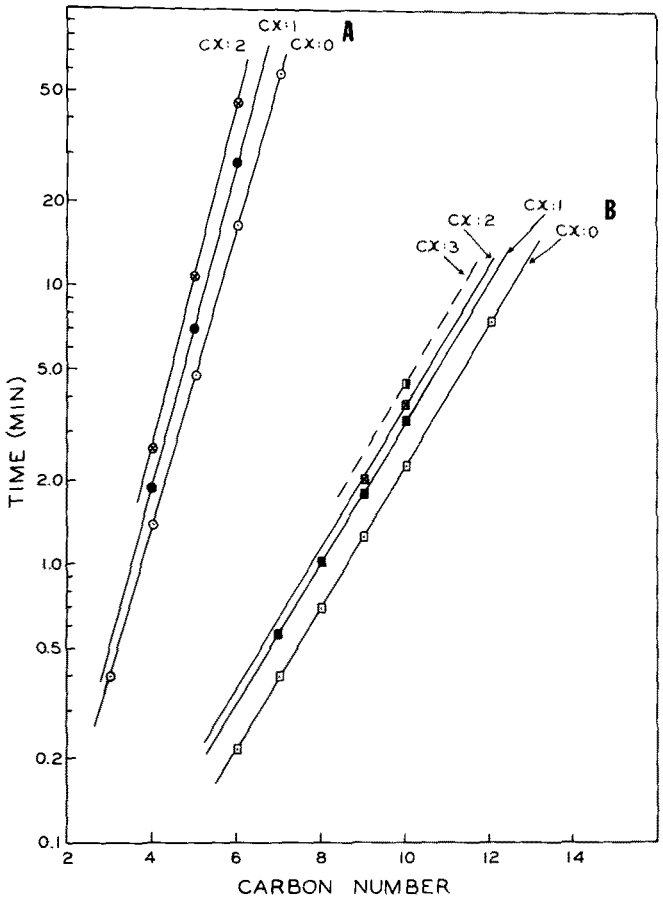

FIG. 2. Retention time of aldehydes by GLC analyses on: A) Carbowax 1500 on Carbopack A; and B) Ap-M on Chromosorb.

Isolation of Carbonylic Volatiles

After the mackerel oils were oxidized, 100 g of the rancid oil was weighed into a 250 ml flask with a magnetic stirrer, and then connected to a high vacuum apparatus through a U trap cooled with liquid nitrogen. During this initial evacuation, a first fraction of free volatiles was trapped. The stopcock between the oil flask and U trap was closed and some nitrogen admitted. The oxidized oil was stirred and heated in a boiling water bath under 100 mm Hg of nitrogen for 2 hr to decompose the peroxides completely (13). The temperature of the water bath was reduced to 40 C, and the flask was evacuated to collect the carbonyls and other volatiles in the same U trap. All the collected volatiles were dissolved in 10 ml methanol.

Estimation of 2,4,7-Decatrienals and Other Aldehydes

Methanol, 90 ml, and the internal standards (1 μmole butanal or dodecanal) were added to the methanol solution of carbonyl volatiles and the solution transferred to a 500 ml flask. A saturated solution of 100 ml freshly recrystallized 2,4-dinitrophenylhydrazine (DNPH) in 5N HCl was added, and the mixture was allowed to stand for 24 hr in darkness (14). The DNPH derivatives of carbonyl compounds were extracted with 200 ml benzene:n-hexane (1:1, v:v), and the dried DNPH derivatives were obtained by evaporation.

Assay by Multiple TLC Spectrophotometric Methods (A)

The TLC procedure previously described (1,15) was employed to separate and identify the DNPH derivatives of decatrienals. The mixture of DNPH derivatives was redissolved in freshly distilled diethyl ether, streaked onto a TLC plate (20 x 20 cm) coated with Kieselguhr G. (E. Merck, Darmstadt, Germany) impregnated with 33% Carbowax 400, and developed with petroleum ether (bp, 100-120 C). The fourth band from the bottom on the TLC plate between the C_4 and C_5 n-alkanal DNPH positions, included decatrienals and was further purified by a second TLC partition using a Silica Gel G (E. Merck) plate (20 x 20 cm) with carbon tetrachloride:n-hexane:ethyl acetate (10:2:1, v:v:v) as the mobile phase. The most mobile band of the second TLC contained the decatrienals, which were isolated by a third TLC separation on a plate (20 x 20 cm) coated with Silica Gel G impregnated with 30% $AgNO_3$ and developed with benzene. The band containing the isolated decatrienals was extracted and decatrienal content was estimated spectrophotometrically at 358 nm in chloroform solution (12).

Assay by TLC-GLC Method (B)

The DNPH derivatives of the monocarbonyls were first separated by TLC on a Carbowax plate as described in the previous section. The preparation was repeated several times until ca. 5 mg DNPH derivative, recovered from the fourth band with diethyl ether, was accumulated. The dried DNPH derivatives of carbonyl compounds were mixed with 35 mg Celite 454, 35 mg dimethylaminobenzaldehyde and 25 mg oxalic acid, and placed in a small scale glass reaction vessel (14). The free carbonyl compounds were regenerated by heating the evacuated vessel to 190 C, and collected by diffusion into a capillary trap containing 100 μl methanol cooled with liquid nitrogen. The methanol solutions containing 2,4,7-decatrienals and other aldehydes were analyzed immediately by GLC on a 5 ft x 3 mm internal diameter (ID) glass column of 10% Apiezon M (Ap-M) on Chromosorb W (100-120) in an Aerograph Hy-Fi Model 600 equipped with a hydrogen flame ionization detector. The operating conditions were 150 C and 30 ml/min helium. A typical chromatogram is shown in Figure 1. Quantitation for 2,4,7-decatrienals was carried out from a calibration curve with correction for recovery using dodecanal as the internal standard.

Assay by Combination GLC Method (C)

The volatile compounds collected in the U trap from the decomposition of peroxides in oxidized mackerel oil also

TABLE II

Percentage of Recovery for Estimation of Butanal, Decanal, Dodecanal, and Decatrienal by Thin Layer Chromatography (TLC) and Gas Liquid Chromatography (GLC)

Aldehyde Added	Recovery (%)		
(0.01 μMole/G Oil)	Combined TLC	TLC-GLC	Combined GLC
Butanal	58	82	98
Decanal	-	-	95
Dodecanal	-	-	92
Decatrienal	53	76	94

TABLE III

Fatty Acid Composition of 4 Oxidized Mackerel Oils

Fatty Acid	Mole %			
	1	2	3	4
14:0	8.1	9.2	9.6	11.4
16:0	16.5	16.8	17.5	18.6
18:0	2.4	2.6	2.6	2.8
Saturates[a]	29.7	32.1	33.0	36.9
16:1	4.7	4.9	5.3	5.6
18:1	11.4	11.8	12.0	12.5
20:1	10.5	10.3	10.8	10.7
22:1	15.0	14.1	14.8	15.0
Monoenes[a]	43.0	42.2	43.9	44.7
Dienes[a]	2.2	2.1	2.2	1.9
18:3ω3	1.6	1.4	1.5	1.3
18:4ω3	4.1	4.0	3.7	3.2
20:5ω3	6.3	6.1	5.2	4.3
22:6ω3	9.9	9.0	7.4	5.1
Polyenes[a]	25.2	23.6	20.9	16.4
Polyene index A[b]	0.752	0.688	0.569	0.384
Polyene index B[c]	0.933	0.845	0.703	0.501

[a]Fatty acids < 1% are not listed here, but are included in the subtotal percentage.

[b]Polyene index A = (18:4ω3 + 20:5ω3 + 22:6ω3)/(14:0 + 16:0 + 18:0).

[c]Polyene index B = Total polyene/(14:0 + 16:0 + 18:0).

were evaluated directly for GLC analyses without reacting with DNPH to protect the carbonyl group. Dodecanal (1 μmole) was added to the sample for quantitation by the same GLC procedure as mentioned in Section B. An Ap-M column was employed to analyze the longer chain carbonyl compounds (C_7-C_{12}). For the short chain carbonyls (C_3-C_7), shown in Figure 1A, the same sample was analyzed on a glass column (12 ft x 3 mm ID) packed with 0.4% Carbowax 1500 on Carbopack A (Supelco Inc., Bellefonte, Pa.) operated at 130 C and 20 ml/min He, in the same Aerograph Hy-Fi GLC apparatus. The aldehydes were identified by their retention times obtained from semilogarithmic linear plots of a number of reference alkanals, alkenals, and alkadienals (Fig. 2). Quantitative results (molar basis) were obtained with relative errors of < 10% from the individual calibration curves. These were based on the peak area ratios with reference to dodecanal standard, and included cross comparison via a peak such as C_6 found in both chromatograms.

Fatty Acid Analyses

Methyl esters of total fatty acids from various rancid oils were prepared by refluxing for 10 min with 7% BF_3 in methanol (16). GLC analyses for determination of all unaltered methyl esters in the samples were carried out on a Perkin Elmer 900 gas chromatograph fitted with a flame ionization detector and a capillary column (46 m x 0.25 mm ID), coated with Silar-5CP (Applied Science Laboratories, State College, PA) and operated at 180 C and 2.5 kg/cm^2 He. Peaks were identified and quantitated as discussed elsewhere (5).

TABLE IV

Volatile Monocarbonyl Aldehydes from 4 Oxidized Mackerel Oils

Aldehydes	Mole/G Oil x 10^{-9}			
	1	2	3	4
Alkanals				
C_3	13.1	62.0	148.4	237.1
C_4	0.85	3.21	8.62	22.5
C_5	1.91	6.14	13.75	31.4
C_6	0.75	1.04	4.06	13.2
C_7	0.15	0.53	2.53	10.5
C_8	-	0.24	1.09	1.64
C_9	-	0.20	0.94	2.05
C_{10}	-	0.18	1.21	2.78
Totals	16.8	73.5	181.0	321.2
Alkenals				
C_4	-	0.14	0.81	2.51
C_5	-	0.27	0.75	3.84
C_6	0.30	0.92	1.74	6.75
C_7	0.10	0.15	0.47	0.91
C_8	-	-	0.39	0.96
C_9	-	-	0.31	0.63
Totals	0.40	1.58	4.89	16.44
Alkadienals				
C_5	-	-	0.10	0.22
C_6	0.14	0.25	0.55	1.32
C_7	-	-	-	0.10
C_9	-	-	-	0.10
C_{10}	-	0.10	0.27	0.42
Totals	0.13	0.34	0.92	2.07
Decatrienals	-	<0.10	0.31	0.71

RESULTS AND DISCUSSION

A number of stationary phases have been investigated for GLC analysis of mixtures of carbonyl compounds containing alkanals, 2-alkenals, and methyl ketones (17). The linear semilogarithmic plots of C_2 to C_{11} homologous aliphatic aldehydes (and methyl ketones) have a parallel in a study of the retention times (18), and GLC analyses have been used to estimate the carbonyl composition in some fish products (14). However, the latter chromatographic method for carbonyl analysis is indirect and the calculation time consuming.

In the study of mackerel oils, the free carbonyls in the volatiles from oxidized oil were determined directly by the combined GLC method (C) using the liquid phases Ap-M and Carbonwax 1500. Representative chromatograms are shown in Figure 1. The Ap-M column was used to estimate the carbonyls with chain length from C_7 to C_{12}, and Carbowax 1500 on the low polarity Carbopack A was employed for aldehydes with a chain less than 7 carbons.

To compare the 3 chromatographic methods, a recovery experiment was carried out using a mixture ranging from n-butanal and 2,4,7-decatrienals, mixed equally from 2 isomers. The results in Table II show that the combined GI C method described herein is satisfactorily efficient with a recovery better than 92% from a study of a mixture of 4 aldehydes. We found GLC to be much simpler and more reproducible than the other 2 procedures, mainly due to the elimination of the DNPH reaction and inadequate recovery from the TLC steps. However, it was disappointing to find that 2-*trans*,4-*cis*,7-*cis*-decatrienal cannot be separated successfully from its isomer, 2-*trans*,4-*trans*,7-*cis*-decatrienal by the Ap-M GLC analysis. Even though the multiple TLC method (1) has been reported to separate these 2 decatrienal isomers, the results in our study with this method have not been satisfactory due to the poor recovery and the incomplete separation on silver nitrate TLC.

Major differences can be demonstrated in the polyenoic fatty acid components of the 4 rancid mackerel oils with peroxide values of 1,15,70, and 151, respectively (Table III). The rates of oxidation for various classes of compo-

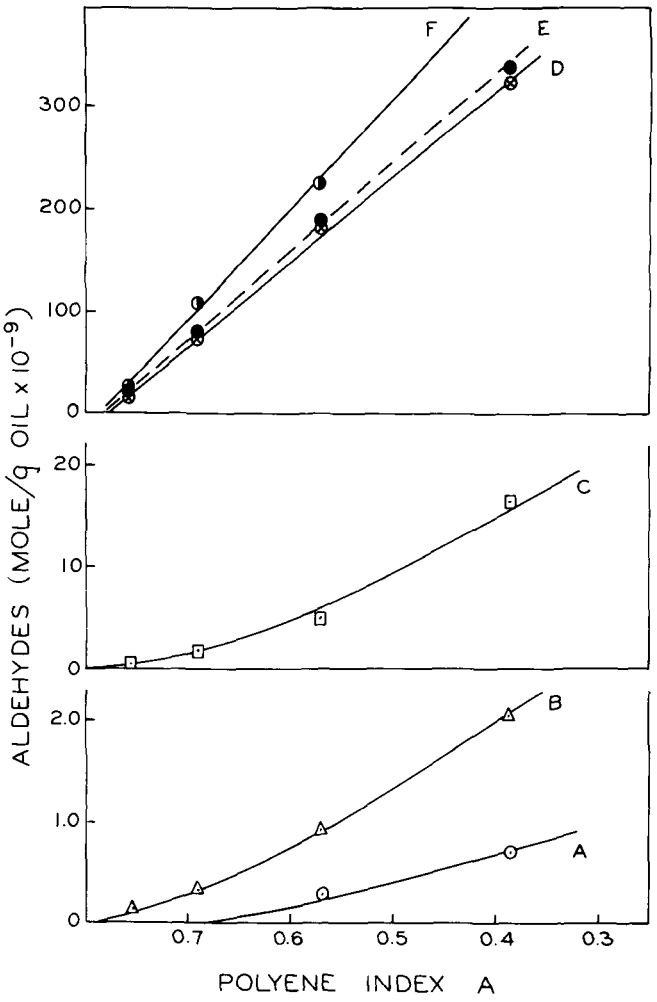

FIG. 3. The formation of (A) 2,4,7-decatrienals, (B) alkadienals, (C) alkenals, (D) alkanals, and (E) total aldehydes in oxidized mackerel oil, as a function of polyene index A.

nent fatty acids cannot be determined from the present limited data. However, as expected, polyenoic fatty acids obviously oxidized faster than monoenoic fatty compounds. For example, in the highly oxidized oil (No. 4), about half of the polyenoic acids were not recovered unchanged, and were presumably oxidized with a decrease from 25 to 13 molar percent; but the monoenoic fatty acids only were changed slightly with a decrease from 43 to 37 molar percent. Although all fish oil polyenoic fatty acids are sensitive to oxidation, the decrease rate of C22:6 to C16:0 has been used as an index of oxidative rancidity of lipids in fish products (19). However, in some fish oils, 20:5ω3 may be a considerably more important component than 22:6ω3 (20), and 22:6ω3 is often a flat peak difficult to quantitate in isothermal gas chromatography. Accordingly, 2 polyene indexes, A and B, have been calculated from the data of Table III and used to follow the oxidation reaction of the mackerel oil. The simpler, polyene index A is preferred as the parameter to monitor the development of rancidity of marine oils, because it includes only the 3 major polyenoic fatty acids 18:4, 20:5, and 22:6. These can be calculated with reasonable accuracy and with minimal problems because they are among the larger gas chromatographic peaks and are free from coincident minor fatty components in most cases of both packed column or open tubular GLC (21,22).

Mckerel are particularly prone to oxidation in frozen storage (23,24), and the peroxide value was found to be 55 in the skin of mackerel frozen at -15 C for 7 weeks (24). However, mackerel oils, which have iodine values as high as 155 (5), and which are as unstable to air and heat as most fish oils, have not been evaluated as thoroughly in studies on oxidation as some other more commercially important oils of marine origin, although an irradiation study of mackerel oil has shown a variety of long chain fission products (25).

The compositions of the monocarbonyl aldehydes from the volatiles of the 4 mackerel oils are listed in Table IV. They were oxidized at 40 C to respective peroxide values of 1,15,70, and 151, and analyzed by the combined GLC method (C). The major products of the alkanal, alkenal, and alkadienal classes, were n-propanal and n-penthanal, n-hexenal, and n-2,4-hexadienal, respectively. The pattern of formation of aldehydes in the oxidized mackerel oil is not unexpected, and is comparable to previous reports for other fish oils (12,26,27). The rates of formation of various aldehydes as functions of polyene index A have been plotted in Figure 3, and pseudolinear relationships have been obtained. The slight variations obtained are presumed to be from the induction period of lipid oxidation. Nonlinearity is most apparent in the plots for unsaturated aldehydes (curves A, B, and C in Figure 3).

Decatrienals were found to be significant only in the 2 more highly oxidized mackerel oils, and the rate of formation of 2,4,7-decatrienals was roughly proportional to the increase of POV (Table I) and the decrease of polyene index A (Fig. 3) after the induction period. This result further supports the previous suggestion (1) that decatrienals are formed from the decomposition of particular oxidized products from polyunsaturated fatty acids with the double bonds in patterns of ω3, ω6, and ω9. Because 2,4,7-decatrienals have not been detected in freshly extracted or moderately oxidized mackerel oils, it should be apparent that the fishy or trainy flavor in most mildly oxidized lipids of mackerel is probably due, not to decatrienals, but to the presence of a complex mixture of carbonyl compounds, as suggested by previous investigators (2,3,12). Mackerel, salmon, and other fish have distinctive odors which sometimes can be distinguished in high quality oils prepared from these fish. In poor quality fish oils, not only are oxidation products from fatty acids present, but a mixture of other chemicals arising from bacterial spoilage, cooking prior to production, etc., also are present. These include amines (trimethyl amine is the classic "fishy" odor), hydrocarbons, and mixtures of nitrogenous and sulfurous breakdown products (25, 28-31).

Comprehensive discussions on lipid oxidation, such as the Symposium on Food in 1961 (32) have stimulated continuing study on the inadequately understood processes of autoxidation. The established and accepted theory of lipid oxidation is that perioxides are the initial and predominant product from the free radical chain reactions and originate from hydroperoxide formation (33). In marine oils, as distinct from vegetable oils where 18:3ω3 is the most highly unsaturated fatty acid, any possible raction mechanism for the formation of 2,4,7-decatrienals is complicated by the role of the additional unsaturation, ω12,ω15, or ω18 in the C_{20} and C_{22} polyenoic acids. Thus, a further kinetic treatment must await characterization of most of the products from specific oxidation of 1 or more of the original polyenoic fatty acids and the determination of the quantitative relationships among intermediates at various stages (33-35).

REFERENCES

1. Meijboom, P.W., and J.B.A. Stroink, JAOCS 49:555 (1972).
2. Badings, H.T., Neth. Milk Dairy J. 19:69 (1965).
3. Badings, H.T., JAOCS 50:334 (1973).
4. Ackman, R.G., J. Fd. Technol. 2:169 (1967).
5. Ackman, R.G., and C.A. Eaton, Can. Inst. Food Technol. J. 4:169 (1971).

6. Pohl, P., and H. Wagner, Fette Seifen Anstrichm. 74:424 (1972).
7. Osbond, J.M., P.G. Philpott and J.C. Wickens, J. Chem. Soc. 2779 (1961).
8. Taylor, W.R., and F.M. Storng, JAOCS 3:1396 (1950).
9. Schwartz, D.P., and O.W. Parks, Anal. Chem. 33:1396 (1961).
10. Bligh, E.G., and W.J. Dyer, Can. J. Biochem. Physiol. 37:911 (1959).
11. Ke, P.J., and R.G. Ackman, JAOCS 50:429 (1973).
12. Wyatt, C.J., and E.A. Day, J. Food Sci. 28:305 (1963).
13. Lea, C.H., and A. Hobson-Frohock, J. Sci. Agr. 16:18 (1965).
14. Golovnya, R.V., and V.P. Uralets, Die Nahrung 16:497 (1972).
15. Meijboom, P.W., Fette Seifen Anstrichm. 70:477 (1968).
16. Morrison, W.R., and L.M. Smith, J. Lipid Res. 5:600 (1964).
17. Golovnya, R.V., and V.P. Uralets, Dokl. Akad, Nauk. 177:350 (1967).
18. Hollstein, E., F. Baumgardt and N. Franzke, Die Nahrung 17:659 (1973).
19. Shono, T., and M. Toyomizu, Sci. Bull. Fac. Agr. (Kyushu Univ. Japan) 26:233 (1972).
20. Ackman, R.G., C.A. Eaton and P.J. Ke, J. Fish. Res. Bd. Can. 24:2563 (1967).
21. Ackman, R.G., and R.D. Burgher, JAOCS 42:35 (1965).
22. Ackman, R.G., Lipids 5:950 (1970).
23. Nagayama, F., S. Imano and Y. Naito, Bull. Jap. Soc. Sci. Fish. 37:415 (1971).
24. Ke, P.J., R.G. Ackman and B.A. Linke, New Series Circular No. 53, Environment Canada, Fisheries and Marine Services, Halifax Laboratory, Halifax, Nova Scotia, Canada, 1975.
25. Dubrarcic, M.F., and W.W. Nawav, J. Agr. Food Chem. 17:639 (1969).
26. Fisher, M.P., and L.A. Wishner, Lipids, 3:88 (1968).
27. Yu, T.C., E.A. Day and R.O. Sinnhuber, J. Food Sci. 26:192 (1961).
28. Shimomura, M., F. Yushimatsu and F. Matsumoto, Kaseisaku Zasshi 22:106 (1971).
29. Gruger, E.H., Jr., J. Agr. Food Chem. 20:781 (1972).
30. Miller, A., III, R.A. Scanlon, J.S. Lee and L.M. Libbey, J. Fish Res. Bd. Can. 29:1125 (1972).
31. Miller, A., III, R.A. Scanlon, J.S. Lee and L.M. Libbey, Appl. Microbiol. 25:952 (1973).
32. Schultz, H.W., E.A. Day and R.O. Sinnhuber, "Lipids and Their Oxidation," Avi Publishing Co., Inc., Westport, Connecticut, 1962.
33. Labuza, T.P., CRC Critical Rev. Food Tech. 2:355 (1971).
34. Morita, M. and M. Fujimaki, J. Agr. Food Chem. 21:860 (1973).
35. Kimoto, W.I., and A.M. Gaddis, JAOCS 51:307 (1974).

[Received February 24, 1975]