Metal-catalyzed Oxidation in Mackerel Skin and Meat Lipids[1]

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ABSTRACT

Kinetic effects of added copper, zinc, and iron compounds have been investigated in the oxidation of lipids in mackerel skin and meat at 60 C using a simple weight gain method. Inorganic Fe(II) and Cu(II) were found to be strong catalysts in mackerel lipid oxidation. The meat lipids were particularly sensitive to oxidation in the presence of Fe(II) and Cu(II) below 5 ppm, and increments above this level did not result in a further increase in catalytic influence. The oxidation of skin lipids increased very rapidly with increased Fe(II) and Cu(II) concentrations of < 10 ppm, and slight increases in prooxidative effects were still recorded when additional metals were added. The oxidations catalyzed by hemoglobin and zinc for skin lipids, and hematin for meat lipids, were proportional on a semilogarithmic basis with the increases in the metal catalysts. The slight variations in fatty acids between mackerel skin and meat samples did not seem adequate to explain the rapid oxidation in skin lipids. Thus, we believe that in the skin lipids one or more fat-solvent-extractable prooxidants, alone or associated with trace metals, were present and were responsible for the high susceptibility to oxidation found for this lipid.

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TABLE I

Proximate Composition and Some Metal Contents in the Skin and Meats of Mackerel

Composition	Skin	Meats
Fats (% by wt)	50	12
Protein (% by wt)	14	18
Water (% by wt)	33	68
Ash (% by wt)	2	1
Amino acid composition (% by wt)		
Total aliphatic AA	33.5	29.4
Glycine	10.8	4.5
Total hydroxy AA	9.2	8.8
Total dicarboxylic AA	22.0	23.7
Glutamic	12.5	13.8
Total dibasic AA	16.8	21.3
Lysine	7.2	9.6
Histidine	2.7	5.5
Total aromatic AA	8.8	9.7
Total S-containing AA	2.6	3.3
Imino acid (proline)	6.6	3.7
Metal contents[a] (ppm, wet sample)		
Zn	19.7	4.0
Cu	1.4	1.1
Total Fe	17.5	10.8
Organic Fe	6.4	14.9[b] 0.2[c]

[a]The contents of Cd and Hg (< 0.05 ppm) and Co, Ni, and Mn (< 0.4 ppm) showed no significant differences between skin and meats.

[b]For the dark meat only.

[c]For the white meat only.

INTRODUCTION

The presence of monoenoic C_{20} and polyenoic C_{20} and C_{22} fatty acids as a characteristic feature of marine lipids has been thoroughly documented since the advent of various chromatographic techniques. Comprehensive reviews of marine lipids as they relate to human nutrition (1) and to food quality (2) include summaries and discussions of the well-known fact that, when exposed to air, marine lipids are particularly liable to oxidation.

Atlantic mackerel (*Scomber scombrus*) contain basically commonplace marine lipids and typical fatty acid compositions (3), but susceptibility to lipid autoxidation prevents the fish from being marketed after reasonable storage periods, either frozen or unfrozen (4). Autoxidation in lipids extracted from whole mackerel has been investigated (5), and the oxidative decomposition of these unsaturated fatty acids and the subsequent formation of monocarbonyls did not appear to differ appreciably from these processes in other marine lipids. However, studies of the lipid oxidation in different muscles within fish stored frozen under various conditions have indicated specific differences in the reaction rate and the kinetic characteristics of this process (6). In addition to the anticipated high susceptibility in the fatter dark meat and in the belly flap, the skin sample (which included the fatty layer underneath the skin) was unexpectedly shown to have the highest rate of lipid oxidation at -15 C, and, in fact, this took place about eight times faster than in meat (7). This unusual difference in reaction rates between mackerel skin and meat for lipid oxidation has also been observed when the extracted lipids were oxidized in thin films at 60 C (6). As the fatty acid composition is not exceptional (6), we were led to believe that one or more fat-solvent-extractable prooxidants probably are present in mackerel skin lipids and that these are responsible for their relatively high susceptibility to oxidation.

In 1971 the catalytic effect of mackerel dark muscle and liver tissues added to vegetable oils was studied (8). Recent research on lipid oxidation promoted by biochemical substances has included catalysis by extracts from various fish samples (9-12). In our recent study (6), we found that the proposed fat-solvent-extractable prooxidants in mackerel skin lipids have a negative temperature coefficient in the natural state under frozen conditions, and thus their catalytic activities in lipid oxidation can be reduced by lowering the frozen storage temperature to -40 C. Alternatively, trace metals could also play an important role in the catalytic oxidation of mackerel skin lipids, either by a direct acceleration of the formation of free radicals (13) or by an indirect activation or complex formation with certain lipids and proteins (14).

The kinetics of metal-catalyzed lipid oxidation have been reviewed by Ingold (15). The transition metals which possess two or more valence states with suitable oxidation potentials both decrease the induction period and increase the rate of lipid oxidation. These metals include iron, copper, cobalt, manganese, nickel, etc., as well as others of minor importance which have been recognized as having activity in the catalysis of lipid oxidation in blends of fish muscles (16) and in some marine oils and lipids (17). In this investigation, we have studied separately the kinetic effects of added copper, zinc, and iron compounds on lipid oxidation in mackerel skin and meat lipids. Possible correlations

with other properties and observations on changes during oxidation at 60 C have also been examined in search of possible causes of the acceleration of the rapid oxidation in skin lipids. In terms of the onset of oxidation in marine lipids, the most important problem in these competitive oxidations between skin and meat lipids, the induction period, has been also examined and compared for various metal-catalyzed conditions.

EXPERIMENTAL PROCEDURES

Preparation of Mackerel Lipids

Atlantic mackerel (*Scomber scombrus*) landed in Nova Scotia, October, 1974, were used for this study. Proximate compositions of the skin sample (including the fat layer underneath the skin) and the meats of fresh mackerel were determined by AOAC methods (18) and are presented in Table I. Amino acids were determined by Spackman's method (19) using chromatography by amino acid analyzer. The content of tocopherols was measured by a combined chromatographic procedure developed by Erickson and his co-workers (20).

Skin and meat lipids for study were extracted by using the method of Bligh and Dyer (21) on the skin and total meats (light and dark muscle) from samples of round mackerel which had been stored in polybags at -40 C for 2 weeks. The average lipid yield from 10 extractions was 50% from the skin samples and 12% from the meats. Each extract was subjected to prolonged high vacuum treatment to remove all traces of solvents and existing volatile compounds.

Analytical data for the two different lipids, determined by the official methods of AOCS (22), are compared in Table II. Oxidative stabilities of the skin and meat lipids at 60 C were estimated by both the AOM (active oxygen method) (22) and a weight gain method (17). TLC and GLC analyses were used for determining the composition of lipids (3) and fatty acids (5) as in our previous studies.

Estimation of Trace Metals in Mackerel Samples

The contents of mercury in mackerel skin and meats, and their lipids, were determined by a semiautomatic atomic absorption (AA) spectrophotometric method (23). For the determination of other trace metals, the skin and meats samples were carefully ashed at 650 C for 24 hr with a slowly increasing rate of temperature. The lipid samples were digested with sulfuric acid and hydrogen peroxide (30%) without preashing (24). The ashed tissue samples were also dissolved in the above-mentioned reagents. The metals in these solutions of decomposed sample were then extracted by methyl isobutyl ketone as the complexes with sodium diethyldithiocarbamate (24). The extracts were directly atomized into the AA spectrophotometer, and absorbances were measured in a Perkin-Elmer 403 AA spectrophotometer at 213.9 nm for Zn, 228.8 for Cd, 232.0 for Ni, 248.3 for Fe, 279.5 for Mn, and 324.7 for Cu as described above (24). The combined iron in hemoglobin and myoglobin in mackerel skin and meat samples was determined photometrically by Saffle's method (25).

Oxidation of Mackerel Skin and Meat Lipids

For the measurement of the rate of lipid oxidation by the weight gain method (17,26), lipid samples (10 g) were accurately weighted out in glass petri dishes (9 cm ID) and placed in a forced hot air oven at 60 ± 1 C. The increased weights of lipid samples were measured daily, the operation of cooling and weighing the samples being completed within 20 min. Six 10 g samples of one lot of skin lipids were oxidized in the petri dishes, and, after measuring the weight gain for all samples daily, the sample for each day was used for the determination of peroxide value (POV),

TABLE II

Analytical Data for Lipids Extracted from Skin and Meats of Mackerel for 2 Weeks at -40 C

Composition	Skin lipid	Meat lipid
Iodine value	93	102
Peroxide value (meq/kg oil)	0.5	0.4
Reflective Index (25 C)	1.4719	1.4754
Oxidative stability at 60 C (hr)		
AOM	152	>200
Induction period	60	>200
α-Tocopherol (mg/g oil)	0.21	0.35
Lipid composition (% by wt)		
Neutral lipids	95	94
Phospholipids	1.1	2.5
Free fatty acids	0.8	2.0
Unsaponified matters	3.1	1.5
Fatty acid composition (mol %)		
Saturates	28.4	30.5
Monoenes	51.0	44.0
Polyenes	20.6	25.5
Polyene Index[a]	0.538	0.768
Metal content (ppm)[b]		
Zn	0.9	0.3
Cu	0.4	0.2
Fe	0.5	0.3

[a]Polyene Index = (20:5+22:6)/(14:0+16:0).

[b]Mn, Ni, Co, and Hg in both skin and meat lipids showed no difference at a level of < 0.1 ppm.

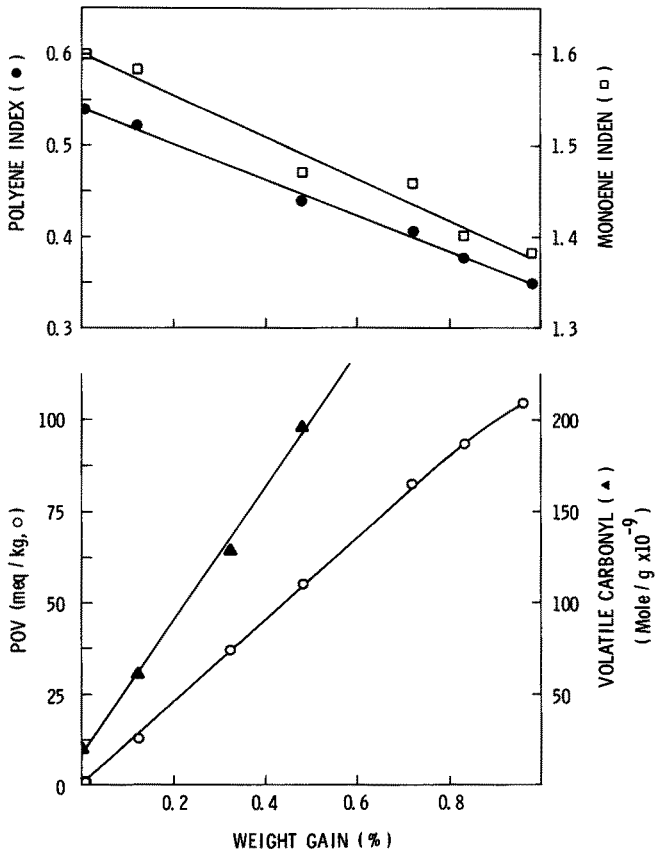

FIG. 1. Changes in lipid oxidation parameters as a function of weight gain in mackerel skin lipid at 60 C. Above: for monoene and polyene indexes. Below: for peroxide values and volatile carbonyls.

carbonyls, and monoene and polyene indexes by GLS methods (5). The relationships between progressive weight gain (% by wt) and these four chemical changes, as a function of oxidation time in the control lipid, are plotted

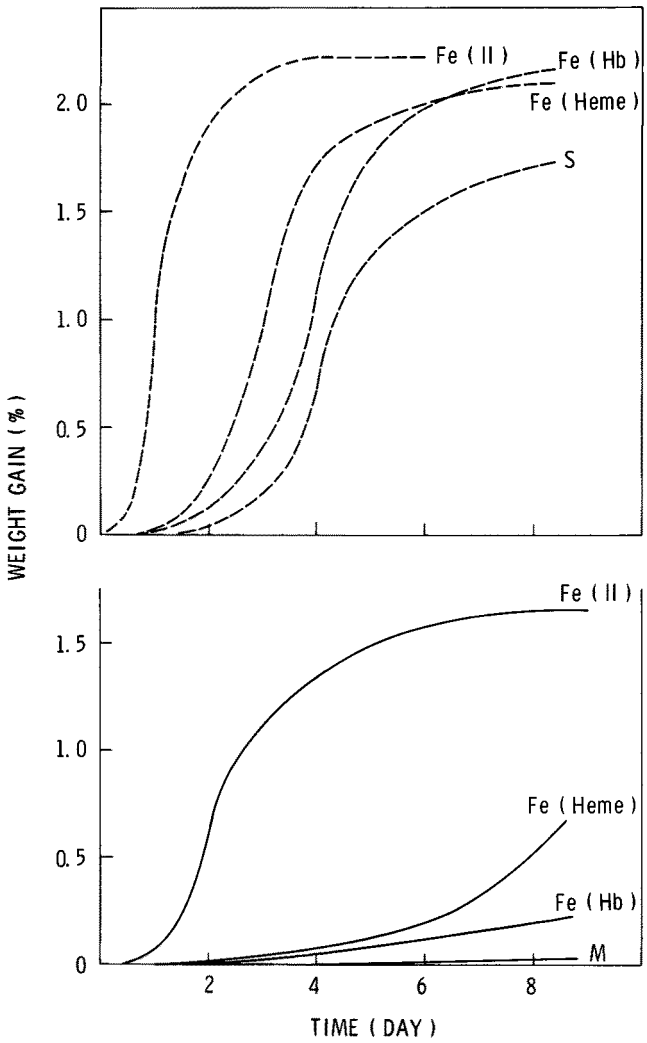

FIG. 2. Catalytic effects of the addition of 10 ppm of Fe (II), Fe (Hb, hemoglobin), and Fe (Heme) on oxidation at 60 C of mackerel skin lipids (S, above) and meat lipids (M, below).

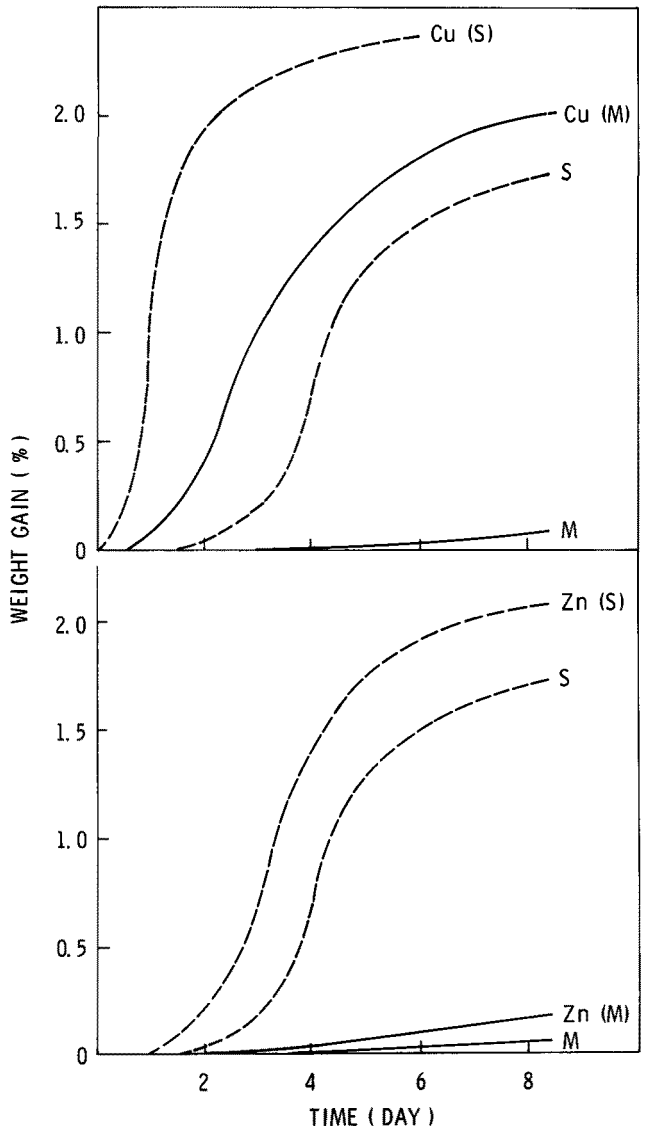

FIG. 3. Catalytic effect of the addition of 10 ppm of Cu (II) (above) and 30 ppm of Zn (II) (below) oxidation at 60 C of mackerel skin (S) and meat (M) lipids.

in Figure 1.

The compounds used for metal-catalyzed oxidation of mackerel lipids were $FeSO_4$, $CuSO_4$, and $ZnSO_4$ (Fisher Scientific Co., Fairlawn, NJ), hemoglobin (ICN Pharmaceuticals, Cleveland, OH), and hemin (Eastman Organic Chemicals, Rochester, NY). These metal-containing compounds were ground into fine powders (ca. 100 mesh), and the quantities required for catalyzing lipid oxidation under various conditions were calculated, based on the metal contents in the compounds. These powdered compounds were accurately weighed and transferred into 20-ml test tubes, each of which contained 15 g of the same lots of skin and meat lipids. After the lipid sample and the metal compound had been thoroughly mixed in the test tube by a Vortex-Genie mixer for 3 min, ca. 10 g of each of these mixed lipid samples was rapidly transferred into the petri dishes and weighed accurately. The uncovered petri dishes with lipid samples were subjected to exposure for oxidation in the oven at 60 C as described above, with a control sample of lipid only.

RESULTS AND DISCUSSION

Numerous variants of the different analytical procedures for monitoring the reaction of lipid oxidation have been proposed. Lea has summarized the methodologies commonly employed in following the oxidation reaction and discussed the details of application to various food lipids (27). From an organoleptic point of view, the rate of oxidation should not only be judged by analyses of primary oxidation products, but also by the determinations of secondary and tertiary products (28), as well as the decrease of unsaturated fatty acids (29). However, the time-consuming nature and unsatisfactory reproducibility of these methods often restrict their use in measuring the rate changes of lipid oxidation, particularly in differential kinetic studies of metal-catalyzed lipid oxidation. Thus, we preferred the simple weight gain method (17,26) used in the present study. The changes in other parameters of lipid oxidation such as POV, volatile carbonyls, and polyene and monoene indexes have been related by plotting the data as a function of the weight gain of the lipids during the oxidation period as shown in Figure 1. Appropriate linear relationships have been obtained, and in our opinion the weight gain method described can be simply and successfully applied for evaluating the catalytic effects of various metals on the oxidation of the two kinds of mackerel lipids.

Proximate composition data for mackerel skin and meat samples is compared in Table I. Mackerel skin, including the subcutaneous fat layer, had four times the fat content of the pooled meats from the same lot of fish. Relatively, the contents of proteins and water in the skin were both lower

than in the meats. In tne composition of amino acids, no significant difference was found except that, relative to the meats, the skin contained twice the amounts of glycine and proline and only half of the content of histidine. Since the catalytic activity of amino acids on lipid autoxidation is not specific to various lipids, data with a difference $> 1\%$ have been listed in Table I for comparison. With regard to the contents of trace metals, total iron in the skin was 70% higher than in the meat sample, but the distribution of organic iron, mainly in heme-compounds, showed some remarkable variations. The highest level was 14.9 ppm in dark meat with 6.4 ppm in skin and only 0.2 ppm in white meat. In both mackerel skin and meats, the contents of Cd, Hg, Co, Ni, and Mn were about the same, but the former contained five times more zinc and 30% more copper than the meat samples. In general, these results were in accord with incomplete data for various species of mackerels (30-32). The results of analyses of lipids extracted from mackerel skin and meats are compared in Table II. The degree of unsaturation of skin lipids, measured both in IV or polyene index, is slightly lower than in the meat lipids. The lipid compositions in mackerel skin and meat lipids do not show much difference, but the fatty acids in the skin lipids do include 7% more monoenes and 5% less polyenes than the meat oils. The content of zinc in skin lipids was three times higher than in the meat lipids, and a similar relationship was obtained for the corresponding tissue samples (Table I). The skin lipid also contains ca. 0.2 ppm of Cu, Fe, etc., more than in the meat lipids, with ca. 30% less tocopherol (Table II). These differences seem inadequate to explain the unusual difference of the reaction rates of oxidation between mackerel skin and meat lipids which we have observed (Table II, Figs. 2 and 3).

The catalytic effects of mixing 10 ppm of Fe or Cu, or 30 ppm of Zn, with the skin and meat lipids on the respective rates of oxidation reaction was investigated as shown in Figures 2 and 3. Comparisons of the effects of three forms of iron (i.e., inorganic, heme, and hemoglobin at 10 ppm) on the oxidation in mackerel skin and meat lipids are presented in Figure 2. These curves indicate clearly that inorganic Fe (II) is a stronger catalyst than organic iron for both kinds of mackerel lipids. Obviously, oxidation in mackerel meat lipids can be accelerated to almost the same reaction rate as pure skin lipids by simply adding 10 ppm of Fe (II). As shown in Figure 3, the catalytic effect on lipid oxidation of 10 ppm Cu (II) is the same as Fe (II). However, the rates of oxidation for both types of mackerel lipids were only slightly promoted when 30 ppm of zinc was added.

Oxidation of mackerel skin and meat lipids catalyzed by various concentrations of Fe, Cu, and Zn are also shown in Figure 4 in terms of induction periods which were estimated from their reaction curves. The catalytic effects of inorganic Fe (II) or Cu (II) on oxidation for both skin and meat lipids are approximately identical, but Cu (II) seemed to be little less effective than Fe (II). It should be noted that mackerel meat lipids are particularly sensitive to oxidation in the presence of inorganic Fe (II) and Cu (II) at concentrations below 5 ppm. Above 5 ppm the oxidation rates were not markedly affected when more Fe or Cu was added. The catalytic influence of Fe (II) and Cu (II) on mackerel skin lipids is slightly different, as shown in Figure 4. The rates of oxidation reaction increase very rapidly when the metal concentrations are increased at < 10 ppm, although increased prooxidative effects could still be observed when more than 10 ppm of metals were used to catalyze the reactions. In addition, the rates of oxidation catalyzed by hemoglobin and zinc for skin lipids, and of hemetin for meat lipids, also increased slowly with concentration and are proportional on a semilogarithmic basis with the increase of concentration of metal catalysts.

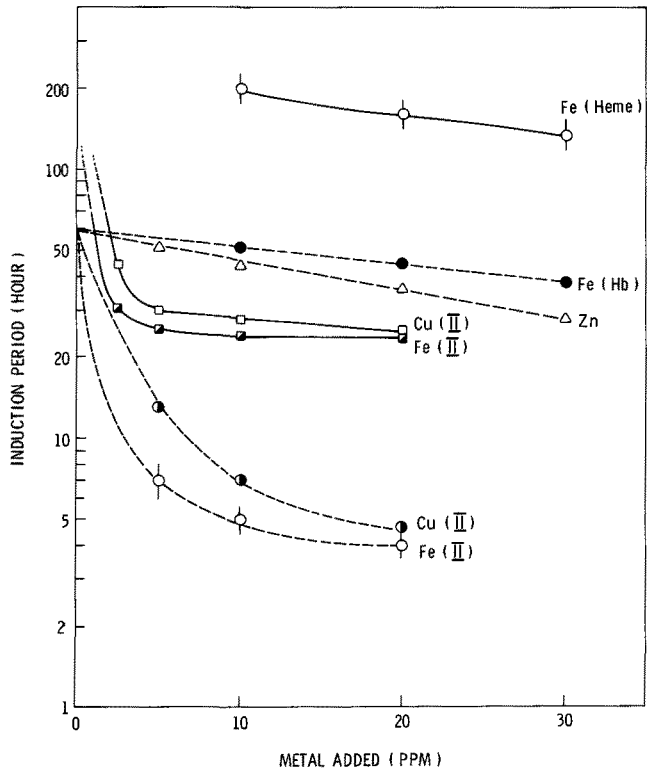

FIG. 4. Variations of induction period in oxidation and mackerel skin (- - -) and meat (——) lipids at 60 C, catalyzed by adding various concentrations of metals.

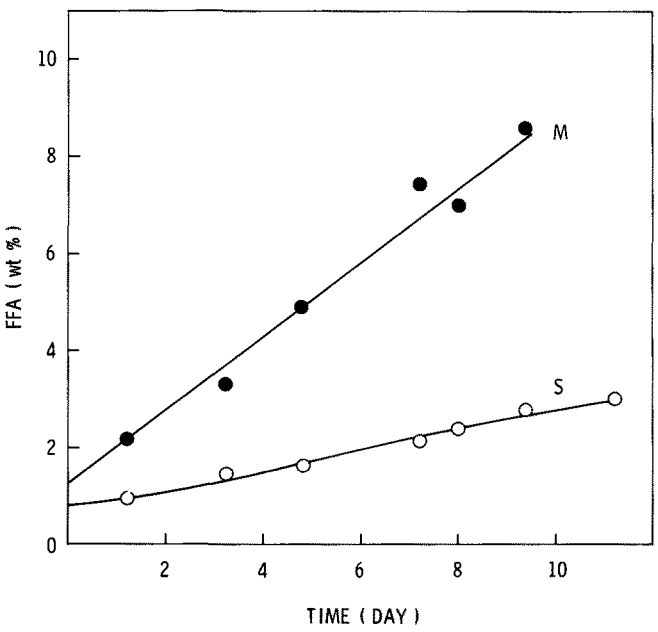

FIG. 5. The formation of FFA in mackerel skin (S) and meat (M) lipids at 60 C during the period of autoxidation.

Lipids hydrolysis with the formation of FFA under frozen conditions has been reported to occur more rapidly in lean fish than in fatty fish (33). In frozen mackerel we have found that the rate of FFA formation was approximately proportional to the fat content in the various parts, except that skin lipids were hydrolyzed much more slowly (6). During the oxidation period at 60 C, the formation rates of FFA in mackerel skin and meat lipids were determined and are presented in Figure 5. The skin lipids were much more slowly hydrolyzed in comparison with the meat

lipids, a result comparable to that for frozen fish. So far, metal-catalyzed lipid hydrolysis in mackerel has not been investigated. However, it has been postulated that FFA interacting with proteins is the driving force for lipid hydrolysis and would affect the oxidation reaction in food lipids (34,35).

In the study described above, inorganic iron and copper have been found to be strong prooxidative catalysts in lipid oxidation, particularly in mackerel meat lipids. The very low levels and small differences of metal contents between skin and meat lipids of mackerel (Table II) cannot fully explain the problem of the rapid oxidation reaction in mackerel skin lipids. As described in our previous reports (6,7), solvent-extractable prooxidants, which may be associated or coupled with some trace metals, could be active in catalyzing the oxidation reaction in skin lipids of mackerel. Since metal ions such as Fe, Cu, and Zn are well represented in biomolecules, and frequently these metal ions in living cells are more concentrated than in the environment as a result of the formation of stable biological complexes (36), the details of the role and reaction mechanism of the trace metals in the oxidation of mackerel skin lipids could be very complex.

In recent studies, various biochemical substances, such as amino acids (10), heme-compounds (8,37,38), organic acids (11,37), pigments (39), and various fish tissues (8,9), have been shown to catalyze the lipid oxidation reaction alone or in association with some trace metals. Sedlacek (13) has demonstrated that some cations can accelerate the formation of secondary oxidation reactions as well as promoting the primary lipid oxidation. Therefore, many details of the processes which could be related to the oxidation potential in two kinds of mackerel lipids still have to be elucidated. In the study of metal-catalyzed oxidation of mackerel lipids, the chief interest lies in how the catalysts function at low levels of oxidation (i.e., within the induction period), since most lipids become unpalatable after the oxidation state has passed into the biomolecular reaction region. Metal catalysts can certainly affect the rates of initiation as well as other reactions known to occur in lipid oxidation. Since we have found differences both in situ and in vitro between two kinds of mackerel lipids, we believe that the complementary data with metal catalysts provides more fundamental information which could lead to a model system for the differential kinetic study of both catalytic and inhibitory effects on metal-catalyzed lipid oxidation.

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