OXIDATIVE DETERIORATION IN FISH AND FISHERY PRODUCTS

Part II - Progress on Studies Concerning Mechanism of Oxidation of Oil in Fish Tissue

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ABSTRACT

Studies showed that the mechanism of oxidation of fish oils in the meat of fish or in fish meal differs from that of extracted oils owing to the activity of biocatalysts in the tissues. The fish meat content and activity of hematin, found to be the primary catalytic agent in such oxidation, was studied. Activities of the proteins of the meat were found to be of lesser importance. Studies were made on naturally-occurring and commercial antioxidants. A mixture of the natural antioxidants citric acid, ascorbic acid, and tocopherol was found to be 80 times as effective, owing to synergism, than was citric acid or ascorbic acid alone. Method of addition of the antioxidant to the test system was found to be of importance in retarding oxidation.

The portion of the program on which progress is reported in this paper deals with the mechanism of oil oxidation while the oil still is associated with the fish tissue or while it is present in fish meal. The mechanism of the oxidation is different under these conditions from that in extracted oils because of the presence in the tissue of certain biocatalysts that affect the course of the oxidation.

Fig. 1 - Colorimetric determination of tocopherol in king salmon oil.

This phase is basic to applications whether involving changes in oil in situ in the meat of stored fish, in fish meals, or in the extracted oil itself because, in all these cases, the oil starts out in the meat and some changes always take place initially in the meat before any manufacturing steps have been started. Accordingly, chief emphasis in the initial stages of this program has been toward elucidating the mechanism of oxidation of oil in fish meat.

HEMATIN CATALYSIS

HEMATIN-COMPOUND CONTENT OF FISH: In the first phase of the work a number of species of fish were analyzed for their content of hematin compounds, substances that have been shown to be powerful biocatalysts for oil oxidation in other foods. Nine species of fish were examined, and the hematin compounds were found to vary in content from 5.4×10^{-5} M in pilchard down to 0.1×10^{-5} M in cod.

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CATALYTIC EFFECT OF HEMATIN COMPOUNDS: The effect of the hematin compounds of fish meat on the catalysis of oil oxidation next was determined. This work was carried out by measuring the oxygen uptake of a salt of an unsaturated fatty acid, ammonium linoleate, when shaken in a Warburg respirometer. With the reaction being carried out at 20° C. and at a pH of 9.0, aqueous extracts of fish were added to the ammonium linoleate substrate and their effect on the rate of oxidation was determined. The fact that the catalysis was due primarily to hematin compounds and not to some other biocatalysts in the fish was confirmed by repeating the experiment in the presence of cyanide, in which case no catalytic effect was observed. Cyanide inhibits hematin catalysis of unsaturated fatty acids oxidation.

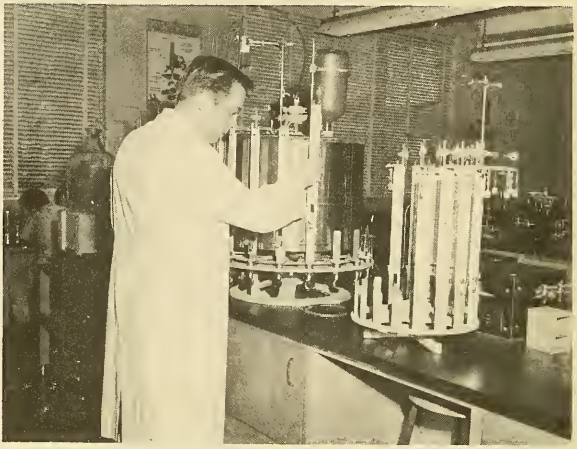

Fig. 2 - Measuring oxidation of tuna meals employing a Warburg apparatus for measurement of oxygen adsorption.

In another series of experiments (1) cubes of fish meat and (2) extracted fish oil from different species were measured for rate of oxygen uptake in a Warburg apparatus.

The results of all these experiments showed a direct correlation between the hematin-compound content of the various species of fish and the catalytic effect on the linoleate oxidation. The rate of oxidation of the fish meat also was correlated with the content of hematin compounds.

Table 1 - Oxidation Rate of Cubes of Light and Dark Meat of Fish in a Warburg Apparatus at 0° C.

Species	Rate of Oxidation	
	Light Meat	Dark Meat
	Microliters of Oxygen Uptake Per Hour Per Gram	
Chum salmon	0	17
Pink salmon	0	13
Tuna	0	13
Sheepshead	0.4	1.5
Lake chub	0.6	1/
Pilchard	7	1/
Rockfish	1	1/
Cod	0.5	1/
Mackerel	8	1/

1/ For these species, no separation of light and dark meat was attempted. The cubes of meat used, however, were mostly light meat.

CATALYTIC EFFECT OF PROTEINS: Some experiments were carried out to determine the catalytic effect of various proteins on the oxidation of fish oils. It was shown that all of the proteins studied had a measurable effect, but that none of these proteins approached hemoglobin in catalyzing the oxidation reaction.

HEMATIN-COMPOUND CHANGES DURING OXIDATION: During oxidation of oil in fish tissue, the hematin compounds that catalyze the oxidation are chemically altered, and their concentration diminishes. Cubes of tuna meat, cut from both the light and the dark tissue, were allowed to oxidize in a Warburg apparatus at 0° C. The content of hematin compound diminished from 2.1×10^{-5} M to 1.0×10^{-5} M in the light

meat and from 67.2 x 10^{-5} M to 49.6 x 10^{-5} M in the dark meat. Spectral absorption curves of aqueous extracts of the samples before and after oxidation showed a shift

Table 2 - Protective Factors for Antioxidants, With or Without Synergists, in a Linolenate Model System

Antioxidant 1/	Protective Factor 2/ in Presence or Absence of Indicated Synergist				
	None	Citrate	Citrate + Ascorbate	Ascorbate	10X Ascorbate
BHA	4.0	4.8	2.0	2.0	9.0
2X BHA	8.6				
BHT	4.9	8.7	1.4	1.4	7.1
2X BHT	15.9				
BHA + BHT	13.5				
NDGA	2.6				
2X NDGA	9.2				
DPPD	2.4				
2X DPPD	54				
Santoquin	8.9				
2X Santoquin	80				

1/ The concentration of antioxidants and synergists was 10^{-4} M in final solutions except that it was 2 or 10 times this amount when 2x or 10x respectively, are indicated.
BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene;
NDGA = nordihydroguaiaretic acid; DPPD = N, N-diphenyl-p-phenylenediamine.

2/ Protective factor = $\frac{\text{Time for system to absorb 300 } \mu 1.0_2}{\text{Time for control to absorb 300 } \mu 1.0_2}$

in maxima from 542 and 578 millimicrons for the unaltered samples to 500 and 630 millimicrons for the oxidized ones. This shift indicates a transformation of oxyhemoglobin (or oxymyoglobin) to methemoglobin (or metmyoglobin).

RATE OF OXIDATION IN FISH MEAT: The rate of oxygen adsorption by cubes of different species of fish were measured in a Warburg apparatus at 0° C. The samples were treated with aureomycin to retard bacterial decomposition. Measurements were made on both the light and the dark meat. The results are shown in table 1.

ROLE OF ANTIOXIDANTS

NATURALLY-OCCURRING ANTIOXIDANTS: Certain antioxidants occur in foodstuffs and are harmless for use as added antioxidants to foods. These include such substances as ascorbic acid, citric acid, and alpha tocopherol. The latter substance occurs in the vitamin E group present in fish oils. Initial work was concentrated on the effect of these types of antioxidants. Oxygen-absorption tests were carried out in model systems in which in most cases an emulsion of (1) one of the fatty acids found in fish-oil glycerides with (2) hemoglobin and a buffer solution was employed. The use of this model system permitted reproducible results to be obtained over a period of time, whereas the use of a fish oil rather than of a pure fatty acid as a test material would have made it impossible to obtain repeat results because of the changes that occur in fish oils with storage and the impossibility of getting new lots of fish oils that are chemically identical to those used in earlier work.

In one test, results using the model system were compared with those obtained employing menhaden oil, and it was found that parallel types of results were obtained. The rate of oxidation was greater and the effect of a given antioxidant was less with menhaden oil, but the comparative effectiveness of different antioxidants was the same in the model system as in the menhaden-oil system. In general, it was found that citric acid and ascorbic acid synergize with tocopherol in inhibiting oxidation. A mixture of alpha tocopherol and citric acid and ascorbic acid, for exam-

ple, was about 80 times more effective in retarding oxidation than was citric acid and ascorbic acid alone.

COMMERCIAL ANTIOXIDANTS: For certain applications to fishery products, it is possible to use some of the more potent antioxidants that are commercially available. A series of tests were carried out employing some of these commercial antioxidants in a model system.

This system consisted of 10 milliliters of linoleic acid (60 percent), 20 milliliters of phosphate buffer (pH 7.0), 0.25 milliliters of Tween 40, and sufficient hemoglobin solution to effect a concentration of 5×10^{-5} M in the final mixture. To this was added the particular antioxidant system under test, and the oxygen adsorption was measured for at least 1 hour in a Warburg apparatus at a temperature of 37° C.

Table 3 - Oxidation Rate of Fish Meals and Freeze-Dried Fish at 30° C.

Sample	Oxidation Rate
	Microliters Per Hour Per Gram
Menhaden meal 1/	10
Tuna meal 1/	3
Freeze-dried:	
Pilchard	300
Mackerel	300
Tuna	28
Pink salmon (light meat)	44
Pink salmon (dark meat)	370
Chum salmon (light meat)	2
Chum salmon (dark meat)	34
Sole	3
Halibut	2
Cod	0.8

1/ These commercial meals had been stored for several months and hence already had gone through the rapid initial oxidation.

The results obtained with the various substances tested are shown in table 2. It can be seen that the antioxidant Santoquin gave the greatest protection against oxidation. This particular antioxidant is not now approved by the Food and Drug Administration for general commercial use.

OXIDATION OF OIL IN FISH MEALS

RATE OF OXIDATION OF MEALS: In this work, both commercial fish meals and freeze-dried fish were used. The latter may be considered as fish meals dried under ideal conditions. The commercial fish meals used in this work had been stored for a period of months before the studies on rate of oxidation were started. They therefore already had gone through the initial high rate of oxidation, which was not the case with the freeze-dried samples. Table 3 shows the oxidation rate for these samples, the moisture content of which had been adjusted to 5 percent. The rate of oxidation was measured in a Warburg apparatus at 30° C.

Table 4 - Oxidation Rate of Tuna Meal 1/ Protected by Various Antioxidants

Antioxidant	Oxidation Rate of Tuna Meal
	Microliters Per Hour Per Gram
Santoquin	3.0
DPPD	3.8
BHA	5.8
BHT	6.7
Propyl gallate	17.0
NDGA	22.0
None (control)	27.5

1/ Yellowfin-albacore mixture.

EFFECT OF COMMERCIAL ANTIOXIDANTS: The oxidation rates of fish meals to which had been added various commercial antioxidants were measured. Antioxidants were added to meals that had been prepared commercially as described in the preceding paragraph. In all cases, the antioxidants reduced the rate of oxygen uptake (table 4).

The slowing of the oxidation rate by any one antioxidant was closely related to the method by which it was added to the meal. Use of a solvent increased the effectiveness considerably (table 5).

The antioxidant effectiveness also was increased by increasing the thoroughness of mixing. Thus in one case where Santoquin was stirred in by hand (employing a solvent), an oxidation rate of 12.0 microliters per hour per gram was obtained, whereas when the antioxidant was added (in a solvent) by grinding twice a value of 3.0 microliters per hour per gram was obtained.

Table 5 - Effect of Using Solvent to Incorporate Antioxidant in Tuna Meal 1/

Antioxidant	Used for Incorporation of Antioxidant	
	No Solvent	Solvent
	Microliters Per Hour Per Gram of Oxygen Uptake	
DPPD	7.0	3.8
BHA	7.4	5.8
BHT	8.0	6.7
Control.	27.5	27.5

1/ Yellowfin-albacore mixture.

SUMMARY

1. Hematin compounds have been shown to play a major role in catalysis of oxidation of fish oils. The content of such compounds, measured in nine species of fish, varied from 5.4×10^{-5} M to 0.1×10^{-5} M.

2. Various combinations of antioxidants were developed for application in retarding oxidation of oil in fish tissue. Some of these were far more effective than were the usual antioxidants used alone or with a single synergist. A mixture of tocopherol, ascorbic acid and citric acid, for example, was about 80 times as effective as was ascorbic acid alone or mixtures of ascorbic acid and of citric acid.

3. The effectiveness of the most widely-used commercial food and feed antioxidants was compared.

4. The rate of oxygen adsorption in fish meals was measured, and certain commercial antioxidants were found to be effective in retarding oxidation. The method of mixing the antioxidant with the meal was found to be of considerable importance in determining the effectiveness of the antioxidant.

USE OF CHEMICALLY-TREATED ICE FOR FISH

"Storage of Fish in Ice Containing Antibiotics or Chemical Storage Products, Annual Report, 1954." Institute of Chemical and Technical Investigations of the Fisheries Department, Bergen, 1955, pp. 11-12.

The experiments were done on cod, one part of which was stored in ice containing aureomycin, and the other, used as a control sample, in pure ice. The temperature range was 0° to -2° C. (32° and 28.4° F.). After 2 days, 97 percent of bacteria were destroyed. Between -0.5 and $+1^{\circ}$ C. (31.1° and 30.2° F.), aureomycin is still more efficient: 99.2 percent of bacteria are destroyed.

The use of foromycene, a chemical storage product, gave similar results.