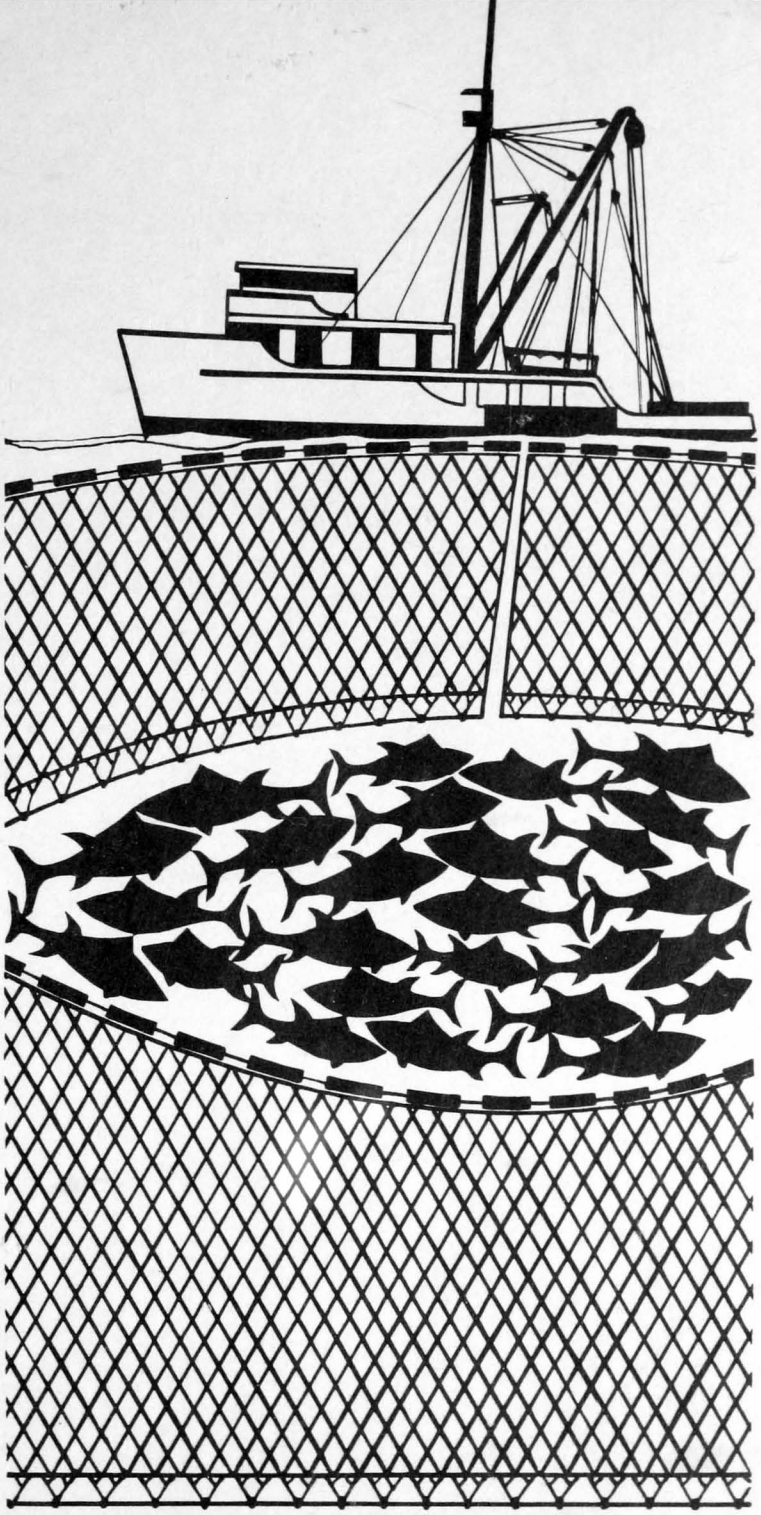

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FISHERY INDUSTRIAL RESEARCH

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EFFECT OF GAMMA RADIATION ON THIAMINASE ACTIVITY IN FRESH-WATER FISH

by

Richard A. Krzeczkowski

ABSTRACT

Sensitivity to gamma radiation of the antimetabolite thiaminase in whole fish was studied so that the potential usefulness of this radiation for controlling the activity of thiaminase in fishery products could be estimated. Although gamma radiation will partially inactivate thiaminase in the species studied, its use for complete inactivation does not appear to be economically feasible.

INTRODUCTION

Thiaminase is an antimetabolite that is indigenous to many species of fish and shellfish (Deusch and Hasler, 1943) and that readily destroys the physiological activity of thiamine (Melnick, Hochberg, and Oser, 1945). When feeds containing thiaminase are ingested by certain animals, a vitamin B_1 deficiency disease, commonly called *Chastek paralysis* (Lee, 1948) results. Thiaminase is regarded as being protein in nature, or at least as containing a protein moiety (Sealock, Livermore, and Evans, 1943) possessing enzymatic characteristics.

Preliminary studies at this laboratory suggested that the activity of the thiaminase of alewife (*Alosa pseudoharengus*), a species that occurs in quantity in the Great Lakes, could be reduced by gamma irradiation. The mode of action, however, was not determined. Possible use of irradiation in processing industrial fishery products encouraged the further study of the capability of gamma radiation to inactivate this enzyme. The general aim of the present investigation, therefore, was to determine the potential usefulness of gamma radiation for inactivating the enzymatic activity of thiaminase in fishery products.

All the thiaminase preparations studied were obtained from whole fish: no attempt was made to determine the effect of irradiation on purified enzyme systems. The variables studied were: (1) irradiation dosage, (2) irradiation temperature, (3) enzyme concentration, (4) pH of irradiated sample, and (5) chemical additives.

I. EFFECT OF DOSAGE

Samples were prepared and irradiated in a manner simulating potential industrial conditions. Commercially, the fish products irradiated for animal food would most likely

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be whole fish or fish wastes. These products therefore were the ones given major attention.

Whole alewife were ground while cold in a meat grinder, first through a plate with ¼-inch holes and then through a plate with ⅛-inch holes. The ground material was mixed, then sealed in TDT (thermal-death-time) cans, and held frozen until irradiated. Before being irradiated, samples were removed from frozen storage while still in the TDT cans, equilibrated to 32° F. in ice water or to the ambient temperature of irradiator (80°-104° F.), and then placed in an insulated cylindrical container.

The samples were irradiated for 0 to 6 hours at a dose rate of 0.85 megarad per hour within the core of a 10,000-curie cobalt-60 source at the Phoenix Memorial Laboratory, University of Michigan, Ann Arbor. During irradiation, samples were maintained either at ambient temperature (80°-104° F.) or at about 33° F. by means of crushed ice packed above and below the TDT cans in the container. Cans were removed from the core at time intervals commensurate with the desired dose, refrozen, and held at 0° F. until analyzed. Except for irradiation, controls were subjected to the same conditions.

Owing to the thiaminase in the alewife, the ground samples contained no thiamine. Presumably, then, the concentration of thiaminase could be measured if the rate of destruction of added thiamine could be followed. Accordingly, known quantities of thiamine hydrochloride were added to potassium chloride extracts of ground samples after irradiation, and the enzymatic activity of thiaminase was determined chemically by use of a modi-

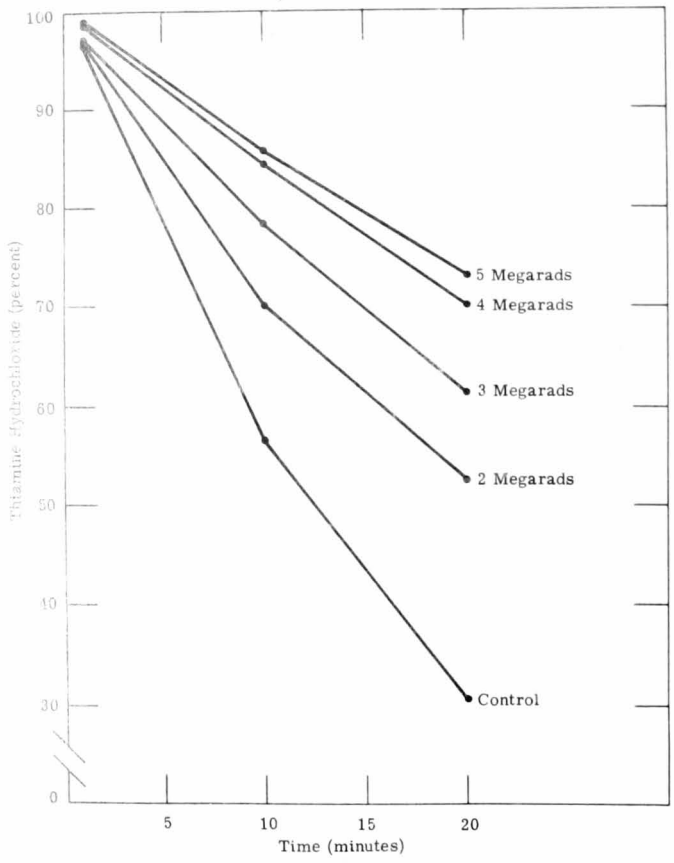

Figure 1.—Effect of gamma radiation at 33° F. on the thiaminase activity of whole alewife at its natural pH (6.9). Thiaminase activity is expressed as a percent of standard thiamine hydrochloride destroyed per unit of time under standard assay conditions.

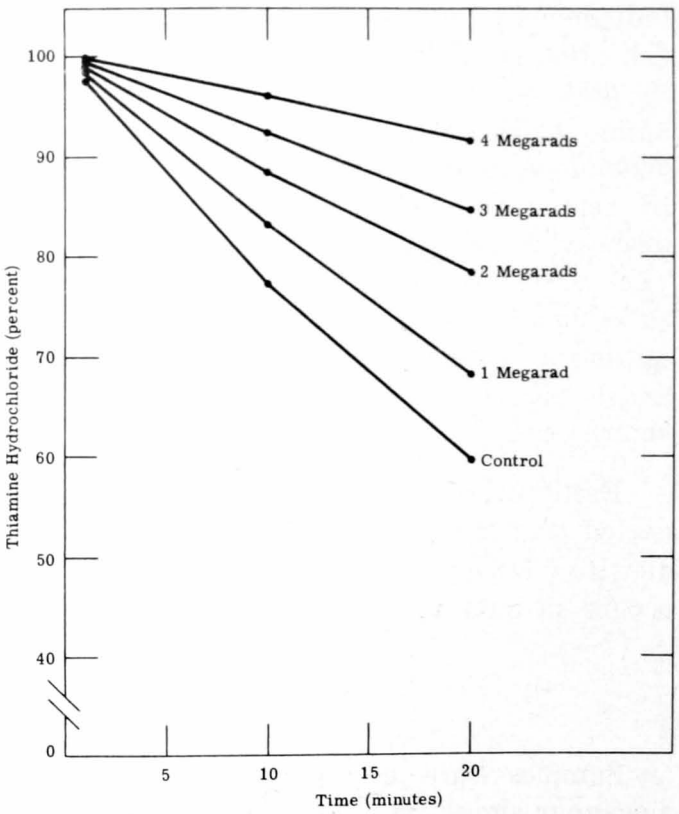

Figure 2.—Effect of gamma radiation at 80°-104° F. on the thiaminase activity of whole alewife at its natural pH (6.9). Thiaminase activity is expressed as a percent of standard thiamine hydrochloride destroyed per unit of time under standard assay conditions.

fied thiochrome method (Gnaedinger, 1965) in which the thiamine was oxidized to thiochrome, a fluorescent compound. The destruction of thiamine in thiaminase extracts was expressed graphically in plots of data obtained by measurement of the fluorescence of thiochrome per unit of time under standard assay conditions (Figures 1 and 2). From the thiaminase-destruction curves, the quantity of thiamine converted per unit of time was computed, and the percentage reduction in thiaminase activity at various dosages and temperatures was calculated.

The added thiamine hydrochloride was destroyed most rapidly in the unirradiated control sample. An increase in dosage resulted in a slower rate of thiamine destruction — that is, in a decrease in thiaminase activity (Table 1). The decrease was more pronounced at the higher irradiation temperature.

Table 1.—Effect of irradiation dosage on thiaminase radiosensitivity in whole alewife

Dosage	Reduction in thiaminase activity of samples irradiated at:	
	33° F.	80°-104° F.
Mrads	*Percent*[1]	*Percent*[1]
1	--	20
2	31	48
3	44	63
4	56	80
5	61	--

[1] Percentage loss of activity relative to that of the nonirradiated control samples.

II. EFFECT OF IRRADIATION TEMPERATURE

The temperatures of irradiation were 80°-104° F. (the ambient temperature of the irradiation facility), 33° F., and below 0° F. The temperature below 0° F. was maintained by packing dry ice around the TDT cans held in the insulated cylindrical container, described earlier. Samples of whole alewife, carp, spottail shiner, bowfin, gizzard shad, and American smelt were prepared for comparative purposes and analyzed as described in the previous section. Table 2 shows the effect of irradiation temperature on thiaminase activity in the various species.

The temperature of the sample at the time of irradiation is evidently an important factor in the sensitivity of thiaminase to radiation. As in the preceding experiment, the greater reduction in activity took place at the higher temperatures.

Table 2.—Effect of gamma radiation on thiaminase activity in various species of fish

Species		Natural pH	Natural thiaminase activity	Reduction in thiaminase activity at:		
Common name	Scientific name			6 Mrads; <0° F.[1]	4 Mrads; 33° F.	4 Mrads; 80°-104° F.
			[2]	*Percent*[3]	*Percent*[3]	*Percent*[3]
Carp	*Cyprinus carpio*	6.75	2,003	0	14	23
Spottail shiner	*Notropis hudsonius*	6.70	1,414	0	27	50
Bowfin	*Amia calva*	6.60	206	0	24	54
Alewife	*Alosa pseudoharengus*	6.90	173	0	56	80
Gizzard shad	*Dorosoma cepedianum*	6.65	112	0	13	43
American smelt	*Osmerus mordax*	6.85	47	Slight	52	95

[1] Samples irradiated in dry ice.
[2] Micrograms of thiamine hydrochloride destroyed in 20 minutes per gram of protein of the untreated raw fish
[3] Percentage loss of activity relative to that of the nonirradiated control samples.

III. EFFECT OF ENZYME CONCENTRATION

Table 2 indicates that the degree of reduction in thiaminase activity of the various species tested is inversely related to the initial amount of thiaminase activity present. Nevertheless, the results are ambiguous, since both the species and the amount of thiaminase

activity varied.

Studies were therefore conducted to determine whether the differences observed in the reduction of thiaminase activity among the species could have depended upon the concentration of enzyme alone. For this purpose, fillets and viscera of alewife were separated from the whole fish. After separation the samples, including those of whole fish, were ground, mixed with thiamine hydrochloride, and assayed for thiaminase. Of the three kinds of samples, viscera showed the highest thiaminase activity (Table 3); fillets, the lowest.

Similar samples were then placed in TDT cans and irradiated at 4 megarads while being held at 33° F.; they subsequently were analyzed for residual thiaminase. The reduction in the amount of thiaminase activity was inversely related to the initial level of activity (Table 3). The differences in residual activity after irradiation appear to be due either to initial enzyme concentration or to stabilizing agents (particularly to the alewife viscera), or to both. Enzyme concentration as a cause of differences was proposed by Lewis, Wells, and Wormall (1961), who demonstrated that urease becomes increasingly self-protective against the effects of X-rays as its concentration is increased.

Table 3.—Effect of enzyme concentration in the alewife on thiaminase radiosensitivity

Kind of sample	Natural pH	Natural thiaminase activity	Reduction in thiaminase activity when samples were irradiated at 4 megarads and 33° F.
		[1]	*Percent*[2]
Viscera	6.9	572	7
Whole fish ...	6.9	185	45
Fillets	6.4	152	58

[1] Micrograms of thiamine hydrochloride destroyed in 20 minutes per gram of protein of the untreated raw fish.

[2] Percentage loss of activity relative to that of the nonirradiated control samples.

IV. EFFECT OF pH

Preliminary studies showed that the natural pH of the various species of fish investigated differed somewhat. Therefore, the effect of pH on thiaminase radiosensitivity was studied in more detail. Samples of whole, ground alewife (prepared as previously described) were adjusted either to pH 8.1 or to pH 4.6 with dilute solutions of sodium hydroxide or hydrochloric acid, respectively. This change in pH alone (without irradiation) did not affect the activity of thiaminase. Adjusted samples, together with a control at natural pH (6.9), were sealed in TDT cans and, in separate experiments, were irradiated at 4 megarads and 33° F. or 4 megarads at the ambient temperature (80°-104° F.).

Table 4 reveals that thiaminase radiosensitivity is dependent, perhaps being highest at the natural pH. Tanaka, Hatano, and Ganno (1959) similarly observed that the radiosensitivity of an enzyme — in their experiments, urease — to gamma rays is influenced by pH.

Table 4.—Effect of pH on thiaminase radiosensitivity in alewife

pH	Reduction in thiaminase activity at 4 megarads and;	
	33° F.	80°-104° F.
	Percent[1]	*Percent*[1]
8.1	26	56
6.9[2]	56	80
4.6	20	33

[1] Percentage loss of activity relative to that of the nonirradiated control samples.

[2] Natural pH.

V. EFFECT OF ADDED CYSTEINE AND SODIUM ACETATE

Hutchinson (1960) showed that cysteine halved the radiation sensitivity of invertase and ribonuclease, whereas acetate buffer increased the radiation damage to these enzymes by from one-and-a-half fold to fivefold. Cysteine and sodium acetate were therefore added to whole alewife so that their effects on thiaminase radiosensitivity could be observed. Samples of whole alewife were prepared as before, except for the addition of 2.4 percent

cysteine or sodium acetate. Samples were irradiated at 4 megarads and 33° F. and analyzed as before. Controls consisted of samples that (1) were irradiated without the additives or (2) were not irradiated but with the additives.

Cysteine completely prevented irradiation destruction of thiaminase activity (Table 5); sodium acetate decreased the radiosensitivity of thiaminase activity by about 25 percent. Cysteine or sodium acetate alone (without irradiation) did not affect the activity of thiaminase.

The preventive effect exerted by cysteine and sodium acetate suggests that SH compounds and acetates, which occur naturally in whole fish, give the enzyme some protection from radiation.

Table 5.—Effect of cysteine and sodium acetate on thiaminase radiosensitivity in whole alewife

Sample	Natural thiaminase activity	Reduction in thiaminase activity of samples irradiated at 4 megarad and 33° F.
	[1]	*Percent*[2]
Alewife	185	56
Alewife + 2.4% sodium acetate	185	27
Alewife + 2.4% cysteine	185	0

[1] Micrograms of thiamine hydrochloride destroyed in 20 minutes per gram of protein.
[2] Percentage loss of activity relative to that of the nonirradiated control samples.

SUMMARY AND CONCLUSIONS

To estimate the potential usefulness of this source of energy for controlling the activity of thiaminase in fishery products, I studied the sensitivity to gamma radiation of thiaminase in fresh-water fish, particularly in alewife. From this study, I have drawn the following conclusions concerning the effect of gamma radiation:

1. Thiaminase sensitivity to the radiation tends to increase directly with dosage level and the temperature of the sample during irradiation.
2. Thiaminase in samples of fish frozen and irradiated while at temperatures held below 0° F. by means of dry ice is extremely resistant to the radiation.
3. The degree of thiaminase inactivation by radiation varies with fish species. This variation is apparently related to enzyme concentration, for thiaminase sensitivity is inversely related to the concentration of the enzyme — at least, in the alewife.
4. A pH above or below the normal pH of the fish appears to decrease the sensitivity of thiaminase to the radiation.
5. The addition of cysteine or sodium acetate to whole alewife tends to protect the thiaminase from the radiation.
6. Complete inactivation of thiaminase is desirable in fish to be used for animal feeding. Thus, it does not appear to be economically feasible to use radiation to inactivate thiaminase in fishery products in that it requires 4 megarads to inactivate only 80 percent of the thiaminase in whole alewife at 80°-104° F.

ACKNOWLEDGMENTS

Hilding G. Olson, Phoenix Memorial Laboratory, The University of Michigan, assisted in the irradiation of the test samples; Robert Buter, Sondra Gunn, Leslie Wurn, and Roann Ogawa gave technical assistance.

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MS #1772

RELATION OF TEMPERATURE, TIME, AND MOISTURE TO THE PRODUCTION OF AFLATOXIN IN FISH MEAL

by

Travis D. Love

ABSTRACT

Numerous instances have been reported in which moldly vegetable meals, when incorporated into feeds, have produced symptoms of acute toxicity in animals. The several toxin principles (aflatoxins) produced by the mold *Aspergillus flavus* have been isolated in crystalline form and chemically identified as lactones, epoxides, or hydroperoxides.

Commercial fish meal has never been reported to contain aflatoxins. In this study fish meal was heavily inoculated with *Aspergillus flavus,* and the concentration of moisture was artificially increased to 18 percent. The meal developed aflatoxins after being held at 28° C. for 35 days. The control meals, which had a normal moisture concentration of 10 percent, did not produce aflatoxins at any temperature studied, though they were heavily inoculated with *Aspergillus flavus* and held for 35 days.

INTRODUCTION

For the past 5 years, deaths from carcinoma and damage to liver following ingestion of moldy vegetable meals by animals have been reported (Sargeant, Sheridan, O'Kelly, and Carnaghan, 1961; British Medical Journal, 1964; Yokotsuka, Sasaki, Kikuchi, Asao, and Nobuhara, 1967). The causative agent has been isolated and identified as a compound belonging to the group of unsaturated cyclic lactones (Pons, Cucullu, Lee, Robertson, Franz, and Goldblatt, 1966; Van Duuren, 1965). The name aflatoxin has been derived from *Aspergillus flavus,* a mold noted for its production of the toxin. The toxin has been shown to be composed of four fractions — B_1, B_2, G_1, and G_2 — all of which fluoresce under ultraviolet light at a wave length of 3,660 angstroms (Cucullu, Lee, Mayne, and Goldblatt, 1966). The toxin is thermostable, since it resists autoclaving at 250° C. and 15 pounds of pressure for several hours (Asao, Buchi, Abdel-Kader, Chang, Wick, and Wogan, 1965).

Although much research has been devoted to aflatoxin in vegetable meals, no study has included fish meals as a medium for aflatoxin production. The literature does not report a single instance where fish meal has been im-

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plicated in aflatoxin poisoning. This paper reports on three experiments made to determine if aflatoxin might nevertheless be produced in fish meal.

In these three experiments, the principal variables were temperature, time, and moisture concentration. The first and second experiments permitted a comparison of the effects of temperature; the first and third, a comparison of the effects of time. All three permitted a comparison of the effects of abnormally high concentrations of moisture in the meals.

I. EXPERIMENT 1 — INCUBATION AT 24° TO 28° C. FOR 5 DAYS

Menhaden meal for the experiments was obtained from local fish-meal plants. The concentration of moisture in the meal as obtained from the plant was about 9 or 10 percent. Three portions of meal were mixed together to form a single sample. Proximate composition was not determined on the meal, since it was accepted as being a regular commercial meal.

The following procedure was used to determine the concentration of moisture needed in the fish meal to permit the growth of *Aspergillus flavus* and its production of toxin. One hundred grams of the mixed fish meal was placed in each of six 500-milliliter flasks. Sufficient water was added to the six flasks to raise the concentrations of moisture in the meals to 10, 14, 18, 22, 26, or 30 percent. Ten milliliters of an inoculum of *Aspergillus flavus*[1] was then added to each flask, and the flasks were allowed to incubate at an ambient temperature of 24° to 28° C. for 5 days. (The culture for the inoculum was prepared by adding a 3-millimeter loopful of the type culture to a 100-milliliter flask of nutrient broth and culturing 5 days at 35° C.)

Any toxin present was extracted and measured according to the method described by both Pons, Robertson, and Goldblatt (1966) and Cucullu, Lee, Mayne, and Goldblatt (1965). Owing, however, to the presence of a considerable amount of polyunsaturated oil, the meal was extracted first with three portions of 100 milliliters each of N-hexane to remove the oil. Pons, Cucullu, Lee, Robertson, Franz, and Goldblatt (1966) have shown that the toxin is not appreciably soluble in N-hexane. Any toxin present was extracted from each fish-meal culture with a solution of 97 percent acetone plus 3 percent chloroform. Evaporation of this extract on the steam bath resulted in a tarry residue. The toxin was extracted from this residue with 100 grams of the acetone-chloroform solution. Most of the residue was insoluble.

In the test for aflatoxins, the soluble semi-purified toxin extracted from the residue was spotted on a thin-layer chromatographic plate, and the plate was set upright in a chamber containing the acetone-chloroform solution at a depth of 2 centimeters. The solution, which wetted the bottom edge of the plate, ascended the plate about 10 centimeters in 20 minutes. At the end of this time, the plate was removed from the chamber and dried in an air oven at 80° C. for 30 minutes. The plate was then examined in a darkroom under ultraviolet light at a wavelength of 3,660 angstroms. Any visible blue-green fluorescence at the dried solvent front was taken as a positive test for aflatoxin. Only the *Aspergillus flavus* culture in the fish meal containing 30 percent of moisture gave a definite positive test for aflatoxin. Determinations for fluorescence were made only at the end of each culture period.

When fluorescence is used to identify a substance, we must establish that the fluorescence is not produced by some other fluorescing substance. In this experiment, I had a negative control that indicated that I was actually seeing aflatoxin fluorescence, since the fish meal with a normal 10 percent of moisture, though inoculated with *Aspergillus flavus*, showed abso-

[1] Pure cultures were obtained through the courtesy of the U. S. Quartermaster Corps Food Research Laboratories, Natick, Massachusetts.

lutely no fluorescence on the thin-layer plate. In contrast, the fish meals with 30 percent of moisture produced the typical blue-green fluorescence described by other researchers. Numerous instances are cited in the literature in which cultures believed to contain aflatoxin were toxic to the test animals. No negative results have been reported when *Aspergillus flavus* was cultured and the cultures were fed to animals. In each instance a fluorescent extract produced by this method was toxic (Allcroft and Carnaghan, 1962; Armbrecht and Fitzhugh, 1964).

II. EXPERIMENT 2 — INCUBATION AT 35° C. FOR 7 DAYS

Experiment 1 was repeated except that flasks were incubated for 1 week in an incubator at 35° C. Under this warmer incubation, aflatoxin was produced in the flasks containing 22, 26, and 30 percent of moisture.

III. EXPERIMENT 3 — INCUBATION AT 24° TO 28° C. FOR 35 DAYS

Experiment 1 was repeated except that the flasks were incubated for 35 days.

In this experiment, a large amount of mold was visible, after 20 days, in the flasks containing 22, 26, and 30 percent of moisture. The ultraviolet examination revealed a very strong fluorescence in the three plates from the meals having 22, 26, and 30 percent of moisture. A very faint but definite fluorescence could be noted on the plate from the meal containing 18 percent of moisture.

CONCLUSIONS

Mycotoxins from *Aspergillus flavus* and related molds will not be found in a good commercial fish meal having a moisture content of 10 percent or less. Fish meal containing 18 percent or more of moisture produced aflatoxins under laboratory conditions when stored for several weeks at 24° C. or higher. Fish meal containing 22 percent or more of moisture may develop aflatoxins in a matter of 5 days at 30° C. Accordingly, fish meal that accidentally becomes moist should be quickly recycled through a plant dryer.

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MS #1781

VARIATION IN THE FATTY ACID COMPOSITION OF PACIFIC HERRING (*Clupea harengus pallasi*) OIL IN ALASKA DURING 1964 AND 1965

by

Paul A. Robisch and Edward H. Gruger, Jr.

ABSTRACT

Industrial users need knowledge about the variability in the fatty acid composition of Alaska herring oil. Accordingly, oil from the 1964 and 1965 catch seasons was analyzed for certain of its chemical properties — individual fatty acid composition, total concentration of the saturated, monoenoic, and polyenoic fatty acids, iodine value, saponification value, free fatty acid value, and peroxide value.

The individual fatty acid components varied to some extent. Ranges in the concentration of the monoenoic fatty acids for the 2 years did not overlap; the average differed by 4.8 percent. Differences in the averages for the saturated and polyenoic fatty acids were only 1.7 percent. Data on iodine value indicated that the total unsaturation of the oil was somewhat greater in 1964 than in 1965; data on the other chemical properties indicated that the oil had not undergone appreciable alteration prior to being analyzed.

The reasons for the above variations could not be determined, since several variables were operating simultaneously.

CONTENTS

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INTRODUCTION

Oil from Pacific herring (*Clupea harengus pallasi*), including that produced in Southeastern Alaska, is generally considered to be a rich source of the C_{20} and C_{22} monoenoic fatty acids. At times, however, these two fatty acids have been abnormally low in samples of herring oil.

The corresponding saturated acids of these monoenoic acids are readily obtainable through hydrogenation for such commercial products as soaps and greases (Georgi and Stucker, 1947). For these and other uses, manufacturers need to know whether the fatty acid composition of the commercially available herring oil is relatively constant or greatly variable.

Lovern (1938b) showed that the degree of unsaturation in the fatty acids of Pacific herring oil was dependent upon the season of capture. Early work by Lovern and Wood (1937) and Lovern (1938a) showed that the fatty acid composition of the oil is related to (1) species, (2) diet, (3) temperature of the water, and (4) salinity of the water.

The purpose of this research was to analyze commercially produced herring oil for the 1964 and 1965 catch seasons to determine the variability of the oil with respect to certain chemical properties — namely, individual fatty acid composition; total concentrations of saturated, monoenoic, and polyenoic fatty acids; iodine value; saponification value; free fatty acid value; and peroxide value. Because of the simultaneous presence of several variables in the catch (see Tables 1-3), we limited this research to these chemical properties.

I. FATTY ACID COMPOSITION

A. PROCEDURE

Described in this section are the samples and the method of determining the fatty acid composition.

1. Samples

Samples of commercial herring oil from fish taken in the waters of Southeastern Alaska were obtained from barge tanks. Each barge tank consisted of two large compartments.

The barge tanks were filled in the following manner: Each individual fish plant stored the oil in a holding tank as it was produced. When the barge tank arrived at the plant, the oil was transferred from the holding tank into the barge. If the production of oil was adequate, the barge would stay at an individual plant from 7 to 10 days and would then move on to a different plant, the above process being repeated until both compartments of the barge were filled. When filled, the barge tanks were sampled by taking oil from five equidistant different levels — starting at the top in each compartment. The five aliquots were combined into one lot.

Since the holding tanks of the individual plants were not necessarily emptied completely each time a barge tank arrived, and since the barge tanks were filled with oil from several individual plants, the samples represented a wide composite of herring oil.

The sampling of the oil varied somewhat during the 1964 and 1965 seasons, both as to the number of samples taken per season and as to the time of year covered. For the 1964 season, 10 samples were obtained; the sampling varied from July 9 to September 16. For the 1965 season, 13 samples were obtained; the sampling varied from August 5 to November 8.

Tables 1 to 3 give data on the total catch, geographical distribution of catch, and sex

Table 1.—Annual catch, 1964-65, of Pacific herring in Southeastern Alaska

Year	Catch
	Tons (English short)
1964	22,127.5
1965	9,267.6

ratio and age of fish as compiled by the Alaska Department of Fish and Game. These data indicate the number and complexity of variables that affected Alaska herring oil during 1964 and 1965.

After the samples were received at the laboratory, they were filtered through diatomaceous earth to remove suspended solid particles. Following filtration, the samples of oil were bubbled with dry nitrogen and stored at −20° C.

Table 2.—Geographical distribution of catch, 1964-65, of Pacific herring in Southeastern Alaska

Location	Relative amount caught in:	
	1964	1965
	Percent	*Percent*
Frederick Sound	44.8	0.2
Upper Chatham Strait	28.9	22.4
Lower Chatham Strait and Cape Ommaney	16.8	69.7
Noyes and Warren Islands	9.5	7.7

Table 3.—Sex ratio and age of fish of Pacific herring, Southeastern Alaska, 1964-65

Year	Fish examined	Sex ratio		Relative number of fish that were:		
		Males	Females	2-5 years old	6-9 years old	6-11 years old
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1964	2,400	32	68	17	--	83
1965	2,500	52	48	40	60	--

2. Determination of Fatty Acid Composition

GLC (gas-liquid chromatography) was used to determine the fatty acid composition (as methyl esters).

a. Preparation of methyl esters.— The oil samples were saponified with alcoholic potassium hydroxide (40 grams per liter), and the resulting potassium soaps were washed free of unsaponifiable matter before being converted to free fatty acids (American Oil Chemists' Society official method Cd 3-25 (Mehlenbacher, Hopper, and Sallee, 1955)). Methyl esters were prepared from the fatty acids by the boron trifluoride-methanol procedure of Metcalfe and Schmitz (1961). The saponification and esterification reactions were analyzed for completion by silicic acid TLC (thin-layer chromatography) (Mangold, 1961).

b. Analysis of methyl esters.—The methyl esters of the samples from the 1964 season were analyzed with a Research Specialties Company Series 600 gas-liquid chromatograph[1], which contained an argon beta-ionization detector and a strontium-90 radioactive source. The column used was made of Pyrex glass, 4 millimeters inside diameter and 232 centimeters long and was packed with 5 percent (by weight) DEGS (diethylene glycol succinate polyesters) supported on 80-90 mesh acid base washed and silanized flux-calcined diatomaceous earth (Anakrom ABS).

The methyl esters of the samples from the 1965 season were analyzed with a Barber-Coleman Company Series 5000 gas-liquid chromatograph, which contained a hydrogen flame ionization detector. The column used was made of Pyrex glass, 4 millimeters inside diameter and 183 centimeters long, and was packed with 5 percent (by weight) DEGS supported on 80-100 mesh acid washed and silanized Chromosorb G.

The analysis involved the identification of peaks, the quantification of peaks, and the application of correction factors.

(1) Identification of peaks.—The GLC (gas-liquid chromatograph) peaks were identified by use of a combination of the following three methods: (1) standards of pure methyl esters were used as references for the C_{14} through C_{24} saturated acids, C_{16} through C_{24} monenoic acids, plus linoleic (18:2), linolenic (18:3), arachinonic (20:4), eicosapentaenoic (20:5), and docosahexaenoic (22:6) acids;

[1] Trade names referred to in this publication do not imply endorsement of commercial products.

(2) equivalent-chain-length values were determined according to the method of Miwa (1963) and were compared to the values reported by Hofstetter, Sen, and Holman (1965) for those peaks for which pure standards were not available; and (3) hydrogenation was used to obtain saturated acids that could be compared with standards.

(2) Quantification of the peaks.—The area percentages of the fatty acids were determined from the GLC recorder curves by multiplying the peak height by the retention time. We found that this approximation method gives results comparable with those obtained by other methods of quantification, such as triangulation. Bartlett and Iverson (1966) also has obtained good results using the approximation method.

(3) Correction factors. — Gerson, Shorland, and McIntosh (1966) and Horning, Maddock, Anthony, and Vandenheuvel (1963) report substantial physical losses of the highly unsaturated fatty acids (20:4, 20:5, and 22:6) when using an argon beta-ionization detector, because of the nonlinearity of the detector. Similar losses were found in the present work for the data obtained on the 1964 oils (GLC's run on an argon beta-ionization detector). Those data obtained on the 1965 oils (GLC's run on a hydrogen flame detector) did not show these losses. Correction factors for the argon beta-ionization data were obtained by comparing the percentages found by analyzing identical samples on both types of detectors. Correction factors of 1.29 and 1.56 were applied to the area percentages for 20:5 and 22:6, respectively, which are reported in Table 4. The other highly unsaturated components (18:4, 20:4, 22:4, and 22:5) were present in too low a concentration to warrant correction.

B. RESULTS AND DISCUSSION

The results of the GLC analyses are shown in Tables 4 and 5. As was indicated earlier, the data in Table 4 for 20:5 and 22:6 have been corrected for nonlinearity to allow direct comparison of the data in the two tables.

The major components for both years are 16:0, 18:1, 20:1, 22:1, and 22:6. Also the overall composition pattern for all 23 samples remains the same. Each major component had a greater range for the 1964 season than for the 1965 season. In 1964, for example, 18:1

Table 4.—Fatty acid composition of Pacific herring oil for the 1964 catch season determined by GLC

Fatty acid (Carbon atoms: double bonds)	Fatty acid composition									
	July 9[1]		July 13[1]		July 24[1]		Sept. 3[1]		Sept. 14[1]	Sept. 16[1]
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
	Area %	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*
14:0	6.9	6.3	6.5	6.7	6.8	6.1	6.0	6.0	6.5	5.6
16:0	14.6	15.0	11.8	13.8	15.1	13.1	16.8	13.9	18.6	15.3
16:1	6.8	7.6	7.3	7.5	7.0	7.2	7.5	7.9	8.0	7.3
17:2 & 17:1[2]	0.8	0.7	0.9	0.8	1.0	0.9	0.7	0.9	0.7	0.7
19:3 & 19:0[2]	1.4	0.9	1.5	1.2	1.4	1.3	0.7	1.1	0.7	0.7
18:0	1.1	1.1	1.3	1.3	1.2	1.3	1.4	1.5	1.5	1.3
18:1	11.8	15.2	11.7	12.2	12.3	14.0	21.7	17.4	25.3	20.5
18:2	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.6	0.6	0.5
18:4	1.3	1.2	1.5	1.2	1.4	1.3	1.2	1.6	1.1	1.3
20:1	18.4	15.2	17.0	19.1	17.4	16.4	12.2	13.2	9.2	13.3
20:4[3]	0.5	0.7	0.7	0.5	0.8	0.8	0.6	0.8	0.6	0.7
20:5[4]	12.5	12.2	15.2	12.2	12.6	15.2	12.7	14.2	11.4	13.0
22:1	14.5	15.2	12.7	14.5	13.6	11.8	8.8	9.2	6.9	9.7
22:5	0.5	0.6	0.6	0.5	0.5	0.5	0.4	0.6	0.4	0.5
22:6[5]	5.5	4.8	7.5	5.2	5.6	6.2	6.3	7.5	5.8	6.8
24:1	0.7	0.6	0.6	0.7	0.9	0.8	0.6	0.9	0.6	0.6
Minor components[6]	2.2	2.1	2.7	2.1	1.9	2.6	1.8	2.7	2.1	2.2
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[1] Date of sampling from the barge.
[2] Indicates peak overlap on recorder chart.
[3] Mixture of two structural isomers.
[4] Area-percent correction factor of 1.29 applied.
[5] Area-percent correction factor of 1.56 applied.
[6] Includes 12:0, 15:0, 17:0, 18:3, 20:2, 20:3, 24:0, 22:3, 22:4.

and 22:1 had a range spread of 13.6 percent and 8.4 percent, whereas in 1965, the range spread was 4.2 percent and 3.3 percent, respectively. Tables 4 and 5 indicate also that two of the major components (18:1 and 20:1) were inversely correlated within the samples.

The data indicate some variation; but the variables involved in the herring catches (Tables 1 and 3) and the wide composite of oil used in filling the barge tanks, prevent us from drawing a conclusion concerning the reasons for the variability of the composition of fatty acids. The data in Tables 4 and 5 merely represent the variation in the different tanks of herring oil as they would be delivered to a consumer.

II. SATURATED, MONOENOIC, AND POLYENOIC FATTY ACIDS

Since two of the major components (18:1 and 20:1) were inversely correlated within the samples, the data in Tables 4 and 5 were summed to give the total percentages for the saturated, monoenoic, and polyenoic fatty acids. These data (Tables 6 and 7) allow direct comparison of the saturated-unsaturated patterns for the individual seasons. To allow easy direct comparison of the total saturated, monoenoic, and polyenoic fatty acids for the two catch seasons, we calculated the data in Table 8 from those in Tables 6 and 7.

III. OTHER CHEMICAL PROPERTIES

In addition to investigating the fatty acid composition of herring oil, we studied other chemical properties: iodine value (Wijs), saponification value, free fatty acid value, and peroxide value. These values supplemented the fatty acid composition data.

The iodine value was used as a check on the reliability of the GLC data, since theoretical iodine values can be calculated from the GLC fatty acid composition data and compared directly with the actual iodine values obtained experimentally. Abnormally high saponification values, free fatty acid values, and peroxide values would indicate that the oil had deteriorated and therefore that the GLC data would be unrepresentative of the oil samples.

A. PROCEDURE

The oil samples were analyzed for iodine value (Wijs), saponification value, free fatty acid value, and peroxide value by the official methods of the American Oil Chemists' Society (Mehlenbacher, Hopper, and Sallee, 1955).

The theoretical iodine values were obtained by multiplying the iodine value for each individual methyl ester by its area percent as given by the GLC data.

B. RESULTS AND DISCUSSION

The chemical constants shown in Table 9 for the 2 years indicate that no large differences occurred in the oil samples. Iodine values, by themselves, do not give any indication as to the individual fatty acid composition of the oil — only the total amount of unsaturation. The higher average iodine value for 1964 indicates that the oil was more unsaturated in 1964 than in 1965. This higher unsaturation in 1964 is also indicated by the data in Table 8.

The theoretical iodine value based on the GLC data reported in Tables 4 and 5 indicates the reliability of the GLC data. The agreement between the average theoretical and experimental iodine values (Table 5) for the 1965 catch season is excellent. The agreement between the two for the 1964 catch season, however, is not as good, even with the application of the correction factors for 20:5 and 22:6. The low theoretical value can probably be explained by the fact that the other highly unsaturated components (18:4, 20:4, 22:4, and 22:5), although present in too low a concentration to warrant correction individually, do warrant correction when combined. Since the low theoretical iodine value was calculated from GLC data obtained from the argon beta-

Table 5.—Fatty acid composition of Pacific herring oil for the 1965 catch season determined by GLC

Fatty acid (Carbon atoms: double bonds)	Fatty acid composition												
	August 5[1]				August 16[1]		August 24[1]		September 21[1]		October 4[1]		Nov. 8[1]
	Sample 11	Sample 12	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23
	Area %	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*
14:0	7.3	6.5	6.6	7.7	6.2	6.6	6.1	6.2	7.1	6.4	6.8	7.5	7.4
16:0	15.4	16.0	17.3	15.5	15.6	16.4	18.3	17.0	15.6	14.8	14.4	15.5	15.3
16:1	6.4	6.6	7.0	6.6	6.6	6.8	7.0	6.8	6.5	6.4	6.2	6.5	6.5
16:2 & 17:1[2]	1.0	1.0	1.0	1.2	1.0	1.1	1.1	1.1	1.1	1.0	0.8	1.0	1.0
16:3	0.8	0.6	0.6	0.8	0.6	0.7	0.6	0.6	0.7	0.6	0.7	0.7	0.7
16:4 & 19:0[2]	1.8	1.6	1.5	1.8	1.4	1.6	1.5	1.4	1.6	1.4	1.4	1.6	1.5
18:0	1.6	1.7	1.8	1.5	1.7	1.7	2.0	1.8	1.6	1.6	1.4	1.4	1.4
18:1	16.0	18.2	18.6	15.6	16.8	16.6	19.0	18.6	15.8	16.0	14.8	15.4	15.8
18:2	0.2	0.3	0.1	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.3	0.5	0.2
18:4	2.5	2.0	2.1	2.4	2.5	2.4	2.5	2.3	2.7	2.8	2.4	2.6	2.6
20:1	12.0	10.5	9.2	11.4	11.0	10.8	7.3	9.6	10.8	11.4	12.4	12.2	12.3
20:4[3]	0.4	0.5	0.4	0.4	0.5	0.4	0.4	0.4	0.6	0.4	0.5	0.3	0.4
20:5	12.6	13.0	14.0	12.0	13.0	12.7	14.1	13.5	13.0	13.5	12.5	12.2	12.3
22:1	12.0	10.9	9.3	11.9	11.6	11.1	9.6	10.0	10.9	11.6	12.6	11.9	11.4
22:5	0.8	0.7	0.8	0.8	0.7	0.8	0.6	0.5	1.0	0.8	0.9	0.3	0.6
22:6	7.0	6.7	6.9	6.7	7.1	6.8	6.6	6.5	7.5	7.8	7.8	7.1	7.5
24:1	1.0	1.0	1.2	1.2	1.2	1.2	1.2	1.1	1.3	1.2	1.2	1.0	1.0
Minor components[4]	1.2	2.2	1.6	2.3	2.3	2.1	1.9	2.3	2.0	2.1	2.9	2.3	2.1
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[1] Date of oil sampling from barge.
[2] Indicates peak overlap on recorder chart.
[3] Mixture of two structural isomers.
[4] Includes 12:0, 15:0, 17:0, 18:3, 20:2, 20:3, 24:0, 22:3, 22:4.

Table 6.—Total area percentage of the saturated, monoenoic, and polyenoic fatty acids in Pacific herring oil for the 1964 catch season

Fatty acid structure	July 9		July 13		July 24		Sept. 3		Sept. 14	Sept. 16
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
	Area %	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*
Saturated	23.4	23.1	20.4	22.7	23.9	21.4	24.9	22.5	27.3	22.9
Monoenoic ...	52.2	53.9	49.3	54.0	51.2	50.2	50.8	48.6	50.0	51.4
Polyenoic	23.9	22.6	29.6	23.0	24.7	27.9	24.0	28.5	22.4	25.0

Note: Date of sampling from the barge tanks (same as Table 4).

Table 7.—Total area percentage of the saturated, monoenoic, and polyenoic fatty acids in Pacific herring oil for the 1965 catch season

Fatty acid structure	August 5				August 16		August 24		Sept. 21		October 4		Nov. 8
	Sample 11	Sample 12	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23
	Area %	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*	*Area %*
Saturated	24.7	24.8	26.4	25.5	24.2	25.5	27.0	25.6	24.8	23.6	23.4	25.2	24.8
Monoenoic	47.4	47.2	45.3	46.7	47.2	46.5	44.1	46.1	45.3	46.6	47.2	47.0	47.0
Polyenoic	26.5	26.4	27.0	26.1	26.7	26.4	27.0	26.4	27.5	28.2	27.1	26.0	26.5

Note: Date of sampling from the barge tanks (same as Table 5).

Table 8.—Comparison of total saturated, monoenoic, and polyenoic fatty acids in Pacific herring oil during 1964 and 1965 catch seasons

Fatty acid structure	1964		1965		Difference
	Range	Average	Range	Average	(1964-1965)
	Area %	*Area %*	*Area %*	*Area %*	*Area %*
Saturated	20.4-27.3	23.3	24.2-27.0	25.0	1.7
Monoenoic . . .	48.6-54.0	51.2	44.1-47.4	46.4	4.8
Polyenoic	22.4-29.6	25.1	26.1-28.2	26.8	1.7

Table 9.—Some chemical constants of Pacific herring oil 1964-65

Chemical constants	Values in:			
	1964		1965	
	Range	Average	Range	Average
Iodine value:				
Experimental . .	146.0-151.8	149.3	143.2-146.0	145.0
Theoretical[1] . .	127.6-151.0	139.2	141.6-151.2	145.9
Saponification value	184.6-190.0	186.4	192.4-195.2	193.3
Free fatty acid value	1.1-2.6	1.7	0.7-2.9	1.5
Peroxide value .	1.8-9.2	5.2	5.9-11.6	8.8

[1] Based on fatty acid composition determined by GLC.

ionization detector, it gives added support to the argument of Gerson, Shorland, and McIntosh (1966) that the highly unsaturated fatty acids undergo GLC losses in favor of the saturated and monoenoic fatty acids. This same problem of linearity may account in part for the greater range spread in the GLC data for 1964.

The saponification value for the 2 years does show a slight difference — the value being higher for 1965 than for 1964. The fact that the saponifiables are mainly fatty acids in the form of triglycerides may account for some of the variation seen in the fatty acid composition.

The free fatty acid and peroxide values are measures of the amount of oxidative deterioration of the oil. Since these values are very low and show little difference among the samples, oxidative deterioration is ruled out as a reason for any variation in the fatty acid composition.

CONCLUSIONS

The individual fatty acid composition of Pacific herring oil varied to some extent in the two seasons of catch investigated. Data on iodine value indicate that the total unsaturation was somewhat greater for 1964 than for 1965. The composition ranges of the monoenoic fatty acids for the two seasons did not overlap and differed by 4.8 percent.

The reasons for the variations mentioned above cannot be determined from the present data, since several variables were operating simultaneously and our experimental approach did not enable us to evaluate them separately. To arrive at more definite conclusions, we would have to continue this work for several more years and use a different investigational approach. We can conclude, however, that during the 2-year period studied, commercial samples of the herring oil did not, in general, vary greatly in the chemical properties investigated.

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Miyauchi, David T.

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Nelson, Richard W.

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Steinberg, Maynard A.

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Stout, Virginia F.

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Tretsven, Wayne I.

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Seattle, Washington

Malins, Donald C.

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1964 Fishery Industrial Research 3(1): 9-21.

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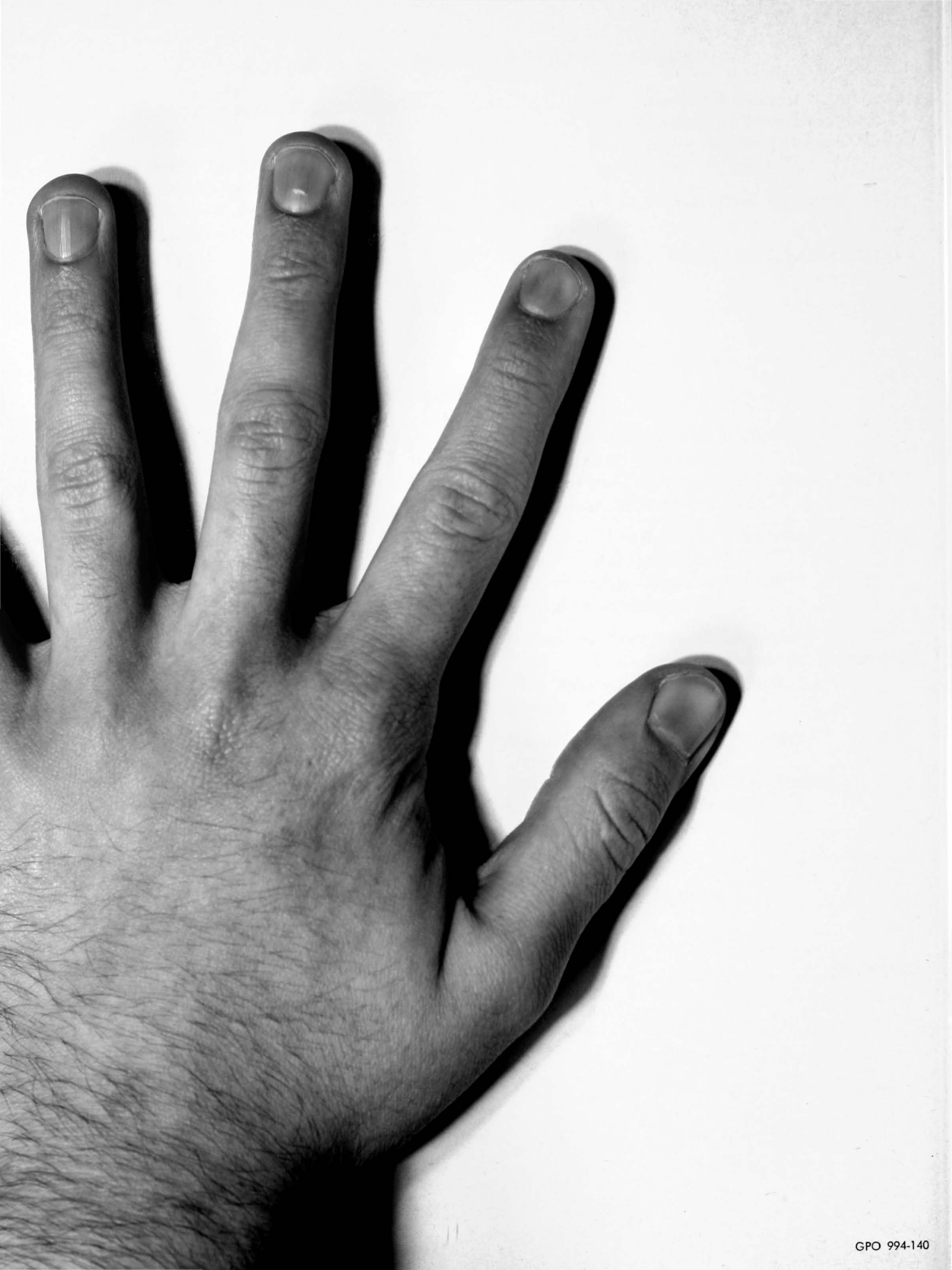

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