

# Annual Report of the Bureau of Commercial Fisheries Technological Laboratory

Seattle, Washington

Calendar Year 1962



UNITED STATES DEPARTMENT OF THE INTERIOR

FISH AND WILDLIFE SERVICE

BUREAU OF COMMERCIAL FISHERIES

Circular 180

Photo is of architect's drawing of new laboratory building under construction in Seattle adjoining present building--due for completion in 1964.

UNITED STATES DEPARTMENT OF THE INTERIOR

Stewart L. Udall, *Secretary*

Frank P. Briggs, *Assistant Secretary for Fish and Wildlife*

FISH AND WILDLIFE SERVICE, Clarence F. Pautzke, *Commissioner*

BUREAU OF COMMERCIAL FISHERIES, Donald L. McKernan, *Director*

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of the  
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Bureau of Commercial Fisheries, Council on Marine Resources

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# Annual Report of the Bureau of Commercial Fisheries Technological Laboratory, Seattle, Washington Calendar Year 1962

by Patricia S. Terao  
Special Assistant, Research Liaison

## BACKGROUND

The Technological Laboratory of the Pacific Region of the Bureau of Commercial Fisheries began its research activities on May 2, 1933, at its present location, 2725 Montlake Boulevard East, Seattle, Wash. This laboratory is one of six of the Branch of Technology, Division of Industrial Research; the other five are located at Ketchikan, Alaska; College Park, Md.; Gloucester, Mass.; Pascagoula, Miss.; and Ann Arbor, Mich.

The laboratory carries out research on the preservation and utilization of fish and shellfish, the chemical properties of constituents, and the application of research findings to new and improved fishery products. By preparing technical bulletins and answering inquiries, the laboratory also provides the fishing industry and the general public with information on fishery products.

Currently three major research programs are underway. The first program aims at

widening markets for fish oils, and consists of organic chemical investigations of the properties and reactions of fish oils, of derivatives from fish oils, and of industrial evaluation of potential uses for the products produced. The second program, in cooperation with the Atomic Energy Commission, concerns the irradiation pasteurization of several species of fish as a means of extending the storage life of chilled (iced) fish. The third program deals with improving the quality of fresh and frozen fish by developing standards of quality. The studies under the latter program at present focus attention on grading methods for judging the quality of halibut as landed by the fishing vessels.

Several smaller projects include experiments on shellfish processing, investigation of species of fish not utilized, and studies on fish spoilage.

## ORGANIZATION

Activities of the laboratory are directed by Maurice E. Stansby, who has been Laboratory Director since 1942.

Research programs are conducted by the Chemical Research Unit and the Preservation and Processing Research Unit; and the nonresearch programs by the Research Liaison Unit and the Administrative Unit. The functions of these four units are described as follows:

(Surnames are used to identify research workers. See section on Personnel for further identification.)

### CHEMICAL RESEARCH UNIT

Programs of the Chemical Research Unit deal with chemical, biochemical, and physical changes in components of fish. Research on fishery industrial products also comes under this unit.

### PRESERVATION AND PROCESSING RESEARCH UNIT

Programs of the Preservation and Processing Research Unit deal primarily with qualitative changes in the fish during handling, preserving, processing, and storing of the product for food purposes. Physical, chemical, and microbiological changes are considered as they affect the product as a whole.

### RESEARCH LIAISON AND ADMINISTRATIVE UNITS

The Research Liaison and the Administrative Units are responsible for the nonresearch aspects of the laboratory programs, such as publications, library, inquiries, publicity, manuscript processing and editing, budgeting, and administrative work. The two units also assist with program planning and coordination of various matters among the four units.

## PROGRAM OBJECTIVES AND 1962 ACCOMPLISHMENTS

### CHEMISTRY OF FISH OILS

Chemistry of fish oils is a continuing investigation in which several approaches are being used to find new and varied uses for fish oils. Most of the research is aimed at utilizing the unique characteristics of fish oil and taking advantage of its unusual chemical properties. Projects at the Bureau's Technological Laboratory in Seattle during 1962 were as follows: (1) Preparation of derivatives, (2) analyses of commercial oils, (3) analyses of oils extracted from fish flesh, (4) preparation and distribution of samples of refined fish oil and derivatives, (5) studies of odors and flavors, and (6) effects of ionizing radiation on lipids of fish. Projects under contract during 1962 were divided into the three categories: (1) Oxidative deterioration, (2) nutritional or pharmaceutical effects of fish oils, and (3) odor and flavor studies.

#### Preparation of Derivatives

Chemical reactions that make use of the characteristic unsaturation of fish oils were studied. An important class of compounds was prepared by developing a method that introduced nitrogen at the double bonds. Malins, Houle, and Wekell prepared a number of nitrogen derivatives of fatty acids that have unique and interesting properties. Nitrate, nitro, acetoxy-nitro, and nitro-nitrate derivatives were synthesized. These compounds can be used as chemical intermediates in syntheses of amino acids, amines, amino nitrates, and amino alcohols. The development of this method makes it possible to exploit the potential of fish oils as starting materials for the synthesis of a variety of nitrogen derivatives, and investigations will continue in this area.

Fatty acids are present in natural triglycerides in random fashion, and very little fractionation can be accomplished. Triglycerides with greater unsaturation can be obtained by re-synthesizing triglycerides from highly unsaturated fatty acids. Gauglitz and Lehman found that the best method was a direct esterification of fatty acids with glycerol, using zinc dust as a catalyst.

Preliminary studies were made on methods of preparing a number of other derivatives from fish oils. Gruger is studying polyurethanes and epoxy esters. Gauglitz and Lehman are investigating carbene reactions. Work is continuing on all of these studies.

#### Analyses of Fatty Acids from Fish Oils

Gruger analyzed commercial menhaden oils, flesh from 25 species of fish and shellfish, and different parts of menhaden for their fatty-acid contents. Commercial menhaden oils were obtained from fish taken from

New York to Florida along the Atlantic coast and from the Louisiana coast in the Gulf of Mexico, and from fish caught during winter, spring, and summer from 1959 to 1961. Major differences were noted in the amounts of myristic (C<sub>14:0</sub>), oleic (C<sub>18:1</sub>), and docosahexaenoic (C<sub>22:6</sub>) acids.

A major difference in the fatty-acid composition of oils from 25 species of fish and shellfish was found in the amounts of eicosenoic (C<sub>20:1</sub>) acid in the different species.

A comparison was made of the fatty-acid composition of oils from different parts of menhaden--body, head, entrails, fillets, bones, roe, and milt. These analyses were made of fish that were 1-3 years old and 4-6 years old.

#### Preparation and Distribution of Fish Oil Samples

Methods for nearly eliminating odors and flavors in fish oils were developed. Adsorptive bleaching of menhaden oil with activated earth and carbon, followed by molecular distillation, gave an oil that was nearly odorless and tasteless.

During 1962, Gauglitz and Hunter used adsorptive bleaching or molecular distillation or both to refine 4,000 pounds of fish oil triglycerides and fish oil derivatives, such as fatty acids and fatty esters. Fractionation via molecular distillation was used to obtain samples with a high percentage of polyunsaturates. Of the materials processed, 2,000 pounds of samples were distributed to other laboratories for research purposes.


Color bodies in the usual commercial tuna oil were not removed by the above method of adsorptive bleaching and molecular distillation. Oil from freshly caught albacore that had not been frozen, however, could be bleached without difficulty.

#### Composition of Oil from Tuna Flesh

Fatty acids in body oils from yellowfin, skipjack, bluefin, and albacore tuna were determined. Roubal compared oils from raw, precooked, and canned tuna as well as oils from light- and dark-meat portions.

The principal difference among the species was in docosenoic acid (C<sub>22:1</sub>), which ranged from 1.6 to 5.4 percent) in albacore and bluefin but was present in only trace amounts in yellowfin and skipjack. In general, the monoenoic-acid content of the light meat ranged from 24-37 percent; oleic acid, 16-22 percent; eicosapentaenoic acid (C<sub>20:5</sub>), 4-13 percent; and docosahexaenoic acid (C<sub>22:6</sub>), 15-22 percent. Fatty acids in the dark meat were roughly parallel to those in the light meat.



Figure 1.--Clay bleaching of fish oils using reactor and vacuum  equipment.

Fatty-acid compositions of oils from tuna canned commercially and in the laboratory were compared. No degradation of higher unsaturated fatty acids was indicated. The fatty acid compositions of the raw and canned products were about the same.

Additional research was carried out on the chemical structure of the monoenoic fatty acids isolated from bluefin body oils of the light and dark meats. The percentage and relative amounts of isomers of the monoenoic acids were also determined.

The project on tuna terminated in June 1962.

## Odors and Flavors

Odors and flavors of fish and fish oils are serious deterrents to increasing their utilization. Contract projects on the problems of odors and flavors of fish oils have demonstrated the complex chemistry involved and the extensive research needed. A new approach was started at the laboratory to investigate fishy odors not only of fish oils but also of other fishery products. Stansby and Kudo are classifying different types of fish odors and flavors and are studying factors that control their formation. Initially, research is being started on the odors and the flavors that develop from oxidation of relatively pure fish oil triglycerides.

## Effects of Ionizing Radiation on Lipids of Fish

Lipids in fish that have been irradiated become discolored and rancid. These adverse effects may be caused by (1) changes in the lipids during irradiation, (2) sensitization of the lipid by ionizing radiation, followed by rapid decomposition during storage, and (3) accelerated deterioration during storage from availability of oxygen that is normally utilized by bacteria.

Since so many reactions take place in such a complex system as fish flesh, it is impossible to determine what has happened. To overcome the difficulty, a purified, highly unsaturated fatty acid was isolated to use in the studies. Stout developed a procedure to produce enough docosahexaenoic acid (C<sub>22:6</sub>) to study.

Autoxidation of the esters of docosahexaenoic acid was used to measure the effects of irradiation. Of the several methods of measurement investigated, the Warburg apparatus to determine manometric oxygen absorption was the best. This determination was done in the absence of light. Irradiation caused little, if any, change if the highly unsaturated sample was held under helium at 0° C.

## Oxidative Deterioration

Oxidative deterioration was investigated at the University of California by Olcott, Shuster,

and Menzel at Berkeley and by Tappel and Roubal at Davis. Studies at Berkeley were on extracted lipids, and at Davis, on lipids in fish flesh. During 1962, Olcott investigated phospholipids and the mechanism of the action of antioxidants. Tappel investigated effects of lipids undergoing oxidation in biological systems.

Antioxidants.--Olcott studied effects of various systems of antioxidants on purified menhaden oil. Activity of antioxidants was affected by concentration and temperature. Amines that act as synergists at one concentration may act as pro-oxidants at another concentration. Some synergistic mixtures are more effective at 60° or 70° C. than at 50° C.

Phospholipids.--Phospholipids are important because they often act as synergists for other antioxidants. Olcott, Shuster, and Menzel studied phospholipids from menhaden and tuna. Menhaden muscle contained 0.55 percent phospholipid, which consisted of 24 percent cephalin, 64 percent lecithin, 6 percent sphingomyelin, and lesser miscellaneous components. Tuna muscle contained 0.5 percent phospholipid, which consisted of 23 percent cephalin, 54 percent lecithin, 8 percent sphingomyelin, 2 percent phosphoinositide, and lesser miscellaneous components.

Oxidation in biological system.--Tappel and Roubal are studying the effects of lipid oxidation on functions of proteins. The first phase will determine whether enzymes or other biological materials are affected by free radicals formed during oxidation of lipids.

## Nutritional and Pharmaceutical Effects

The nutritional effects are being investigated from two standpoints: Positive effects of their roles in lipid metabolism and possible negative effects of oxidized oils. A third project is an investigation of whether or not fish oils have a healing effect on wounds.

Fish oils and lipid metabolism.--Studies on the role of fish oils in lipid metabolism are being made by Peifer, at the Hormel Institute, University of Minnesota. Two projects are involved: (1) Effects of marine oils on distribution of lipids in the blood-vascular system and (2) influences of hormonal regulation on metabolism of marine-oil components. Past results showed that oils from a number of species of fish effectively lowered cholesterol levels in hypercholesteremic animals. Both extracted oils and oils in fish flesh have this cholesterol-depressant activity. Lowered cholesterol levels in these animals were accompanied by a more favorable balance between cholesterol and phospholipids in the blood. Changes also were



associated with changes in the distribution of classes of lipids in the circulating lipids and of specific fatty-acid components in tissues. These effects of fish oils are due to more than just total unsaturation. For instance, a wide spectrum of fatty acids is more effective than a mixture of 85 percent pentaenes and hexaenes. Attempts are being made to determine the specific factor or factors that are effective.

Nutritional effects of oxidized marine oils.-- In the project that Oldfield is investigating at Oregon State University, marine oils were oxidized to different levels and were fed to rats and pigs at levels of 10 percent and 15 percent. Both food intake and efficiency of the feed decreased at high levels of oxidation. When  $\alpha$ -tocopherol acetate or ethoxyquin was added to the oxidized material, each was an effective antioxidant; and growth of the animals was as good as when unoxidized oil was fed. One of the important observations was the rapid oxidation which occurred after the oils were added to the diets. It was found that higher the level of oxidation of oil, greater the oxidation in the diet. Thus, oil with a peroxide value of 61 was added to the diet and allowed to stand at room temperature, and after 24 hours it had a peroxide value over 1,300. Ethoxyquin added to the diet prevented this oxidation.

Applied research on the effect of certain components of fish on healing of skin lesions.-- Winkelmann, dermatologist at the Mayo Clinic, Rochester, Minn., studied the effects of fish oils on wounds and burns. In no instance did fish oils, triglycerides, or the unsaponifiable fraction have any greater healing effect than mineral oil.

### Odors and Flavors in Fish Oils

Two contract projects at Hormel Institute covered different aspects of odors and flavors found in fish oils. Different approaches were used in the two projects.

Studies on the odors of fish oils.--Chipault was concerned with the origin and the nature of the compounds responsible for undesirable odors and flavors. The study included separation of odors and attempts to associate them with different components of the oils, isolation and identification of the highly volatile compounds present, and research on autoxidation of purified methyl esters from fish oils. This project ended June 1962.

Investigation of chemical compounds causing fishy flavors in marine oils.--Mangold is concerned with defining "fishy" flavors and odors in terms of chemical structure. A synthetic approach is being used in which

unusual lipids are to be prepared and their behavior studied under conditions that will determine whether they or similar compounds might be precursors of compounds causing fishy flavors. Unsaturated aldehydes are being prepared and formed into lipids for these studies.

### CHEMICAL COMPOUNDS FORMED DURING FISH SPOILAGE

Most of the work reported on spoilage was done on spoiled fish; however, changes that start in fish immediately after death have a definite effect on quality of the fish. These post-mortem changes are being studied with two objectives in mind: (1) To find means to slow undesirable reactions and thus increase the period that the fish are of good quality and (2) to establish criteria or objective tests that can be used to determine how fresh the fish are.

### Proteolytic Activity of Fish Muscle Tissue

The proteolytic activity of fish muscle is important because of its probable effect on quality. Groninger purified and characterized the proteolytic enzyme(s) of albacore muscle so that we could better understand its nature and subsequently make it possible to suggest the role it plays in the muscle during post-mortem changes. Albacore was selected for the experiment since it is a commercially important species and since fresh specimens can be obtained.

Aqueous extracts of muscle were purified by a combination of salting-out with ammonium sulfate and diethylaminoethyl-cellulose chromatography. As a result of these techniques the specific activity (proteolytic activity per unit weight protein) was increased approximately hundredfold.

The characteristics of the enzyme extract from albacore muscle were determined. These characteristics were found to differ from those reported for cod muscle by Siebert (in Heen and Kreuzer, *Fish in Nutrition*, Fishing News Ltd., London, 1962, p. 80-82). Thus, different species of fish apparently have different muscle proteolytic enzymes.

The activities of the enzyme extract toward the soluble proteins of albacore muscle show that under certain conditions the muscle enzyme extract can attack muscle protein. Future work should give a wider perspective of the effect of muscle proteolytic enzymes on quality.

### INVESTIGATION OF PRESERVATION AND PROCESSING VARIABLES

Development of new or improved preservation and processing methods is essential for increasing the utilization of our fishery

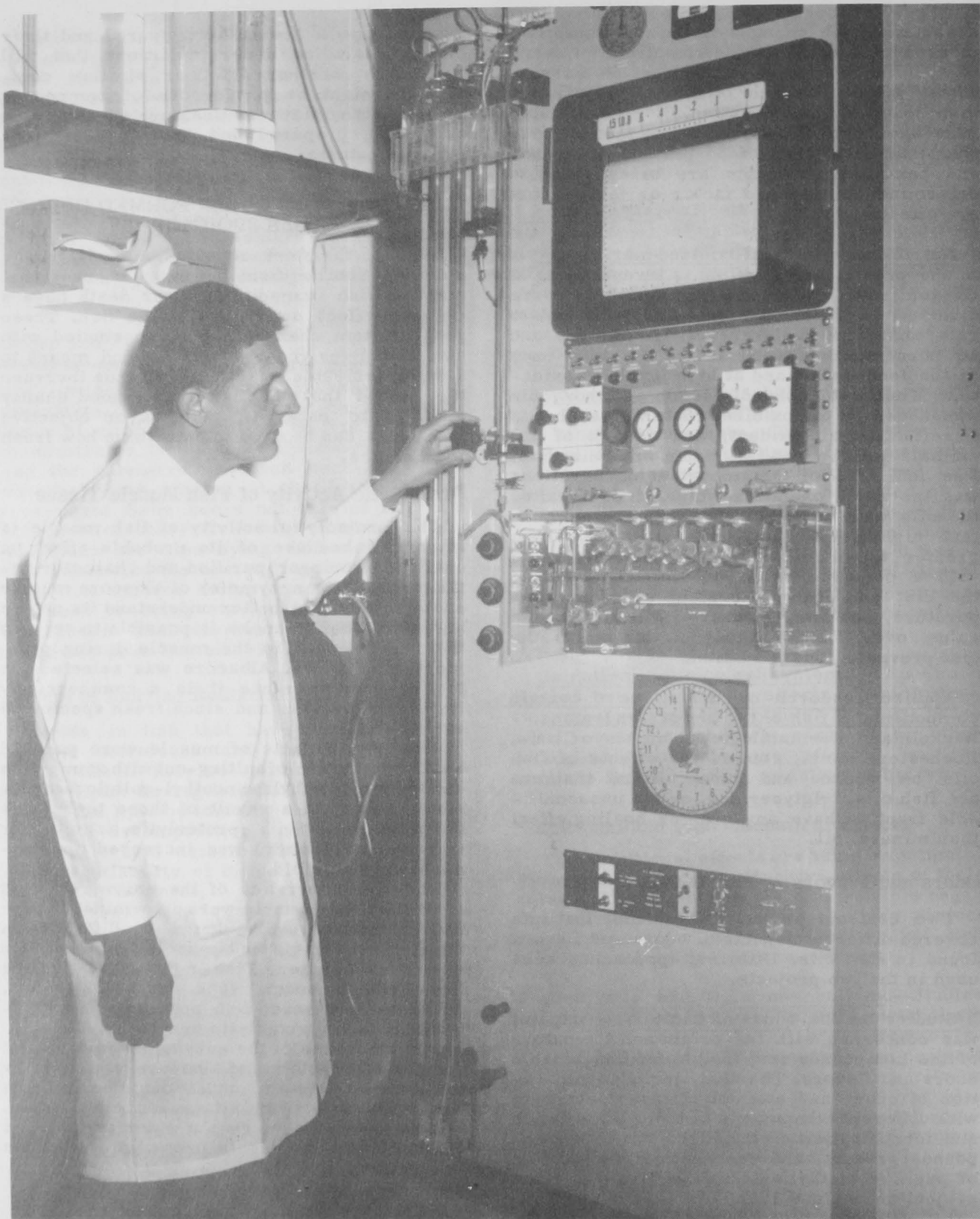


Figure 2.--Amino acid analyzer fabricated at the Bureau's Technological Laboratory, Seattle.

resources. The program objectives are as follows: (1) To develop objective quality tests, (2) to improve existing preservation and processing methods, and (3) to investigate new processing methods such as irradiation and freezing at sea in the North Pacific. Findings are applied to improve the domestic fisheries and to diversify processing methods for species not fully utilized. Contractual studies have been planned to determine the possible and immediate application of engineering and mechanical improvements that will solve problems of production efficiency in the Northwest fisheries.

### Processing and Acceptability of Fishery Products--Dungeness Crab Meat

Research studies on the problems involved in producing quality packs of fresh and frozen Dungeness crab meat were carried out by Nelson during 1962. Studies on processing methods and plant technique were somewhat limited because of an extremely poor crab fishing season. Measurements of draining time prior to packing, however, provided information relative to the variability of the drained weight of crab meat.

A paper reporting the results of a study of the proximate composition of Dungeness crab meat was prepared for publication.

Heat pasteurization of Dungeness crab meat as a means of extending the shelf life of the product was investigated. Depending upon the time and temperature of treatment, an acceptable pasteurized crab meat can be prepared that can be held at refrigerated temperatures for considerably longer periods of time than can regular fresh crab meat.

### Evaluating the Quality of Fishery Products--Texture

Texture measurement by an objective method is desirable in evaluating the quality of fishery products both in research and in quality control. An instrument designed by Dassow and McKee to measure resistance of fish muscle to shear was constructed at the laboratory; this instrument proved useful as a tool for determining texture. Research studies by Nelson included a study of the changes in king crab texture during frozen storage. In addition to the paper entitled "Development of an instrument for evaluating texture of fishery products," which was published in Food Technology, a supplemental report covering the construction details of the instrument was prepared.

### Oxidation Evaluation

A study of EMQ (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline) as an antioxidant on salmon flesh was carried out. Barnett found

that EMQ increased the storage life of frozen salmon and appreciably improved the color retention.

### Radiation-Pasteurization of Fishery Products

The Atomic Energy Commission is supporting the Bureau's project to develop a practical procedure for the radiation-pasteurization of Pacific crab meat and fillets of sole and flounder. Dungeness and king crab meat were studied in the former group, and petrale sole and halibut were used for the initial studies of the flatfish species. Samples of these products were irradiated at dose levels between 0 and 0.6 megarads. The storage characteristics of these radiation-pasteurized samples held at 33° F. and 42° F. were studied by sensory (Stoll), bacteriological (Eklund), and chemical tests (Spinelli). Results of limited experiments completed by Miyauchi and coworkers during the second half of 1962 indicate the following:

1. The optimum irradiation levels for producing samples having minimum radiation odors and flavors and with extended refrigerated storage life appear to be from 0.2 to 0.4 megarads for Pacific crab meat and about 0.4 megarads for fillets of petrale sole and halibut.

2. The radiation-pasteurized samples have a shelf life at 33° F. and 42° F. from two to six times greater than the unirradiated control samples.

3. For this radiation-pasteurization process, vacuum-packed samples have storage characteristics superior to air-packed samples.

4. In order to obtain maximum benefit from the radiation-pasteurization process, low refrigerated storage temperatures should be used. Samples stored at 33° F., for example, were of higher quality for a longer storage time than were those stored at 42° F.

### DEVELOPMENT OF VOLUNTARY STANDARDS FOR FRESH AND FROZEN FISHERY PRODUCTS

The general objectives of the standards project are to develop yardsticks of quality measurement and to control quality by the use of voluntary standards. These standards provide a means of processing and marketing fishery products at a defined quality level.

### Development of Voluntary Standards for Frozen Sole and Flounder Fillets

U.S. grade standards for grades of frozen sole and flounder fillets, applicable to Atlantic and Pacific coast species, were completed and officially published (April 1962, first issue). Supplementary inspectors' instructions,

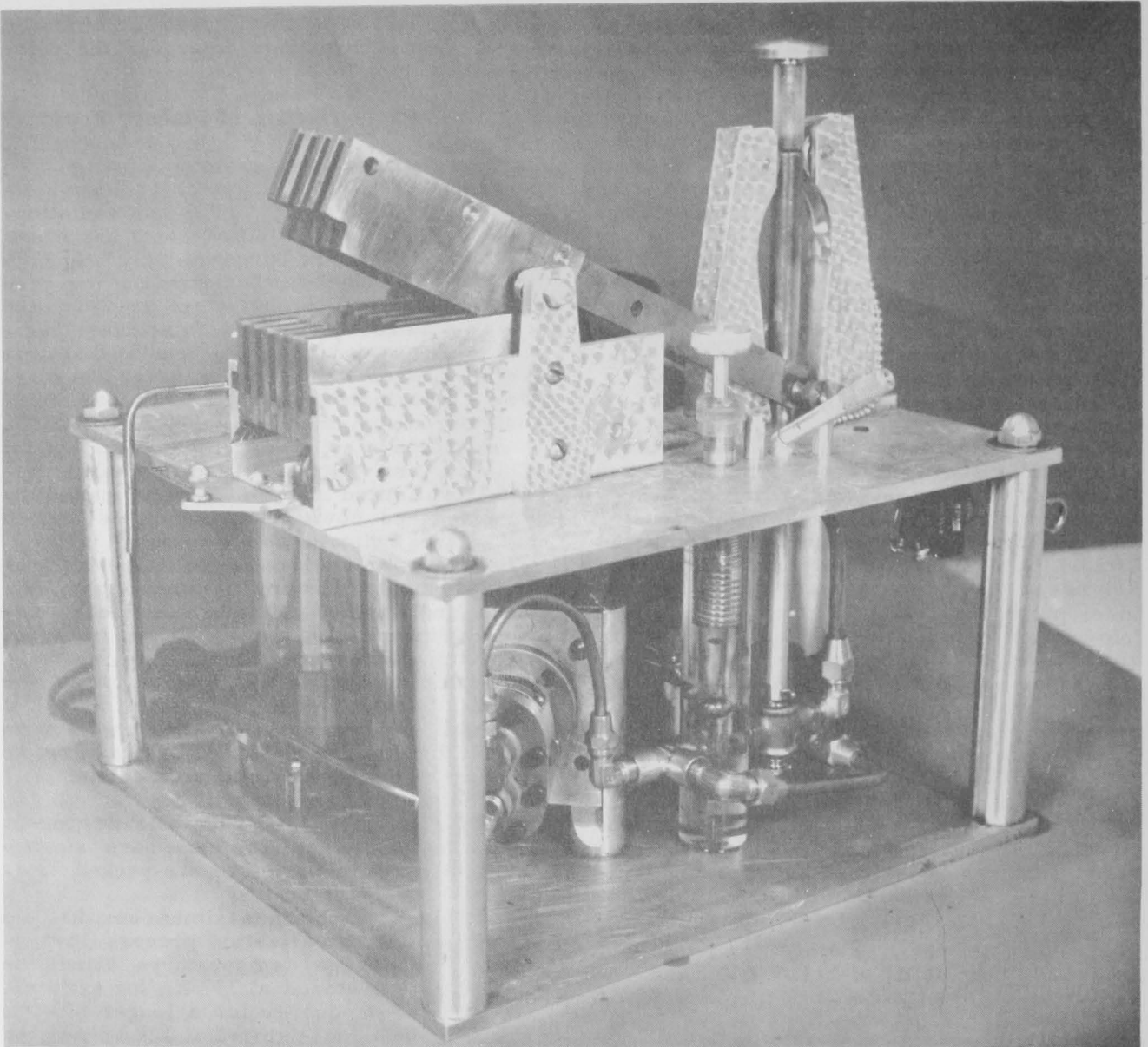


Figure 3.--Instrument constructed at the Bureau's Technological Laboratory, Seattle, for evaluating the texture of fishery products.

to assure the uniform interpretation and application of these standards, were developed for use by the official Fishery Product Inspectors of the U.S. Department of the Interior.

The abnormal "milky" and "jellied" conditions of sole and flounder, which are problems under the U.S. grade standards, were further investigated by Patashnik. The "milky" condition was found in several Pacific coast species and was observed as localized pockets of milky white fluid. These milky products resulted from the enzymatic proteolysis of the muscle tissue by specific protozoan parasites. The "jellied" condition, recognized by the slimy, jellylike, glossy appearance of the fish fillets flesh, was restricted to deep-water Dover sole. It was found to involve

mainly higher than normal amounts of moisture and lower than normal amounts of protein, but did not involve any identifiable parasites in the fish flesh.

#### Bacteriological Methods of Quality Control

Objective methods for evaluating the quality of fish and fishery products are needed for purposes of grading, quality control, and experimentation. As changes in microbiological flora on the surface of the fish are closely associated with quality, techniques of sampling the surfaces were developed and compared by Tretsven. Samples consisting of rinses, scrapings, swabbings, and excised portions were taken from various fish; the

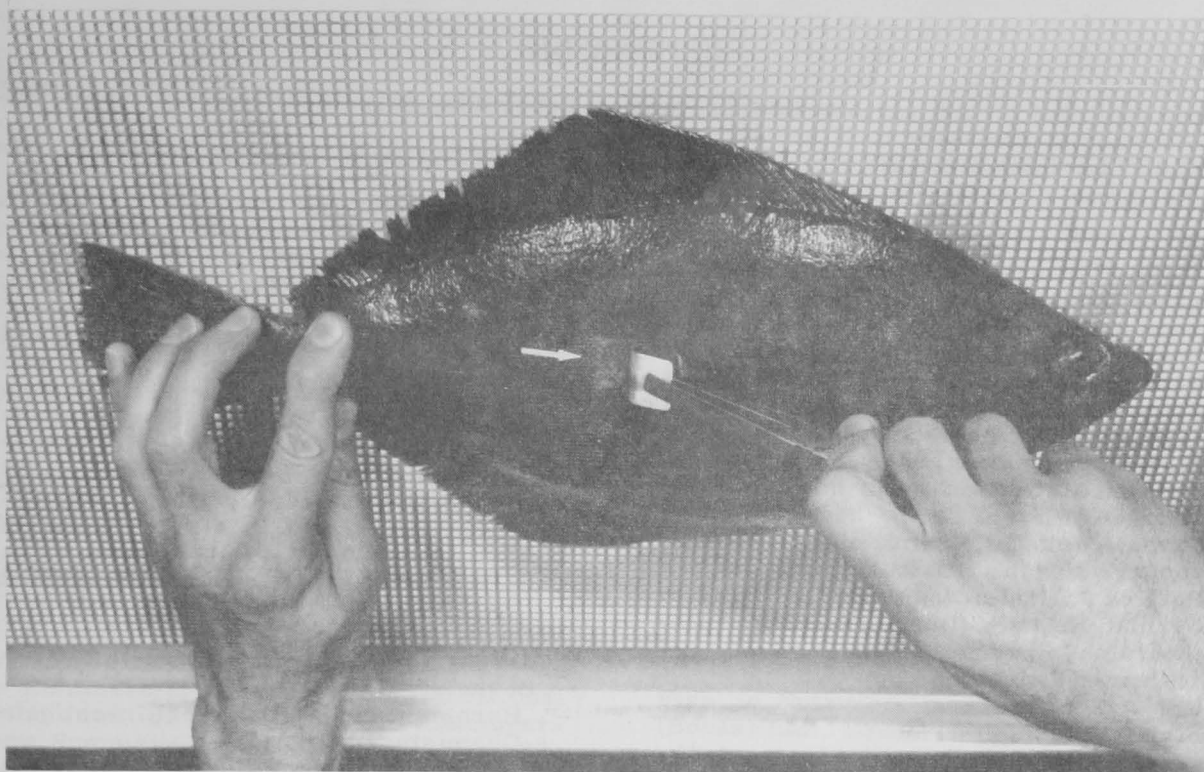


Figure 4.--Obtaining a scrape sample from a 5 square centimeter area on the surface of sole.

samples were analyzed; and the results were compared. The excised-surface sample was used as a standard, and other methods of sampling were compared with it. Bacteria counts obtained by rinsing represented 8 percent, by swabbing represented 35 percent, and by scraping represented 46 percent of the bacteria count of the excised-surface method of commercial cod. These four methods of sampling appeared to be the most practical and were developed further for determining both the amounts of slime and the number of micro-organisms on fish.

#### Development of Voluntary Standards for Fresh and Frozen Dressed Halibut

Standards and grading specifications for fresh and frozen dressed halibut are needed to define quality and promote fair dealing and quality improvement at the dock and processing level. A work program was outlined to develop these standards and specifications. The initial accomplishments were as follows:

1. The methodology was determined by Patashnik for evaluation of the texture of fresh and thawed halibut.

2. A procedure was developed by Tretsven for sampling slime of the landed fresh halibut. The relation of slime to quality, bacterial counts, and storage life of the chilled dressed fish will be evaluated.

3. Grading procedures now used by the industry at dockside were observed in Wash-

ington and Alaska ports by Rafn. Data were obtained and tabulated for the evaluation of existing industry methods of grading halibut and their possible application to a proposed U.S. grade standard.

#### INSPECTION AND CERTIFICATION SERVICE

Inspection and certification services are performed in accordance with industry need and requests, applying the latest standards of grade and condition and quality specifications for examination and certification of fishery product quality.

Peaks in inspection activity conformed to patterns established in previous years. March, with its heavy Lent-induced fresh and frozen fish purchasing by institutions, and October, with its usual high volume in canned salmon inspections, were consistent with previous corresponding months as the most active of the year. The normally lagging period during July, August, and September, however, provided record volumes in tonnage inspected and in revenue. The inspection of canned food products increased at the rate projected by the trend shown in past years. Revenue for the calendar year exceeded that of any prior year by 15 percent. The increase in the number of requests for certification of export shipment was significant.

## PERSONNEL

Maurice E. Stansby, Laboratory Director

### SEATTLE LABORATORY PROGRAMS

#### Organic Chemistry and Biochemistry

Neva L. Karrick, Program Leader

#### Organic Chemistry Project on Fish Oils

Chemists:

Edward H. Gruger, Jr., Project Leader

Donald C. Malins

Erich J. Gauglitz, Jr.

Virginia F. Stout

Clifford R. Houle

Lawrence W. Lehman

George Kudo

John C. Wekell (Intermittent)

Subprofessional positions:

Patrick J. Hunter

Norman K. Allen (Intermittent)

#### Biochemical Project on Fish Spoilage

Biochemist: Herman S. Groninger, Jr.,

Project Leader

#### Analytical Studies

Subprofessional Position: Mabel A. Edwards

#### Preservation and Processing

John A. Dassow, Program Leader

#### Preservation (mainly by irradiation)

Chemists:

David T. Miyauchi, Project Leader

Melvin W. Eklund

John Spinelli

Home Economist: Nancy V. Stoll

Subprofessional positions:

Dave H. Wieg

Murian H. Owens

#### Processing

Chemical Engineer: Richard W. Nelson,

Project Leader

Chemist: Harold J. Barnett

Food Technologist: Lynne G. McKee (Retired June 1)

#### Inspection and Certification Service

Agricultural Commodity Graders:

A. Morris Rafn, Project Leader (Also under Standards)

John J. Conley (Also under Standards)

Frank T. Poysky

#### Standards Development

Agricultural Commodity Graders:

A. Morris Rafn, Project Leader (Also under Inspection)

John J. Conley (Also under Inspection)

Chemical Engineer: Max Patashnik

Chemist: Wayne I. Tretsven

#### Research Liaison

Patricia S. Terao, Special Assistant, in Charge

Chemists:

Alice S. Hall (Part time)

Martin Heerdt (Part time)

#### Administrative Unit

Supervisory Clerk--Vacancy

Clerk-stenographers:

Maxine W. Densmore

Gretchen V. Lindbergh

Procurement Clerk: Helen M. Robertson

Clerk-typists:

Juliane A. Nakawatase

Virginia Pong (Part time)

Physical Science Aids:

Bradford J. Reed

Marion L. Burnside

### CONTRACT AND COOPERATIVE PROGRAMS

#### University of California

Oxidative Changes in Fish Oil

Berkeley, Calif.

Harold S. Olcott, Principal Investigator

Chemists:

Daniel B. Menzel

Constance Y. Shuster

Physical Science Aid: Robert D. Jensen (Intermittent)

Davis, Calif.

A. L. Tappel, Principal Investigator

Chemist: William T. Roubal

#### University of Minnesota

Fish Oils and Lipid Metabolism, Including Cholesterol Depressant Research

James J. Peifer, Principal Investigator

Odor Research

Helmut K. Mangold, Principal Investigator

J. R. Chipault, Principal Investigator (Terminated June 30, 1962)

#### Oregon State University

Nutritional Properties of Oxidized Fish Oil

J. Oldfield, Principal Investigator

#### Mayo Association

Skin Curing Properties of Fish Oils

R. K. Winkelmann, Principal Investigator

#### Columbia University

Studies on Fractions of Oxidized Cod Liver Oil

Hans Kaunitz, Principal Investigator

#### Marine Chemurgics, Inc. (Morehead City, N.C.)

Studies on the Lipids of Different Parts of Menhaden

Theodore M. Miller, Principal Investigator

#### Bureau of Commercial Fisheries Technological Laboratory (College Park, Md.)

Feeding Fractions of Ethyl Esters of Fish Oils to Chickens

Kam Leong and David Miller, Principal Investigators

## PUBLICATIONS

### TECHNOLOGICAL LABORATORY, SEATTLE

Dassow, John A., Lynne G. McKee, and Richard W. Nelson.

Development of an instrument for evaluating texture of fishery products. *Food Technology*, vol. 16, no. 3 (March), p. 108-110.

Abstract: A compact texture instrument of moderate cost, simple design, and reasonable precision that uses the principle of hydraulic force and measurement of gage pressure was constructed. The instrument is designed so it can be adapted with various devices for shear, tensile strength, or penetrometer measurements. Tests using a shear jaw of five movable blades showed that values correlated with increasing toughness as noted by sensory observations when the Dungeness crab samples were tested.

Karrick, Neva L.

Progress in marine oil research conducted and sponsored by the United States Bureau of Commercial Fisheries. *International Association of Fish Meal Manufacturers News Summary*, no. 10 (December), p. 92-104.

Abstract: The fish oil program of the Bureau's Technological Laboratory, Seattle, from 1958 to June 1962 is reviewed. 48 references.

McKee, Lynne G., and Richard W. Nelson.

Hydraulic press for laboratory preparation of fish press cake. *U.S. Fish and Wildlife Service, Commercial Fisheries Review*, vol. 24, no. 12 (December), p. 14-16. [Also as Separate No. 663.]

Abstract: A hydraulic press for preparing fish press cake in the laboratory was constructed. The press consists essentially of head, hydraulic jack, movable and stationary platens, and stand. The features of the press are its simple construction, light weight, portability, and capacity to handle sufficient material at one time to charge a laboratory meal dryer.

Miyauchi, David.

Application of centrifugal method for measuring shrinkage during the thawing and heating of frozen cod fillets. *Food Technology*, vol. 16, no. 1 (January), p. 70-72.

Abstract: A centrifugal method for measuring shrinkage of fish meats during freezing, thawing, and heating was adapted. The precision of the results was satisfactory considering the heterogeneity of the raw material. Use of the centrifugal method in studies with Pacific cod (*Gadus macrocephalus*) fillets gave the following results:

(1) Agreement of thaw-drip determinations between two fillets from the same fish was good; however, variation was considerable among fish. (2) During storage at 0° F., the cook-drip increased by a small but

significant amount during the first several months and then remained almost constant throughout the 12-month test period. (3) Thaw-drip and cook-drip increased with storage temperature.

Patashnik, Max.

Accuracy of net-weight determinations for frozen glazed halibut steaks. *U.S. Fish and Wildlife Service, Commercial Fisheries Review*, vol. 24, no. 10 (October), p. 5-8. [Also as Separate No. 658.] Also *Frosted Food Field*, vol. 35, no. 5 (November), p. 38-39.

Abstract: A check was made on the accuracy of the net-weight determination for frozen glazed halibut steaks as ascertained by the method given in the current U.S. Standards for Grades of Frozen Halibut Steaks. The possibility of simplifying the method was investigated.

Stansby, Maurice E.

Proximate composition of fish. (Presented at FAO Fish in Nutrition Conference.) *In Fish in Nutrition*, p. 55-60. Fishing News (Books) Ltd., London, England.

Abstract: Large variations occur in proximate composition, both from species to species and also from fish to fish of the same species. In addition, large variations occur in composition of different parts of the fish. The protein content of fish, although averaging about 19 percent, may vary from 6 to 28 percent. Oil content may vary from 0.2 to 64 percent, ash from 0.4 to 1.5 percent, and moisture from 28 to 90 percent. A classification into five categories with respect to oil and protein contents of fish is proposed. Most species fall into the category of low oil and high protein content. Need for proximate composition of fish is currently greater than in the past because of the trend for heart specialists to recommend diets requiring either low fat or a minimum of saturated fat, both of which can be obtained by using generous proportions of fish in the diet. Other needs for proximate composition data occur in various other fields of fishery technology, such as for calculating yield in the processing of various products.

Speculations on fishy odors and flavors. *Food Technology*, vol. 16, no. 4 (April), p. 28-32.

Abstract: Many types of fishy odors and flavors occur in fish; there is no predominant odor that is "the" fishy odor. These odors may range from those characteristic of individual species of fish through altered odors resulting from such changes as oxidative deterioration of fish oils and release of specific compounds during bacterial or enzymatic degradation. The mechanisms of reactions and the nature of the chemical

compounds causing fishy odors are still imperfectly understood, and more research is needed to clarify the nature and causes of these various fishy odors.

Thurston, Claude E.

Physical characteristics and chemical composition of two subspecies of lake trout. *Journal of Fisheries Research Board of Canada*, vol. 19, no. 1 (January), p. 39-44.

Abstract: Trout taken from deep waters of Lake Superior are of two subspecies--a fat one and a lean one--that vary greatly from each other in physical characteristics and in chemical composition. The fat species has a smaller head, larger body, and lighter color. The percentage of oil does not reach 20 percent in the fillets of the lean species, whereas it may reach 67 percent in fillets of the fat species, which has the highest known oil content of any fish.

### SEATTLE LABORATORY IN COOPERATION WITH OTHER LABORATORIES

Kaunitz, Hans, Donald C. Malins, and Donald G. McKay.

Studies of the generalized Shwartzman reaction produced by diet. II. Feeding of fractions of oxidized cod liver oil. *The Journal of Experimental Medicine*, vol. 115, no. 6 (June), p. 1127-1136.

Abstract: A series of fractions of oxidized cod liver oil were obtained by molecular distillation and were fed to pregnant rats in an attempt to correlate observed induction of the generalized Shwartzman reaction with chemical structure of the dietary lipid. Incidence of the Shwartzman reaction was higher in animals fed fraction III. The specific lipid material(s) causing development of the generalized Shwartzman reaction remains to be found.

Leong, K. C., D. G. Snyder, G. M. Knobl, and E. Gruger.

Feeding of fish oil and ethyl ester fractions of fish oil to broilers. *Poultry Science*, vol. 41, no. 5 (September), p. 1658.

Abstract: An 8-week feeding experiment was conducted to compare the possible utilization by broilers of fish oil and fish oil fractions and to determine the effect of these oils on the flavor of the cooked meat.

Mangold, Helmut K., Rudolf Kammereck, and Donald C. Malins.

Thin-layer chromatography as an analytical and preparative tool in lipid radiochemistry. 1961 International Symposium on Microchemical Techniques, Symposium vol. 2, p. 697-714.

Abstract: The use of thin-layer chromatography in combination with techniques of radiochemistry is described. Lipids were labeled by reacting them with diazomethane-C<sup>14</sup> or acetic anhydride-C<sup>14</sup>. The

labeled derivatives were fractionated and quantitatively analyzed by the consecutive use of thin-layer adsorption chromatography and paper partition chromatography.

Patashnik, Max, Anthony Frascatore, and A. Morris Rafn.

United States standards for grades of frozen sole and flounder fillets. U.S. Fish and Wildlife Service, Bureau of Commercial Fisheries Standards, April, First Issue.

Abstract: The grade is determined by observing the product in the frozen, thawed, and cooked conditions and is evaluated by numerical scoring. Points are deducted for variations in quality. "U.S. Grade A" is the quality of frozen flounder or sole fillets for which the total score is not less than 85 points; "U.S. Grade B," less than 85 points but more than 70; and "Substandard," less than 70 points.

Richardson, T., A. L. Tappel, and E. H. Gruger.

Polyunsaturated fatty acids in fish mitochondria. (Presented at FAO Fish in Nutrition Conference.) In *Fish in Nutrition*, p. 150-152. Fishing News (Books) Ltd., London, England.

Abstract: The unsaturation of mitochondrial fatty acids increased as follows: chicken liver < beef heart < rat liver < catfish liver < carp liver < salmon liver < salmon heart. Linoleic and arachidonic acids accounted for 22.3 percent, 51.6 percent, 45.2 percent, 4.5 percent, 20.6 percent, 4.9 percent, 2.7 percent of total fatty acids in these preparations, respectively. Linolenic acid was not detectable in mitochondria from chicken liver, beef heart, rat liver, and catfish liver. Molar ratios of EFA (essential fatty acids) to cytochrome c ranged 70 to 1,051. Generally, fish mitochondria were low in total essential fatty acids, but contained high levels of C<sub>20:5</sub> and C<sub>22:6</sub> fatty acids. Chicken mitochondria contained a C<sub>22:6</sub> polyene (2.6 percent). Mitochondrial linoleic acid was low in salmon liver (0.9 percent) and salmon heart (1.1 percent) and was undetectable in catfish liver. Mitochondrial fatty acid patterns of hearts and livers of pelican, cormorant, grebe, murre, scoter, and fur seal were similar to those of fish. The main differences were the lower average of total fatty acids and the higher average of stearic and arachidonic acids in the mitochondria of birds and seal. The research indicates that fish mitochondria have no compulsory requirement for linoleic acid or linolenic acid, fish apparently do not concentrate EFA in mitochondria, and classical EFA do not function in mitochondrial electron transport.

Richardson, T., A. L. Tappel, L. M. Smith, and C. R. Houle.

Polyunsaturated fatty acids in mitochondria. *Journal of Lipid Research*, vol. 3, no. 3 (July), p. 344-350.



**Abstract:** Fatty acids of mitochondria from hearts and livers of fresh-water and marine fish, marine birds, fur seal, and from sweet potato have been analyzed by gas-liquid chromatography. Cytochrome patterns, determined from difference spectra, were similar for all animal species; the quantities of cytochromes varied slightly, heart mitochondria yielding higher values than did the liver mitochondria in each species. Fish mitochondria showed a slightly lower cytochrome content than did bird and mammalian mitochondria; sweet potato particles contained very little cytochrome. Fatty acid patterns and degree of unsaturation were similar in mitochondria from fish and from birds and seals that eat fish.

Thurston, Claude E., George Kudo, and S. R. B. Cooke.

Function of tuna oil fatty acids as collectors in the flotation of iron ore. *Society of Mining Engineers*, vol. 223, no. 4 (December), p. 350-352.

**Abstract:** Unfractionated fatty acids from tuna oil and five fractions from these fatty acids were tested for their ability to concentrate a specularite ore from northern Michigan. The best results were obtained with a fraction precipitated from acetone at -5° C. and having an iodine number of 192. This fraction gives results equal to those obtained with tall oil (currently used for iron ore flotation) when one pound per ton at a pH of 7 was employed.

## CONTRACTORS OF SEATTLE LABORATORY

Mangold, Helmut K., and Rudolf Kammereck.

New methods of analyzing industrial aliphatic lipids. *The Journal of the American Oil Chemists' Society*, vol. 39, no. 4 (April), p. 201-206.

**Abstract:** After isolation by thin-layer chromatography, lipid classes were further fractionated according to chain length and degree of saturation by complementary methods, such as paper chromatography or gas chromatography or both, which permit rapid analyses of complex mixtures of industrial lipids.

Olcott, H. S., J. Froines, and C. Y. Shuster. Muscle lipids of tuna. (Presented at FAO Fish in Nutrition Conference.) *In Fish in Nutrition*, p. 146-147. Fishing News (Books) Ltd., London, England.

**Abstract:** Some newer methods of analyses were applied to a study of the muscle lipids of tuna fish with particular reference to the phospholipid components. Preliminary data were obtained with a small number of samples of albacore and yellowfin tuna muscle. The total lipid content ranged from 0.4 percent to 10 percent of the total net weight. Neutral lipid accounted for most of that over

0.25 percent. Of the phospholipid fractions, the lecithins were the most abundant (40-60 percent); the cephalins were present in amounts of one-third to one-half as much (17-25 percent), and phosphoinositides accounted for less than 5 percent. A considerable portion (10-30 percent) of the lecithin and cephalin fractions were in the form of plasmalogens. Preliminary fatty acid determinations indicated that palmitic acid accounted for most of the saturated fatty acids in the neutral fat and in the lecithins but that stearic acid was the more abundant in the cephalins.

Peifer, James J.

Comparative effects of marine oils, marine oil fractions and whole fish meals on hypercholesteremic rats. (Presented at FAO Fish in Nutrition Conference.) *In Fish in Nutrition*, p. 282-283. Fishing News (Books) Ltd., London, England.

**Abstract:** In an attempt to establish the chemical nature of the fatty acid components causing the observed activity of marine oils on hypercholesteremic rats, the esters prepared from menhaden oil fatty acids were separated into a series of fractions differing in their relative degrees of unsaturation. These marine oil ester fractions were found to be more effective than were common dietary fats and oils in reducing the blood and liver lipids of the hypercholesteremic rat (*Federation Proceed.*, 20, 93, 1961). The relative effects of the ester fractions and the dietary fat supplements were not directly related to the total unsaturation of these oils. The menhaden esters were far more effective in reducing the tissue lipids of the rat than could be predicted on the basis of the total unsaturation of these fractions.

Peifer, James J., F. Janssen, R. Muesing, and W. O. Lundberg.

The lipid depressant activities of whole fish and their component oils. *The Journal of the American Oil Chemists' Society*, vol. 39, no. 6 (June), p. 292-296.

**Abstract:** Hypercholesteremic rats were used to investigate the lipid depressant activities of lyophilized whole fish prepared from menhaden, silver salmon, mullet, and ocean perch. Ingestion of the whole fish supplements promoted a significant reduction in the circulating levels of cholesterol and phospholipids and in the TC/TP (total cholesterol/total phospholipids) ratios of blood lipids from the rat. The nonlipid components, isolated from the fish, had no apparent influence on the distribution of lipids in the blood and liver tissues.

Privett, O. S., and Christense Nickell.

Determination of structure of unsaturated fatty acids via reductive ozonolysis. *The Journal of the American Oil Chemists' Society*, vol. 39, no. 9 (September), p. 414-419.

**Abstract:** An improved method of reductive ozonolysis for the determination of structure of unsaturated fatty acids is reported. The ozonization is carried out at -60 to -70° C. by adding the sample dissolved in pentane to a .02-.03 M pentane solution of ozone. The reduction is effected by the Lindlar catalyst at 0° C. in pentane or in other solvents, such as the methyl esters of short-chain fatty acids or in dimethyl phthalate, and the aldehydic fragments are analyzed by gas-liquid chromatography.

Richardson, Thomas, and A. L. Tappel.

Swelling of fish mitochondria. *The Journal of Cell Biology*, vol. 13, no. 1 (January), p. 43-54.

**Abstract:** The physical properties of fish liver and rat liver mitochondria were compared as a function of temperature and osmotic pressure. The data indicate that fish mitochondria are more flexible and swell at a more rapid rate over a 0° to 30° C. temperature range, whereas the rates of swelling at 30° to 40° C. are comparable. All the data indicate that under comparable conditions the fish mitochondrial membranes are more flexible and presumably more permeable and labile than rat mitochondrial membranes. The findings are discussed in relation to the general metabolic implications and the possible contributions of the membrane constituents to membrane behavior.

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