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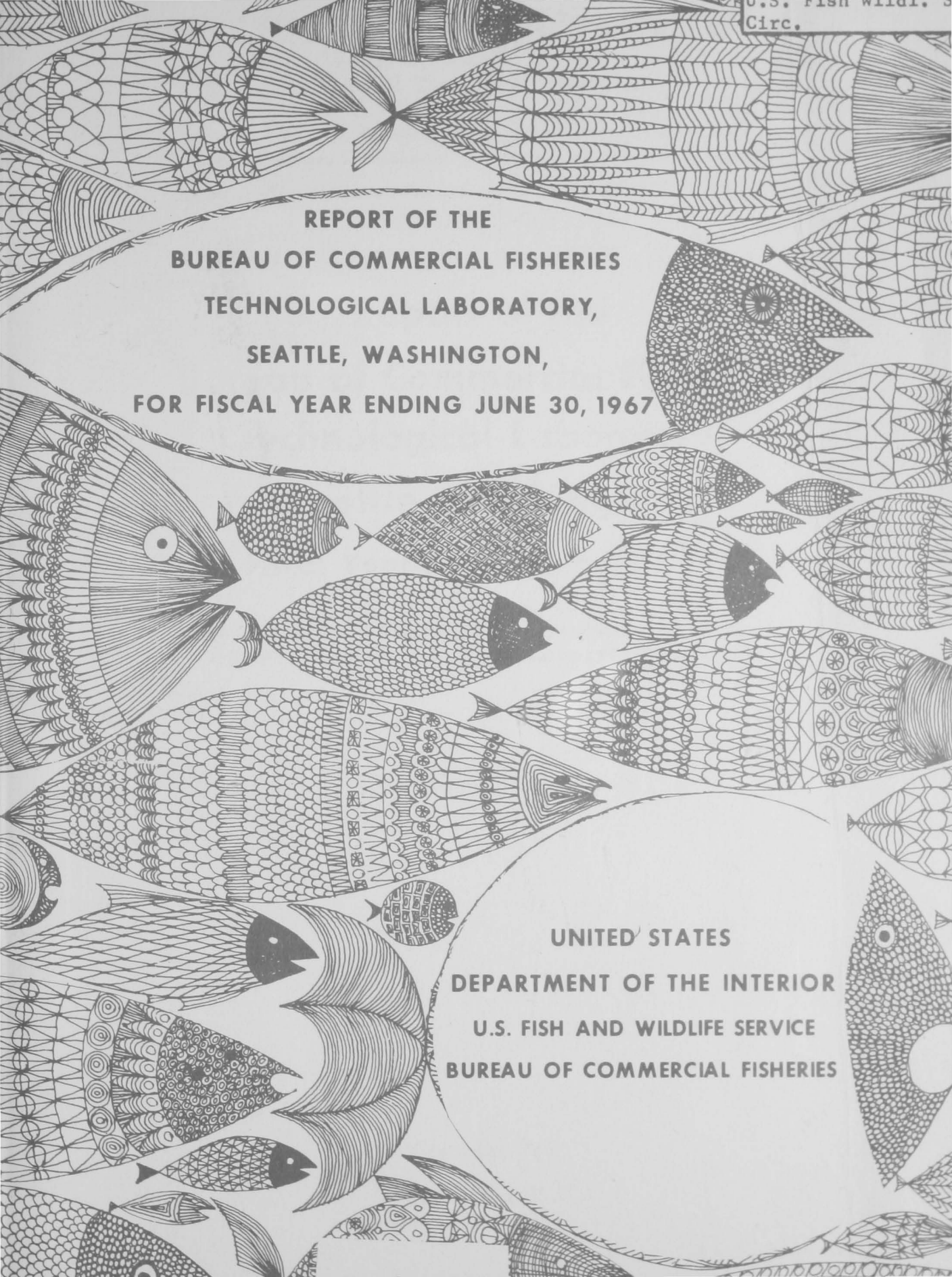
REPORT OF THE BUREAU OF COMMERCIAL FISHERIES TECHNOLOGICAL LABORATORY, SEATTLE, WASHINGTON, FOR FISCAL YEAR ENDING JUNE 30, 1967



UNITED STATES
DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE
BUREAU OF COMMERCIAL FISHERIES

UNITED STATES DEPARTMENT OF THE INTERIOR
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Russell E. Train, *Under Secretary*
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for Fish and Wildlife, Parks, and Marine Resources
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Dayton L. Alverson, Acting Director, BUREAU OF COMMERCIAL FISHERIES

Report of the Bureau of Commercial Fisheries Technological Laboratory, Seattle, Washington,

for Fiscal Year Ending June 30, 1967

MAYNARD A. STEINBERG, Laboratory Director
JOHN A. DASSOW, Assistant Laboratory Director

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CONTENTS

Report of the Bureau of Commercial Fisheries Technological Laboratory, Seattle, Washington, For Fiscal Year Ending June 30, 1967

MAYNARD A. STEINBERG, Laboratory Director
JOHN A. DASSOW, Assistant Laboratory Director

ABSTRACT

The accomplishments of the Technological Laboratory for the fiscal year ending June 30, 1967, are described. They include work on the: protection of frozen salmon by antioxidants, development of packaging techniques for air-shipping live Dungeness crabs, use of condensed phosphates for improving the water-holding capacity of fresh fish fillets, utilization of fish oil for industrial and food products, relation of flavor to post mortem breakdown of nucleotides in fish and shellfish, and studies on the radiation preservation of fishery products.

HIGHLIGHTS OF THE LABORATORY PROGRAM DURING FISCAL YEAR 1967

GENERAL

Early in the fiscal year, the BCF (Bureau of Commercial Fisheries) Technological Laboratory in Seattle was reorganized, and the BCF Food Science Pioneer Research Laboratory was formed as a separate laboratory to investigate the oxidation of fish oils. The new laboratory has six researchers, including Maurice Stansby, the Laboratory Director. The Technological Laboratory, now comprised of 25 on the research staff, was regrouped under a new Laboratory Director, Maynard A. Steinberg, formerly Assistant Laboratory Director of the BCF Technological Laboratory at Gloucester, Mass. Both Laboratories continue to occupy the fourth floor of the new fishery research building and share the space for common administrative and operating functions.

With the new, but somewhat smaller, Technological Laboratory, it was essential to reassess the Laboratory's research and development role in relation to regional and national fishery needs and developments. The increasing need for expansion and strengthening of the domestic fisheries was recognized in a revised statement of the aims of the Laboratory:

1. The development of knowledge that will improve the handling, processing, preservation, distribution, and utilization of fishery products.
2. The demonstration to industry of this knowledge in the form of methods and techniques that result in the efficient production from our total resource of a wide variety of consistently high-quality food and industrial products.

RESEARCH HIGHLIGHTS

The Laboratory program was organized into six program areas in accordance with the field assignments and current contractual research for the U.S. Atomic Energy Commission:

1. Preservation, Utilization, and Process Engineering.
2. Chemistry of Fishery Products: Fish Oils and Pesticide Residues.
3. Chemistry of Fishery Products: Biochemical Investigations.
4. Radiation of Fish: Application of Radiation-Pasteurization. Processes to Pacific Coast Fishery Products.
5. Pathogens in Radiation-Pasteurized Fishery Products.
6. Fishery Inspection Services.

Preservation, Utilization, and Process Engineering

Studies of the methods for holding and shipping live Dungeness crabs by air were started in response to the problem of high mortality of crabs during commercial trial shipments by

industry. Preliminary results indicated that keeping the crabs at 40° to 45° F. and using an improved shipping container resulted in high rates of survival.

Treatment with an antioxidant, ethoxyquin, was shown to retard the undesirable oxidative changes and fading of the normal color in frozen salmon.

Freezing and storage of Dungeness crab meat in the form of frozen blocks was evaluated and appeared to be quite feasible as a means of preservation.

The limited water-holding capacity of the fresh and thawed fillets of a number of species of fish has long been a problem, owing to the unsightly drip formed and the accompanying loss in weight of the fillets. Chemical studies of the use in fish flesh of condensed phosphates, which are common additives in foods like meats and dairy products, demonstrated that the water-holding capacity was markedly improved by the addition of small amounts of tripolyphosphates.

The potential recovery of soluble proteins extracted from whole fish or fish wastes was shown in laboratory tests in which soluble fish proteins were precipitated by hexametaphosphate, removed from solution, and dried.

Initial studies on "problem" species such as Pacific hake and several species of rockfish showed that these fish could be used in modified frozen fish blocks and processed specialty products.

Chemistry of Fishery Products: Fish Oils and Pesticide Residues

Increasing world needs for edible fats and oils have been related to current fish-oil studies. The development of new fisheries in relation to the manufacture of fish protein concentrate will produce new sources of fish oil in large volume; therefore, our laboratory broadened its research to include the chemistry of upgrading edible fish oils and the development of fish-oil derivatives for use in food.

Laboratory work on food additives from fish oils was begun with the development of monoglycerides.

A unique and economical method for synthesis of nitrated fatty acid esters from fish oils was developed to permit the use of these fish-oil derivatives in various industrial chemical processes. In 1967, the two chemists who did the research received a U.S. patent for the method.

The Laboratory made a limited survey of the residue levels of chlorinated-hydrocarbon pesticides in some Pacific fish and shellfish. Pesticide residues in the eight species analyzed to date were substantially lower than the tolerance for residues in beef. This first survey showed that pesticides in fish and shellfish do not pose a health problem for the consumer.

Chemistry of Fishery Products: Biochemical Investigations

A study of the enzymes causing the degradation of the nucleotides in the muscle of certain species of fish indicated that a common food additive, EDTA (ethylenedinitrilotetraacetic acid), might be used to inhibit the changes affecting loss of flavor and would therefore improve the flavor of the fillet during prolonged storage.

Radiation of Fish: Application of Radiation-Pasteurization Processes to Pacific Coast Fishery Products

Previous research showed that a low radiation dose of 0.1 to 0.2 megarad was sufficient to kill 99 percent of the spoilage bacteria on the fish flesh. The radiation dose thereby extended the shelf life of the packaged fresh product at 33° F. from 2 to 5 weeks, a period up to five times longer than the shelf life of the nonirradiated fish. This work was done on halibut, petrale sole, English sole, Pacific ocean perch, and king crab and Dungeness crab meat. In the past year, similar tests with true cod indicated that the shelf life was extended two to three times or up to 3 weeks of storage for vacuum-packed fillets stored at 33° F. Fresh Pacific oysters irradiated at 0.2 megarad and stored at 33° F. kept up to 10 days longer than the nonirradiated oysters. Whole cooked Dungeness crab (in the shell) were irradiated at 0.2 megarad and kept 15 days at 33° F. or 5 days longer than the nonirradiated. If eviscerated, the irradiated crab could be stored up to 25 days at 33° F. in marketable condition.

Tests with irradiation pasteurization of fillets in large wholesale packages were promising and indicated that the storage life was extended from 7 to 14 days at 33° F. when the fillets were repacked into retail packages after an initial 7 to 14 days of storage at 33° F.

Experimental shipments of irradiated fish and crab meat were made under commercial conditions to distributors and retailers in the West and Midwest to demonstrate the shelf-life extension and the commercial feasibility of the irradiation preservation method. Most of the people in the industry felt that the process will be of significant benefit to their operations.

Continuing research on practical methods for estimating the quality of irradiated fish demonstrated that measurement of free glucose could be a useful criterion.

Loss of liquid or drip from both irradiated and nonirradiated fish fillets is a problem in certain species during refrigerated storage. This drip can be minimized by treatment of the fillets with condensed phosphate, a common

and inexpensive food additive. A belt-type spraying machine for the application of phosphate solution was built and tested. It demonstrated clearly that the application of a phosphate spray is effective in reducing drip.

Detailed microbiological studies of the surviving microflora of irradiated sole fillets have shown that the predominant spoilage flora were lactobacilli, a common food-spoilage organism, and that aerobic bacteria of health significance--e.g., coliforms--were suppressed by the radiation treatment.

Pathogens in Radiation-Pasteurized Fishery Products

Detailed microbiological studies are underway to ensure that the recommended processes for commercial use of radiation pasteurization of fishery products are safe and that they preclude any public health hazard from Clostridium botulinum. Current research demonstrated that the outgrowth of type E Cl. botulinum does not occur in inoculated packs of irradiated or nonirradiated petrale sole fillets held for 72 days at 38° F. At higher temperatures, outgrowth and toxin production can be demonstrated for both irradiated and nonirradiated fillets.

Fishery Inspection Services

Lot inspection, the primary inspection service performed by the Bureau in this region, continued to grow steadily as indicated by the annual volume of products inspected. More than 9 million pounds of fishery products were inspected during the year in the region.

REPORT FROM THE LABORATORY DIRECTOR

An annual report of a laboratory should be more than an inventory of work accomplished and of reports presented or published. It seems particularly desirable for this year that we review our aims. The reorganization of the Laboratory and the change of Director early in the fiscal year provided the impetus for this review. As you read our Laboratory objectives in the Highlights section, I hope you note that we have a strong orientation toward the direct application of our research and toward developmental studies leading to the increased utilization of fishery resources. A few examples have been given in the research highlights, and more appear in the detailed summaries of the programs. In those instances where either laboratory experiments or preliminary tests are promising, we believe that the applied studies must be continued until both we and the interested members of the fishing industry agree that our phase of the work is done and that the next move is up to industry.

On the other hand, I would not want to have you believe that our Laboratory has forsaken the longer range and more difficult research involving the chemistry, biochemistry, and microbiology of our fishery species in relation to use. This work, even when it cannot be expanded to the degree we believe desirable, provides the root structure for any laboratory concerned with new ideas and the solution of old and knotty problems. Examples of the current research in these fields are evident to some degree in the program summaries; however, one should read the detailed reports listed under Laboratory Publications for a better understanding of these more basic laboratory studies. The publications and reprints are available on request to those interested in the details of the research.

LOOKING BACK ON THE RESEARCH YEAR

In reviewing the research accomplishments for the year, I am pleased to report that our program appears to be in balance, with both applied and basic results in evidence.

On the applied side, important contributions have been: (1) the study of essential factors in handling and shipping live Dungeness crab, (2) studies of the use of tripolyphosphates and their effect in preventing excessive loss of drip in fresh fillets, (3) technical support given to industry in using Pacific hake for fish meal and in producing a high-quality meal, and (4) trial shipments to demonstrate commercial feasibility of the radiation-pasteurization process for fresh fillets of three species of Pacific trawl fish.

On the more basic or laboratory-research side, our Laboratory has contributed significantly to the state of knowledge in the fields of the chemistry and microbiology of fish muscle. Studies of the degradation of the nucleotides in fish muscle and the enzymes involved have shed some light on the important subject of flavor change in fish and shellfish. The award of a patent for the organic synthesis of nitrated fatty acid esters from fi oils is a significant contribution toward greater industrial uses of fish oils. The detailed analytical determination of the pesticide residues in fishery products has been essential to demonstrate the significance of the problem, which happily is not serious, judging from the findings this year. On the microbiological side, the detailed study of the surviving microflora of irradiated pasteurized fish and the determination of the biochemical characteristics of types B and F Cl. botulinum from Pacific Coast habitats have supplied knowledge fundamental for safe process development. Further, the

research has yielded knowledge dividends applicable to the general field of fish and food preservation.

THE PERSPECTIVE AHEAD

Finally and to return to my initial theme, in this report let us also look toward future accomplishments. First and of greatest potential significance are those future contributions that will expand our domestic fisheries and increase our use of the resources available to our fleets. Perhaps our greatest step forward will come through the development of methods by which whole fish can be converted to high-quality and appealing edible protein and oil products. Closely related to this is our plan, now underway, for the broad development of improved and modified fish products in which species like hake, rockfishes, and arrowtooth flounder may be used fully for food despite characteristics, such as soft texture of the flesh or poor keeping quality, that to date have limited their use for food.

In fresh fish production, we can look ahead to the completion of the many aspects of the irradiation pasteurization process and can predict that this process will see large-scale commercialization as a means of marketing premium fresh fish on a wider scale than ever before. Linked with this development will be the completion and application of techniques for preventing the loss of the "just-caught" flavor that every fisherman knows. I hope you will look forward to seeing progress reports on these and other developments in our future annual reports.

INDUSTRY PROBLEMS - A BASIS FOR RESEARCH

National fishery problems are characterized by a moderate decline in domestic landings during recent years and an increasing dependence on imported fishery products. The domestic demand has increased in accordance with our growth in population; however, per capita consumption has remained between 10.5 and 11.0 pounds in the last several years. FAO (Food and Agriculture Organization) has announced that the United States dropped from fifth to sixth place in world fishery production in 1966. FAO's Yearbook of Fishery Statistics for 1965 showed that the leading fishery nations are increasing their catches and landings every year in contrast to the U.S. decline. The background for this picture is quite complex and beyond the scope of this summary; however, there is no question but that improved harvest technology, process engineering, and product developments are important approaches if the U.S. fisheries are to be revitalized.

This somewhat dismal national fishery situation must be used as the backdrop for our evaluation of regional fishery problems and the selection of research areas suitable to our laboratory capabilities and responsive to the needs of the industry. The following lists this region's needs and potentials as we view them technologically:

1. Increase domestic landings.--Now, only the trawl fisheries can achieve really significant increases in the landings for Washington and Oregon. Major increases in fisheries such as those for salmon and halibut do not appear likely. Other fisheries of this region include those for Dungeness crab, oysters, clams, shrimp, albacore tuna, smelt, herring, and fresh-water species that are important to specific areas and segments of the industry. They also hold no promise for substantial increases of the magnitude needed--10, 50, or 200 million pounds per year.

2. Expand the trawl fisheries.--Several accepted fillet species such as petrale sole, true cod, and lingcod appear to be limited already. Pacific ocean perch, dover sole, and sablefish, however, are accepted species with potential for substantial expansion. The species little used in our present trawl fishery--rockfishes, Pacific hake, walleye pollock, arrowtooth flounder, starry flounder, and other flatfishes--have the marked potential for expansion.

3. Develop new and improved preservation methods.--The species with marked potential for expansion are all "problem" species, commonly regarded as scrap fish good for reduction or animal feed but hardly worth bringing in to the dock at the present price and demand. All are difficult to preserve properly for food use by existing fishery methods, both on the vessel and in the plant.

4. Improve efficiency and mechanize handling methods.--Mechanization techniques and large-volume handling methods must be developed and adopted; otherwise, it is economically impractical to harvest and use the fish at the low price essential to compete with other fish or meat proteins.

5. Diversify production.--Fishery products do not compete effectively with other animal protein foods and do not provide the quality, convenience, and variety expected by the consumer. Much-improved and new processing concepts, methods, and food applications are required to improve the competitive position of existing products, such as fresh and frozen fillets, and to use the problem species in an expanded fishery. Other diversified food industries provide

a wealth of technology and engineering that could be applied to fisheries. New knowledge and techniques arise from the advancements in food science--protein and oil chemistry, biochemistry of enzymes, and microbiology of food preservation.

6. Develop and apply quality control.--Small fisheries and limited production operations do not permit modern quality control. In the projection of an expanded and diversified fishery, the modern production facilities will require laboratory and technical control to ensure the product quality needed.

These needs, linked closely on a regional and national level to domestic fishery problems, provide the basis for our research orientation. The current research programs are planned with these important industry needs in mind.

ORGANIZATION AND STAFF

Each of the following programs is directed by a program leader who reports to the Laboratory Director for review of research priorities and project recommendations. Program leaders exercise considerable autonomy within their research units with respect to research assignments and intermediate goals.

Maynard A. Steinberg, Laboratory Director
John A. Dassow, Assistant Laboratory Director
Patricia S. Terao, Administrative Officer

Program: Preservation, Utilization, and Process Engineering

Richard W. Nelson, Research Chemical Engineer - Program Leader
Max Patashnik, Chemical Engineer
John A. Dyer, Chemical Engineer
Wayne I. Tretsven, Research Chemist
Harold J. Barnett, Research Chemist
Alice S. Hall, Research Chemist
Barbara J. Kemp, Research Chemist
George Kudo, Research Chemist
Patrick J. Hunter, Engineering Technician (Chemist)

Program: Chemistry of Fishery Products: Fish Oils and Pesticide Residues

Erich J. Gauglitz, Jr., Research Chemist - Program Leader
Virginia F. Stout, Research Chemist
Clifford R. Houle, Research Chemist
Lawrence W. Lehman, Research Chemist
John C. Wekell, Research Chemist

Program: Chemistry of Fishery Products: Biochemical Investigations

Herman S. Groninger, Research Chemist - Program Leader

Program: Radiation of Fish: Application of Radiation-Pasteurization Processes to Pacific Coast Fishery Products

David T. Miyauchi, Research Chemist - Program Leader
John Spinelli, Research Chemist
Fuad Teeny, Research Chemist
Gretchen A. Pelroy, Microbiologist
John P. Seman, Jr., Microbiologist
Dave H. Wieg, Physical Science Aid

Program: Pathogens in Radiation-Pasteurized Fishery Products

Melvin W. Eklund, Research Microbiologist - Program Leader
Frank T. Poysky, Food Technologist
David I. Wieler, Microbiologist

Program: Fishery Inspection Services

A. Morris Rafn, Supervisory Fishery Products Inspector
George A. Berkompas, Fishery Products Inspector

Clerical-Stenographic Staff

Margaret G. Hodgins, Secretary
Dolores A. DeWitt, Clerk-Typist
Isabell L. Diamant, Clerk-Stenographer
Gretchen V. Lindberg, Clerk-Stenographer
Helen M. Robertson, Procurement Clerk

Laboratory Services

Laura G. Lewis, Biological Aid (Fisheries)

In addition to the above full-time staff, the Laboratory employs student scientific aids and has provided limited facilities for visiting scientists.

PHYSICAL FACILITIES

The Technological Laboratory occupies space jointly with the Food Science Pioneer Research Laboratory on the fourth floor of the new fishery research building completed and occupied in early 1965. Specialized equipment, instrumentation, and facilities include gas chromatographs for lipid research, recording visual and infrared spectrophotometers, refrigerated and preparative ultracentrifuges, an electron magnetic resonance spectrometer, a refrigerated environmental laboratory, freeze-dryer, plate freezer, a large molecular still, and specialized facilities for research involving hazardous solvents.

The Technological Laboratory has a separate pilot-plant building with 2,600 square feet of floor space. This building, constructed in 1936, is used for a processing plant and a pilot plant, for providing freezing and cold-storage facilities, for fishery inspection services, and for an isolation microbiological laboratory for research involving Cl. botulinum.

A research cobalt-60 Mark II irradiator supplied by the Atomic Energy Commission is located nearby at the College of Fisheries, University of Washington.

The Laboratory operates no vessels, but it conducts specific studies at sea aboard either the BCF exploratory fishing vessel, John N. Cobb, or commercial fishing vessels under cooperative arrangements.

PRESERVATION, UTILIZATION, AND PROCESS ENGINEERING

Richard W. Nelson, Program Leader

This program provides industry with information that is of direct assistance in improving the quality of landed fish and of fishery products. The program also works toward the better utilization of the fishery resources that are now harvested and provides incentive for harvesting the unused resources through the development of new and attractive products. Our aims are to improve the competitive position of the domestic industry and to provide the consumer with a variety of high-quality seafood products.

We have begun to probe new areas such as the shipping of live Dungeness crabs to distant and heavily populated markets and the formulation of frozen fish blocks using diced or coarse-ground fish.

In a cooperative project with the BCF Technological Laboratory at Ann Arbor and the Tectrol Division of the Whirlpool Corporation, we are studying how controlled atmospheres may extend the shelf life of fresh fish.

EFFECT OF ETHOXYQUIN ON THE OXIDATION OF FROZEN SALMON

The evaluation of the effectiveness of ethoxyquin as an antioxidant when applied to king salmon flesh was completed early this year. Results showed that a 0.1 percent concentration of ethoxyquin effectively retarded the oxidative deterioration of salmon held in frozen storage. A 0.01 percent concentration of ethoxyquin was ineffectual as an antioxidant.

At the higher concentration (0.1 percent), ethoxyquin retarded the fading of the normal salmon color and, in some instances, actually improved the color. This phenomenon did not occur when ethoxyquin was used at the lower concentration.

The 0.1 percent concentration of ethoxyquin imparted a flavor described as medicinal (bitter) by some taste-panel members but as sweet by others.

A manuscript entitled "The antioxidant effect of ethoxyquin on frozen salmon flesh" is being prepared for publication.

FROZEN DUNGENESS CRAB MEAT BLOCKS

Dungeness crab meat stored frozen in cans often develops off-flavors and becomes tough. To overcome this problem, we began a study to determine if freezing the meat in blocks would slow these changes.

Crab meat was frozen in blocks flooded with glaze water by means of a technique similar to that used for freezing king crab meat. The blocks were cut into 1-pound portions that were glazed and overwrapped with aluminum foil. These blocks were stored at 0° F. and periodically examined for changes in flavor and texture.

Evaluation of the samples at 1 and 3 months showed that only moderate changes in flavor and texture had occurred.

At the end of 6 months of storage, noticeable changes in both flavor and texture were evident; however, we did not consider these changes to be as extensive as those normally occurring in the canned products. The block-preservation method appears to produce a better product than that stored in cans.

More work is planned to complete the evaluation of this method for processing Dungeness crab meat.

AIR SHIPMENT AND LIVE-HOLDING STUDIES

This year we began studying how to improve methods for shipping live Dungeness crabs by air to distant markets.

Initial work on the program was restricted to observing and evaluating industry practices. This work included observations on vessel operations, inplant handling, airline handling procedures, and retailing procedures.

Because live Dungeness crabs that are to be shipped by air are held mainly in areas where usable sea water is not always available, early laboratory work was centered around the development of a closed-system (recycled sea water) live tank. We have found that the optimum temperature for holding live crabs in a closed live-tank system is 45° to 50° F. In addition to the work on the live-tank facilities, we began laboratory studies concerning the effects of varying temperatures, shipping-container requirements, icing requirements of the packaged crabs, and suitability of available packing materials.

From the data gathered in our studies, we have concluded that shipping temperatures of 35° to 45° F. and times not exceeding 2 days out of water are most suitable for transporting live Dungeness crabs.

Figure 1.--Packaging live Dungeness crabs for shipment.

We also have evaluated a number of containers and container designs. Several containers, including one designed by Laboratory personnel, have been found suitable.

Tests on packaging materials to protect the live crabs during shipment have shown that moist cellulose and burlap are cheap and effective.

Banding the claws reduced damage to the crabs during shipment, but the process is time consuming and may not be economically feasible.

The crabs can be cooled during shipment by chemical ice (gel-ice) at a ratio of about 1 pound of ice per 3.5 pounds of crabs.

STUDY OF THE USE OF CONDENSED PHOSPHATES FOR THE IMPROVEMENT OF PROCESSED FISH QUALITY

Effect of Condensed Phosphates on the Water-Holding Capacity of Fish Tissue

The aims of this study are to determine the effects of condensed phosphates in improving the WHC (water-holding capacity) of fish tissue and to provide information on the influence of such factors as species variations, fish quality, and processing, which may affect these treatments.

The results of experiments in which samples of raw minced cod and English sole tissue were treated with solutions containing 0.01M to 0.20M TPP (tripolyphosphate), PP (pyrophosphate), or NaCl, showed that a measurable improvement in WHC was obtained only in samples treated with phosphate concentrations higher than 0.02M. Maximum improvement in WHC was obtained with 0.04 to 0.05M phosphate. In similarly treated cooked samples, the relation between water retention and phosphate concentration was about linear up to 0.03M. Higher concentrations of phosphate produced only slight improvements in the water retention of the tissue.

As Mahon[1] had reported that combinations of polyphosphate and NaCl were more effective than polyphosphate alone in improving water retention, samples were treated with solutions containing various concentrations of TPP and PP in 1 percent and 2 percent solutions of NaCl. These experiments showed that in samples cooked after treatment, the combinations of NaCl and polyphosphate were slightly less effective than the polyphosphate alone. In samples held in the raw state, only the combinations containing 2 percent NaCl were more effective than polyphosphate alone.

On a comparative basis TPP was slightly more effective than PP in improving WHC.

[1] Mahon, J. H. 1962. Preservation of fish. U.S. Pat. 3,036,923.

It was also noted that the degree of improvement produced by a given treatment was dependent on the species of fish used.

Inorganic Tripolyphosphatase and Pyrophosphatase in Fish Tissue

To determine if there was a significant breakdown of TPP or PP by the inorganic phosphatases of fish tissue, we prepared whole tissue homogenates from English sole fillets and assayed for TPPase and PPase activity. During a 1-week incubation period, only a small amount of polyphosphate breakdown occurred. This breakdown was due to PPase activity. TPPase activity in extracted actomyosin was negligible.

Complexes of Soluble Fish Proteins With Condensed Phosphates

Laboratory experiments have shown that soluble proteins can be precipitated from acidified solutions with condensed phosphates such as hexametaphosphate. The precipitation is quantitative; and by varying conditions such as pH, protein concentration, or the chain length of the phosphate, we can obtain complexes with various protein-to-phosphate ratios. The aims in this work are to produce such complexes from soluble fish proteins, to study their physical and chemical properties, and to determine if they can be used by food processors. Another interesting possible use for this reaction is the selective precipitation of waste protein from fish-processing plants to reduce pollution and recover a valuable byproduct.

Laboratory tests have shown that 98 percent of the proteins in a 1-percent solution of soluble fish proteins can be precipitated when the solution is made 0.08M with respect to sodium hexametaphosphate and the pH is lowered to 4.3 with hydrochloric acid. The precipitate is granular and can easily be removed from solution by centrifugation. The wet precipitate may be dried and dissolved in salt solutions buffered at pH 7.0. It is not readily soluble, however, after it has been dehydrated with organic solvents such as isopropanol or acetone. The dehydrated complex is hydrolyzed by both pepsin and papain.

Results of preliminary experiments in which 5 percent of the dry ingredients of biscuit and cake dough was replaced with the complex indicate that the complex may be used to improve the texture of such products.

Expanded use of underused species offers the greatest possibilities for increasing landings in our region. We have therefore directed our research toward learning more about the characteristics of the flesh of several of these species when processed in various ways. These characteristics will determine for each its greatest potential use--i.e., whether the flesh can be used nearly unaltered for conventional products or whether it must be substantially altered in texture or other characteristic for use in specialty products.

Hake

The Pacific hake is available in commercial quantities; and the BCF Exploratory Fishing and Gear Research Base, Seattle, Wash., has developed the specialized harvesting gear to catch these fish.

Production of fillet blocks from hake does not appear to be feasible now because of the high incidence of a myxosporodian parasite that softens the texture of the cooked flesh. We are monitoring the incidence of this organism to determine whether it is decreasing, as we might expect as harvesting continues and the hake population is reduced.

We began studies of the use of hake in minced, ground, and sausage-type products. Several sausage products suitable for canning have been prepared and show promise.

Black Rockfish

Although rockfish of several species are landed and used in the fresh-fillet market, they lack flavor and have poor keeping quality both fresh and frozen. As a possibility for encouraging more use of rockfish, we are developing an improved frozen fish block. Rockfish fillets are either coarsely ground or packed as whole fillets after being blended with additives to improve WHC, enhance flavor, and retard rancidity. Results appear promising from the standpoint of initial taste-panel acceptance of oven-fried, breaded fish portions prepared from the blocks. We need further work to determine the best combination of antioxidants to provide protection against rancidity.

Anchovy

Several hundred pounds of anchovy caught off the Washington coast were evaluated for possible use in food products. Although the fish was iced within 12 hours after capture, deterioration from proteolytic enzymes in the viscera occurred within 1 to 2 days. In addition, sensory and chemical tests (2-thiobarbituric acid or TBA) indicated a high degree of oxidative rancidity within the same period. Several anchovy products were prepared; but none was acceptable because of poor texture, flavor, and appearance.

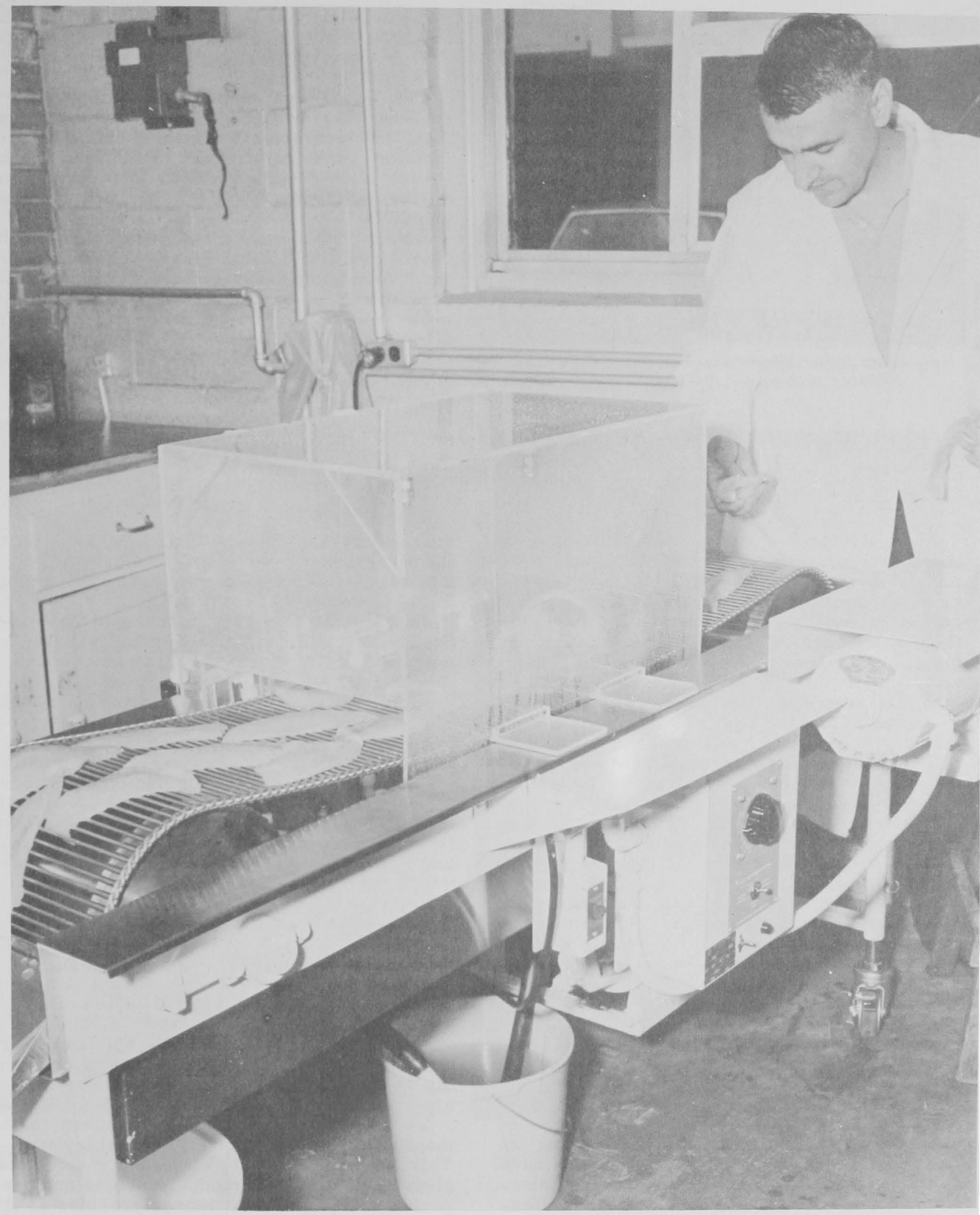


Figure 2.--Spray treatment of fresh fillets with a solution of sodium tripolyphosphate to improve quality.

Figure 3.--Experimental frozen block prepared from minced rockfish flesh.

SEPARATION AND PURIFICATION OF OIL IN SOLVENT PROCESSING OF FPC (FISH PROTEIN CONCENTRATE)

The economics of the utilization of FPC require that a high-grade oil be recovered from the solvent-oil mixture obtained during the manufacture of FPC. This Laboratory is developing methods to recover most of the oil from the flesh of both lean and oily fish.

Several batches of FPC were made in the laboratory from Pacific hake taken from off the Washington coast and from Puget Sound. Solubilities of hake oil in isopropanol-water mixtures at various temperatures were determined as an aid in determining optimum extraction and oil recovery procedures.

CHEMISTRY OF FISHERY PRODUCTS: FISH OILS AND PESTICIDE RESIDUES

Erich J. Gauglitz, Jr., Program Leader

Food scientists predict that the world demand for edible fats and oils will continue to increase. The present limited use of fish oils could be significantly expanded by using chemical alteration and stabilization to improve their flavor and odor. When market conditions are favorable, however, large quantities of fish oil are used in Canada and northern Europe for the manufacture of margarine.

A goal of our fish-oil studies is to develop methods of producing high-quality edible fish oils by investigating the relation between product quality and the chemical and physical changes that occur during processing, handling, and storage of oils. Simultaneously, we are developing chemical reactions and procedures for the use of fish oils or fish-oil derivatives in both edible and industrial applications. Pesticide residues in Pacific Northwest species are being surveyed to determine the extent of contamination and whether normal processing will effectively reduce the residues so that the public continues to receive a wholesome product.

DISTRIBUTION OF LIPIDS IN FISH AND SHELLFISH

Information on the chemical properties of a species is of great value in promoting its use for food or industrial purposes. We have examined anchovy taken from different locations off the Washington coast, from different water temperatures, and at different times of day (i.e., night hauls versus day hauls). Fatty-acid analyses showed no significant differences in composition as a function of these variables. We noted that as the length and weight of the fish increased, the percent of oil in the flesh also increased.

Samples of Pacific hake from the Puget Sound (Port Susan) fishery were analyzed with respect to the fatty-acid composition of the lipids.

Along with the above species of fish, samples of king and Dungeness crab were also analyzed. We made these analyses to determine if the greater textural and oxidative stability of the flesh of king crab during frozen storage was related in an obvious way to differences in lipid composition. Although the two species of crab did differ in the classes and types of lipids present, the differences were not sufficiently great in themselves to explain the phenomenon.

FOOD ADDITIVES FROM FISH OIL

A new project for the development of emulsifiers from fish oil was started. Currently, vegetable-oil monoglycerides are important commercial emulsifiers. The analogous fish-oil monoglycerides were chosen for initial study in this Laboratory.

Two essential phases are involved in the monoglycerides project: (1) Laboratory work--This work includes developing a method that gives high yields of monoglycerides and preparing emulsions with monoglycerides as the emulsifying agent; and (2) Industrial contacts--It is vitally important to the project to determine what characteristics the food industry

requires of both monoglycerides and emulsions. Furthermore, it is important that we find out what tests and quality-evaluation procedures industry uses so that we may duplicate them in the laboratory. The type of tests we perform in our laboratories becomes inportant when we attempt to sell the industry on these compounds because we must provide data they recognize and understand.

Since this program began, we have learned about both monoglycerides and emulsion technology through library research and laboratory work. We have found a method of preparing monoglycerides in about 90 percent yields from fish oil and have just begun to prepare emulsions using these monoglycerides. At present the preparation of an emulsion appears to be more art than science; hence it is difficult to reproduce our results with the current techniques. This problem, however, should be overcome shortly as we gain more experience.

Future work will be centered about the two phases cited above. We expect that industry will tell us what kind of emulsions are currently being produced and what type of monoglyceride emulsifier is being used. What we learn from industry will be incorporated in our monoglyceride program. For example, we expect that at first we will attempt to incorporate fish-oil monoglycerides (both hydrogenated and nonhydrogenated) into existing emulsion recipes for evaluation--i.e., merely substitute the fish-oil monoglyceride for current vegetable or animal monoglycerides.

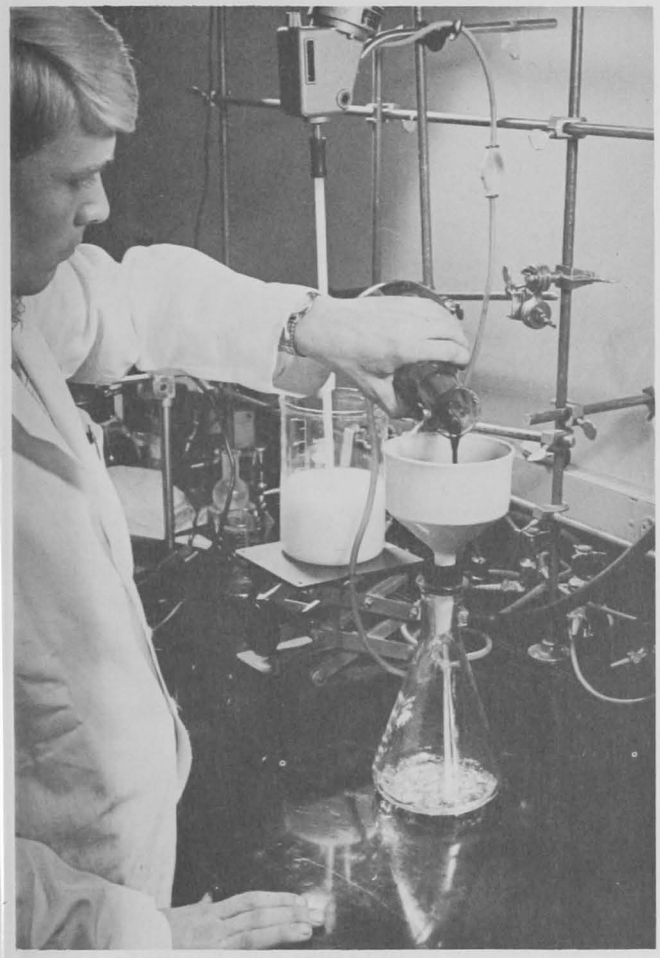


Figure 4.--Purification of monoglycerides preparation by decolorizing charcoal.

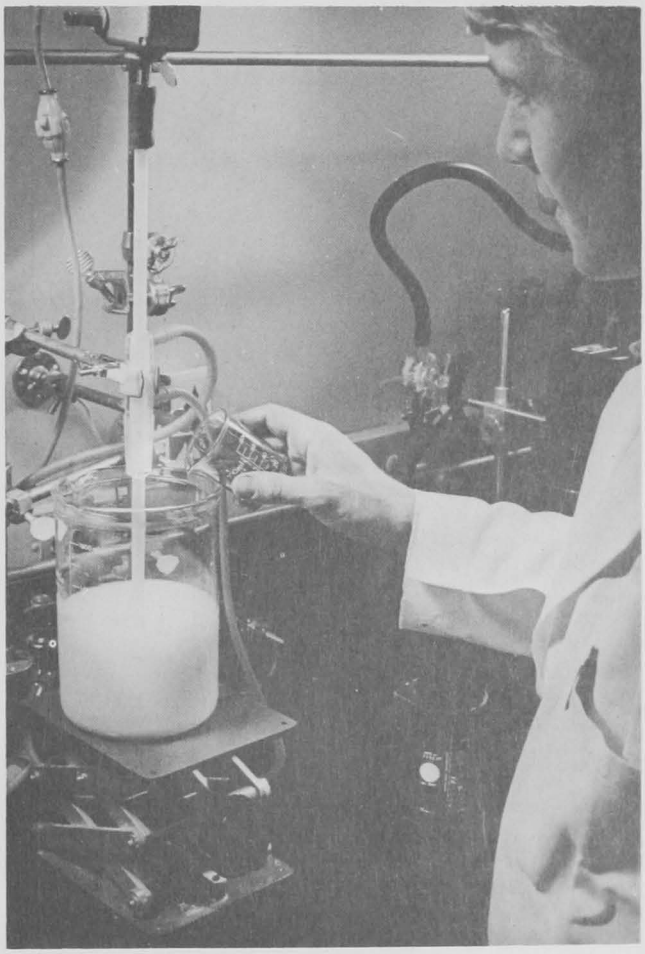


Figure 5.--Preparation of an emulsion using fish-oil monoglycerides as the emulsifying agent.

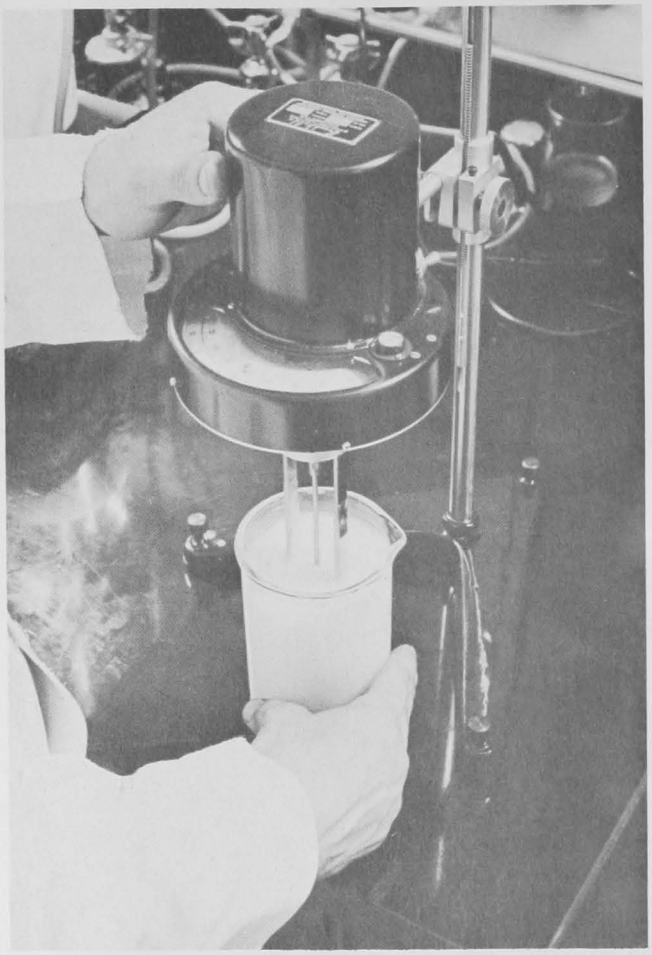


Figure 6.--Evaluation of an emulsion by measuring viscosity and flow characteristics.

NITROGEN DERIVATIVES

Research on nitrogen derivatives from fish oils has ended. Final data were submitted to the Solicitor's Office of the Department of the Interior to substantiate the claims in a patent "Synthesis of unsaturated nitrate esters," by J. C. Wekell, C. R. Houle, and D. C. Malins. Some of the earlier work on nitration of fish oils is now available in U.S. Patent 3,305,567, "Nitrated fatty acid esters," by Clifford R. Houle and Donald C. Malins, assignors, granted February 21, 1967.

The invention described in the current application outlines a unique and economical method for the synthesis of unsaturated nitrate esters using acetyl nitrate as the nitrating agent. The reaction results in a direct conversion of fatty alcohols to the corresponding nitrate esters. Nitrate esters can serve as substitutes for halides in various industrial syntheses, as oil or gasoline additives to act as "scavengers," and as blocking groups for reactive hydroxyl groups.

The patent that was issued covers the production of nitro- and nitrate-ester compounds from unsaturated esters and triglycerides similar to those found in fish oils. The process uses a mild, effective, and easy way to prepare nitrating reagent and avoids many of the undesirable side products and hazardous production problems of previous methods. A few of the potential uses for compounds covered in the patent are as bactericides, disinfectants, and corrosion inhibitors.

PRODUCTION OF EDIBLE FISH OIL

With the probable exception of hake, commercial FPC (fish protein concentrate) will probably be made from oily pelagic species such as anchovy, herring, and sardine. These species are usually abundant and readily caught. For these reasons, fish oil will become a major byproduct of the FPC process. Fish oils produced in most existing reduction plants cannot meet the sanitary requirements

for food use. Fish oil derived as a byproduct of the FPC process will undoubtedly satisfy these requirements.

Our research efforts are directed to the development of recovery methods to separate high-quality oil from the isopropanol-water-oil mixture resulting from the preparation of FPC. We will evaluate these oils to determine how much more refining may be necessary to produce a food-grade product.

Recovery of a high-quality fish oil from the Pacific hake is beginning to show definite promise. About 65 to 70 percent of the total oil from hake containing 5 to 6 percent fat can be recovered in a fairly simple cooling operation. Analysis of this oil indicates that it is a good starting material for further processing to meet food-grade requirements. The remaining oil can also be recovered and would probably be suitable for industrial applications.

Countercurrent extractions of hake at ambient temperature were inefficient in removing oil under the extraction conditions that were employed. Calculations based on theoretical considerations indicate that drastic increases in the solvent-to-fish ratio would be necessary to accomplish the desired results. In contrast, countercurrent extractions of hake at elevated temperatures were effective in removing oil of excellent quality.

Figure 7.--Controlling temperature during a six-stage countercurrent solvent extraction of ground hake.

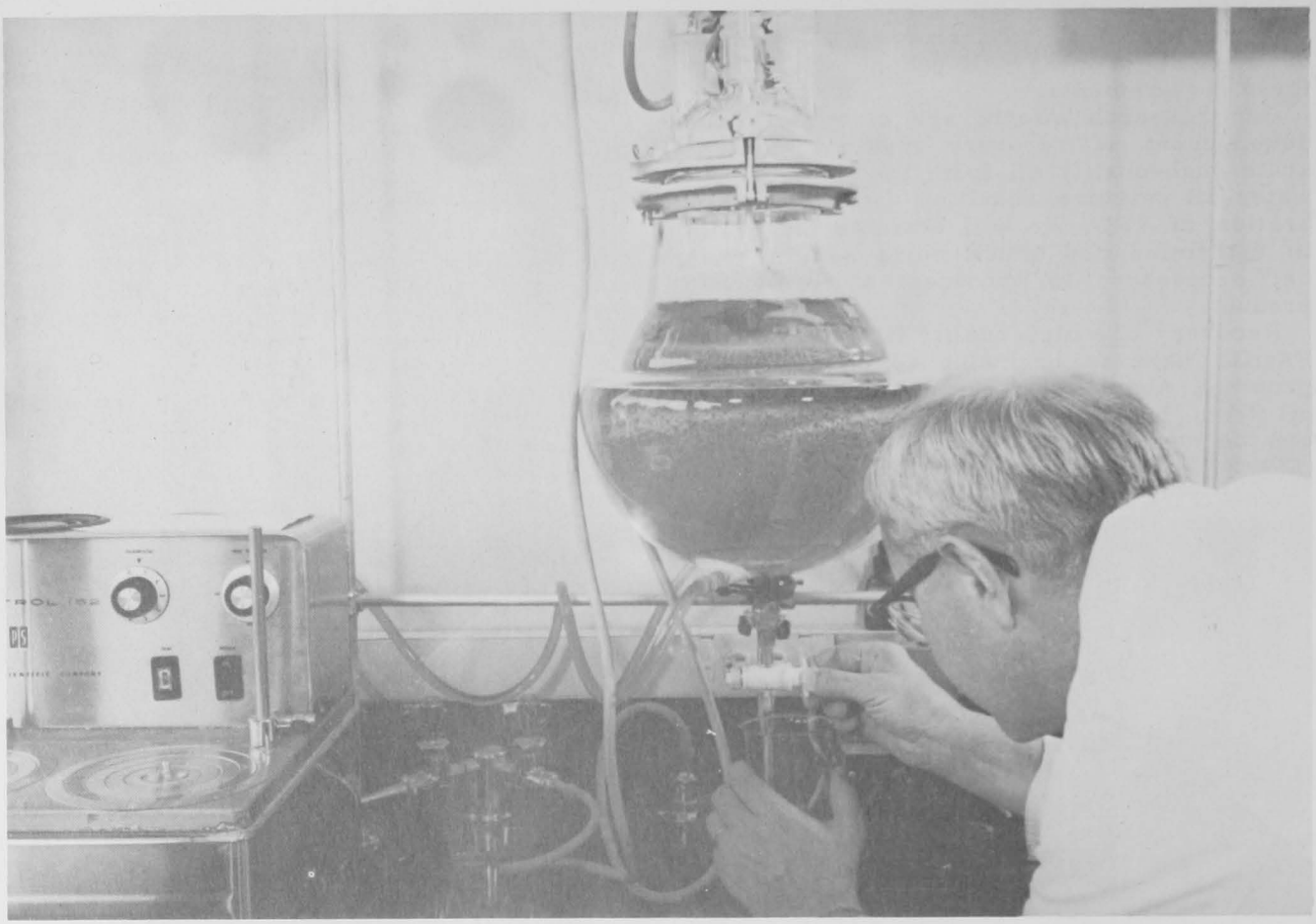


Figure 8.--Sampling of miscella during solvent extraction of Pacific hake.

PESTICIDE RESIDUES

A knowledge of the content of pesticide residues in aquatic animals is essential to ensure that the public consumes wholesome fishery products. The initial aim of our pesticide research has been to survey residue levels in aquatic food resources of the region and, if necessary, to develop means of reducing these residues.

Chlorinated-hydrocarbon pesticides have been in use for over 20 years. Although their use has declined as more specific pesticides have been developed, their marked resistance to degradation has resulted in an amazing accumulation of these compounds in the environment. Furthermore, certain compounds are converted into stable toxic derivatives; these are additional residues of obvious significance in food products. In contrast, most of the newer pesticides such as malathion, parathion, and sevin are not persistent. Consequently, we have directed our efforts toward measuring residues of the more important chlorinated-hydrocarbon pesticides and their persistent metabolites--namely, DDT, DDE, DDD, dieldrin, and endrin.

Local fish and shellfish have been analyzed for pesticides. This analysis requires extraction of the residues, purification through several steps, and finally identification and quantification of possible pesticidal substances. Because very low concentrations of pesticides are found (less than 1 milligram per kilogram), extreme care must be taken at all stages to prevent contamination and to remove interfering substances. Also, extremely sensitive analytical techniques must be used.

Normally, the pesticide residues are identified by gas chromatography. The presence of these materials is further confirmed by means of thin-layer chromatography and liquid-liquid partition chromatography. In these ways, data have been obtained for hake meal and for the following fish, listed according to volume of landings: Dungeness crab, English sole, yellowtail rockfish, ocean perch, anchovy, true cod, hake, and starry flounder. As had been expected, the residue levels for all products were substantially lower than the tolerance for beef (the closest product with an established tolerance). Table 1 shows the ranges of residue levels.

Table 1.--Pesticide residues in fishery products of the Pacific Northwest /Tolerance for beef is 7 p.p.m./

Species	Range of Residues			
	DDE	TDE	DDT	Total
	P.p.m.	P.p.m.	P.p.m.	P.p.m.
Hake (meal)	0.267-0.299	0.046-0.051	0.074-0.085	0.330-0.420
Dungeness crab	0.027-0.040	0.011-0.013	trace-0.013	0.046-0.083
English sole*	0.009-0.053	0.013-0.088	0.010-0.058	0.034-0.199
Yellowtail rockfish*	0.017-0.037	trace-0.013	trace-0.018	0.020-0.050
Ocean perch*	0.012-0.013	trace-0.004	0.012-0.014	0.029-0.036
Anchovy	0.058-0.172	0.072-0.244		0.131-0.416
True cod*	0.005-0.006	0.006-0.007	0.004	0.015-0.017
Hake	0.040-0.111	0.041-0.141	0.042-0.223	0.123-0.475
Starry flounder*	0.018	0.030	0.013	0.061

*Fillets

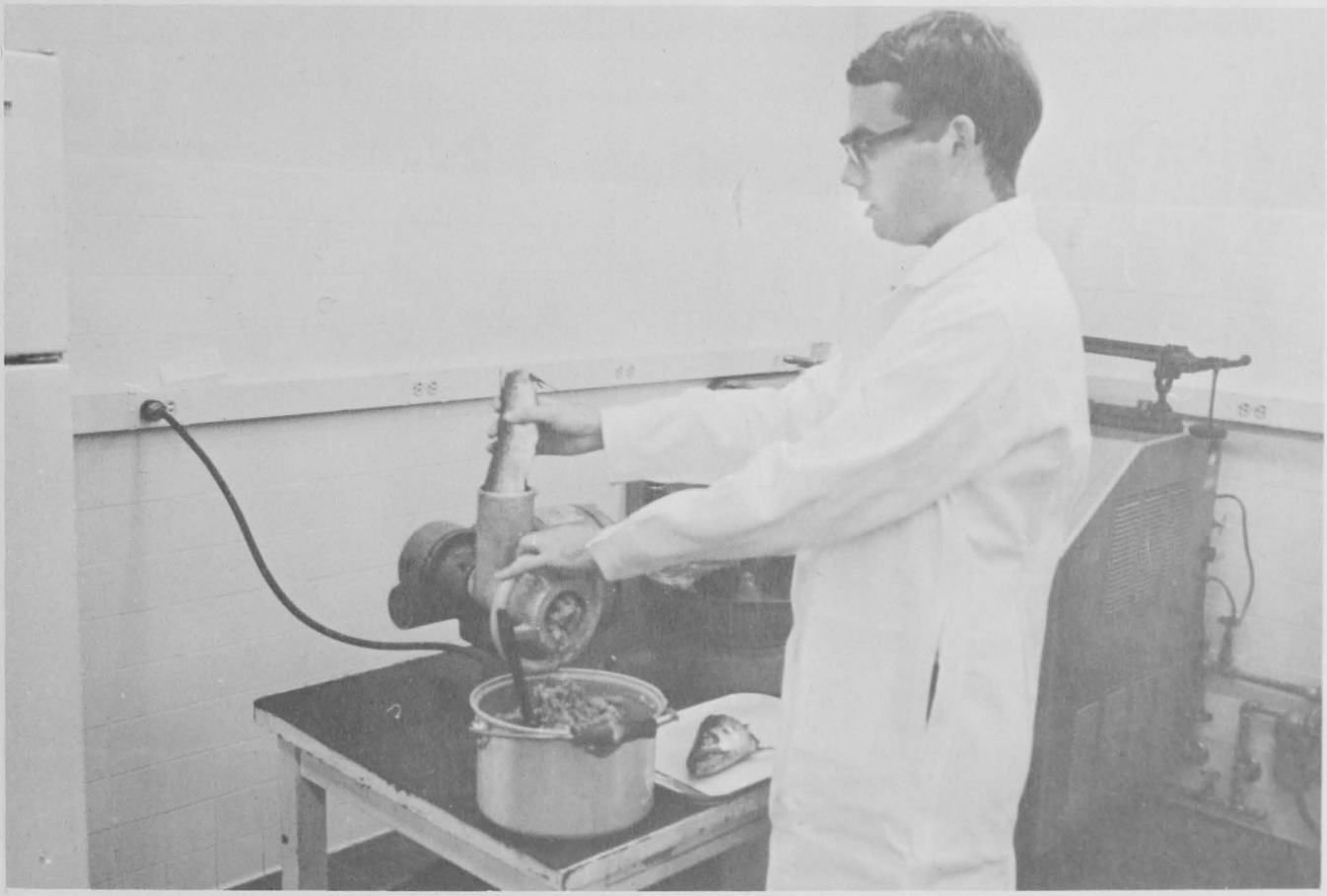


Figure 9.--Preparation of fish for pesticide analysis.

Figure 10.--Purification of extracts of pesticide residues.

Figure 11.--Gas chromatographic analysis of pesticide residues in fishery products: identification and measurement of individual residues.

CHEMISTRY OF FISHERY PRODUCTS: BIOCHEMICAL INVESTIGATIONS

Herman S. Groninger, Program Leader

The aim of the current research is to study the enzymes of fish and crab muscle with particular emphasis on the characterization and control of some enzymatic changes that affect the quality of fishery products. The nucleotides and the enzymes associated with their breakdown soon after the death of the animal were studied during the year, because the nucleotides and the products of degradation are associated with both loss and improvement of flavor.

FISH

Inosine monophosphate is the major nucleotide in stored fish muscle, and it is important because it enhances flavor. During refrigerated storage of fish muscle, the enzyme-catalyzed degradation of inosine monophosphate continues. This rate of degradation is a factor related to the quality of the fishery product in which the muscle is used.

Studies of muscle inosine monophosphate showed that the rates of its breakdown among the different species of fish range widely. Black cod, true cod, starry flounder, Dover sole, English sole, and sand sole had fast rates. Anchovy, Sebastodes sp., hake, lingcod, and rock sole had slow rates. Our earlier studies showed that a food additive (ethylenediamine-tetraacetate) could be used to control the breakdown of inosine monophosphate in certain species. In species with very rapid breakdown rates, the endogenous inosine monophosphate was degraded before the ethylenediamine-tetraacetate became effective.

As yet, we see no direct relation between inosine monophosphate breakdown rate and quality; however, the flavor-enhancing effects of inosine monophosphate can be shown in the products made from a number of species. Because inosine monophosphate affects flavor, the control of its breakdown is an important quality-stabilization measure.

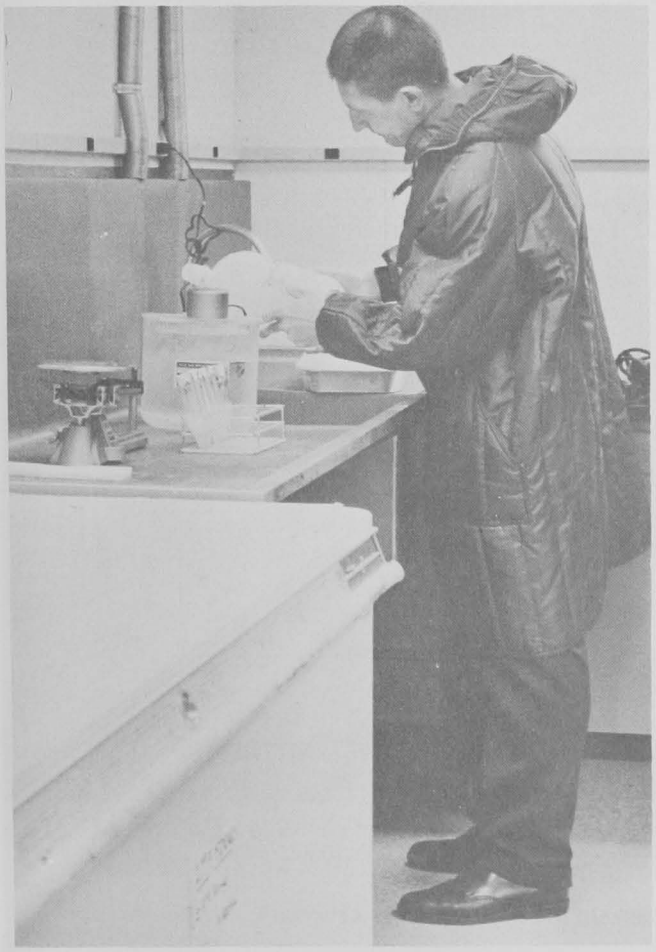


Figure 12.--Dialysis of fish enzymes in the cold laboratory.

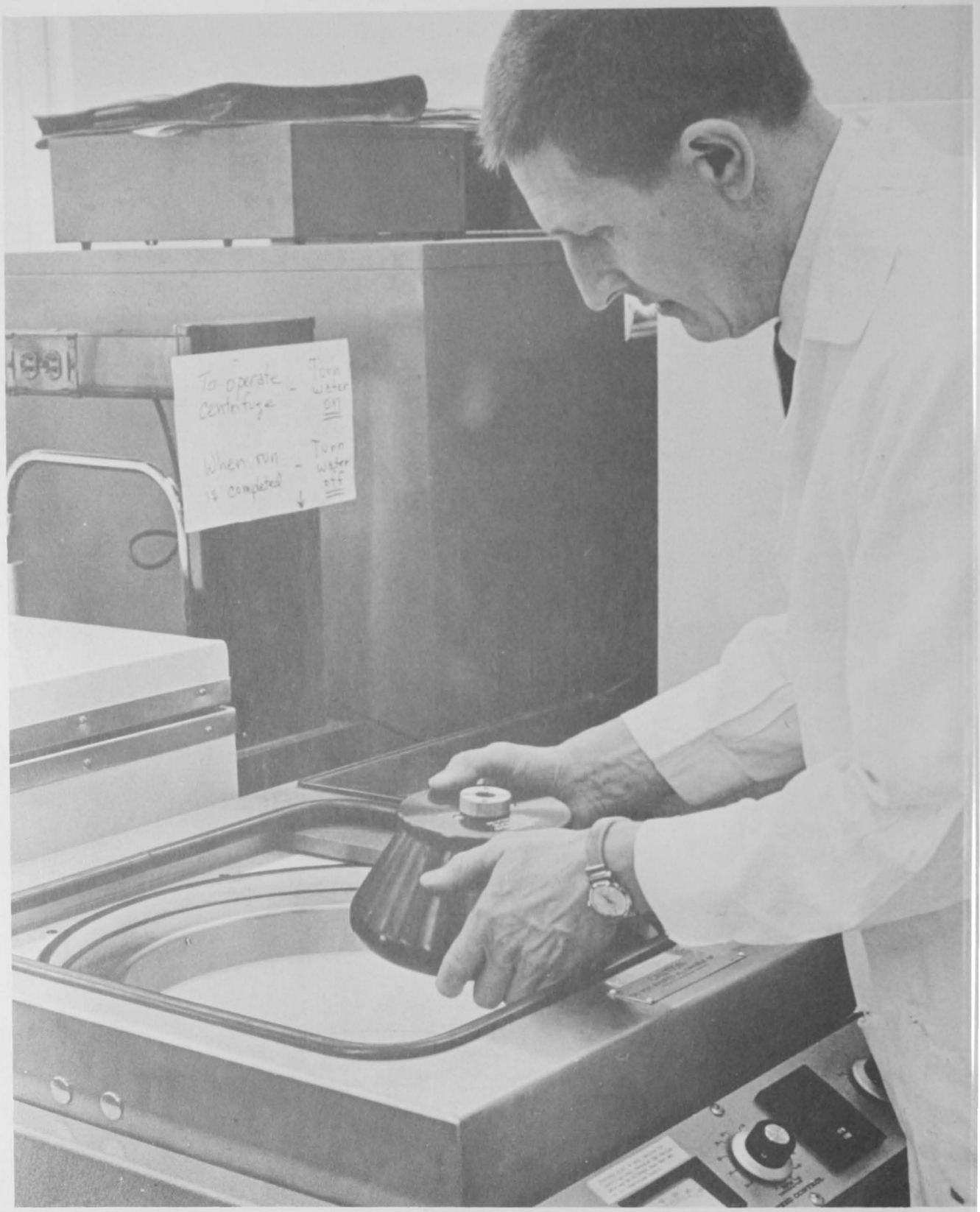


Figure 13.--Centrifugation of enzymes using a preparative ultracentrifuge.

CRAB

Studies on the nucleotide contents of crabs showed that king crab muscle had about one-half as much total nucleotide as fish or Dungeness crab. Cooking crab muscle at temperatures and for periods of time similar to those used in commercial practice broke down the nucleotide to nucleoside. In general, the effect of the double cook (10 minutes at 156° F. + 4

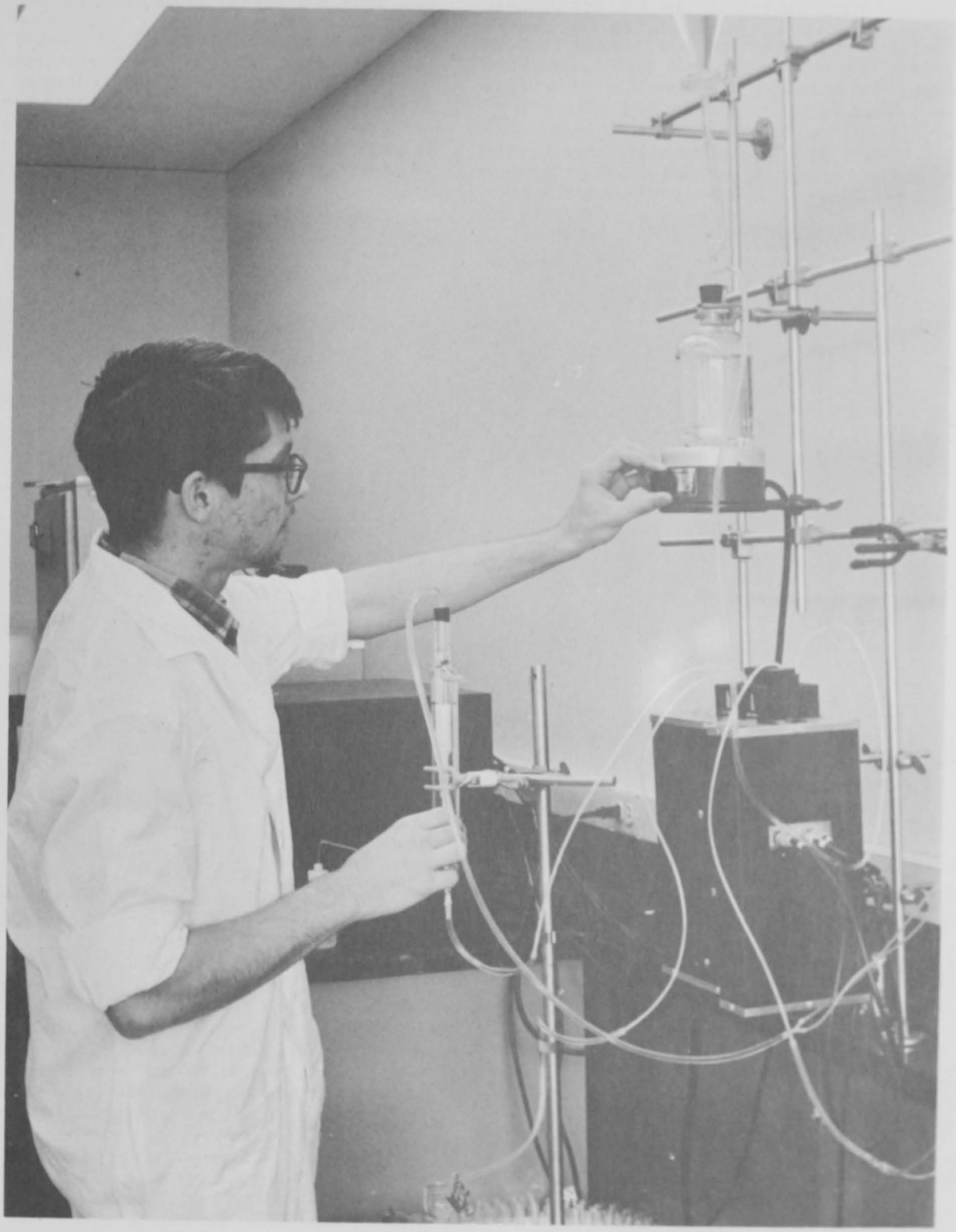


Figure 14.--Fractionation of the nucleotides of crab meat and fish flesh.

minutes at 212° F.) was similar to the single cook (20 minutes at 212° F.) except that during the double cook a greater proportion of the total nucleotide was converted to nucleoside.

Inosine monophosphate did not accumulate in either raw or cooked crab, as the breakdown of inosine monophosphate appeared to be relatively faster than the conversion of adenosine monophosphate to inosine monophosphate. Therefore, inosine monophosphate is present in crab at subthreshold flavor levels and does not appear to be an important factor in enhancing flavor.

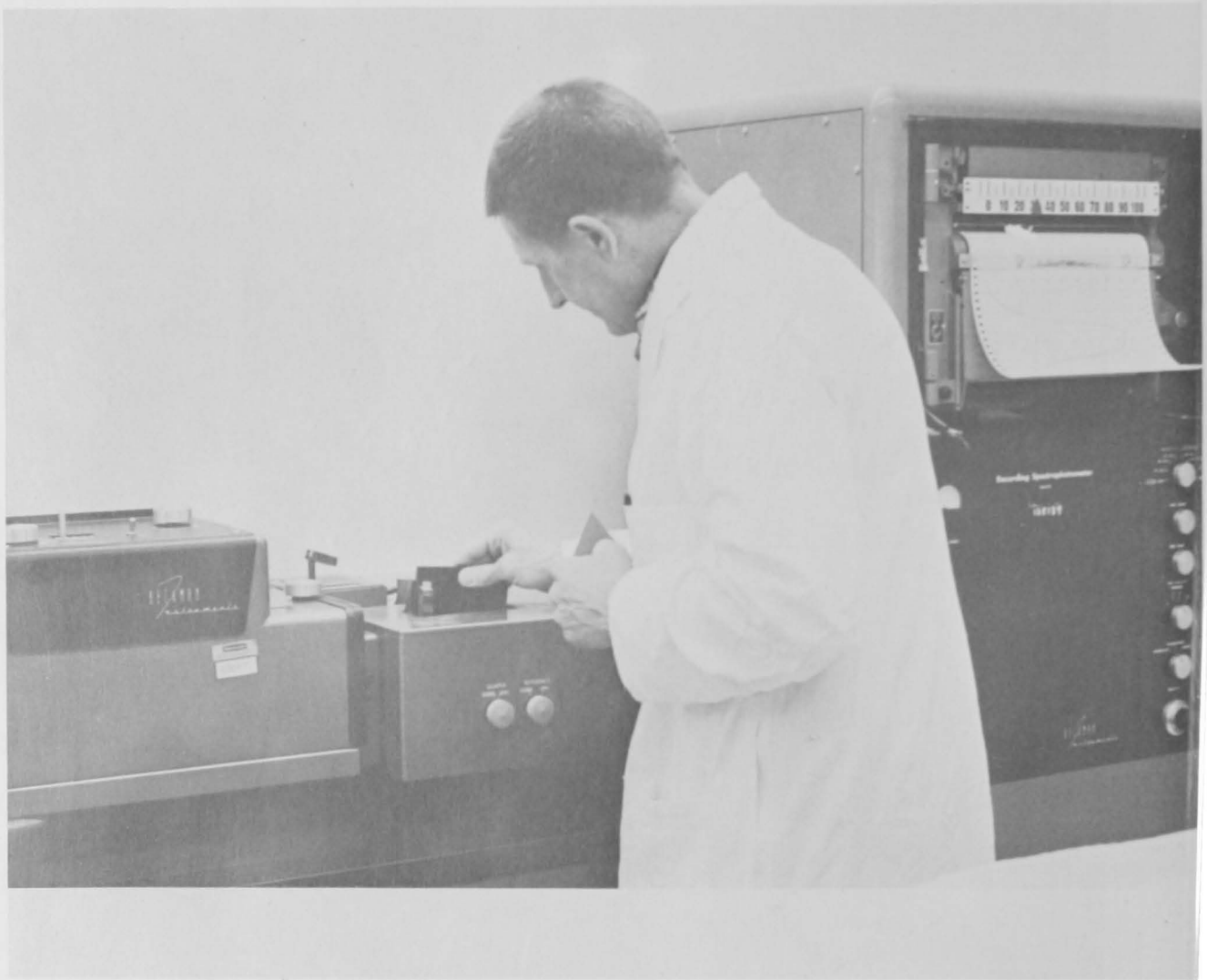


Figure 15.--Determination of the ultraviolet absorption spectrum of a nucleotide fraction from crab meat.

RADIATION OF FISH: APPLICATION OF RADIATION-PASTEURIZATION PROCESSES TO PACIFIC COAST FISHERY PRODUCTS

David T. Miyauchi, Program Leader

The object of this program is to develop radiation-pasteurization techniques that will extend significantly the fresh shelf life of Pacific Coast fishery products. Our research has been directed toward obtaining experimental data necessary for the preparation of a petition to the U.S. Food and Drug Administration for commercialization of radiation processing of fish and fishery products.

STORAGE-LIFE STUDIES

Radiation-pasteurized marine products can be marketed in several ways. Our storage-life studies included products packed in retail packages and those packed in large wholesale units, which later were repacked into retail packages.

We found radiation pasteurization to be effective in prolonging the storage life of the fishery products studied. A radiation dose of 0.1 to 0.2 megarad was sufficient to give certain products--halibut steaks; fillets of petrale sole, English sole, and Pacific ocean perch; and king and Dungeness crab meats--packed in retail packages a shelf life of from 2 to 5 weeks at 33° F. This shelf life is from two to five times as long as the shelf life of corresponding nonirradiated control samples. True cod fillets vacuum packed in heat-sealable polyester pouches and irradiated at 0.1 and 0.2 megarad had a shelf life two to three times greater than the 4-to-7-day shelf life of the nonirradiated control samples. With Pacific oysters that were either vacuum packed in cans or air packed in glass jars, a radiation dose of 0.2 megarad extended the shelf life of the samples held at either 33° or 42° F. by at least 10 days over that of the nonirradiated control samples; a dose of 0.1 megarad extended the shelf life of the oysters stored at 33° F. by about 10 days but provided no significant shelf-life extension at 42° F. Irradiating cooked, whole Dungeness crab in the shell at 0.2 megarad increased the shelf life at 33° to about 15 days from about 10 days for the nonirradiated control samples. The viscera had been a source of off flavors in the meat during storage, and eviscerating the cooked crab prior to irradiation further increased the storage life to about 25 days.

In studies to determine the feasibility of irradiating fillets in large wholesale packages for repacking later into retail packages, we found that, with fillets of sole and Pacific ocean perch, an oxygen-impermeable package gave the best results. The #10-size C-enameled can proved most suitable for maintaining product quality and providing convenience of handling. Fillet tins lined with polyethylene bags were satisfactory, provided air pockets between the bag and the metal container were eliminated by careful packing. Polyethylene bags alone, the commonly used material for marketing fresh Pacific Northwest fish fillets, were not suitable for holding irradiated fillets for more than a week.

The fillets irradiated in the wholesale packages at 0.2 megarad were stored for periods of 7 to 14 days at 33° F., repacked into retail packages, and then had an additional storage life equivalent to the normal storage life of the nonirradiated control fillets. Thus, radiation pasteurization of fish fillets in wholesale units would permit the fillets to reach the marketing area in good condition and still have sufficient shelf life remaining for retail marketing.

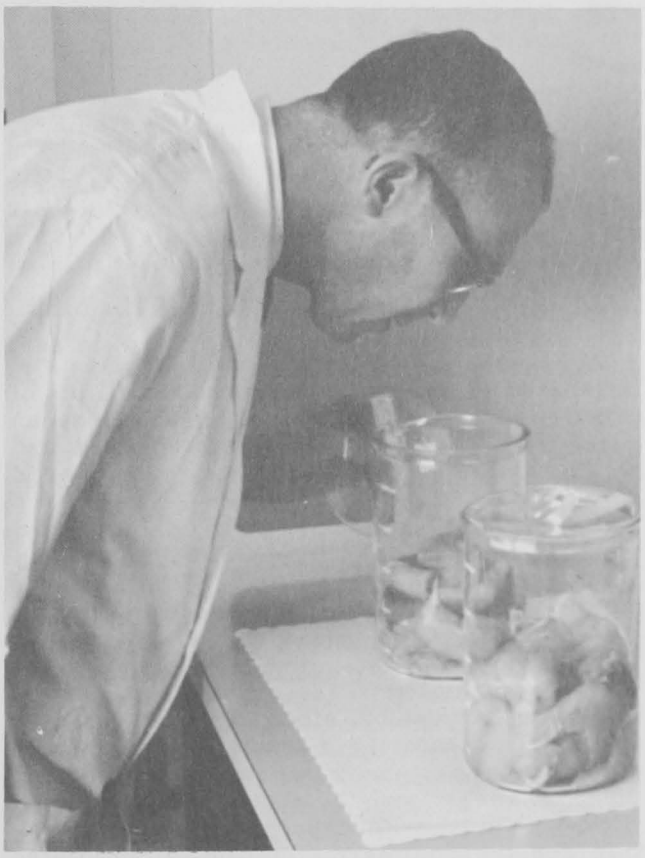


Figure 16.--Judging the odor and appearance of radiation-pasteurized petrale sole fillets after various periods of storage at refrigerated temperatures.

Figure 17.--A panel of judges evaluating the quality of cooked, radiation-pasteurized Pacific ocean perch fillets.

SHIPPING STUDIES ON IRRADIATED FISH

During the past year, about 2,000 pounds of irradiated Dungeness crab meat and fillets of petrale sole, Pacific cod, and Pacific ocean perch were shipped to nine distributors and retailers located in Seattle, Yakima, and Spokane, Wash.; Oakland, Los Angeles, and San Diego, Calif.; Denver, Colo.; Kansas City, Mo.; and Chicago, Ill. Our aims in making these shipments were to: (1) evaluate the suitability for irradiated fish fillets of packing materials and containers currently used in the industry, (2) determine how shipping and storing irradiated fillets under commercial operating conditions affect quality, (3) have industry evaluate the marketability of irradiated fillets that had been stored for periods up to 2 weeks, and (4) obtain data to support petitions to the Food and Drug Administration for the commercialization of irradiated flounder species.

Among the packaging materials readily available to the fish processors, either the 12-pound or 25-pound fillet tins or water-resistant cartons containing an oxygen-impermeable plastic-bag liner were found to be the most suitable for shipping and storing irradiated fish fillets. A heat-shrinkable saran bag, which is used for packaging turkeys, gave good results as a liner; but polyethylene bags, because of their high-oxygen permeability, were not suitable because fillets adjacent to the film became rancid and slightly discolored after 7 to 10 days of storage at 33°F.

Product temperatures were continuously recorded during transit and storage on all shipments. They ranged from 28° to 41° F. Below-freezing temperatures were occasionally recorded during refrigerated truck shipments. The 41°F. temperature was recorded in a restaurant "walk-in" cold storage room, which had a mean temperature of 35°F. The temperature range of the nine distribution points was 32° to 37°F. The temperature data indicate that these distributors were following good commercial handling practices and that the irradiated fish were not exposed to temperatures that would permit outgrowth of pathogens.

Our experience with these shipments shows that fish shipped by reliable carriers reaches its destination without delay and in excellent condition. Good-quality fillets irradiated at 0.1 megarad have a shelf life of at least 14 days; bacterial counts on these fillets ranged from 100 thousand to 10 million per gram after 14 days of iced storage. Fillets irradiated at 0.2 megarad were always found to be in good condition after 14 days of iced storage; total plate counts on these fillets ranged from 10 thousand to 1 million per gram.

There were comments both pro and con regarding the color of the irradiated fillets, which tend to become slightly pink. West Coast fish distributors thought this would benefit sales; but in the Midwest, where flounder is considered to be a white fish, one distributor thought the color change might cause sales resistance. Comments regarding irradiated fillets have generally been encouraging, and most industry people feel that radiation pasteurization of fish will significantly benefit their operations.

FREE SUGARS AS QUALITY INDICES FOR IRRADIATED FISH

The free glucose and ribose contents have been determined in Pacific ocean perch and English sole fillets irradiated at 0, 0.1, 0.15, and 0.2 megarad and stored at 33° and 42° F. The objects of these experiments were

to: (1) determine the relative rates at which these sugars are consumed by the microflora of irradiated and nonirradiated fish and (2) establish whether these measurements have use in the objective assessment of the quality of irradiated fish. In nonirradiated fish, both the glucose and ribose content of the fish tissue declined rather uniformly, and at the point of spoilage about 25 percent of the original sugars remained in the tissue. In stored irradiated fish, the glucose content of the fish declined at a relatively uniform rate, and only 10 to 20 percent of the original glucose remained in the tissue when spoilage was detected. The ribose content of the stored irradiated fillets, however, remained almost constant until incipient spoilage was detected. The ribose then declined rapidly and was not more than 30 to 40 percent of the original at the time the fish was definitely spoiled.

Results from these experiments indicate that a measure of the free glucose of irradiated fish could be a useful criterion in objectively estimating the quality of the fish. Although the measurement of free ribose alone is not a useful index of quality, a high ribose-to-glucose ratio might indicate if fish had been irradiated.

SPRAY APPLICATION OF CONDENSED PHOSPHATES ON FISH FILLETS

Whole fish and fish fillets are commonly treated with condensed phosphates prior to being frozen. This treatment reduces drip loss during and after thawing. Our work with irradiated fillets shows that treating fillets prior to irradiation not only reduces drip loss significantly but also helps maintain the natural color and texture of the fillets.

We devised a spray method to replace the usual dip method of applying phosphate solutions to fillets. Dipping has several inherent disadvantages: the solution quickly becomes fouled with bits of fish tissue and soluble material that encourage the growth of bacteria, and the concentration of the solution must constantly be monitored and adjusted. These problems can be overcome with proper filtering and monitoring devices, but they are better overcome by elimination of the problem. We therefore designed and had built a spraying machine that will continuously and uniformly spray solutions of phosphates, glazes, and antioxidants onto fillets. The machine is equipped with pressure-regulated, variable-position spray heads and a variable-speed conveyor belt. Normally, 500 pounds of fish fillets per hour can be treated with this unit. Trial runs and pilot-plant studies have demonstrated the feasibility and utility of this method for applying protective coatings to fillets.

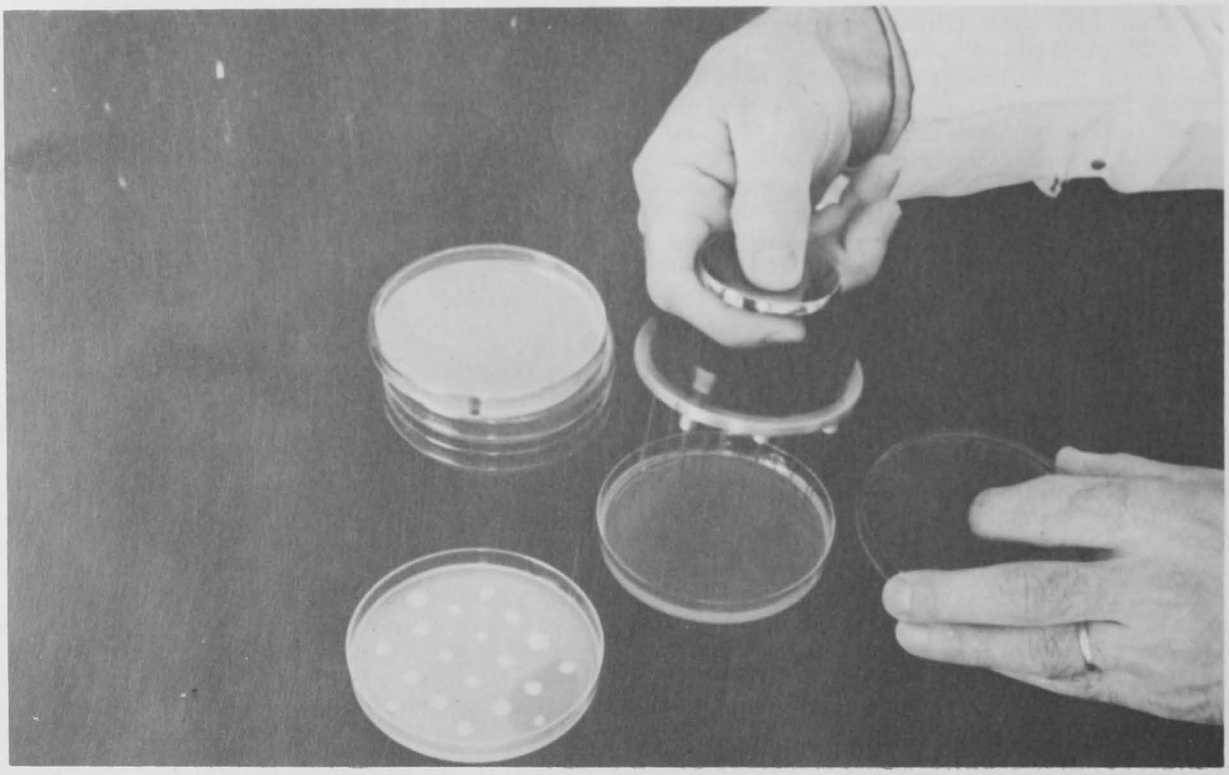
CHANGES IN THE MICROFLORA OF VACUUM-PACKAGED PETRALE SOLE FILLETS STORED AT VARIOUS TEMPERATURES

The spoilage flora of irradiated fishery products stored at refrigeration temperatures are significantly different from those of nonirradiated seafoods. Because radiation-pasteurized seafoods, like other perishable foods, can be mishandled by exposure to high temperatures during shipment and storage, it is important to determine whether they might become a health hazard before they become unacceptable because of deteriorated appearance and off odors.

We made studies to determine how elevated storage temperatures affect the composition of the microflora of irradiated and nonirradiated petrale sole fillets. Fillets were vacuum packaged in mylar polyethylene bags and irradiated at 0, 0.1, 0.2, and 0.3 megarad and stored at 33°, 38°, 42°, 50°, 60°, and 72° F. The fish were monitored throughout storage for spoilage, total bacterial count, coliform count, enterococcus count, and the presence of coagulase-positive Staphylococcus. Generic changes in the aerobic flora were determined by the identification of nearly 14,000 microbial isolates. The identification of such a large number of isolates was made possible by the use of a special multipoint replicating device that was developed in this laboratory. The predominant spoilage flora at the time of spoilage of the nonirradiated fish stored at 42° F. and below were Pseudomonas. When the nonirradiated fish was stored above 42° F., the predominant spoilage flora were lactobacilli. Coliforms and enterococci showed outgrowth at the higher storage temperatures but were suppressed by the radiation treatment. No coagulase-positive Staphylococcus were found in any of the irradiated samples.

EFFECT OF LACTOBACILLI ON THE GROWTH OF STAPHYLOCOCCUS AUREUS AND CLOSTRIDIUM BOTULINUM

There is a significant difference in the microflora at the time of spoilage of vacuum-packaged, irradiated fish as compared with the microflora of air-packaged, irradiated fish. Vacuum-packaged fish are superior in quality and have a longer storage life. The predominant organisms at the time of spoilage of irradiated, vacuum-packaged fish are lactobacilli that are capable of growing at refrigeration temperatures. These lactobacilli may have a preservative effect in themselves, in that their products of metabolism can inhibit the growth of other organisms. Certain species of lactobacilli have been shown to



Figures 18 and 19.--Transfer of microbial cultures with multipoint inoculating device.

inhibit the growth of S. aureus and the growth and/or toxin production of Cl. botulinum. The purpose of this study was to determine whether the lactic acid bacteria found in irradiated fish are also able actively to suppress the growth of these food pathogens.

About 2,600 Lactobacillus cultures were tested for their ability to prevent the growth of S. aureus. These organisms were isolated from nonirradiated and irradiated (0.1, 0.2, and 0.3 megarad) petrale sole fillets after various periods of storage at 33°, 42°, and 60° F. The isolates represented several distinct types based on an arbitrary grouping using biochemical and colonial characteristics.

Of the total number of isolates studied, 57 percent were able to inhibit S. aureus. The number of inhibitory organisms found increased as the radiation dose given the fish samples increased. The temperature at which the fish was stored did not affect the percentage of inhibitory organisms found at any point during the storage period.

Experiments are in progress to determine whether these lactobacilli can effectively inhibit the growth of Cl. botulinum and, if so, to determine the mechanisms of the inhibition.

PATHOGENS IN RADIATION-PASTEURIZED FISHERY PRODUCTS

Melvin W. Eklund, Program Leader

The object of this program is to ensure that the commercial use of pasteurization doses of ionizing radiation to preserve fishery products precludes public health hazards from the bacteria Cl. botulinum.

To accomplish this, we have studied: (1) incidence and concentration of Cl. botulinum type E cells in the marine environment, (2) new types of Cl. botulinum isolated at our laboratory from the Pacific Coast of North America, and (3) outgrowth of Cl. botulinum type E in nonirradiated and irradiated fishery products packaged and stored under conditions simulating those found in commercial operations.

Work already completed shows that Cl. botulinum type E is common in marine sediments collected from the Pacific Coast of the United States. The biochemical characteristics of strains of Cl. botulinum types B and F isolated from the Pacific Coast of the United States are in many ways the same as those of type E.

Preliminary studies have been completed to determine the outgrowth of type E in irradiated petrale sole fillets stored at refrigerated temperatures. We have shown that toxin production does not occur in irradiated or nonirridated petrale sole fillets during a storage period of 72 days at 38° F. This is more than four times the maximum storage life of irradiated petrale sole fillets at 38° F.

More detailed experiments are in progress at higher storage temperatures to evaluate the degree of safety that exists in irradiated fishery products with respect to Cl. botulinum.

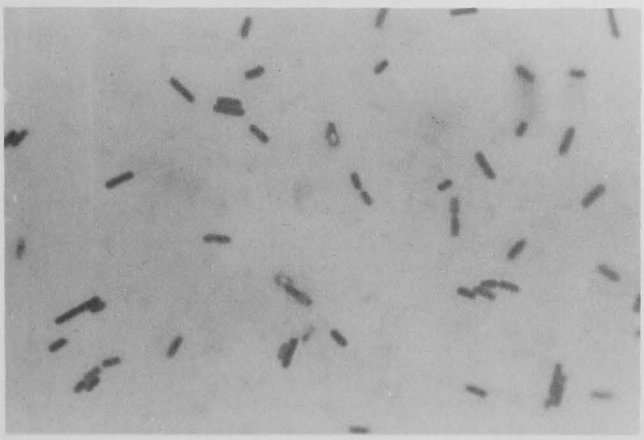


Figure 20.--Vegetative cells and spores of Clostridium botulinum type F isolated from the Pacific Coast of the United States.

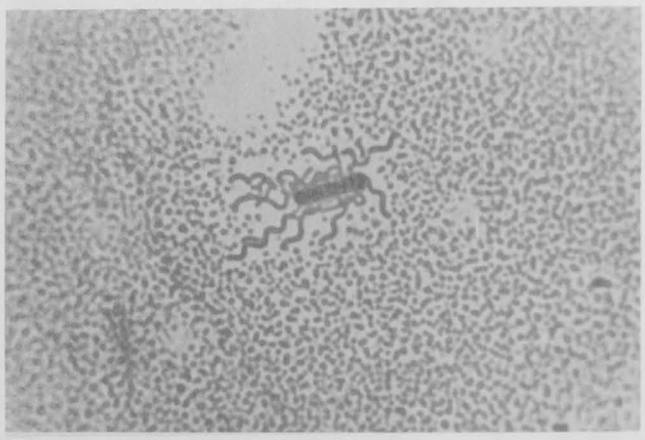


Figure 21.--Peritrichous flagella of Clostridium botulinum type F cells isolated from the Pacific Coast of the United States.

FISHERY INSPECTION SERVICES

A. Morris Rafn, Supervisory Inspector

The U.S. Department of Interior's Fishery Products Inspection Service began in the Pacific Northwest in 1959. From its beginning it has grown steadily in the volume of products inspected. The primary activity in this region is lot inspection. One plant, which processes king crab products, is under continuous inspection. Most processing plants in this region are small, and none produces breaded products. There is a trend toward the consolidation of plants which may make it easier for them to take advantage of the USDI continuous inspection program.

A total of 9,398,875 pounds of fishery products was inspected during this fiscal year. The lot inspection consisted of fresh, frozen, canned, salted, and spiced fish. The inspection requests were from Federal and State procurement offices, hospitals, schools, and food brokers. A total of 300,000 pounds of frozen king and coho salmon was inspected for export to Europe.

The headquarters for this unit is located at the Bureau's Technological Laboratory, 2725 Montlake Blvd. East, Seattle, Wash. A suboffice is located at Bellingham, Wash.

Figure 22.--Examination of canned salmon for compliance with buyer specifications.

PAPERS PRESENTED AT MEETINGS AND MISCELLANEOUS TALKS

DYER, JOHN A.

Considerations for reduction of salmonella in fish reduction plant operations. Bureau of Commercial Fisheries Salmonella Workshop for Fish Meal Industry, Seattle, Wash., May 11.

The Bureau's process for FPC. Annual Meeting of Pacific Fisheries Technologists, Ocean Shores, Wash., Mar. 19-22.

EKLUND, MELVIN W.

Salmonella in fish reduction plants. Bureau of Commercial Fisheries Salmonella Workshop for Fish Meal Industry, Seattle, Wash., May 11.

Review of analytical methods in food microbiology: Clostridium botulinum. Round Table, Annual American Society for Microbiologists Meeting, New York, N.Y., May 3.

GAUGLITZ, ERICH J.

Properties and applications of fish oils. Military-Industry Conference, U.S. Army Natick Laboratories, Natick, Mass., Oct. 18-19.

GRONINGER, HERMAN S.

Degradation of fishery products: Muscle nucleotidase activity and inhibition during processing and storage. Annual Meeting of Pacific Fisheries Technologists, Ocean Shores, Wash., Mar. 19-22.

HALL, ALICE S.

Career opportunities for women in Federal Government. Rainier Beach High School, Seattle, Wash., Feb. 2.

Career opportunities for women in Federal Government. Roosevelt High School, Seattle, Wash., Feb. 13.

Career opportunities for women in Federal Government. Nathan Hale High School, Seattle, Wash., Feb. 13.

Career opportunities for women in Federal Government. Garfield High School, Seattle, Wash., Feb. 15.

Career opportunities for women in Federal Government. West Seattle High School, Seattle, Wash., Feb. 20.

Career opportunities for women in Federal Government. Sealth High School, Seattle, Wash., Feb. 25.

NELSON, RICHARD W.

Air shipping fresh fish. Bureau of Commercial Fisheries - United Air Lines Seminar for Northwest Fish Processors and Fishermen, Seattle, Wash., Dec. 2.

Freezing troll-caught salmon at sea. Bureau of Commercial Fisheries Meeting with Salmon Trollers and Industry, Seattle, Wash., Dec. 16.

Chilling conditions in handling fresh fishery products--How the chilling conditions are attained. Class in Marine Refrigeration at Ballard High School, Seattle, Wash., Mar. 7.

Freezing procedures in handling fishery products. Class in Marine Refrigeration at Ballard High School, Seattle, Wash., Mar. 8.

Air shipping live Dungeness crab. Annual Meeting of Pacific Fisheries Technologists, Ocean Shores, Wash., Mar. 22.

PATASHNIK, MAX.

Quality effects of freezing and frozen storage of fish. Class in Marine Refrigeration at Ballard High School, Seattle, Wash., Mar. 8.

STEINBERG, MAYNARD A.

FPC--Its place in an increasing population. Bellingham Chamber of Commerce Meeting, Bellingham, Wash., Sept. 21.

Radiation preservation of foods. Rotary Club Meeting, Bellevue, Wash., Dec. 27.

Radiation preservation of foods. Annual Meeting of Pacific Fisheries Technologists, Ocean Shores, Wash., Mar. 19-22.

Standards and inspection of fishery products. Annual Meeting of Pacific Fisheries Technologists, Ocean Shores, Wash., Mar. 19-22.

Fish oil program. Meeting of National Fish Meal and Oil Association, College Park, Md., Mar. 28.

FPC--A progress report. Bureau of Commercial Fisheries Dinner Meeting with Industry, Norselander Restaurant, Seattle, Wash., May 3.

Greenland halibut. Bureau of Commercial Fisheries Dinner Meeting with Industry, Norselander Restaurant, Seattle, Wash., May 3.

Health aspects of fish processing. Bureau of Commercial Fisheries - Industry Salmonella Workshop for Fish Meal Industry, Seattle, Wash., June 7.

STOUT, VIRGINIA F.

A modern American woman. "Women of Achievement" Tea, Past Presidents' Assembly, Seattle, Wash., Nov. 15.

TRETSVEN, WAYNE I.

Chilling conditions in handling fresh fishery products--Factors involved in quality changes. Class in Marine Refrigeration at Ballard High School, Seattle, Wash., Mar. 7.

Preservation, utilization, and process engineering

BARNETT, HAROLD, AND RICHARD W. NELSON.
1966. Recent technological studies of Dungeness crab processing. Part 4--Preliminary report on salt uptake and heat penetration in whole-cooked crab. U.S. Fish Wildl. Serv., Fish. Ind. Res. 3(3): 13-16.

BARNETT, HAROLD J., RICHARD W. NELSON, AND JOHN A. DASSOW.
1967. Technological studies of Dungeness crab processing. Part 3--Laboratory experiments in the control of drain time. U.S. Fish Wildl. Serv., Fish. Ind. Res. 3(4): 11-17.

Chemistry of fishery products: Fish oils and pesticide residues

GAUGLITZ, ERICH J., JR.
1967. Properties and applications of fish oils. Activ. Rep. 19: 104-108.

HOULE, CLIFFORD R., AND DONALD C. MALINS.
1967. Nitrated fatty acid esters. U.S. Pat. 3,305,567 (Feb.), 3 pp.

Radiation of fish: Application of radiation-pasteurization processes to Pacific Coast fishery products

MIYAUCHI, DAVID, JOHN SPINELLI, AND GRETCHEN PELROY.
1966. Application of radiation-pasteurization processing to Pacific Coast fishery products. U.S. At. Energ. Comm., Div. Tech. Inform., Sixth Annu. AEC Food Irradiator Contract. Meet. (Oct. 1966), Sum. Accomplishments, CONF-661017, pp. 3-8.
1966. Application of radiation-pasteurization processes to Pacific crab and flounder. Final summary for the period November 1965 through October 1966. U.S. At. Energ. Comm., Div. Tech. Inform., Annu. Rep., TID-23835, 110 pp.

SPINELLI, JOHN.
1966. Development and utilization of objective indices for fishery products. American Seafood Distributors, July, pp. 1-8.
1967. Degradation of nucleotides in ice-stored halibut. J. Food Sci. 32: 38-41.

Pathogens in radiation-pasteurized fishery products

EKLUND, MELVIN W.
1966. Incidence of Clostridium botulinum on the Pacific Coast of the United States. Shellfish Sanit. Res. Plann. Conf., 1965 Proc., pp. 70-72.

EKLUND M. W., AND F. POYSKY.
1967. Incidence of Cl. botulinum type E from the Pacific Coast of the United States. In M. Ingram and T. A. Roberts (editors), Botulism 1966, Proceedings of the Fifth International Symposium on Food Microbiology: Moscow, July 1966, pp. 49-56. Chapman and Hall Limited, London.

EKLUND, M. W., D. I. WIELER, AND F. T. POYSKY.
1967. Outgrowth and toxin production of nonproteolytic type B Clostridium botulinum at 3.3 to 5.6 C. J. Bacteriol. 93: 1461-1462.

EKLUND, M. W., F. T. POYSKY, AND D. I. WIELER.
1966. The significance of Clostridium botulinum type E in the application of radiation-pasteurization processes to Pacific crab meat and flounder. U.S. At. Energ. Comm., Div. Biol. Med., Annu. Rep., 90 pp.
1966. The significance of Clostridium botulinum type E in the application of radiation-pasteurization processes to Pacific crab meat and flounder. U.S. At. Energ. Comm., Div. Tech. Inform., Sixth Annu. AEC Food Irradiat. Contract. Meet. (Oct. 1966), Sum. Accomplishments, CONF-661017, pp. 123-127.

HOBBS, G., ANITA STEIBRS, AND M. W. EKLUND.
1967. Egg yolk reaction of Clostridium botulinum type E in different media. J. Bacteriol. 93: 1192.

PELROY, GRETCHEN A., AND MELVIN W. EKLUND.
1966. Changes in the microflora of vacuum-packaged, irradiated petrale sole (Eopsetta jordani) fillets stored at 0.5 C. Appl. Microbiol. 14: 921-927.

PELROY, GRETCHEN A., JOHN P. SEMAN, JR., AND MELVIN W. EKLUND.
1967. Changes in the microflora of irradiated petrale sole (Eopsetta jordani) fillets stored aerobically at 0.5 C. Appl. Microbiol. 15: 92-96.

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