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Report, Bureau of Commercial Fisheries Technological Laboratory, Pascagoula, Mississippi,

for fiscal years 1967 and 1968

UNITED STATES DEPARTMENT OF THE INTERIOR

U.S. Fish and Wildlife Service
Bureau of Commercial Fisheries

Circular 327

Cover Photograph.--Gulf fish on conveyor belt being unloaded from vessel.

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U.S. FISH AND WILDLIFE SERVICE

BUREAU OF COMMERCIAL FISHERIES

Report Bureau of Commercial Fisheries Technological Laboratory, Pascagoula, Mississippi,

for fiscal years 1967 and 1968

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ABSTRACT

The activities of the Technological staff at Pascagoula concern product and process development, containerization, chemical composition of fishery products, pesticide residue analysis, and microbiological analysis. Presented are results of research on new and improved methods of preventing the development of flesh browning in snapper, rancid odors and flavors in Spanish mackerel, adverse texture changes in frozen oysters, blue discolorations in crab meat, green discolorations in frozen raw breaded shrimp, and adverse changes in canned shrimp during storage. A countrywide study of the shipment of iced fish in leakproof containers is described. New attempts in increasing the iced storage life of shrimp through the use of bacteriostatic agents are discussed. Developments in the sanitary handling of fish meal are presented, as are the results of a study to mechanize the handling of various types of industrial fish.

Report
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INTRODUCTION

During fiscal years 1967 and 1968, most of the fisheries in Region 2 were financially profitable.

The domestic shrimp industry, largely located in Region 2, became the first U.S. fishery to reach a $100 million exvessel evaluation. In spite of increasing shrimp imports, the consumer demand equalled the supply, and the price continued to move upward. The production of breaded shrimp is estimated to continue at nearly 100 million pounds for the year.

Florida calico scallops have become a factor in the national production of scallops. Several vessels from Florida and Georgia are now landing calico scallops at St. Augustine and Cape Canaveral, Fla. One of the vessels carries automatic processing equipment--shell-cutting machinery and shucker-eviscerator units.

Blue crab processors continued to be plagued with light landings, labor shortages, and consumer resistance to high retail prices.

Menhaden landings, although higher than those off the east coast of the United States, decreased 11 percent over the previous year.

Snapper, one of the Region's most desirable species of fish, usually marketed as whole fresh fish partially drawn (guts removed), played a part in the increasing demand for fresh iced fish, either as fillets or dressed whole fish.

As the Technological Laboratory at Pascagoula moved into its 10th year of operation, industry requested more and more technological advice. In keeping with Bureau policy, we gave these requests our careful attention and made every effort to provide information. In some instances, we had to divert manpower for a quick study if the problem was new and seemed to affect a substantial portion of the industry.

The Laboratory staff continues to survey and monitor technological research granted to the States under Public Law 88-309, "Commercial Fisheries Research and Development Act of 1964." The staff is occasionally requested to report on the progress of an Economic Development Administration project that has technological significance.

During fiscal year 1967 we evaluated the program of the Laboratory and altered it somewhat. We halted the microbiological program and the chemistry program and diverted the funds to a more practical and timely program of assistance to the Region's industry. The purpose of the program is to increase the economic gain of our fisheries through the development of new products, processes, and handling techniques.

This report summarizes the results of research carried out in the past 2 years under both the old and the new research plans.

PROCESSING AND PRODUCT DEVELOPMENT

In developing our program of technological aid to the fishing industry of Region 2, we determined that a number of the serious problems of the industry lay in the field of processing and product development. We selected a number of species of both edible fish and shellfish and of industrial fish that could benefit in increased production and economic value if certain technological problems were solved. The reorganized program logically developed into two subsections, (1) edible fish and shellfish and (2) industrial fish.

EDIBLE FISH AND SHELLFISH

We felt that increased economic benefit to the fishing industry would result from research on several important species in the industry. Reports on the research conducted so far concerning problems associated with the snapper, Lutjanus spp., Ocyurus sp.; Spanish mackerel, Scomberomorus maculatus; oysters, Crassostrea virginica; blue crab, Callinectes sapidus; shrimp, Penaeus spp.; tuna, Thunnus alalunga; and Coho salmon, Oncorhynchus kisutch. Studies on the containerization of fishery products are also described, as are those aimed at developing new methods of processing fishery products.

SPECIES

The following sections describe the research efforts that are being made to solve problems preventing greater utilization and economic benefit of the several species.

Snapper

We examined the present market potential for snapper fillets and steaks because large stocks of snapper have been discovered in the Caribbean, and they merely need a potential market to be profitably used. The market potential appeared to be quite promising provided that solutions were available for problems of rapid browning of the fleshy side of the fillet and discoloration of the red skin pigment.

Preparatory to beginning work on the problem of the browning of snapper steaks and fillets, we visited the major Mississippi, Alabama, and Florida snapper producers and processors. While on the tour, we learned that, in addition to the browning and discolorations, the snapper producers had a problem with the shrinking and curling of the skin when Caribbean and yellowtail snapper were cooked. We conducted a short experiment to try to find how to eliminate the skin-curling. We cut fillets of both species into 1-inch cubes, dipped them in a 10-percent solution of each of the chemicals listed below, and cooked them for 30 seconds in a microwave oven to encourage skin curling. The chemicals used were sodium chloride, calcium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium carbonate, sodium citrate, citric acid, acetic acid, and sodium tripolyphosphate. The only promising additive was citric acid.

Since the use of citric acid appeared to be beneficial in solving this problem we experimented further to find the best conditions for its use. Several Caribbean and yellowtail snappers were dipped whole for 1 minute in a 10-percent citric acid solution; some were not dipped. After being frozen several weeks, the fish were then thawed, deheaded, steaked, and broiled. The nondipped controls of both species curled excessively, whereas the citric acid treated steaks did not curl at all. The excellent texture and appearance of the treated steaks was indicated by the organoleptic score they received from our taste panel. We then notified industry of this relatively easy and inexpensive way to eliminate the skin shrinkage of certain snapper species.

In an effort to solve the browning problem in frozen snapper steaks and fillets, we put up a test pack of snapper steaks and fillets. We decided to attack the browning problem first by using antioxidants. We felt that the inability of antioxidants to penetrate the fish flesh and skin might be responsible for the inadequate protection of fishery products with antioxidants found by other workers in this field; therefore, we devised a semiautomatic injection system that uses hypodermic needles and an automatic syringe. We successfully used this system to inject solutions of seven different antioxidants into the fish prior to steaking or filleting them. To test the efficiency of the system we incorporated an oil-soluble dye in the mixture of (a) vegetable oil and (b) emulsifying agent, water, and vegetable oil. Fillets and steaks were both subjected to a series of injections. A fairly even coverage was obtained with about 4 ml. of solution. Oil injections--such as might be used for BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), Tenox 4, and Tenox 6[1]--were not at all satisfactory since the antioxidant did not penetrate the fillet evenly. For this reason all oil soluble materials were injected in an oil-water emulsion.

We obtained 1,400 pounds of fresh, gutted, iced yelloweye snapper from a local fishery. The chemical additives used in this experiment were BHA, BHT, propyl gallate, Tenox 4, Tenox 6, ascorbic acid, and Na_2EDTA (the disodium salt of ethylenediaminetetraacetic acid). We used three different methods to apply each of these chemicals: (1) dipping the steaks and fillets; (2) injecting the fish before steaking or filleting; and (3) injecting the fish before either steaking or filleting them and then dipping the steak or fillet. All of the steaks and fillets, after being glazed, were stored in wax-coated boxes with wax-paper separators. We also processed control packs of steaks and fillets.

To ascertain whether atmospheric oxygen or oxygen in the water glaze was playing a role in the browning of snapper flesh, we put into vacuum sealed Cryovac bags several boxes of control fillets and froze them. We also dipped fillets in citric acid, Tenox 4, and Tenox 6, and packaged them in Cryovac vacuum bags to ascertain whether the additional protection of the vacuum package would prove beneficial.

We evaluated the pack organoleptically as well as chemically. The chemical tests included free and total ribose determinations on the meat, free amino acid content of the meat, and reflectance measurements to determine the degree of browning that had taken place. Our taste panel scored the pack after it had been stored at -10°F. for 9 months. The panel scored 45 percent of the total pack as inedible. The fillets that received the highest organoleptic score after 9 months of storage were the ones that had been treated with citric acid and packaged in Cryovac vacuum bags. Fillets injected with ascorbic acid received the lowest score. By the end of 9 months of storage we could see that antioxidants were not efficient in controlling the browning of the snapper flesh. The

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

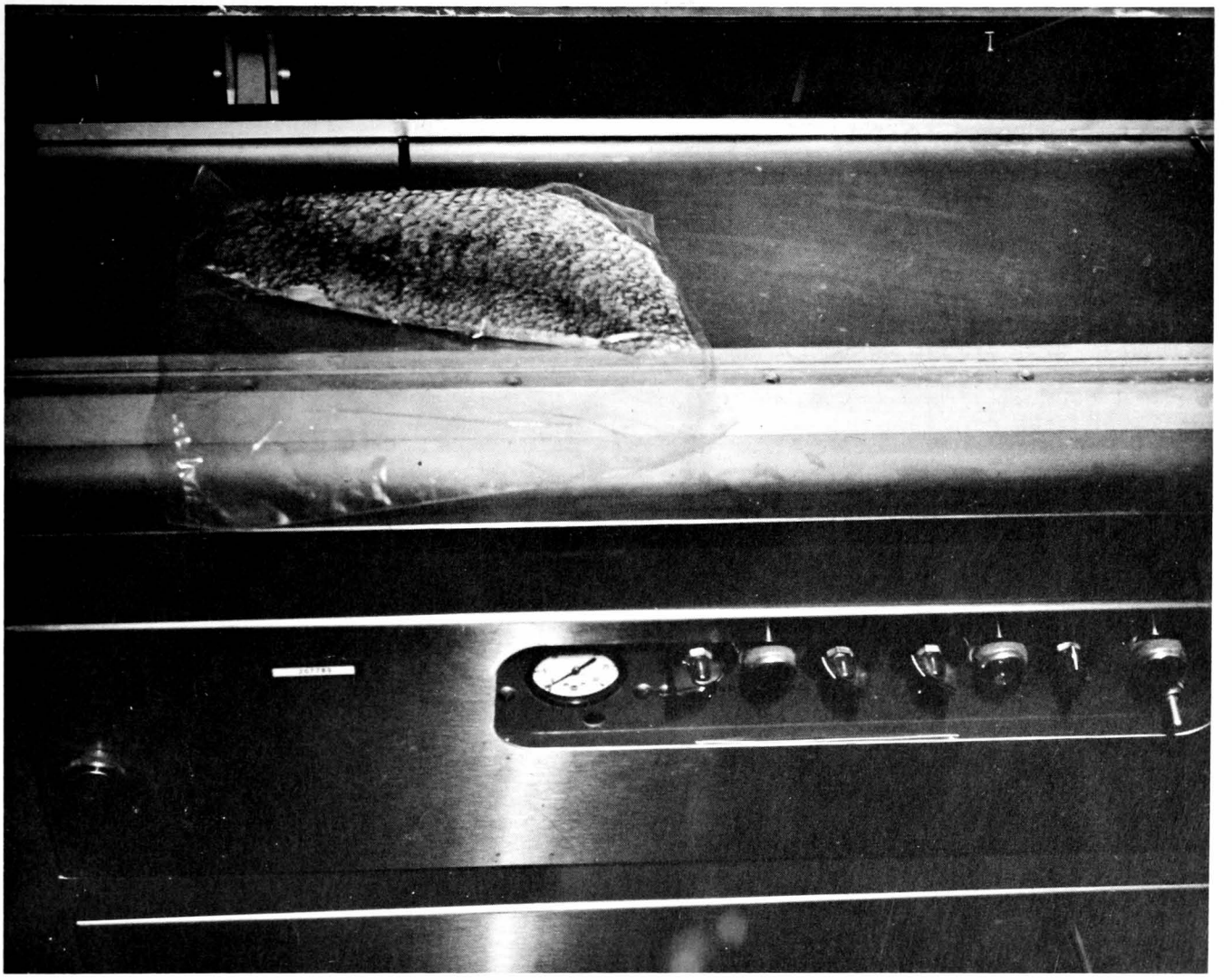

Figure 1.--Processing snapper in vacuum bags to prevent browning.

fillets that had been packaged in Cryovac vacuum bags had much better quality organoleptically (taste, appearance, texture) than the ones that were glazed and stored in the wax-coated cartons. This finding indicates that the browning reaction is partially of an oxidative nature but not one which can be controlled by ordinary antioxidants.

We learned from our initial review of the literature that the flesh has amino acids and certain sugars that can react and cause the browning. Armed with this information and the fact that antioxidants did not seem to inhibit the browning of the snapper flesh, we put up another test pack of snapper that we treated with chemicals that suppress the reaction of amino acids with sugars. The chemicals that we used in amounts not exceeding Food and Drug Administration additive allowances were TDP (3,3'-thiodipropionic acid), glutathione, Na_2 EDTA + PG (propyl gallate), and TBHQ (mono-tertiary-butylhydroquinone). We used only fillets in this pack. The chemicals were injected into the fillets, which were packaged in Cryovac vacuum sealed bags. We also processed controls, which were untreated. The pack was evaluated organoleptically as well as chemically. The chemical tests on the flesh included free and total ribose determinations, free amino acid determinations, and reflectance measurements.

After 6 months of storage at -10° F., the flesh of the fillets that had been treated with TDP and glutathione was nearly as light in color as that of a freshly filleted fresh snapper. The flesh of the control fillets, the TBHQ fillets, and the Na_2 EDTA + PG fillets was very brown at the end of 6 months of frozen storage. According to the free ribose data, the chemicals TDP and glutathione had almost completely inhibited the reaction between the amino acids and sugars present in the flesh. This inhibition is the reason for the light-colored flesh of the fillets treated with these two chemicals. Because the fillets treated with TDP and glutathione were given the highest organoleptic score by our taste panel, we felt that we had a good product that could be produced for a minimum in extra cost.

The next step was to determine the consumer appeal of the package and of the fillets and the prospective price in the retail market. These are the first questions that a prospective processor would ask when told of the product.

Because our Laboratory staff has less than 25 people who could be classed as representative consumers, we had to arrange to have members of the surrounding community serve on our consumer taste panel. One hundred people whom we contacted at random agreed to participate in return for a supper of snapper.

We gave these people frozen samples to try. We had obtained, from a local source, 550 pounds of fresh yelloweye snapper, which we scaled and filleted. These fillets were packaged in Cryovac bags, frozen, and given to the samplers. After cooking the product by methods left to their discretion, each family filled out a 10-question form. From these questionnaires we learned that about 58 percent of the panel would pay between $1.00 and $1.50 per 12 oz. package for snapper fillets if they were available to the public. This stated price is about what these fillets would have to sell for at the retail level. All of the families thought that the fillets were packaged very attractively. Many families remarked that the clear Cryovac bag not only allowed them to see both sides of the fillets to give them a better idea of the quality, but also gave them an idea of the amount of fish to purchase. Ninety-three percent of the panel also indicated on their questionnaire that these frozen fillets were equal to, or better in taste than, fresh iced fish. As a new fishery product, this forzen Cryovac-packaged snapper was received extremely well by the general public in this area.

Spanish Mackerel

Development of rancidity in oily fish during frozen storage has long been a problem to the fishing industry. This rancidity has resulted in shorter shelf life of certain species and a loss of profits to the industry. Antioxidants have brought some relief from rancidity. The greatest use of antioxidants to retard rancidity has been with foods of other than marine origin. The fishing industry has used antioxidants in a limited way to preserve fish meal or certain lean species of fish such as haddock and cod. Because of rancidity, fatty fish species such as Spanish mackerel and striped mullet, Mugil cephalus, have had large losses in profit and in repeat sales.

Several researchers have shown recently that rancid odors and flavors could develop with or without oxygen. They found that minute quantities of copper or iron will induce the development of rancid odors and flavors. It would seem that the development of rancidity in certain species might be twofold in nature--either through the oxidation of the oil in the normal manner or through metal ion catalyzed reactions.

We realized the tremendous help a solution to the rancidity problem would be to the fishing industry. In January 1968, we started a project that we hope will offer some relief to the industry. One thousand and one hundred pounds of Spanish mackerel were received from Marathon, Fla., by air. The fish, which were less than 24 hours old and in perfect condition, were treated with a variety of antioxidants by (1) injecting, (2) dipping, and (3) injecting and dipping. The fish were treated either as whole fish or as fillets. Fillets were packed in vacuum and with an ice-water glaze. Whole fish were glazed. The antioxidants used were Tenox 4 (BHA and BHT) and Tenox 6 (BHA, BHT, PG, citric acid, and propylene glycol). Na_2 EDTA was also used in combination with Tenox 4 and Tenox 6 or by itself. Organoleptic and chemical evaluations have been made at 2-week, 1-month, and 3-month intervals to date. Results from the 2-week samples were used as a baseline denoting extremely fresh fish. Our taste panel scores for all samples on the 2-week draw were 3.5 or better. After 3 months of frozen storage, organoleptic tests showed all samples still to be in good condition with scores of 3.0 to 4.4. These scores, however, were a slight decrease over the corresponding initial scores. The major producers of Spanish mackerel estimate that 6 months of storage time elapse before Spanish mackerel has noticeable rancidity. After the 3-month draw, our expert taste panel detected no rancidity. The taste panel preferred mackerel packed in vacuum or treated with EDTA after 3 months of storage.

Chemical tests included FFA (free fatty acid) and peroxide values. All samples had increased peroxide values and FFA content over the 3 months' storage time. Comparison of peroxide values with organoleptic scores are not significant, inasmuch as samples with better organoleptic scores had higher peroxide values. FFA content followed a trend similar to that followed by peroxide values.

The data collected after 3 months of frozen storage show that samples treated with Na_2 EDTA received better organoleptic scores and had lower peroxide and FFA values than the controls.

Oysters

From talks with several members of the oyster industry, we found that they would like to be able to freeze "fat" oysters and have them retain their fresh-as-raw-stock taste, texture, and appearance for 6 months or longer. This freezing capability would provide a supply of good oysters during the season when the oysters are lean, or thin. Although frozen oysters have been marketed for 20-25 years, the length of time they can be stored and thawed in a high quality condition is only about 1 month.

Figure 2.--Injecting Spanish mackerel with a new chemical to retard the development of rancid odors and flavors.

During the latter part of the 1967 oyster season, we put oysters in cans which were then vacuum sealed and frozen. The variables of surrounding liquid medium used were tap water, oyster liquor, sodium chloride (5 percent), glycogen (1 percent), and the polyphosphate Freez-Gard (0.25 percent). After being sealed, the oysters were frozen to -28° F. in a blast

freezer. They were transferred to a walk-in freezer and stored at -10° F. for examination at 1, 3, 6, 9, and 12 months of storage. Additional oysters were IQF (individually quick frozen) and glazed in the above liquids, sealed in Cryovac bags, and stored in the same manner as the canned oysters.

The frozen canned oysters and glazed Cryovac oysters were tested for organoleptic rating, pH, and glycogen content immediately after freezing and with each subsequent draw.

All packs stored 1 month showed little difference from newly purchased raw oysters in taste, texture, and appearance. Those oysters stored for 3 months and 6 months had a wide range of organoleptic scores--the sodium chloride treatment had the highest score and the glycogen treatment the lowest score. Results of the study indicate that sodium chloride solution is best for packing oysters whether they are frozen in cans or sealed in vacuum bags. Six months appears to be the maximum length of time that oysters can be frozen and still be acceptable in taste, texture, and appearance when compared to fresh raw oysters. This method of wet packing frozen oysters does result, however, in the desired increase in storage life.

Figure 3.--Frozen raw oysters on the half-shell.

pH values remained rather constant through the experiment. The glycogen content of the different packs varied considerably but could not be correlated with organoleptic ratings, type of liquid medium, or length of storage.

Blue Crab

The pasteurized and canned blue crab meat industry has been plagued for many years with the sporadic blueing of the picked meat. We have started research to determine the cause and to solve this problem. We suspect that what one segment of the industry calls blueing, another calls blue-greying (iron sulfide discoloration) and that even several types of "blueing" occur. Many workers believe that the discoloration is caused by different factors and thus may not be the same problem in each instance. For example, an occasional spot will form in the blue crab meat. This blue is very discrete and does not affect the entire contents of the can. On the other hand, a blue-grey discoloration sometimes affects the entire contents of the can. Still another variation in this phenomenon is a definite grey color associated with a break in the enamel of the can--obviously the age-old problem of iron sulfide discoloration.

We turned our attention to the blue or blue-grey discoloration. A review of the literature shows the cause to be attributed to: (1) metal ions, such as copper and iron, forming a complex with the proteins, (2) low-quality raw material, (3) overheating of the canned product, and (4) the presence of excessive sulfur in the female crab during certain seasons of the year. Several "cures" have been suggested, but none seems to work.

After a careful study of the literature, we decided to study first the copper and iron complex theory. We performed three experiments.

1. We conducted an experiment that called for including chelating agents to tie up the metals (e.g., Na_2 EDTA) and for including acids (e.g., lactic and citric acids) to lower the pH. Research workers had suggested that each of these actions is an effective remedy. The crab meat was treated with each chemical, sealed in cans, and pasteurized according to commercial practice. After storage for 1 year at 40° F., none of the cans, including controls, had blueing. Some of the additives had a detrimental effect on the crab meat, whereas others caused no ill effects. A similar experiment with chelating agents and acids was conducted simultaneously with cans that had the inside enamel purposely scratched. Results showed some "rusting" in the can seams throughout all treatments in both experiments. We saw no blue-grey discoloration, however, in "scratched" cans with the exception of those treated with citric acid. Those treated with EDTA and lactic acid did not discolor to a noticeable degree.

2. We made another experiment to determine if the pasteurizing time and temperature affected the color of pasteurized crab meat. We heated cans of crab meat for 5, 10, 20, and 30 minutes at 170°, 180°, and 190° F. The cans were stored 12 months at 40° F. and examined periodically. Results show that pasteurizing at 170° F., regardless of time at this temperature, had no effect on the color. Crab meat heated to 180° F. was slightly discolored throughout the can; again, there was no appreciable difference between packs pasteurized for different lengths of time at this temperature. Pasteurization at 190° F. caused the greatest amount of discoloration, which again was independent of time of pasteurization. The conclusion from this work is that overheating does cause some blue-grey discoloration; therefore, pasteurization temperatures should be held to a minimum. Pasteurization times should be adjusted so that they suitably reduce hazardous bacterial counts at the lowest temperature possible. Length of time at the pasteurizing temperatures does not affect the color.

3. Our third experiment was made to learn how the quality of the raw crab meat affected the quality of the pasteurized crab meat. We obtained fresh picked crab meat and pasteurized portions after 0, 1, 3, 6, and 10 days storage of the picked crab meat at 40° F. We pasteurized the cans using commercial practices including refrigerated storage (40° F.). We made bacteriological, organoleptic, and color analyses at regular intervals. The discoloration increased in the pasteurized meat as the time of "aging" (before pasteurization) increased. Storage time of the pasteurized meat has little to do with discoloration. The crab meat "aged" 10 days before pasteurizing commenced, however, was spoiled after 1 month of storage.

Shrimp

Since the shrimp industry of Region 2 is so large and manufactures such a wide array of products, we found several problem areas connected with various facets of the industry. Research on shrimp processes and products which we conducted is described in the following paragraphs.

Frozen raw breaded shrimp.--An industry problem of rather large economic proportions was brought to the Laboratory by several of the largest producers of frozen raw breaded shrimp. Frozen raw breaded shrimp were being rejected by distributors, brokers, retailers, and customers with regularity, owing to the appearance of green spots on the breading surface. After careful examination, several detrimental qualities were obvious. The green appearance consisted of large flat areas of three different shades of green (dull blue-green,

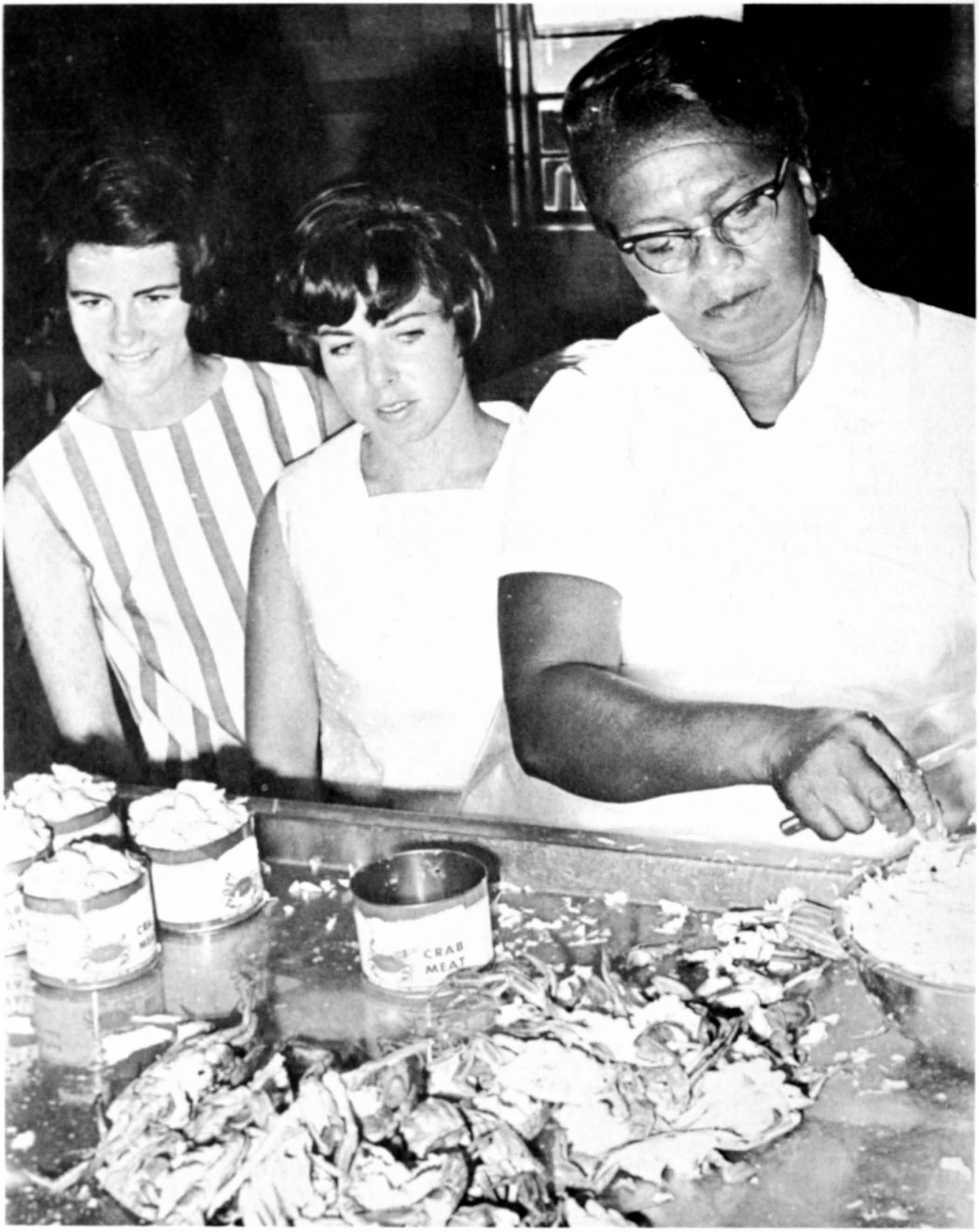

Figure 4.--Picking crab meat in the time-honored way.

bright green, and bright blue), originating at the shrimp-breading interface.

We made a microbiological and chemical analysis of the shrimp. No organisms on the shrimp, either bacteria or molds, were found capable of producing the various colors. The shrimp were found to have a high pH and a strong smell of ammonia. Chemical analysis of the batter and breading from the shrimp revealed a high content of iron, chromium, cobalt, nickel, and copper. Analyses for iron and other trace minerals in colored and non-colored breaded shrimp suggested the possibility of colored ammonia complexes. Considerably more than the expected amount of iron found in the samples was in the ferrous state. Ferrous ion produces a grey-green color when complexed with ammonia.

Upon closer examination we found magnetizable black particles on the cut surfaces of the shrimp, and these particles were the clue to the problem. To recover the black particles, we digested several samples of breading from colored shrimp with NaOH (sodium hydroxide) and filtered the digest mixture. The recovered black particles were placed on ammoniacal shrimp. These were then processed by a hand batter and breading operation after which they were frozen. After several days in frozen storage, the shrimp were allowed to thaw momentarily. The temperature fluctuation was repeated a second time. The characteristic green colors appeared after the second thaw.

We made a plant visit to survey the manufacturing practices, as well as to draw a number of samples. Sources of water to the plant and online samples were analyzed for trace minerals. The major source of contamination with metal residue was not found because online samples contained residues but closed-water supplies did not. The possibility arose that metal residues were airborne; therefore, air samples were taken with an air-sampling vacuum pump. The air samples contained significant amounts of cobalt, manganese, and nickel. Apparently, metal dust carried into the plant by air currents was settling on open water tanks, shrimp, breading, and batter. Then, through a complex reaction at the shrimp breading interface which takes place at temperatures near freezing, metal ions are produced that combine with ammonia to produce various colored compounds. We made recommendations regarding the elimination of the airborne contaminants.

Canned shrimp.--During the past biennium we processed a test pack of canned shrimp in the laboratory. We were trying (1) to control the formation of struvite in canned shrimp and (2) to give the shrimp a firmer, more desirable texture. A possible new food additive was added to the pack in the liquid medium at levels of 0.25 percent, 0.50 percent, and 1.00 percent. We processed this pack under simulated commercial conditions. To provide optimum conditions for struvite to form, however, we stored the pack under refrigeration so that we could evaluate the capability of the additive as a preventor of struvite. We found that if the shrimp are to be stored for 1 year or less, that the 0.25 percent level of additive produced the best textured shrimp with little or no struvite formation. If the shrimp are to be stored for 1 year or longer, however, the 0.50 percent level of additive produced the best textured shrimp with little or no struvite.

In cooperation with the American Shrimp Canners Association, we helped put up a test pack of canned shrimp at a New Orleans shrimp canning plant. The purposes of putting up this pack were also twofold: namely, (1) to control struvite formation in canned shrimp and (2) to give the shrimp a more desirable texture. This pack had the same additive mentioned previously. The additive was added to this pack at levels of 0.25 percent and 0.50 percent in the liquid medium. To ascertain the effect of size on the usefulness of the additive, we used both small and jumbo shrimp. We divided the pack into three equal lots and stored one lot in a conventional warehouse, another in an air-conditioned warehouse, and the third lot in the conventional warehouse for 3 months and then rotated to the air-conditioned warehouse to be stored there for 3 months. The 3-month rotating cycle of this third lot was continued throughout the storage period. After 18 months of storage, we found that the cans of shrimp that (1) contained the 0.50-percent level of additive and that (2) had been stored in an air-conditioned warehouse for this period of time had the best texture in packs of both sizes of shrimp. We found also that struvite seldom formed in these packs.

Shrimp texture.--To gain more knowledge about how marine organisms destroy shrimp connective tissue, we developed a method to isolate and purify the insoluble collagen of shrimp. By learning more about the nature of shrimp collagen from a chemical standpoint, we felt we could better understand its destruction, which is accompanied by softening of texture.

The method used for the isolation of insoluble shrimp collagen was a modified procedure of Veis, Anesey, and Cohen. This procedure involved extraction of homogenized shrimp meats with different buffers. All of the foreign protein was removed by these extractions, and the pure insoluble collagen was left. After isolating and purifying insoluble shrimp collagen, we then made several chemical and physical analyses of it. The analyses included determinations of hexose, amide nitrogen, amino acids, total nitrogen, and x-ray diffraction pattern. Insoluble shrimp

Figure 5.--Processing raw shrimp in a modern manner.

collagen has an unusual amino acid composition. It contains the amino acid tryptophan, which is not found in any other collagen. It displays the highest lysine content of any reported collagen. Shrimp collagen contains about one-third the amount of hydroxyproline normally found in other collagens. This low hydroxyproline content suggests that shrimp collagen is not as structurally sound as other collagens. This structural weakness accounts for the fact that shrimp connective tissue, of which collagen is the major constituent, is relatively easily destroyed by the enzyme systems of micro organisms.

PRESERVATION

Methods of preservation of fishery products which allow increased quality, yield, and storage life are always in demand. During fiscal years 1967 and 1968, we experimented with the use of nitrofurans and radio sterilization to ascertain their effectiveness in achieving one or more of these goals.

Nitrofurans

Nitrofurans are organic chemical compounds, many of which have bacteriostatic properties. In other words, compounds of this type tend to prevent the growth and multiplication of bacteria. The Japanese have demonstrated the effectiveness of many nitrofurans in inhibiting bacterial growth on fishery products. Furylfuramide has been shown to be the greatest inhibitor of bacteria growth of all the nitrofuran compounds. This compound, although not as yet approved by FDA (U.S. Food and Drug Administration), has been approved by the Ministry of Welfare of Japan for use in fishery products such as kamaboko (fish sausage). Furylfuramide has been used successfully for 14 years in Japan; however, there is no record of any nitrofurans having been used to preserve food in the United States.

Approval of CTC (chlortetracycline) has been suggested by a number of workers as a food preservative for seafoods. This antibiotic was withdrawn from the approved list of additives for foods by FDA because resistance of bacteria to CTC was thought to have increased. Therefore, we saw a need for an effective preservative which would not lead to the formation of resistant bacteria.

We made studies to determine if nitrofurans were effective against micro-organisms associated with fresh shrimp and picked fresh crab meat. Other workers have shown that nitrofurans were very effective against many species of bacteria associated with seafood.

Shrimp were dipped into solutions containing various concentrations of furylfuramide and then iced as in commercial practice. We made bacteriological, organoleptic, and chemical analyses to determine if the chemical was effective in extending the iced shelf life. Results indicated that application of furylfuramide extended the shelf life by 2 or 3 days. Bacteriological data showed little change in the number of the overall flora; however, we think that furylfuramide prevented the growth of certain spoilage bacteria, resulting in the better organoleptic score. Organoleptic analysis is still the best criteria of quality; however, the chemical analyses tended to confirm the organoleptic results.

We made an additional experiment to determine if furylfuramide suppressed the growth of proteolytic bacteria. Results show some suppression of growth. When furylfuramide is coupled with CTC, the suppression of growth is more pronounced.

We made an in vitro study to determine if furylfuramide would inhibit the natural flora of shrimp. Flasks of nutrient broth containing furylfuramide were inoculated with the natural flora of shrimp. We incubated the flasks and stored them at 22° C. (72 F.). The turbidity of the broth was measured at intervals to assess the growth rate. Results indicated that 5 p.p.m. of furylfuramide prevented growth over a 60-hour period. Isolated proteolytic bacteria were subjected to the same treatment as the natural flora, and the results were generally the same as those in the mixed culture.

Apparently furylfuramide does prevent the growth of proteolytic bacteria but not of certain other species of bacteria. As far as quality of seafood is concerned, proteolytic bacteria are the most devastating group. Accordingly, the use of furylfuramide should be thoroughly investigated to determine its in vivo effectiveness in preventing the growth of proteolytic bacteria.

We made the same type of study on nitrofuryl acrylamide as we did on furylfuramide. We found that nitrofuryl acrylamide was generally ineffective. The Japanese had approved this chemical as a preservative for fishery products; however, commercial use demonstrated it to be ineffective.

As was indicated earlier, furylfuramide is not now approved by the FDA for food use in this country; consequently, it cannot be put in commercial foods destined for human consumption. Efforts should be directed, however, to petition the FDA for approval of this chemical.

Radiosterilization

The Technological Laboratory at Pascagoula had an agreement with the U.S. Army to develop acceptable radiosterilized (irradiated) fishery products from tuna and salmon. Although we and the Army gave priority to tuna and salmon, we also considered other species as

basic materials for the preparation of convenience items. Species such as Spanish mackerel and mullet, Mugil spp., offer excellent possibilities as basic ingredients because of their abundance, low cost, and nutritional value.

The work is in its first year, and only albacore tuna and coho salmon have been studied to date. The products were developed and prepared at Pascagoula and frozen for shipment to U.S. Army Research Laboratories, Natick, Mass. They were irradiated at 4.5 megarad and returned to Pascagoula for evaluation.

The first product we developed was a precooked breaded salmon steak. We tested 10 different breading and batter materials; tried eight different food coatings that were used to prevent moisture loss during cooking; and established the optimum temperature of the oil used for cooking. A manufactured breading-batter formula was determined to give the desired color when the breaded steak was heated in oil at 360° F. for 3 to 4 minutes. Addition of food-coating (methylcellulose) materials did not prevent moisture loss appreciably. This finding may have been not significant, because the control breading formula did contain a small amount of methylcellulose. A consumer-type taste panel rated this irradiated product as acceptable.

The second product we studied was nonirradiated broiled and baked salmon. Raw 3/4-inch steaks were heated in an open-air oven until the temperature of the center of the steak reached 170° F. The broiling and baking procedures produced a steak covered with a soluble-protein curd that was unsightly and that could not be removed completely. We tested three additional methods of enzyme inactivation: (1) microwave heating, (2) heating the individual steaks in plastic bags in a boiling water bath, and (3) heating in raw steam. All methods produced the same results as those previously mentioned. We abandoned our attempts to develop this product further.

Fish cakes were prepared from tuna and salmon, using two formulas that had been developed for cod fish cakes. After several modifications, we found that a formula consisting of cornmeal, gelatin, water, and seasoning was acceptable. The taste panel rated

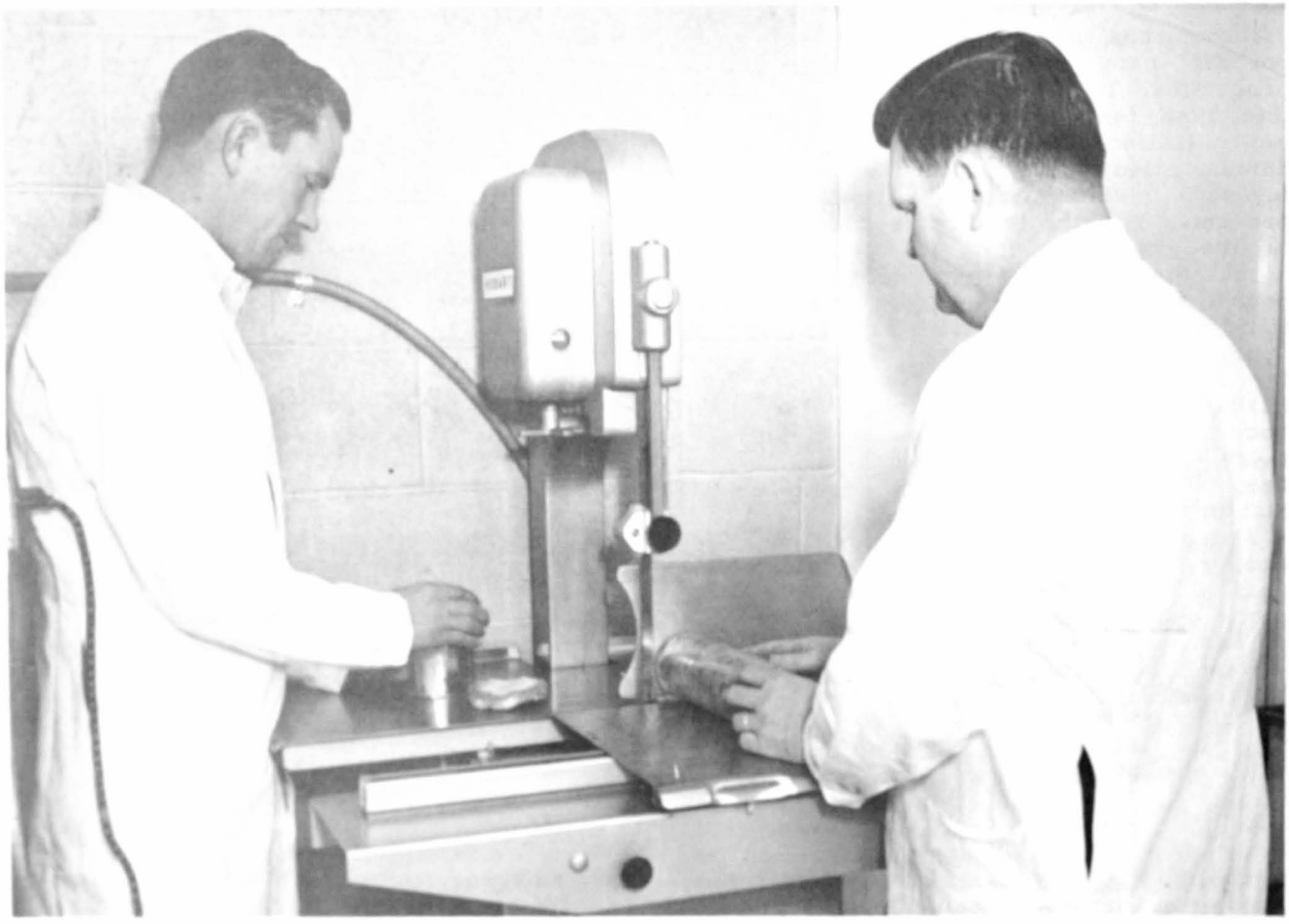

Figure 5.--Canning precooked patties destined to be processed by the new method of irradiation sterilization.

the irradiated salmon cakes higher than they rated the tuna cakes; however, both received very high organoleptic scores. Liquid hickory smoke was added to each of the above products. The "smoked" product was rated equal to or better than the comparable product without smoke. The smoke flavor apparently tends to mask the irradiation flavors and odors. In fact, smoked products produced the highest panel scores of all items developed.

A fish loaf was prepared from tuna and salmon. We tested 10 different formulations, including 1 that was prepared separately with tomato sauce. The two products receiving the highest panel scores before being irradiated were produced in large quantities for irradiation. The irradiated products were acceptable to the panel; salmon rated the highest. The products (both tuna and salmon) with tomato sauce rated lower than those without the sauce.

CONTAINERIZATION OF FISHERY PRODUCTS

We presented results of a Bureauwide study of iced seafood shipping containers at the National Fisheries Institute meeting held in San Francisco in April 1968.

We began this study in an effort to find an economical and efficient means of shipping small lots of fresh iced seafoods in leakproof containers. Included in the study were boxes of several designs. The most notable difference among the designs was whether the box was of one-piece styrofoam construction with lid (commercial box, Seattle crab box) or of breakdown multiple-piece construction with lid (Pascagoula box, Gloucester box). We did this study because common carriers refused to accept wooden boxes or barrels which have to be re-iced and which leak ice water.

Table 1 indicates the box is yet to be designed that will satisfy completely the needs of the fresh iced seafood industry. The present boxes generally allow the seafood to reach its destination in good condition but are themselves received in poor condition. Because these containers are relatively expensive, it would be economically desirable to design a box that would withstand repeated usage.

We felt that it was necessary to establish a method for efficiently sanitizing a container if it were to be used as a multiple-trip iced seafood box. Therefore, a large shipment of fresh fish was made in several commercially constructed polystyrene containers as well as in the common wooden fish boxes. We examined these containers bacteriologically before and after the shipment and after thorough cleaning

Table 1.--Results of the Bureau of Commercial Fisheries and National Fisheries Institute containerization study

Type container	Boxes shipped	Boxes: Received with good fish	Badly damaged	Percentage damaged	Lost or delayed 7 days or more
	Number	Number	Number	Percent	Number
Commercial box	42	35	30	71	4
Gloucester box	6	5	0	0	1
Pascagoula box	43	35	26	60	5
Seattle crab box	2	2	0	0	0
Total	93	77	56	60	10

Type container	Breakdown of damage by shippers: Boxes damaged by: Truck	REA[1]/rail	REA air	Air freight	Bus
	Number	Number	Number	Number	Number
Commercial box	1 of 1	5 of 5	13 of 15	11 of 16	0 of 3[2]
Gloucester box	-	0 of 2	0 of 1	0 of 3	-
Pascagoula box	1 of 1	4 of 6	12 of 16	9 of 16	0 of 3
Seattle crab box	0 of 1	-	-	0 of 1	-
Total	2 of 3	9 of 13	25 of 32	20 of 36	0 of 6

[1] Railway Express Agency
[2] Cardboard torn and dirty
Remarks: The only bad fish found were in broken boxes 4 days in transit or in good boxes delayed 7 days or more.

and sanitizing. The fish were in transit 4 days. We used the swab technique to make bacteriological counts (total aerobic counts). We cleaned the boxes with a high-pressure warm-water detergent cleaning gun. The boxes were sanitized with a chlorine compound solution in the same high-pressure cleaning gun. Results indicated that we removed 97 percent of the bacteria from the polystyrene boxes and 93 percent from the wooden boxes. It must be pointed out that all boxes used were new and had not been used commercially; therefore, the wooden boxes were not soaked with fish juices, water, and holdover bacteria. This newness accounted for a high removal of bacteria from the wooden box. As the wooden boxes continue in use, they will undoubtedly become increasingly difficult to clean and sanitize. The polystyrene boxes are impervious and appear to permit easy cleaning while offering good insulation.

Figure 7.--Sanitizing a new type of reusable container.

INDUSTRIAL FISH

The problem we felt deserved the most attention in the industrial fish fishery was that of accidental contamination of the finished products with molds and bacteria. We conducted two studies, one on mold toxin contamination and one on Salmonella contamination.

MOLD TOXIN

The staff at this Technological Laboratory noticed a large number of reports on animal carcinomas and deaths from the consumption of moldy vegetable meals. These toxins were produced by the mold species Aspergillus flavus and were designated "aflatoxins". The question arose whether aflatoxin might be produced in fish meal that had been accidentally water soaked.

A series of simple studies was designed to (1) discover whether such a growth might occur and (2) determine the moisture and temperature limitations on production of the toxin. From the Army Research Laboratories, Natick, Mass. we obtained live cultures of a known toxin-producing variety of A. flavus. To determine toxin production, we used a method developed by the Southern Regional Research Laboratory, U.S. Department of Agriculture, New Orleans. This method uses thin-layer chromatography with identification of the isolated toxin under ultraviolet "dark" light.

The experiments showed that under laboratory conditions aflatoxin would not be produced in normal fish meal (with less than 10 percent moisture) up to 35 days when held at temperatures of 83° F. At temperatures of 75° to 85° F., toxin could be produced in 35 days in fish meal that contained 18 percent moisture or more. In fish meal containing 30 percent moisture, A. flavus could produce the toxin in only 5 days at 83° F. At 95° F., toxin was produced in fish meal in only 7 days at a 22-percent moisture level. We pointed out to the industry that to avoid the production of aflatoxin, the fish meal that has accidentally become wet must be immediately recirculated through the dryer until its moisture content is 10 percent.

SALMONELLA

Of late the fish meal industry has become acutely aware of the overall salmonellosis problem in general and of Salmonellae contamination of fish meal in particular. Because research by both the Bureau of Commercial Fisheries and the U.S. Department of Agriculture has shown that average numbers of Salmonellae cannot survive the time-temperature conditions of the normal commercial drying procedure, the presence of Salmonellae in fish meal can only result from product contamination subsequent to the drying operation or from gross contamination of the raw material. These facts have been published in the recent "Code of Good Manufacturing Practices" developed for industrial fishery byproducts (Agriculture Research Service 91-51, May 1965). Industry, however, felt they needed help to translate scientific findings to commercial practice. This being the case, we believed that a project detailing the nature of (1) the Salmonellae problem, (2) the factors

that influence salmonellosis, and (3) the control methods that have proven successful in similar industries would be of value to the fish meal industry.

After FDA promulgated the recent regulation forbidding the interstate shipment of animal feed ingredients contaminated with any species of the bacterial genus Salmonella, we formulated a project that involved an operational procedure review of each fish meal producing facility. While at a plant, we instructed plant management on the scope of product contamination and necessary preventive control measures.

To date, we have visited 33 fishmeal producing facilities in Regions 2, 3, and 4. Before wide audiences, we have made formal presentations of instructive material. In these presentations we emphasized that we ask them to follow certain guidelines, which cannot be considered as clear-cut rules. At each plant, after we made a detailed inspection of all operational procedures, we suggested those measures that the facility should take to produce a Salmonellae-free product.

Figure 8.--Preparation of materials to be used for sanitizing box cars to prevent recontamination of fish meal during shipment.

DEVELOPMENT OF PROTOTYPE MECHANICAL DEVICE: ONBOARD FISH PRESS

Due to the ever-increasing need for protein for human and animal consumption, we foresaw that every effort must be made to utilize all protein sources that are reasonably available to us.

Shrimping today is one of the largest fisheries in the world. The Gulf States' catch of shrimp was more than 195 million pounds in 1965 and 179 million pounds in 1966. We estimate that for each pound of shrimp caught, 6 pounds of industrial fish are caught--or an average for the 2 years of 1 billion pounds of fish. These industrial fish are thrown overboard, because space is at a premium on most shrimp boats. In addition, a number of unutilized species of anchovies and herring are available in the waters of the Gulf of Mexico and South Atlantic for which industry has no efficient procedures now. With these facts in mind, we are attempting to develop an onboard vessel press to remove the bulk of the moisture from the fish and thus produce a semiprocessed product that can be stored in a small area.

We fabricated a heavy-duty drum with perforated screens and a concave and convex piston that would exert a pressure of 10,000 pounds to squeeze moisture from several species of fish. The device pressed 60 percent of the moisture from the raw anchovylike fish; however, only as little as 5 percent of the moisture could be removed for some other species of fish. As the experiment proceeded it became quite obvious that we would have to cook the large fish before we pressed them. We felt, however, that press drying of raw anchovylike fish could become a practical and economical process at sea.

Another phase of this project was to investigate the practicality of a direct heat-drying operation to make fish meal at sea. This study involved drying whole, small fish without pressing out either the oil or the water. We fabricated a gas-fired rotating oven. Samples were subjected to 250° F. for up to 12 hours. Very little water was removed. More experimentation will be necessary before a satisfactory method of drying whole fish can be developed.

COMPOSITION OF FISH AND SHELLFISH

The project entitled "Composition and Nutritive Value of Fish and Shellfish" was concluded during the biennium. Species studied were Chesapeake Bay blue crab, Dungeness crab, Cancer magister; brown shrimp, Penaeus aztecus; ocean perch, Sebastes marinus; alewife, Alosa pseudoharengus; Atlantic croaker, Micropogon undulatus, and striped mullet.

We analyzed not only the proximate composition of these species but also their amino acid, lipid, mineral, steroid, and vitamin contents. We sampled the various species

bimonthly for 1 year. We divided the different species into two lots by sex when possible and randomly when not. Certain species such as blue crab and Dungeness crab were separated into three samples--body meat, claw meat, and offal.

A short summary of the essential findings follows below. A large portion of the amino acid data (concerning shrimp, blue crab, and ocean perch) has been published in previous reports available from this Laboratory.[2] All species analyzed showed seasonal variations in the quantities of various amino acids.

For samples that were divided into different types of tissue (blue crab and Dungeness crab), we quantitated the differences in the amino acid content of the body meat, claw meat, and offal at any one sampling time. Amino acids known to predominate in certain metabolic reactions were found in larger quantities in the tissues in which these functions were performed. In both crab species, products of catabolism such as ornithine and urea were more abundant in the offal than in the body and claw meat. Amino acids such as lysine and arginine were predominant in the body and claw meat. Amino acids common to all three samples and that did not vary significantly throughout the sampling period were aspartic acid, cystine, hydroxyproline, phenylalanine, proline, serine, threonine, and tyrosine. The seasonal changes in amino acid content of the blue crab appear to coincide with reported reproductive cycle changes. Dungeness crab samples were collected only for the length of the commercial season (4 months); therefore, differences noted in the amino acid content of Dungeness crabs could not be related to life cycle processes for this reason.

Amino acids which either increased or decreased in croaker or alewife over the sampling period were aspartic acid, serine, proline, isoleucine, leucine, lysine, histidine, and arginine. The amino acid content of mullet fillets showed the largest significant changes from March to June, a period of tissue buildup before the start of accelerated gonad maturation.

Trace mineral analysis of the several species with an atomic absorption spectrophotometer has been completed for the entire sampling period. We analyzed samples for calcium, magnesium, zinc, copper, manganese, cobalt, iron, and molybdenum. We determined the percent recoveries and accuracy of the method because this instrument had not previously been used to determine the mineral content of fishery products.

[2] Love, Travis D. and Mary H. Thompson. Annual Report, Bureau of Commercial Fisheries Technological Laboratory, Pascagoula, Mississippi, fiscal year 1965. U.S. Fish. Wildl. Serv., Circ. 251.

Love, Travis D. and Mary H. Thompson. Report, Technological Laboratory, Bureau of Commercial Fisheries, Pascagoula, Mississippi, for fiscal year ending June 30, 1966. U.S. Fish. Wildl. Serv., Circ. 262.

PESTICIDE RESIDUES IN FISH AND SHELLFISH

Laboratory work on pesticide residues was confined to the effect of heat processing on nine chlorinated hydrocarbons added to canned shrimp. The pesticides used were BHC (1,2,3,4,5,6-Hexachlorocyclohexane), aldrin (1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo,exo-5,8-dimethanonaphthalene), heptochlor (1(or 3a),4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene), heptachlor epoxide (1,4,5,6,7,8,8a-Heptachloro-2,3-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene), DDE (2,2-bis-(p-Chlorophenyl)-1,1-dichloroethylene), DDD (2,2-bis-(p-Chlorophenyl)-1,1-dichloroethane), DDT (Dichlorodiphenyltrichloroethane, mixture of p,p' and o,p' isomers), dieldrin (1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene), and endrin (1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,endo-5,8-dimethanonaphthalene).

We analyzed the experimental test pack of shrimp that had been stored 1 year at -4° C. (26° F.). We found that storage time did not affect the recovery of added pesticides to any extent. Percent recoveries after 1 year of storage were slightly higher than the recoveries after 1 week. The increased recovery after 1 year of storage was within the experimental error of the procedure.

We analyzed the cans from the pack six times during the year's storage period. The largest average percent recovery found was 80 percent of the added DDE; the lowest, 23 percent of the added DDD. Owing to the poor recovery or to the disappearance of the added pesticides, we did not discern any breakdown of pesticide from its original form to other forms.

A control pack that consisted of heat processing water and added pesticides without shrimp meat did not show any better recoveries than did those cans packed with shrimp. Those packs to which lower levels of the pesticides were added had a better percent recovery than did those to which higher levels were added. When pesticides were added in quantities of .5 and .1 p.p.m., the recoveries from the controls were 14, 117, 71, and 104 percent for heptachlor, DDE, DDT, and dieldrin, respectively. When heptachlor, DDE, DDT, and

dieldrin were added to water and processed at the 3, 7, 7, and 3 p.p.m. level, respectively, recoveries were 16, 90, 68, and 93 percent. The higher level of added pesticides did not result in a higher amount recovered, which should have occurred if the analysis procedure was at fault; in most instances the amount recovered was lower. The recovery of added heptachlor and DDT did not decrease, nor did the recovery of their degradation products, DDE and heptachlor epoxide, increase. The above results indicate that a reaction between some part of the can and the added pesticides is dependent on the quantity of pesticides in the can. Substantiation was thus obtained in support of an earlier theory that a reaction between the pesticides and the metal can was accelerated by heating.

The project ended June 30, 1967.

U.S.D.I. INSPECTION SERVICE CONSULTATION

MICROBIOLOGICAL ANALYSES OF SAMPLES

This Laboratory provides technological advice to the U.S.D.I. (United States Department of the Interior) inspection personnel and to the inspected plants in Region 2. Members of the staff are occasionally called upon to visit the inspected plants and provide on-the-spot advice on a troublesome problem.

It is a regional practice that new products offered for production under the U.S.D.I. shield must first be thoroughly examined before permission is granted. This examination always includes a bacteriological analysis for total plate count, most probable numbers of coliform group present, and a determination of the numbers of Escherichia coli in the frozen seafood. A taste-panel evaluation of the cooked product follows the bacteriological analysis. Table 2 shows results of the samples examined during the year.

Table 2.--Requests for inspection to use U.S.D.I. shield

Plant	Product	Packages examined	Results
		Number	
A	Breaded shrimp cubes	6	Refused
	Breaded shrimp portions	6	Refused
	Breaded shrimp sticks	6	Refused
	Small stuffed shrimp	6	Granted
	Large stuffed shrimp	6	Granted
	Stuffed flounder fillets	6	Granted
B	Peeled and deveined shrimp	8	Granted
C	Shrimp sticks	6	Refused
	Shrimp cakes	6	Refused
	Stuffed shrimp	6	Refused
	Shrimp portions	6	Refused
	Stuffed shrimp	6	Refused
	Stuffed flounder fillets	6	Refused

In addition, lot inspections are frequently performed when the requesting plant is near our Laboratory. Table 3 shows lot inspections made during the year.

FROZEN RAW BREADED SHRIMP

A member of the Pascagoula staff visited all inspected plants in Regions 2, 3, and 6 for the purpose of acquainting management with the new stringent regulations for "Good Manufacturing Practices" promulgated by the FDA. He inspected each plant and informed the manager of possible danger spots that might allow the finished product to be bacteriologically contaminated. Talks were presented at joint FDA/BCF breaded shrimp sanitation workshops in Brownsville, Tex., and Los Angeles, Calif. As a result of information gained from these operation procedure reviews, the microbiology staff and inspection personnel have written a publication dealing with sanitation guidelines for the breaded shrimp industry.

Table 3.--Lot inspections

Location of warehouse	Product	Packages	Time
		Number	Hours
Baton Rouge, La.	Peeled, deveined shrimp	45 5-lb. packages	8
New Orleans, La.	Menhaden fish solubles	31 samples	40
Bayou la Batre, Ala.	Fresh iced raw headless shrimp	6 5-lb. packages	3
Bayou la Batre, Ala.	Frozen raw headless shrimp	5 5-lb. packages	3
Mobile, Ala.	Frozen raw headless shrimp	12 5-lb. boxes	8
Pascagoula, Miss.	Frozen raw headless shrimp	-	4
Pascagoula, Miss.	Frozen raw headless shrimp	-	6

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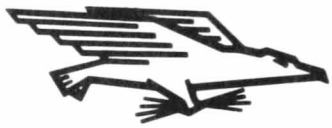

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