(7) Availability of financing from other sources.

If, on the basis of the above evaluation, a project appears to be substantial in character and design, it will be recommended for approval at either 50 or 75 percent federal funding. Such projects may be funded at the 75 percent level if they are regional, national or international in scope. If such projects are of limited local (intrastate) scope, they may not be funded at more than the 50 percent federal level.

The excellent response of the fishery administrators and biologists of the states to the PL 88-309 program has been most gratifying. This continued interest in the program will help insure sound, well-planned projects that will lead to the development of the commercial fishery resources in the respective states and subsequently in the nation.

FISHERIES TECHNOLOGICAL RESEARCH IN THE GULF OF MEXICO BY THE BUREAU OF COMMERCIAL FISHERIES

By TRAVIS D. LOVE

ABSTRACT

The research function at the Pascagoula Technological Laboratory is described. The staff, the equipment, and the microbiology and chemistry programs are briefly discussed.

Biochemical studies of fish and shellfish are a major program at the laboratory. The deterioration of fresh iced shrimp was studied recently by following the increase in pH, analyzing the amino acid, hydroxyproline, and evaluating the variation in the nitrogen values of fractions eluted by different solvents.

Microbiological studies accompanied the biochemical studies. Daily total plate counts demonstrated the increase in microorganisms as the shrimp became less acceptable as a food. Fluctuations in the types of organisms present were shown to be related to the changes that took place in the iced environment as the salinity—and the marine bacteria—decreased.

Microscopic studies on histological sections of the shrimp flesh showed that collagen in the connective tissue deteriorated as tissue integrity was lost. Bacterial invasion of the tissue accompanied destruction by the enzymes.

The study has culminated in an Industry-Government Symposium, where research results will be presented.

INTRODUCTION

Technological research in the Bureau of Commercial Fisheries is concerned with the study of marine products from the point of catch to the ultimate consumer. Handling, processing, storage, and transportation of seafoods are studied through applied research projects at the Pascagoula Technological Laboratory. A more basic type of research probes the biochemistry of protein degradation. Microbiological studies follow changes in bacterial population as the seafoods are processed and handled. The following research projects are current at Pascagoula.

I. MICROBIOLOGY

A. *Botulism Studies*

Several times in the past, botulism has been implicated in deaths following ingestion of seafoods. Type E *Clostridia botulinum* is considered to be the usual causative organism in marine products. Following the outbreak in 1963, the Pascagoula Laboratory began a study of

Author note.—Travis D. Love, Laboratory Director, Bureau of Commercial Fisheries Technological Laboratory, Pascagoula, Mississippi.

the basic physiology of the organism in order to determine a means of control. The type E organism was said never to occur south of the 38th parallel in a marine environment. Under an AEC contract, we are now engaged in a survey of the Gulf of Mexico to determine the extent of the organism's occurrence. Type E bacteria were isolated from our first samples of mud and water taken from Galveston Bay!

B. *Salmonellae Studies*

Salmonellae dysentery bacteria have been a problem in all processed foods. A study is underway at Pascagoula to determine methods for control of the organism. We are using time-temperature studies to evaluate the heat treatment needed to pasteurize marine products containing the bacteria.

C. *Bacteriological Standards for Frozen Seafood*

State and federal regulatory agencies, such as the Food and Drug Administration and the public health services, are attempting to set up mandatory bacteriological standards for seafoods at the consumer level. Their approach is to use one broad standard to cover all seafoods. Our studies indicate that variations between different products, and often between different lots of the same product, are tremendous. We hope by our data to show the Association of Food and Drug Officials of the United States Committee that individual standards are needed for each food and, thereby, to aid them in setting up realistic standards for the commercial seafood industry.

II. CHEMISTRY

A. *Studies of Chemical Composition and Nutritive Value*

Since the laboratory began operations seven years ago, the study of the chemical composition of fish has been a major project. Originally, the project determined oil, protein, ash, and moisture of the numerous species used by the catfood industry. Our samples, gathered at the docks of the catfood-canning plant, represented all the fish going into this product. Seventeen species were analyzed on a quarterly basis for five years so that cyclic changes in the life of each species could be identified. These data are published quarterly and are available free.

Owing to numerous requests for information about seafoods suitable for human consumption, this project has now been broadened so that all six other technological laboratories of the Bureau can submit food fish and shellfish from their area for analysis at Pascagoula. We are now analyzing these seafoods for trace minerals and hope later on to analyze them for vitamins.

With the addition of a Beckman Amino-Acid Analyzer[1] to our equipment, the Pascagoula biochemists began a study to determine the exact amino-acid content of several species of fish and shellfish. The graphic record that the instrument gives of the amino acids in each species is valuable for estimation of the nutritional value of the seafood. This analysis has been included in the project we are now undertaking to determine amino acids in fish from Boston to Seattle to Key West.

B. *Pesticides*

Following the newspaper publicity on fish kills caused by pesticides in the Missisippi River, the Pascagoula Laboratory was assigned the task of determining the possibility of pesticides being present in processed seafoods. In order that our results indicate what effect, if any, commercial processing has on the final level of pesticides in the seafood, our samples will cover the entire span of the product from catch to consumer. By means of the gas-liquid-chromatography equipment, with electron-capture device and automatic graphic recorder, the pesticide chemists are working with amounts as low as parts per billion. This program is coordinated with the Interagency Pesticide Committee; all plans and data are funneled through a single coordinator.

[1] Use of trade names does not imply endorsement of commercial products.

C. *Chemical Reactions in Processed Seafoods*

As we would expect, biochemical changes continue in fish even after they are processed into the canned, frozen, or fresh product. One of the most pressing problems in the Gulf is the unfavorable changes in texture, flavor, and appearance of canned shrimp. A project is now underway to determine the biochemical mechanism producing these unfavorable changes. Pascagoula chemists, using various additives and processing conditions, supervised the canning of a large sample of shrimp. Samples are being withdrawn on a scheduled basis for determination of loss of protein and changes in texture and for organoleptic evaluation.

One of the changes occurring is that of iron sulfide discoloration of the interior of the can, the shrimp, and the liquor. Discoloration occurs when the tin plating migrates from the can walls, under certain pH conditions, and leaves bare iron to react with the sulphur in the product. Research at Pascagoula has shed much light on this mechanism, and certain processing changes have been proposed to help control it.

III. "CRASH" PROJECTS AND SEAFOOD SYMPOSIA

Since the scheduled projects are operating on a relatively long-term basis, it is appropriate to point out how the Pascagoula Laboratory has been of service to the industry on pressing, immediate problems. We provide this service by diverting our manpower to the solution of special problems that arise suddenly. The data obtained may or may not result in a government-industry symposium, where they are discussed.

A. *The Mullet - Utilization Project*

Florida has huge stocks of under - utilized mullet, *Mugil cephalus.* In answer to a growing demand by the fishermen, the Florida Conservation Department proposed a Product and Development Marketing Program to be carried out jointly with our laboratory. Accordingly, the Pascagoula Laboratory, processing a pilot canning plant, was asked to develop a canned mullet product. Several types of pack were produced, but they met with poor taste-panel acceptance until a boneless, skinless, fish-flake-type pack was developed. Samples of and methodology for this product were furnished to several plants in Florida, which are now selling the canned product to State institutions.

One of the marketing problems with this species is that its common name, mullet, has fallen into disrepute in some markets. The Florida Conservation Department has sponsored a publicity drive to call the product "Lisa," its Spanish name. This expedient, however, has not as yet met with complete success, since few people will buy a fish with which they are not familiar.

B. *The Chloresterol Study*

Most people are acquainted with the large-scale five-year study made by the American Heart Association whereby a controlled diet was furnished to middle-aged males in several large cities in an attempt to determine the role of diet in arterioschlerotic heart disease. These researchers found that chemical analysis data for chloresterol in fish and shellfish were practically nonexistent. The Bureau of Commercial Fisheries was notified that unless, in a few weeks, such data could be supplied, these seafoods must be left off the test diet. Pascagoula was asked to start a crash program to determine the presence and amount of chloresterol in seafoods.

Seafood samples were expressed by air from all over the United States, and the Pascagoula Laboratory diverted researchers to the chloresterol-analysis program. After approximately 30 days of intensive effort, enough data were obtained to permit the American Heart Association to keep fish and shellfish on the diet.

C. *The Study of Fresh Iced Shrimp*

Since the introduction of the U. S. Department of the Interior

Voluntary Standards of Grade in Fishery Products, concern has grown over the quality of the raw material received for further processing. Standards for U. S. Grade A Frozen Raw Headless Shrimp require that the raw material as received from the boat be of very high quality. To assist the industry to meet these Standards, the Pascagoula Technological Laboratory planned a study of fresh iced shrimp to determine what happens biochemically to the shrimp to render them less acceptable.

Pascagoula researchers made short trips aboard shrimp trawlers operating from Fort Myers, Florida, to Pascagoula, Mississippi, to obtain samples of known history of all three commercial species. Pink shrimp, *Penaeus duorarum*, were obtained from the white sandy bottoms of the Dry Tortugas beds; white shrimp, *Penaeus sitiferus*, from the muddy bottoms off the mouth of the Pascagoula River; brown shrimp, *Penaeus aztecus*, from the muddy, trashy bottom near the mouth of the Mississippi River. The data will show later what type of bottom influences the microbiological flora and, ultimately, the quality of the shrimp.

The catch, as emptied on the deck of the trawler, was beheaded and divided into two lots. One lot was washed by the method commonly used on shrimp boats; the other lot was washed exceptionally well with copious amounts of water. Both lots were mixed with ice in the ratio of two parts ice and one part shrimp and stored in second-hand wooden shrimp boxes. All samples were returned to the laboratory, where daily withdrawals were made for chemical, microbiological, histological, and organoleptic analysis.

Microbiological data. — The total bacteriological plate counts on the brown and white shrimp from muddy, trashy bottoms, were initially higher than those on the pink shrimp. An example of this difference is shown in the next slide, which demonstrates that the white shrimp from the muddy bottom started with a higher count and reached a level of one million per gram much quicker than did the pink shrimp.

The second factor our data revealed as affecting the keeping quality of shrimp was the thoroughness of washing after the shrimp were beheaded on the trawler. Results show that the bacterial count rises very high during the second week of storage if the washing is not performed with extra care.

The third factor affecting the shrimp was the manner of icing. Our method of mixing the ice 2:1 with the shrimp and allowing some melting of the ice permitted the shrimp to remain as Grade A longer than is usual under normal commercial practices. The next slide will show how long the shrimp will last under these conditions.

One of the most interesting facts developed from our examination of daily samples is that there are several major population changes in the bacterial flora on the shrimp. During analysis, successive populations of different genera would grow to a peak and fall off; then a higher peak from the succeeding genera would follow. Slides 3 and 4 illustrate this growth pattern. In the terminal stages, where the shrimp are barely acceptable as food, the Achromobacter become the predominant genus, constituting approximately 80 percent of the total plate count.

Chemical data. — The chemical studies are aimed at two objectives —first to determine how the enzymes, natural and bacterial, tear down proteins; and second, what damage there is to the connective tissue (Slide 5). This slide illustrates graphically how the shrimp connective tissue is tied together. In our studies, three fractions of the protein were followed during degradation: (1) the nonprotein-nitrogen portion composed of amino acids and small peptides; (2) the salt-soluble fraction, composed of enzymes, muscle proteins, and a portion of connective tissue; and (3) the acid-soluble fraction, composed of other portions of the connective tissues. A micro-Kjeldahl-nitrogen value was obtained for each portion each day so that the degradation of the shrimp tissues could be followed.

For the purpose of this discussion, it is sufficient to say that the nitrogen values show that the shrimp deteriorate biochemically, parallel

with the rise and fall in bacterial population. (Slides 6, 7, and 8.) These slides illustrate the three fractions as degradation takes place and reflect the downward trend of the nitrogen values.

An additional analysis, that for hydroxyproline, also reflects the destruction of connective tissue. As the collagenase enzyme of the marine bacteria tears apart the collagen of the connective tissue, hydroxyproline is released and appears in the three fractions mentioned above (Slides 9 and 10). These slides show a portion of the data and illustrate the increase in hydroxyproline. The destruction of the connective tissue results in soft shrimp that is especially unfit for canning.

pH has been used as an indicator of the quality of shrimp by many firms in the canning industry. Our pH values were carefully checked and rechecked, since they appeared to be higher than those commonly used. The starting pH at Day 1 was 7.0 for the pink and the brown shrimp, but it rose to 7.3 for the well-washed white shrimp. All three species of shrimp reached a pH of 8 before the taste panel noted an "off" flavor. (Slides 11, 12, and 13.) These slides illustrate the rise in pH as the shrimp are aged in ice.

At the beginning of this study, we decided that we would try to show the deterioration of shrimp tissues by means of histological preparations—we wanted to dramatize the effects produced by both intrinsic, or autolytic factors, and extrinsic, or bacterial factors. Although we cannot histologically demonstrate which of these factors caused the greater damage, the results clearly show the overall damage caused by a combination of these factors.

The methods used in this study were intended to show differences in the connective tissue and the relation of this tissue to the other tissues of the shrimp. The reasons for directing our methods primarily toward connective tissue were two-fold: (1) since this tissue serves as a supporting tissue, its loss will result in the loss of overall tissue integrity, which, in turn, results in a soft, or mushy shrimp; and (2) the overall composition of this tissue includes such specific elements as collagen and elastin, which are subject to attack by collagen-and elastin-specific enzymes from both land and marine microorganisms.

The following slides dramatize the degradative or spoilage processes that take place in the tissue of shrimp during prolonged ice storage:

Slide No. 14 — Hindgut of a 1-day shrimp
Slide No. 15 — Same area in a 14-day shrimp
Slide No. 16 — Outside of 1-day shrimp
Slide No. 17 — Outside of 7-day shrimp
Slide No. 18 — Outside of 14-day shrimp
Slide No. 19 — Post-mortem bacterial invasion
Slide No. 20 — Post-mortem bacterial invasion
Slide No. 21 — Post-mortem bacterial invasion

In conclusion, I hope that this brief discussion has brought into focus some of the problems that must be solved in the handling, processing, and storing of fish and shellfish if the consumer is to be offered a Grade A product.

SOME EFFECTS OF ENDRIN ON ESTUARINE FISHES

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ABSTRACT

Laboratory experiments were conducted to determine the acute and chronic effects of endrin to estuarine fishes. Short-term bioassays in flowing seawater determined 24-hour LC_{50}'s for spot (*Leiostomus xanthurus*), mullet (*Mugil cephalus*), menhaden (*Brevoortia patronus*), longnose killifish (*Fundulus similis*), and sheepshead minnows (*Cy-*