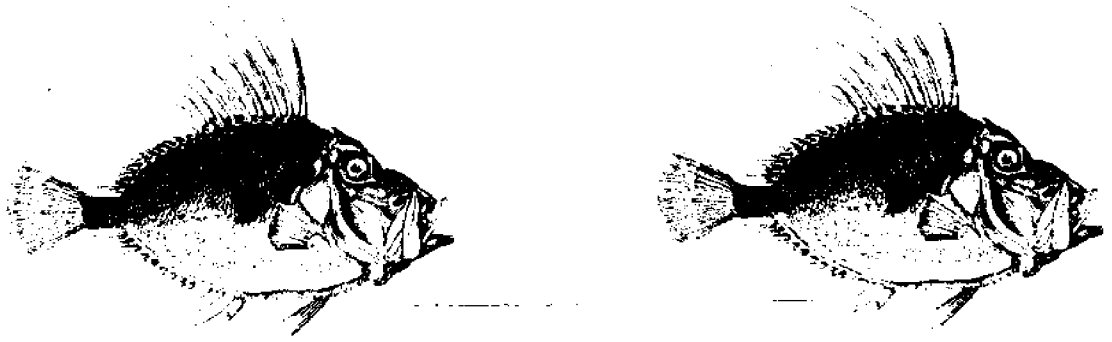
SGR-94

Tropical and Subtropical Fisheries Technological Society of the Americas

Conference Proceedings
Thirteenth Annual Conference
October 16-18, 1988
Gulf Shores, Alabama



Florida Sea Grant College Program
University of Florida
Gainesville, Florida

THIRTEENTH ANNUAL CONFERENCE

TROPICAL AND SUBTROPICAL FISHERIES TECHNOLOGICAL SOCIETY OF THE AMERICAS

OCTOBER 16-19, 1988
GULF SHORES, ALABAMA

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SEAFOOD TECHNOLOGIST
ALABAMA/MISSISSIPPI SEA GRANT CONSORTIUM

PROCEEDINGS
COMPILED BY
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FLORIDA SEA GRANT COLLEGE PROGRAM
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SEPTEMBER 1989

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ABSTRACTS

The following abstracts represent conference presentations not submitted as full length papers. The Tropical/Subtropical Fisheries Technological Society encourages publication of all presentations, but publication is an author's option.

A MESSAGE FROM THE PACIFIC FISHERIES TECHNOLOGISTS

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INTRODUCTION

Good morning ladies and gentlemen of the Tropical and Sub-tropical Fisheries Technologists (TSFT). It is indeed a pleasure to extend my greetings to you on behalf of the 250 member Pacific Fisheries Technologists (PFT). Together with the Atlantic Fisheries Technologists (AFT), we three sister organizations share the common goal of providing the media for exchange of technical and scientific information among fisheries technologists of our respective regions.

PFT was started 40 years ago by west coast food technologists working in industry, government and academia. Its membership largely consists of residents of Alaska, British Columbia, California, Hawaii, Japan, Mexico, Oregon and Washington. PFT holds an annual meeting in rotation among British Columbia, California, Washington, Alaska and Oregon. This sequence may be broken if the membership inserts a special location. The 25th annual meeting was thus held in Honolulu, HI.

Informality characterizes PFT, both in its structure and function. The executive committee consists of the President and Secretary-Treasurer from the host region, area representatives from 7 regions, a representative-at-large, and the immediate past President and Secretary-Treasurer. The entire executive committee, except for the last two positions, are elected anew at the annual meeting which is usually held during early spring to coincide with the University spring breaks.

In order to foster the candid exchange of ideas, PFT does not publish abstracts or proceedings and selects secluded locations for its meetings. PFT, thus, provides an uninhibited forum for industry, government and academic representatives to freely air their views. Its lack of continuous executive structure, however, prevents PFT from developing a systematic plan and major decisions are usually passed on rather than being acted on.

FISHERIES OF PACIFIC COAST - PAST AND PRESENT

Commercial landings of fish and shellfish in the Pacific region are the largest in the nation. The total landings of this region in 1987 was 2,508,916 lbs. with a combined value of $1,389,454,000 (Fisheries of the United States, 1987, Current Fisheries Statistics No. 8700).

Table 1 lists the 1987 commercial landings of 5 Pacific coast states. Alaska landings are disproportionately large. Thus, the value of Alaska

landings is over 5 times greater than that of California, 10 times that of Oregon and over 6 times that of Washington.

Table 1. 1987 Fish Landings of PFT Member States

State	Thousand pounds	Million dollars
Alaska	1,697,547	941.7
California	451,663	173.2
Hawaii	16,088	29.1
Oregon	138,545	95.3
Washington	205,073	150.2

Table 2 lists the value of 1987 ex-vessel landings at 50 top U.S. ports within the Pacific region.

Table 2. Value of Fish Landed in Pacific Coast Ports in 1987

State	Major Port	Million dollars
Alaska	Kodiak	131.1
	Dutch Harbor	62.7
	Cordova	41.9
	Petersburg	36.9
	Sitka	33.6
	Ketchikan	22.8
Washington	Bellingham	27.4
	Seattle	21.9
	Westport	15.8
	Blaine	15.2
	Ilwaco	13.1
Oregon	Astoria	24.6
	Newport	21.9
	Coos Bay	19.1
California	Los Angeles	55.6
	Bodega Bay	13.4
	Eureka	12.5
	Fort Bragg	12.5

The statistics reported above, however, represent a rather recent development. Historically, California, Oregon and Washington played far more important roles in the region's fisheries than is indicated in these statistics. Many of you know or have heard about the rise and fall of the sardine industry in California. The tuna industry also had

a hay day in California in the 50's and 60's. We owe our basic understanding of the post-mortem biochemical changes in fish flesh to scientists working with tuna. These include the process of lipid oxidation, role of heme protein, histamine formation, spoilage indicators such as total volatile acids (TVA) and bases (TVB).

Another center of fisheries research is the Seafood Laboratory of Astoria, Oregon. It is located at the mouth of the Columbia River which once supported a thriving salmon industry and a busy port which landed varieties of fish including tuna. Technical problems and needed solutions were always waiting at this Lab's door step. The Oregon moist pellet developed at Astoria is still the standard of the salmonid nutritionists.

A major center of fisheries utilization research, however, was in Seattle. The National Marine Fisheries Service (NMFS), once known as the Bureau of Commercial Fisheries, had a full compliment of scientists in all aspects of fisheries technology. They were housed in a well equipped laboratory located next to the College of Fisheries of the University of Washington (UW). UW also launched an ambitious Food Science and Technology program in the College of Fisheries in the 50's. These two groups were quite active and served as the backbone of PFT for years. Research on highly unsaturated fish lipid, the process of nucleotide degradation, the nature of psychrophilic bacteria including that of non-proteolytic Clostridium botulinum, research on seafood irradiation and fish protein concentrate (FPC) were carried out by this group.

Another governmental group that made a significant contribution was the Technological Services Branch Laboratory of the Canadian Department of Fisheries and Oceans at Vancouver, British Columbia. This group worked most closely with the fishing fleet and the harvesting sector and was responsible for the development of many onboard handling methods such as the refrigerated sea water (RSW) chilling system.

FUTURE OF FISHERIES OF PACIFIC COAST

In Table 3, I have listed the seafood technological issues of the Pacific region. This list was excerpted from funding priorities identified in the Saltonstall-Kennedy program for 1987 (Federal Register 52: 20962-20972, June 3, 1987).

Table 3. 1987 NMFS Funding Priority for Research and Development in Support of Fishing Industry of Pacific Region

Region	Project
Pacific Islands	1. Improve yield of "sashimi"-grade tuna. 2. Reduce undesirable biochemical changes in oceanic pelagic fish.
California	1. Develop fishery for underutilized ground fish. 2. Provide seafood quality assurance and control from net to table. 3. Develop fishery for coastal pelagic species.
Pacific Northwest	1. Improve flatfish processing technology. 2. Develop harvesting and processing technology to improve seafood quality. 3. Develop whiting industry. 4. Conduct product development research for salmon.
Alaska	1. Develop flatfish processing technology. 2. Develop by-catch utilization technology. 3. Develop "surimi" product standards. 4. Evaluate seasonal variation of fish flesh quality. 5. Develop alternate pink salmon products.

As can be seen, major fisheries related activities are moving northward. If we include the emerging aquaculture industry of British Columbia, the need for technological input is clearly located in the northern Pacific region.

This region, however, lacks the technological infrastructure to cope with the anticipated future needs. NMFS has a utilization research group in Kodiak, Alaska. In 1981, the Alaska State Legislature established the Fishery Industry Technology Center (FITC) within the University of Alaska to enhance the technological competitiveness of Alaska's fishing industry. The University of British Columbia is reviving the seafood science and technology program, previously housed in the Technical Services Branch Laboratory. These groups are still new or small and may take years to realize their full potential.

We are seeing the change. Yesterday's technological need is replaced with the new and more complex ones. Research and development activities are also moving from one region to another. Nevertheless, if our charge

is to develop and strengthen the U.S. fishing industry to its fullest potential, our task has merely begun.

Thank you again for the opportunity to introduce PFT to you and I personally extend an invitation to all of you to attend our annual meetings. The next meeting is scheduled at the Hotel Captain Cook, Anchorage, Alaska from February 12 to 15, 1989. For further information, please contact:

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MODEL SEAFOOD SURVEILLANCE PROJECT: AN UPDATE

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As described at the 12th Annual Conference of the Tropical and Subtropical Fisheries Technology Society of the Americas in November, 1987, the National Oceanic Atmospheric Administration (NOAA) is conducting a Congressionally mandated Project to design an improved program of seafood certification and surveillance, entitled the Model Seafood Surveillance Project (MSSP). This Congressional mandate requires NOAA to conduct such a study that incorporates the HACCP system and coordinates and consults with the Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), the states and industry.

As the Project begins its second year, a progress report of the Project's activities is in order.

The study was originally requested by Congress in order to address the problem of increased consumer concerns and media attention over the safety, wholesomeness, and economic disparities of seafood for sale in the United States. Consumer organizations and the media are quick to point out that fishery products do not receive mandatory inspections similar to that of meat and poultry products that are inspected by the USDA. These organizations make allegations that an individual is six times more likely to get sick from eating seafood than from other protein flesh foods, based upon interpretations of the 1982 Centers for Disease Control (CDC) report on food borne disease outbreaks. Also, the media has concentrated on cases of product misrepresentation in which fish were sold under different specie names. This media examination into perceived consumer problems has been expanded with a variety of investigative television reports and major media coverage. For example, in 1987 West German officials found a parasite problem with some of their domestic fishery products. This was blown into a full scale public health issue by German television, resulting in a major (over 50%) loss of sales of fishery products. In 1988, a Canadian television magazine program similar to that of Sixty Minutes produced a controversial report on parasites in fishery products and the danger to the seals on the Atlantic Coast. Also in 1988, The Wall Street Journal carried an article on the safety of seafood products. All of these activities have been compounded with local newspapers, television, and radio spots which profess to be doing investigating reporting into the public health problems associated with seafood.

These activities have prompted numerous and various Congressional actions. In 1986, Congress asked the General Accounting Office (GAO) to conduct a survey. They also, in 1986, supplied NOAA with the

funding to conduct this seafood surveillance and certification design project. In 1987, Representative Dorgan issued a bill calling for mandatory inspection of seafood products under the USDA. Also in 1987, there were a variety of red meat and poultry hearings in the Senate and the House of Representatives which detailed the fact that fishery products are not inspected as meat and poultry are. Again in 1987, two other bills offered by Representative Stenholm and Senator Leahy were introduced. Representative Stenholm's bill called for extensive research into public health problems with food products. Leahy's bill called for inspection of seafood and the setting of water quality standards.

The GAO report, which was Congressionally requested in 1986, was issued in 1988. They concluded that "...there does not appear to be a compelling case at this time for implementing a comprehensive mandatory federal seafood inspection program similar to inspections used for meat and poultry..." However, they did believe that continuing attention and support are needed for a number of initiatives, including the development of the seafood surveillance model, which could be used as a basis for seafood inspection if such a program were to be desired.

Our Congressional charge then, within NOAA Fisheries, is to conduct the Model Seafood Surveillance Project, base it in on the Hazard Analysis Critical Control Point (HACCP) concept, and consult with FDA, USDA, the states and industry. Congress specifically stated in their appropriations that we were to use the HACCP concept. In our Project, we define Critical Control Point as a process step failure which may result in an unacceptable health or economic fraud risk.

The objective of our Project is to design an improved inspection system that provides reasonable consumer protection. We do intend to base it upon the HACCP concept and we also intend to give equable treatment to domestic, imported, and exported product.

Our approach, as demonstrated in Figure 1, is to divide consumer hazards in the consumption of foods, including seafoods, into three main categories: (1) product safety issues, (2) food hygiene issues, and (3) economic fraud issues. All of these affect product that is to be used for food.

Our Congressional deliverable will be a nationwide surveillance and certification system that is based upon these three issues. We intend to analyze the options available for such a system, perform appropriate economic analyses, and give a NOAA recommendation as to the option of choice. This will then be forwarded to Congress in answer to their charge. A complete description of the Project can be found in the proceedings of the 12th Conference of this society.

Within the Project process we have numerous components and partners. For product safety issues, we are working with the National Academy of Sciences (NAS) through a two year contract. We are also working with CDC by funding part-time personnel to code and issue

reports for the 1983-86 foodborne outbreak disease data. We are working with the industry through HACCP workshop programs (which will be further described in detail), National Fisheries Institute (NFI), selected Sea Grant personnel, and of course National Marine Fisheries Service (NMFS) staff. For food hygiene and economic fraud issues, again we are working with the industry through HACCP workshops, NFI, and our NOAA staff. And, for economic analysis, we have contracted to NFI, who is using the firm of Kearney-Centaur to perform the economic analysis of the individual HACCP models.

The HACCP workshops, as mentioned above, are being conducted by NFI's National Fisheries Education Research Foundation under a Saltonstall-Kennedy grant. Workshops are being conducted for processing, vessels, imports, wholesale, and retail. Out of these workshops a report is issued which describes the HACCP model plans to be used within that (those) commodity(ies) or industry(ies). These reports are submitted to industry for review; the design of the HACCP model is then tested in plants using on-site investigative techniques. This testing produces a large volume of data, which is analyzed. The results are forwarded to a steering committee, which had been previously nominated at the workshop. The steering committee generates a report in which they either make the request for a retest or submit it for industry review. This report is the basis for a quality assurance manual for the individual plants or industries to use and also will be the basis for which that commodity is outlined in the Congressional report.

Our entire Project intends to conduct 22 processing workshops (including imports), 9 vessel workshops, 5 distributor/wholesaler workshops, and 3 retailer workshops. We intend to do on-site testing of approximately 261 plants, 81 vessels, 30 distributors/wholesalers, and 27 retailers.

Our status as of October 15, 1988 indicates that we have completed 15 workshops, which includes 33 differing commodity products. Within these workshops, we have developed critical control point models, identified hazards associated with the end use of the product, and drafted research and regulatory recommendations. At each of these 15 workshops the participants have been asked to evaluate the workshop process. An evaluation summary indicates that we have had a total of 324 participants, 218 of which responded to the questionnaire for a 67% respondent value. This summary does not include the breaded shrimp workshop as they used a different format for evaluation. When we asked the participants if the meeting was well organized and if the objective was met, if the training materials were adequate, and if their understanding of HACCP was okay, we had 41% strongly agreed with those points and 54% said they agreed. We only had 4% respondents tell us that they disagreed with those three points.

Two commodities were completed as of October 15: the breaded and cooked shrimp. There are also two commodities in which site testing is

currently on-going: fresh and frozen fish for the four regions of the northeast, northwest, southeast, and southwest; and the raw shrimp model.

We have let the NAS contract and they are beginning work with an anticipated public meeting in January, 1989.

We have CDC processing their 1983-86 data in order to update the report of foodborne outbreak diseases; that data is to be in NOAA Fisheries in December, 1988.

Once all commodity and industry workshops, testing, and reviews are completed: (1) NOAA Fisheries will generate the options, (2) add to these options the NAS safety evaluation and the economic analysis of each model, (3) make our recommendation, and (4) report to Congress.

As the breaded shrimp and the cooked shrimp models are completed and have been reviewed by their respective steering committees, a synopsis of their recommendations follows: In the breaded shrimp industry the workshop participants developed some research recommendations which include: (1) more rapid and reproducible sulfite methodology, (2) better identification of filth and extraneous material in finished products, (3) determine the levels of pesticides/herbicides in foreign pond-raised shrimp, (4) look for chlorine alternatives, (5) determine the efficacy of microwave cooking in the destruction of microbial pathogens, and (6) conduct investigations concerning microbial pathogens of increasing importance, i.e., Listeria and Yersinia.

The cooked shrimp industry developed research needs very similar, i.e., (1) more rapid and reproducible sulfite methodology, (2) determine the effectiveness of sodium tripolyphosphate to minimize water loss, (3) research the safety of gas packed, vacuum packed, and modified atmosphere packed product, (4) determine effectiveness of sodium hydroxide as a processing aid, and (5) re-evaluate criteria for rejecting and accepting decomposed subsamples.

Regulatory recommendations from the breaded shrimp industry suggest: (1) standardization of state codes and regulations with a provision for federal preemption, (2) development of foreign Memorandum of Understanding (MOUs), (3) tightening the FDA Defect Action Levels (DAL's) for decomposition, (4) tightening the FDA microbial DAL's, and (5) development of specific Good Manufacturing Practices (GMP's).

The cooked shrimp industry's regulatory recommendations are looking for: (1) a standard of identity for cooked shrimp with a sunset provision, (2) product microbial guidelines to assure production under GMP's, and (3) strengthened decomposition criteria.

Within the breaded, cooked, and raw shrimp models we had 30, 31, and 24 operational steps, respectively. There were 9 critical control points in the breaded shrimp industry, 6 in the cooked shrimp industry, and 9 in the raw shrimp industry. Finally, we had 64% of the breaded

shrimp industry participate, 38% of the cooked shrimp, and 20% of the raw shrimp. (All of these figures are based upon the production values listed in the NOAA fisheries statistics database.)

With respect to shrimp testing, 9 out of the 49 plants that produce breaded shrimp (as listed in the NOAA Fisheries' statistics database) or 18% were sampled for on-site model testing. Within the cooked shrimp industry, 9 out of the 55 plants, or 16%, were tested.

Within the breaded shrimp processed critical control points, the workshop determined that there were, as stated before, 9 critical control points. These 9: purchasing and receiving, thawing, peeling, food additives, holding, battering and breading, hand battering, check percent of flesh, and racking, were examined in plants along with all other operational steps. From the testing, it was determined that there were some additional critical control points such as: size grading, weighing, labeling, and repack. These critical control points have been approved by the steering committee and are being forwarded to the industry for review.

The cooked shrimp process critical control points, in which there were 6 from the workshop, also were tested. It was determined from this testing procedure that an increase in the number of critical control points would be generated. In fact, the steering committee has added: size grading; thawing; cooling; head/peeled/deveined/grade after cooking; inspection and grading after cooking; pack, weigh, and labeling; refrigeration after cooking; chilling after cooking; and re-pack. The peeling/deveining machine step which was indicated.to be a critical control point at the workshop was not found to be critical within the testing procedure.

The steering committee also made a recommendation for sanitation critical control points. The breaded shrimp industry indicated that: (1) storage, label, and use of chemicals, (2) transfer and use of ice on unlike products, (3) food handlers washing facility, (4) absence of effective in-plant sanitation program, (5) water source, and (6) appropriate supervisors being held accountable for the cleanliness compliance of their employees, be considered critical points in sanitation compliance. The cooked shrimp industry agreed with all of the above and added another critical control point: cooked or finished products are not adequately separated by space or time from possible raw product contamination.

The fresh and frozen fish HACCP models are divided into four regions: northeast, southwest, southeast, and northwest. There were a varying number of operational steps for the production of their commodities, i.e., 25, 22, 26, and 26 steps, respectively, with 8, 5, 11, and 5 critical control points, respectively. Varying industry participation was found: 12% in the northeast, 36% in the southwest, 3% in the southeast, and 31% in the northwest, to give an overall nationwide participation of 19%.

Within the process of testing fresh and frozen finfish models, 4% of the plants in the northeast, 6% in the southeast, 13% in the southwest, and 5% in the northwest will be invited to participate in the process. There will be also two aquaculture plants in the southeast to determine their operational critical control points.

The fresh and frozen finfish workshops generated some research and regulatory recommendations. Research recommendations include: (1) establish a national data bank of fish protein patterns for species identification, (2) determine thermal death times of microbial pathogens by microwaving, (3) determine consumption patterns, both commercially and recreationally, (4) gather baseline data on levels of parasites per species, (5) develop a rapid practical test for scombrotoxin and also for ciguatoxin and, (6) develop a methodology for determination of added phosphates. Regulatory recommendations included: (1) give inspectors embargo authority, (2) increase penalties for species substitution, (3) offer the state the option to run the program under the federal guidelines, and (4) have a periodic review of all regulations to determine if they are still, in fact, current with technology and practices in the processing industry.

The MSSP fiscal year (FY 89) activities are going to be modified in order to generate an interim report to Congress. With the completion and economic analysis of the HACCP models for breaded, cooked, and raw shrimp, along with fresh and frozen finfish, the MSSP intends to deliver an interim report to Congress in the fall of 1989. This report will present the completed models, detail the approach for imported products, and outline the remaining work. In addition to the interim report, the intent is to continue in-plant testing and conduct vessel workshops. Also, upon receipt of the 1983-86 CDC foodborne outbreak disease reports, a summary will be completed in the attempt to determine where the real problems lie in seafood consumption. NAS will be supporting this effort with the continuation of their work in looking at the product safety aspects of seafood consumption.

SUMMARY

Our program has found that there are problems in the consumption of seafood, but they are limited. We feel that the media distortion on these problems will continue and the Congress will take action. We will complete our study in an appropriate fashion in order to deliver the most practical and economically feasible system for mandatory seafood surveillance and certification based upon the HACCP concept.

In summary, it is the MSSP's intent to fulfill our Congressional and industry obligations by completing our study. MSSP intends to deal effectively with imports and also intends to provide for state inspection under the federal developed guidelines. In fulfilling our Congressional and industry obligations, we feel that we will also fulfill the public expectations of an increase in consumer protection and reduction of the anxiety in the consumption of seafood products.

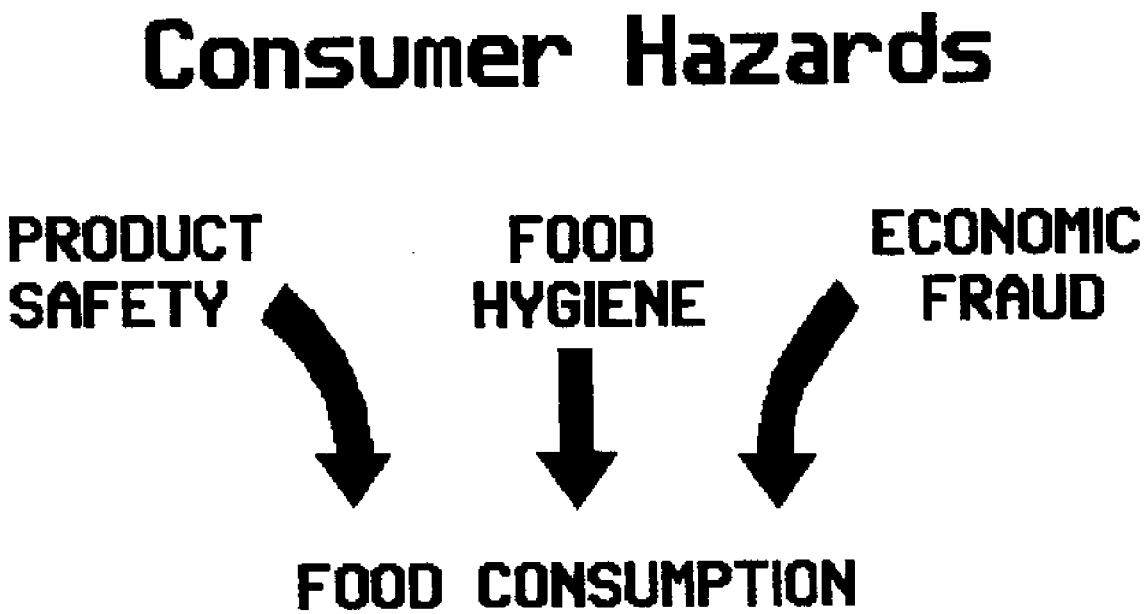

Figure 1. Potential Consumer Hazards in the Consumption of Seafoods.

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INTERSTATE SHELLFISH SANITATION CONFERENCE
DEVELOPMENT OF A SHELLFISH PLANT EVALUATION FORM

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INTRODUCTION

The Interstate Shellfish Sanitation Conference (ISSC) is composed of federal and state regulatory and advisory officials along with representatives of the molluscan shellfish industry. Its most basic mission is to enhance safety in the consumption of raw clams, mussels and oysters. The Food and Drug Administration (FDA) provides federal oversight for this cooperative effort and has final ruling over the National Shellfish Sanitation Program (NSSP) guidelines under which the participating states must operate. Uniformity and standardization of inspection and evaluation criteria are important within this national program.

One step taken by the ISSC at its 1986 Conference was the initiative to develop standardized forms to evaluate shellfish plants and shellfish growing waters. Other necessary steps to improve Program uniformity include federal/state training on the use of evaluation forms under Program guidelines and a move toward the best, reasonable uniformity in decision criteria and actions taken based upon the evaluations. This report will note the progress in the development of the shellfish processing plant evaluation form and further recommendations and actions by the Conference in this area.

PROCESS AND PROGRESS

Seeing the need for improved uniformity in the application of NSSP guidelines, the ISSC formed the Check Rating and Standardization Committee at the end of its 1986 Conference. The Committee was composed of state officials , industry representatives, FDA and NMFS personnel, and one international regulatory official. During early 1987 various sanitation and inspection forms and evaluation systems were reviewed, including both FDA and National Marine Fisheries Service (NMFS) processing plant inspection forms and some state inspection forms. At the 1987 Conference, the Committee drafted a form which it felt adequately covered the inspectional criteria of the NSSP Manual, Part II, as revised in 1986. The form had its basis in a model presented the previous year by the FDA to a committee which had been working to revise Part II of the NSSP Manual.

The form is a list of 48 items which should be reviewed and scored by the regulatory official. The items were weighted by the Committee according to public health relevance on a point scale from one to five, with an item weighted at 5 points as an item of very significant public health relevance. There are nine items noted as critical control points which are not scored, but each must be in compliance with the Manual for

an acceptable plant status rating. The Committee has recommended that points be deducted from the maximum score of each item based upon the degree of non-compliance with Manual guidelines (partial scoring).

Partial scoring has been one of the primary considerations of the Committee, and there is some concern that this will lead to increased subjectivity and non-uniformity. Some Committee members have said that an "all or none" scoring system is better. In either scoring method though, it is the uniformity and effectiveness of the training which should allow the rating official to either deduct a partial score or decide the "all or none" score of an item that may be somewhat out of compliance. Furthermore, up to this point the Committee has not determined what the recommended pass/fail criteria should be. Whether it is a raw score or possibly the number of more serious 4 or 5 point items out of compliance which will determine the acceptable status of the plant has not yet been recommended.

The 1987 Conference adopted the plant evaluation form for use in a voluntary, pilot trial by some states in 1987-88. The purpose of the pilot test was to evaluate how the form worked in a plant setting, to determine its harmony with the revised Part II of the NSSP Manual, to collect data on scores, and to gather comment. During later 1987, FDA's Northeast Technical Services Unit (NETSU) conducted the voluntary pilot test with several FDA regional shellfish specialists and state officials using the form at 28 plants, five of which were evaluated jointly by FDA and the state official. Comments were sent to NETSU, and a report was compiled and sent to the ISSC Board and Check Rating and Standardization Committee leaders in March, 1988. Following review and comment on the March report, FDA's NETSU issued a final report on the plant evaluation form pilot test along with recommendations in June, 1988.

At its 1988 Conference, the full Committee reviewed the report and comment on the pilot test. Several changes were made in the evaluation form, but the basic format, scoring and comment approach remained. It was suggested and agreed upon that the form and instructions be used to evaluate the plant, but that the report to be filed by both FDA and state personnel should more closely resemble FDA's current inspection form 483. Only those items (of the 48 item total) which are deficient should be recorded by both score and comment on the actual report forms. This system should reduce unnecessary paperwork. Because the evaluation and report form revisions proposed by the Committee are currently being performed by the FDA, the forms could not be included in this paper.

The Committee submitted full recommendations to the ISSC and FDA for a more complete and final pilot test of the plant evaluation form during 1988-89. Included in the recommendations were a proposed training sequence and suggestions for involvement of several personnel to design reasonable sampling into the pilot test. The Committee also developed a draft statement on the intent and purpose of the pilot test to those who would participate and an evaluation questionnaire for completion by all participants.

NEXT STEP

The Committee recommendations are under review by the FDA. Whether or not the FDA will complete the training/standardization and pilot test evaluations of selected plants prior to the 1989 Conference is unknown at present. After two years of development and an initial pilot test, the Committee believes that it is very close to a usable plant evaluation form. It is hoped that data and comment from the second, more complete pilot test can be used to finalize the form at the 1989 ISSC Conference. If that phase is completed and the form is finalized, the next crucial step by the Committee will be the discussion and recommendation on plant pass/fail criteria previously mentioned, i.e., how the form will be used by the FDA and states to determine individual plant acceptability. Committee recommendations on the form and the decision criteria used to determine plant status or rating will be voted upon by Conference delegates prior to national Program use.

ECOLOGICAL ASPECTS OF *LISTERIA*

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CLASSIFICATION AND HABITAT

Bergey's Manual of Systematic Bacteriology (20) currently lists the genus *Listeria* with "Regular, Nonsporing, Gram-Positive Rods." Eight species are included in the genus: *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. murrayi*, *L. grayi*, and *L. denitrificans*.

L. monocytogenes has been isolated from clinical infections, feces of healthy humans and animals, and the environment (20). *L. ivanovii* was first isolated from sheep in Bulgaria (27). *L. innocua* and *L. grayi* have been found in the environment and in human or animal feces (20).

L. seeligeri has been isolated from plants, soil, and sheep feces. *L. welshimeri* and *L. murrayi* have been associated with soil and decaying plants. *L. denitrificans* was first isolated from cooked ox blood (20).

Listeria monocytogenes:

Until the mid 1960s, *L. monocytogenes* was thought to be exclusively associated with clinical infections of humans or animals. It has since been isolated from numerous species of mammals and birds, including zoo, laboratory, wild, and domesticated animals, and house pets (10). The organism has been isolated from intestinal tracts of healthy humans, rodents, swine, and poultry (22); it has been recovered from decaying vegetation, ticks, pond-reared trout, crustaceans, stream water, silage, sewage, and soil (10). It has also been associated with food products, including milk, cheese, meat, fruits, vegetables (25), and seafood.

L. monocytogenes has been found on all continents except Antarctica (10). It grows at temperatures from 3 to 45°C, with an

optimum growth temperature of 38°C (18). It is capable of growth at a pH of less than 5.6 (7) and is often associated with moist environments.

The isolation of Listeria from decaying vegetation and its ability to multiply at low temperature and to survive for long periods in soil imply a saprophytic existence in the plant-soil environment which may serve as a natural habitat or reservoir (27, 28). Since ingestion is considered a primary means of transmission, the alimentary canal is an important portal of entry and exit for listeriae (4). Animal-to-animal transmission by the fecal-oral route therefore seems likely.

Soilborne organisms can be widely distributed by fecal carriers or application of manure as fertilizer. An ecological cycle can then be established which involves the shedding of Listeria from infected animals with subsequent contamination of water, soil, vegetation, and fishery products. Feeding of contaminated silage can lead to reintroduction into animals and contamination of milk and meat products (25).

LISTERIOSIS

Listeriosis is recognized most commonly in ruminants; it occurs frequently in the temperate zone but rarely in the tropics (11). A seasonal occurrence is evidenced by the presentation of most cases in winter and early spring. Common source epidemics of listeric encephalitis in ruminants are usually associated with feeding of spoiled silage. The bacteria are able to survive the harvesting process and to reproduce in storage of silage which does not develop a normal pH (4.0 to 4.5) (3, 4).

However, it cannot be implied that each instance of listeric infection following silage feeding results from ingestion of the organism. There is evidence that in listeric encephalitis of ruminants, exposure is via the upper respiratory tract, whereas oral exposure results in abortion in pregnant animals (9). A cow with listerial mastitis or encephalitis could become infected from soil, surface waters, dirty floors, or other environmental sources indirectly associated with the fecal-oral cycle (4).

After the mid 1970s, increases in human and animal listeriosis coincided. The increase seen in animals may have been associated with changes in agricultural practices such as big bale silage production (8). These increases also coincided with increased gastrointestinal disease in humans and use of untreated sewage sludge on agricultural land (26).

ENVIRONMENTAL ISOLATION OF *LISTERIA*

Animals, soil, vegetation:

Isolation of *Listeria* from a nonagricultural, residential, suburban community (28) indicated that the organism was associated with areas other than those involved in agricultural pursuits or use of farm animals. *L. monocytogenes* was isolated following overwintering of decaying moist vegetation, indicating the psychrotrophic properties of this organism. No bacteria were isolated in early fall when surface soil was relatively dry. On the other hand, the ability of *L. monocytogenes* to survive without significant loss for weeks in both moist and dry environments has also been reported (16).

In West Germany, *L. monocytogenes* was found to be present in a high proportion of plants, soil samples, and feces of deer and birds (26). The organism was isolated in greatest numbers from uncultivated soil and mud samples where the moist environment appeared to enhance its survival and multiplication. Fewer strains were isolated from uncultivated soil at a depth of 10 cm. The lowest numbers of bacteria were present in areas used for agricultural purposes. The bacterium was isolated from leaves both on the ground and from shrubs 50 cm above the ground. Isolation of *Listeria* from all sources was independent of the incidence of listeriosis in domestic animals in the same area (26).

Birds have been suggested as possible sources of listeriae in silage. Gulls and rooks often forage for insects among freshly cut grass wilting in fields. Gulls may act as vectors, transferring organisms in sewage from one place to another without becoming overtly infected and, therefore, play a significant role in initial contamination of grasses used for silage (8).

Fecal specimens from seagulls feeding at sewage sites were reported to have a higher rate of carriage of *Listeria* spp. than those from gulls feeding elsewhere (8). Birds feeding on sewage had a fecal carriage of listeriae of 26%. Fecal specimens from gulls feeding at other sites had a carriage rate of only 8%. No seasonal difference was found.

Fecal specimens from rooks normally presented a low incidence of listeriae; however, on one occasion which coincided with the nesting season and peak period for listeriosis in sheep, 12 of 20 specimens contained *Listeria*. From these, 8 strains of *L. monocytogenes*, 5 strains of *L. innocua*, and 1 strain of *L. seeligeri* were isolated (8).

Waste products:

It has been reported that of the total sludge produced at sewage works in England and Wales, approximately 20% is disposed of at sea, 40% is applied to agricultural land, and 40% is applied to other land or incinerated (24). The most popular method for disposal of liquid sludge is application to land, which poses potential risk to human and animal health. Where disease outbreaks have been associated with spreading of sewage sludge, the majority were due to spreading of raw sludge or night soil.

Counts of L. monocytogenes in sewage sludge have been reported to range from 800 to >18,000 per liter (23). These numbers were of concern because of the practice of spraying sludge on agricultural land. Results of survival time studies for sewage sludge sprayed on land showed no detectable decrease in numbers of L. monocytogenes at 8 weeks following spraying.

In 1986, Al-Ghazali and Al-Azawi (2) reported the isolation of L. monocytogenes from a sewage treatment plant in Baghdad, Iraq. Listeria was isolated from all test samples at each stage of treatment. Highest numbers were recorded in raw sewage sludge, while the lowest were observed in sludge cake. Digested sludge also showed a decrease in the number of bacteria. Low numbers recorded in sludge cake during the summer period coincided with low moisture content, which was less than 3.7%. The pH of the sludge cake ranged from 6.1 to 8.6.

L. monocytogenes has been isolated from effluents from sewage treatment plants (2), poultry packing factories, abattoirs, and cattle markets (23). Surface waters receiving such effluent could become a route for recycling these bacteria.

Recently, fish and crustaceans have been reported to harbor or be infected by L. monocytogenes. Consumption of shellfish or raw fish has been implicated in the development of listeriosis in New Zealand (15). This has become a major concern of the food processing industry.

It is possible that certain aquaculture practices could lead to contamination of fish and seafood products meant for human or animal consumption. The practices are the same as those used in agriculture, i.e., use of wastewater or manure as fertilizer. Although use of manure is not a common practice in the United States, it is widely employed in third world countries from which the United States imports most of its shrimp. Examples of manuring in aquaculture are the application of pig and duck manure and human waste in China. In New Zealand, where spiny rock lobster are being raised for export, goats graze near pond areas (17). The combination of fish feed and animal excrement added to aquaculture systems provides conditions for growth of pathogens which may then accidentally enter the food chain.

Processing plants:

In addition to the direct routes of contamination already mentioned, unsanitary conditions in the processing plant can lead to contamination of food products. The ability of L. monocytogenes to grow at low temperatures raises concern over its potential as a post-process contamination agent for refrigerated foods (16).

In 1988, Knight et al. (13) reported the isolation of Listeria from a food processing plant. Swab test samples from sanitized surfaces were positive for L. monocytogenes. Some suggested sites where Listeria may be found were unchlorinated water supplies, cool wet areas, equipment legs, rusted framework, drains, refrigeration units, air-handling systems, conveyers, and etched stainless steel surfaces.

Recent findings of the Food and Drug Administration (FDA) have raised questions regarding the focus of contamination (internal vs. external) of Listeria in seafood products and the means of acquisition of the organism (pre- vs. post-processing). Studies conducted at the FDA Fishery Research Branch on laboratory-infected fresh shrimp and contaminated market samples have suggested that contamination is external. However, it remains to be shown whether contamination of imported frozen products occurs during processing or from unsanitary aquaculture practices.

SUMMARY

Listeria is present in the environment worldwide. Its reservoir is a complex interaction of soil, leaf litter, sewage, silage, and infected animals, principally sheep, goats, cattle, domestic fowl, and wild birds (1). The ability of the organism to survive extreme environmental conditions is of utmost importance. The persistence of Listeria in both agricultural and nonagricultural areas and its potential for surviving in other environments should not be ignored, especially with regard to potential foodborne sources.

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LACTIC ACID USE IN SEAFOOD MICROBIAL CONTROL

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Lactic acid is an established food processing aid performing many functions, including antimicrobial activity, on many foods. However, there is little information available in this area concerning seafood. Therefore, the bactericial effects of lactic acid was tested on crab meat, oysters, and shell-on and peeled shrimp. Lactic acid concentrations, ranging from 1.5, 3.0, and 6.0 percent, were used to treat samples at 1, 10, and 120 minutes. In addition, crab meat and shrimp samples were inoculated with Listeria monocytogenes and treated with lactic acid. Experimental results indicate that lactic acid reduced the microbial levels of crab, oyster, and shrimp meats as well as the pathogen levels of the inoculated samples.

Materials and Methods

Sample Procurement

Florida based firms were the source for the headless shell-on and peeled shrimp (Tampa Maid, Inc., A.P. Bell Fish Co., Singleton Shrimp Co. Inc., and Bee Gee Shrimp Co. Inc.); cooked and picked blue crab meat (Sea Sweet Co. Inc.) and shucked oysters (Leavins Seafood Co. Inc.). All samples were chilled and transported to the laboratory. In most instances the product was received within 24 hours post-processing. The raw shrimp were obtained in the frozen form which is typical for over 80 percent of the incoming product for domestic processing. Shrimp samples were thawed slowly in refrigeration prior to treatments. The blue crab meat was obtained on the day of processing as 'fresh' picked and chilled from the whole cooked crab. The fresh, raw oysters were obtained on the day of shucking.

Lactic Acid Treatments

Shrimp (shell-on and peeled), blue crab meat and oyster meats were dipped (complete submersion) in 1.5, 3.0, and 6.0 percent lactic acid for 1, 10, and 120 minutes. Control (untreated) samples were exposed to 0 percent lactic acid (distilled water) at the same pre-determined exposure time intervals as the experimental treatments. All dips were performed at room temperature (23-25°C).

Inoculation Techniques

Peeled shrimp, were inoculated with 10^8-10^9 per ml of Listeria monocytogenes, (ATCC 15313) at room temperature for 10 minutes and drained for 10 minutes. Following the inoculation, half the Listeria contaminated shrimp were treated with a 1.5 percent lactic acid dip for 10 minutes. Bacterial counts for total heterotrophic bacteria and Listeria monocytogenes were taken (zero reference point). The control half (non-dipped) and treated shrimp were then frozen (-20°C). Periodically, (days 7, 14, 21, and 28). frozen samples were removed from the freezer and enumerated for total heterotrophic plate count and Listeria without allowing for thaw. The intent was to monitor for bacterial consequences to post-treatment while in frozen storage.

Sample Preparation

Experimental and control samples were taken from the freezer, one at a time to prevent thawing, and deposited into a preweighted Waring blener jar, the weight of meat recorded, then an equal weight-volume of phosphate buffered saile (pH 7.4) added. The mixture was homogenized (10-15 second burst) and the resulting homogenate was then diluted with 10 percent peptone water from 10^{-1} to 10^{-5}. These dilutions were used for determining the aerobic plate count and Listeria count.

Total Aerobic (Heterotrophic) Plate Count

Standard plate count agar (Difco) was used for the determination of total aerobic plate count (FDA, 1978) in the shrimp, blue crab, and oyster samples. The pour plate method was used and plates incubated at 37°C for 48 hours.

Listeria Count

Listeria monocytogenes was enumerated using surface plating on modified McBride agar (MMA). Listeria colonies from MMA were selected with 45° oblique trans-illumination (FDA, Bacteriological Analytical Manuel, 6th Ed.). Ten typical (blue-blue-grey) colonies formed by Listeria per plate were picked and streaked for seperation on trypticase soy agar with 0.6% yeast extract for confirmation.

Confirmatory test included: catalase reaction (positive), Gram stain preation and morphology (gram-positive coccoid to diptheroid-like rods), characteristic tumbling motility (when examined microscopically using a 0.85% saline wet mount), umbrella shaped growth when grown in SIM motility medium at 27°C, B-hemolysis on sheep blood agar, carbohydrate fermentation (dextrose, positive; esculin, positive; maltose, positive; rhamnose, positive; mannitol, negative; and xylose, negative), nitrate reduction (negative), and Voges-Proskauer (positive). The results were compared with typical reactions for Listeria monocytogenes acording to FDA's Bacteriological Analytical Manuel (6th Ed.) for Listeria isolation. All colonies identified as L. monocytogenes were transfered twice on tryptose agar slants and incubated for 24 hours at 34°C; the last transfer included two tubes. The Listeria growth in both were harvested in a total of 3 ml of Difco FA buffer to a 16 * 125 mm screw cap tube. This tube was then heated at 80°C for 1 hour, and the Listeria were pelleted by centrifugation. Approximately 2.2 ml of the supernate was removed, and the Listeria resuspended in the remaining buffer and used in the slide aggulation reaction using Difco sera.

Sensory Assessments

Based on product comparisions with informal yet experienced panelist to determine flavor and texture acceptability.

Results and Discussion

Shrimp, both shell-on and peeled, were exposed to lactic acid concentrations of 1.5, 3.0, and 6.0 percent. Exposure times were set at 1, 10, and 120 minutes. Experimental results were similar for both the shell-on and peeled shrimp (Fig's. 1,2). Observed results were that untreated shrimp had the highest bacterial counts per gram meat, as would be expected. With a 1.5 percent lactic acid concentration, a significant reduction in bacterial counts occured. Lactic acid concentrations of 3.0 and 6.0 percent also yielded a decrease in bacterial counts, with the lowest bacterial counts at a 6.0 percent concentration, although decreases were not as large as at the 1.5 percent concentration. In all instances, a 1.5 percent concentration had the largest reduction in bacterial counts, with a 6.0 percent concentration having the lowest bacterial counts overall. Concentration results showed that as lactic acid concentrations increased, bacterial counts per gram meat were reduced.

Fig. 1

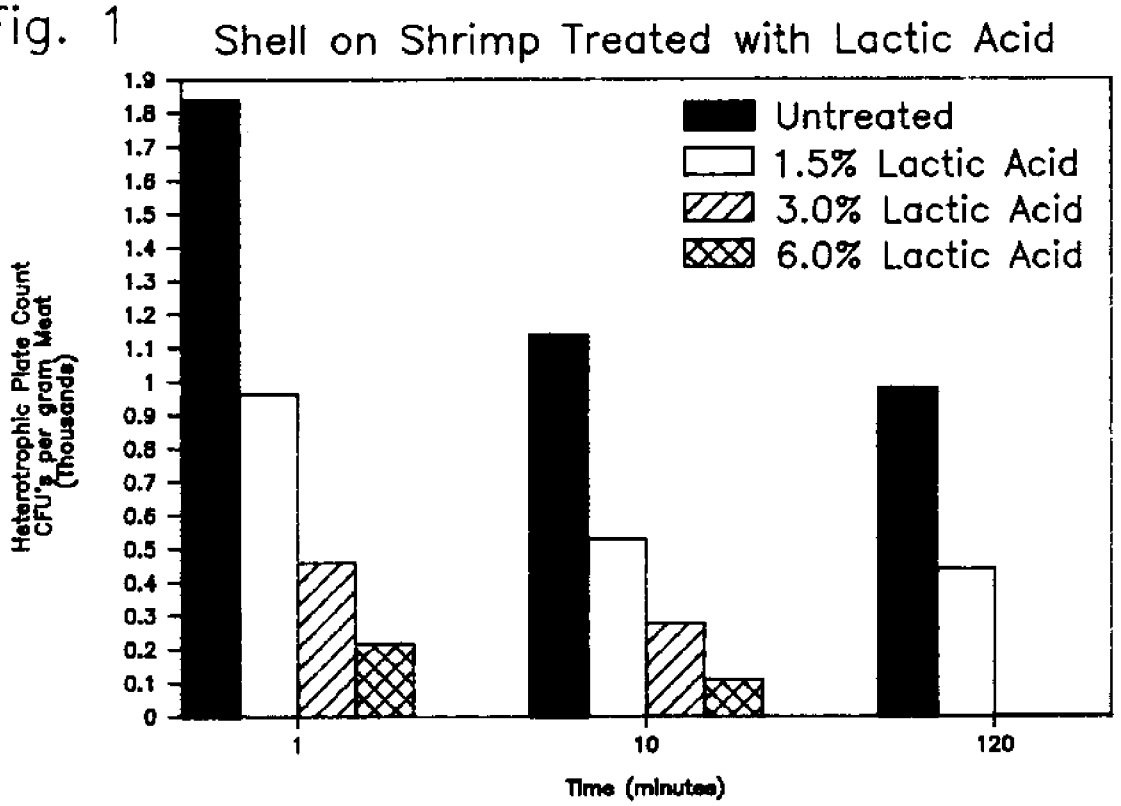
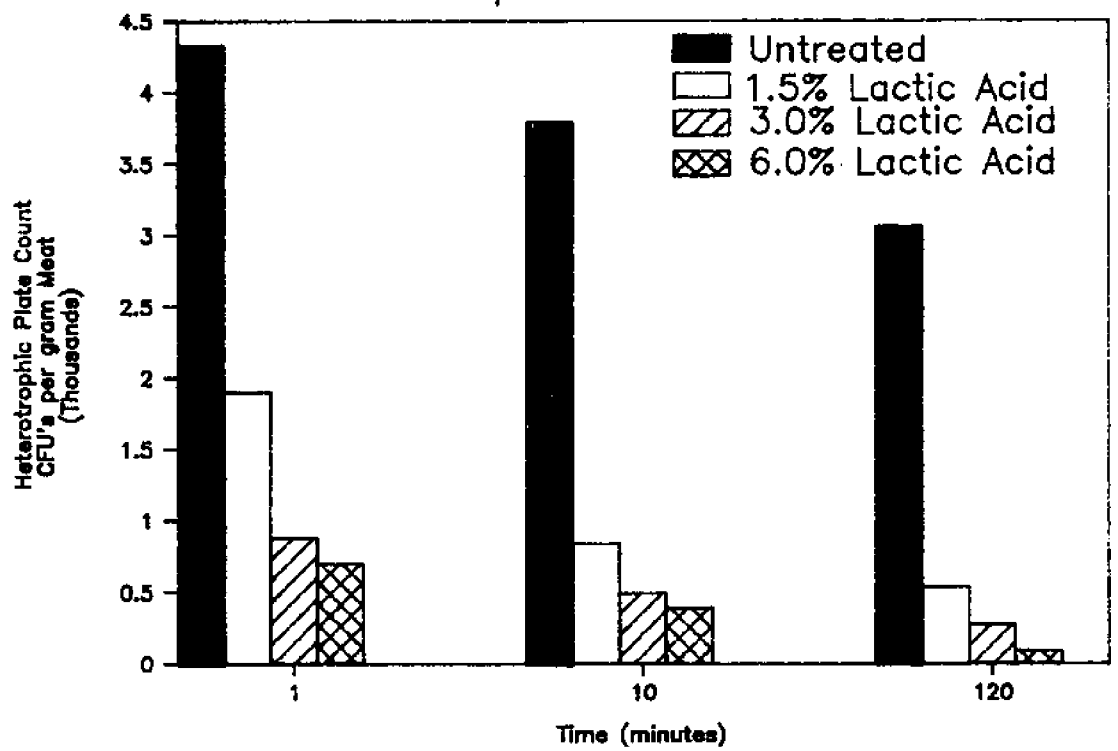

Shell on Shrimp Treated with Lactic Acid
Untreated
1.5% Lactic Acid
3.0% Lactic Acid
6.0% Lactic Acid
Heterotrophic Plate Count CFU's per gram Meat (Thousands)
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
10
120
Time (minutes)

Fig. 2


Peeled Shrimp Treatment with Lactic Acid
Untreated
1.5% Lactic Acid
3.0% Lactic Acid
6.0% Lactic Acid
Heterotrophic Plate Count CFU's per gram Meat (Thousands)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1
10
120
Time (minutes)

Exposure time was also influencial upon bacterial counts per gram meat. An untreated 10 minute exposure soak showed a decrease in bacterial counts when compared to bacterial counts for an untreated 1 minute exposure soak, while an untreated 120 minute exposure soak further decreased bacterial counts over an untreated 10 minute exposure soak. Likewise, a 1.5 percent 1 minute exposure soak was higher in bacterial counts than for a 1.5 percent 10 minute exposure soak, with a 120 minute exposure soak at 1.5 percent having the lowest bacterial counts. These observations were consistent when comparing 1, 10, and 120 minute exposure time soaks with 3.0 and 6.0 percent concentrations against one another, respectively. Exposure time results showed that as exposure time soaks increased, bacterial counts per gram meat were reduced.

Crab and oyster meats (Fig's. 3,4) were also separately tested using the varying lactic acid percent concentrations and exposure time soaks. Experimental results from these tests followed similar patterns observed while using the shell-on and peeled shrimp.

Results from the individual concentration and exposure time experiments with shrimp, crab and oyster demonstrated an inverse relationship with regard to bacterial counts. That is, as both concentration and exposure time were increased, there was a greater decrease in bacterial count reduction per gram meat. For this reason, it is a combination of both concentration and exposure time which is most effective in reducing bacterial counts.

After examining normal bacterial flora reaction to various lactic acid concentrations and exposure times, it was then essential to determine if human pathogens, namely Listeria monocytogenes, would also be susceptible to the effects of lactic acid concentration and exposure time as had the normal bacteria flora. Bacterial counts for Total heterotrophic plate count and the survivability of Listeria monocytogenes on frozen shrimp were examined. Looking at Total heterotrophic count (Fig. 5), there was a gradual reduction of heterotrophic bacterial counts over the entire testing period for untreated frozen shrimp, which was due to storage at -20°C, without any drastic heterotrophic bacterial count reductions. Upon addition of a 1.5 percent lactic acid concentration with a 10 minute exposure time, herterotrophic bacterial counts dropped slightly at the zero time reference, which was expected from earlier studies. However, seven day reference samples showed a marked reduction in heterotrophic bacterial counts for the treated frozen shrimp, with gradual decreases in heterotrophic bacterial count reductions following for the remainder of the testing period, much like what is seen for the untreated frozen shrimp. Total Plate Count results showed that a 1.5 percent lactic acid concentration and 10 minute exposure time was capable of reducing heterotrophic bacterial counts, but when combined with frozen storage, a major reduction in herterotrophic bacterial counts can be achieved.

Fig. 3 Crab Meat Treatment With Lactic Acid

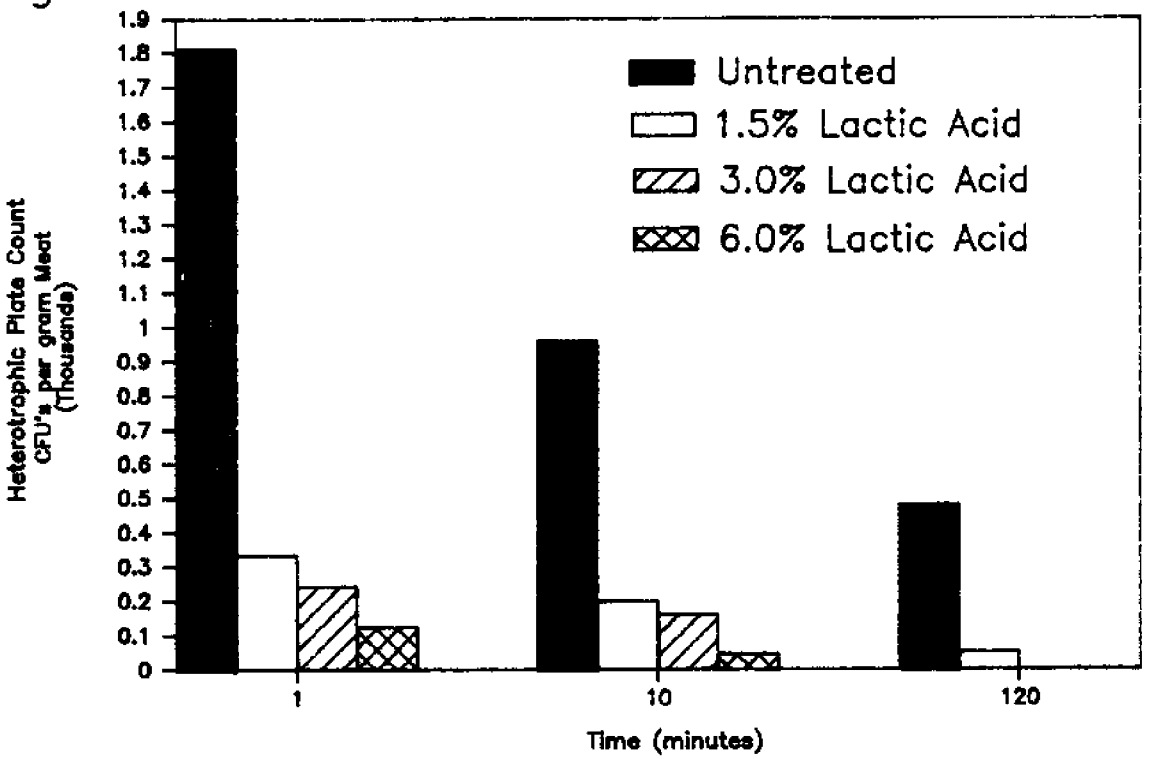


Fig. 4 Oyster Meat Treatment with Lacitc Acid

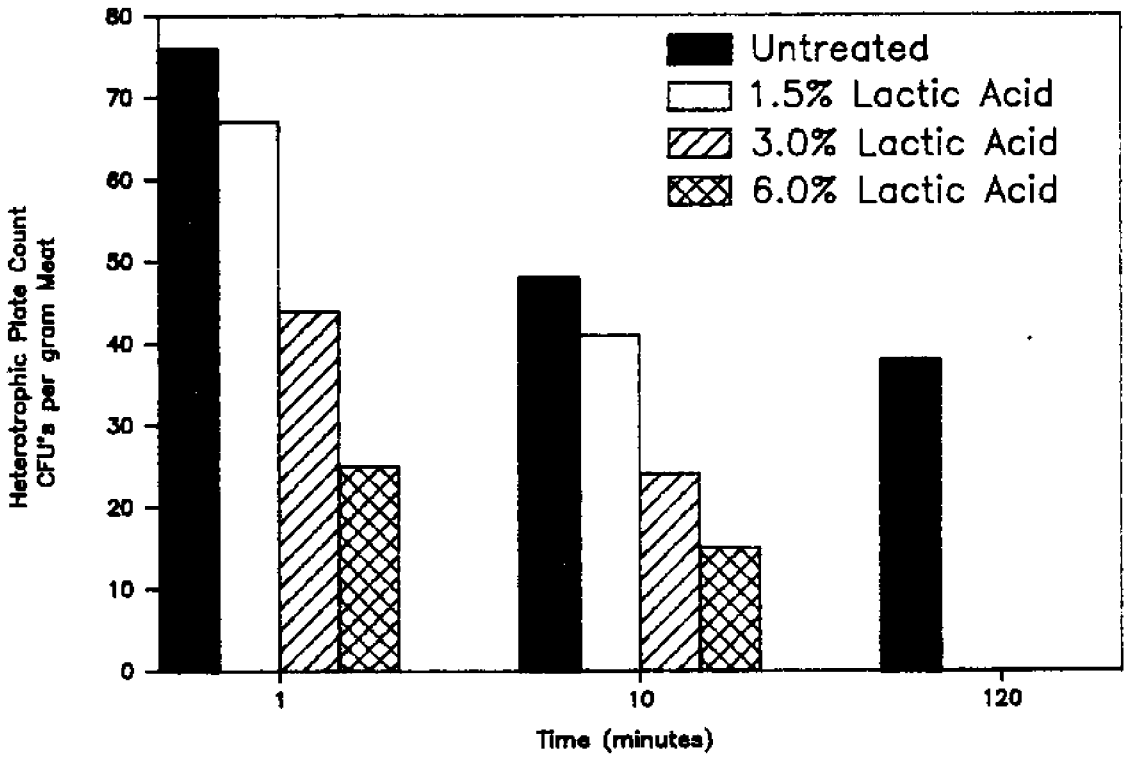


Fig. 5

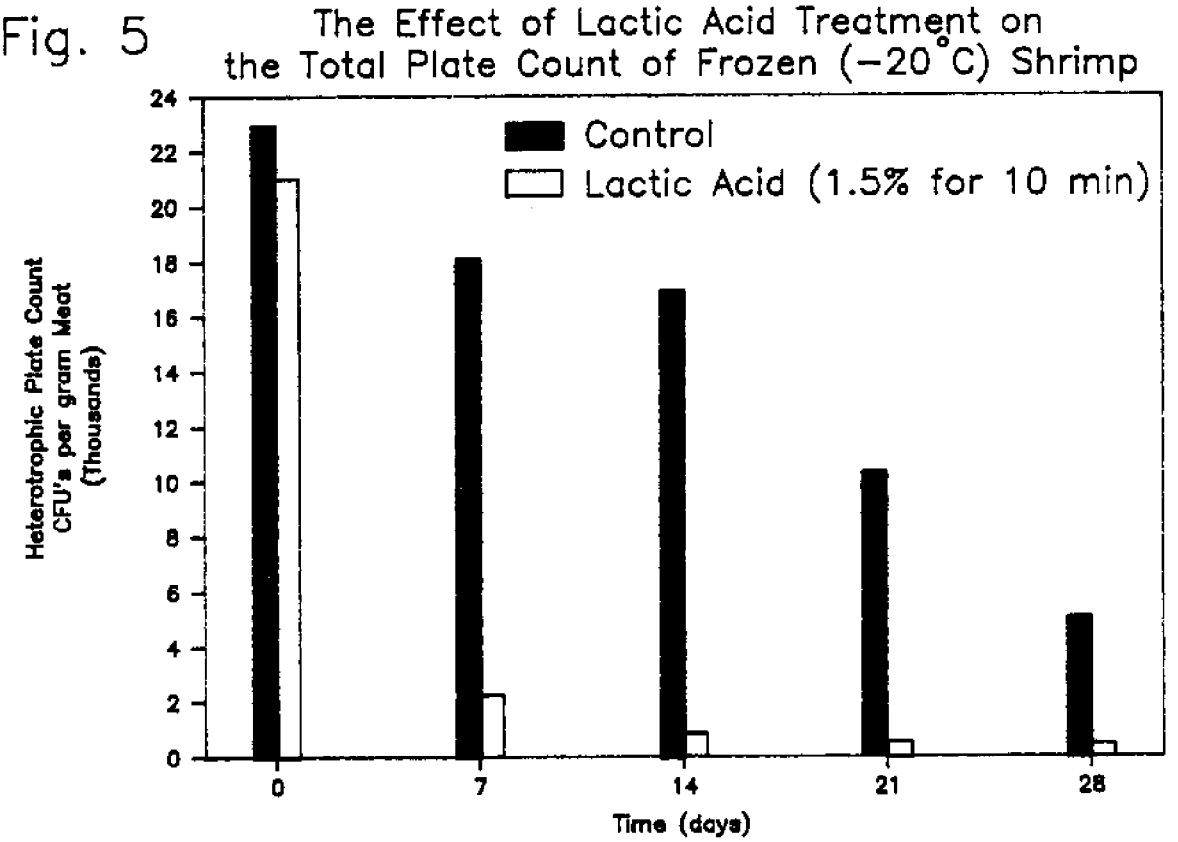
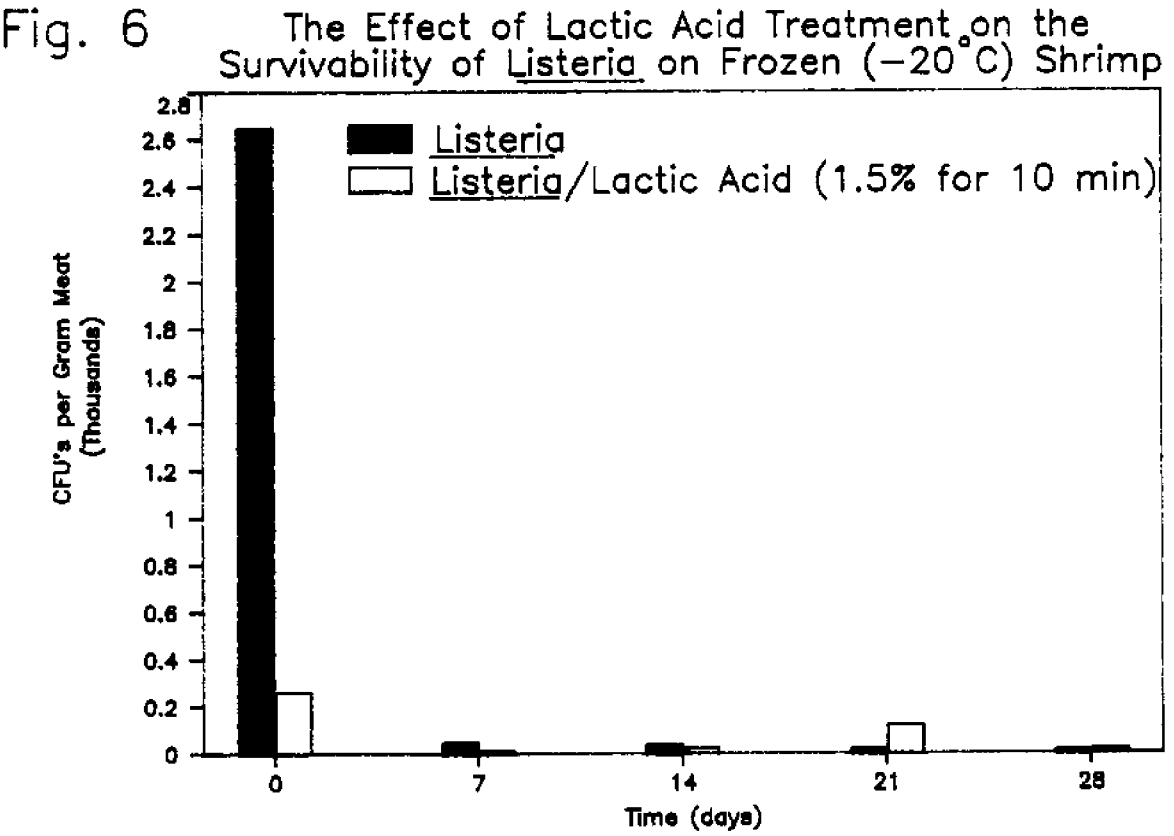

The Effect of Lactic Acid Treatment on the Total Plate Count of Frozen (−20°C) Shrimp
Control
Lactic Acid (1.5% for 10 min)
Heterotrophic Plate Count CFU's per gram Meat (Thousands)
24
22
20
18
16
14
12
10
8
6
4
2
0
0
7
14
21
28
Time (days)

Fig. 6


The Effect of Lactic Acid Treatment on the Survivability of Listeria on Frozen (−20°C) Shrimp
Listeria
Listeria/Lactic Acid (1.5% for 10 min)
CFU's per Gram Meat (Thousands)
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
7
14
21
28
Time (days)

Listeria survivability results (Fig. 6) indicated a high inoculation of Listeria at the zero reference point. Seven day reference samples of frozen shrimp showed a dramatic reduction in Listeria counts, due to Listeria's inability to survive at a temperature of -20°C. Listeria counts steadily dropped slowly throughout the remaining test period, with continued exposure to frozen conditions. When Listeria inoculated samples were subjected to a 1.5% lactic acid concentration at a 10 minute exposure time, Listeria counts were reduced substantially at the zero reference point. Listeria counts further decreased at the seven day reference point and remained steady for the duration of the experiment. There was a slight increase at the twenty-one day reference point, but with results from the fourteen and twenty-eight day reference points showing the same amount of Listeria reduction, this was concluded to be an error in dilution of the sample.

The largest decrease in Listeria count reduction, whether untreated or treated with 1.5 percent, took place within one week of storage at -20°C. Storage of samples for one week at -20°C was more efficient in reducing Listeria counts than a 1.5% lactic acid 10 minute exposure at the initial start of the experiment. However, at any reference point during the experiment, inoculated samples treated with 1.5% lactic acid for a 10 minute exposure showed a larger decrease in Listeria reduction than samples not treated with the lactic acid.

Before starting Listeria work with lactic acid, several sensory test were conducted to evaluate lactic acid effect on product appearance, odor, flavor, and texture. All varying lactic acid concentrations were used with either a 10 or 120 minute time exposure.

Ten minute exposure soak results of shell-on and peeled shrimp (Fig's. 7,8) indicated that a 1.5 percent lactic acid concentration was acceptable in all catagories of evaluation and was indistinguishable from the control (untreated). Shell-on shrimp at 3.0 percent lactic acid was acceptable in three catagories but unacceptable in texture; Meat where the head had been removed showed a slight hardening and was undesirable. Peeled shrimp at 3.0 percent lactic acid was acceptable for appearance only. The odor had a scant acidic smell, the flavor had a superficial vinegary taste, and meat texture was slightly hard where the head had been removed. With 6.0 percent lactic acid, all evaluation catagories were unacceptable for either shell-on or peeled shrimp. Shell-on and peeled shrimp meats had a slight translucent appearance thought to detract from product value, odor was acidic, a mild acidic taste pervaded the flavor, and meat texture was hard where the shrimp heads had been removed.

Fig. 7

10 min. soak time SHELL–ON

Lactic acid %	Appearance	Odor	Flavor	Texture
0%	2–3	2	2–3	2
1.5%	2–3	2–3	2–3	2–3
3.0%	2–3	2–3	2–3	5
6.0%	5–6	5–6	4	6

2–Like 4–Neutral 6–Dislike

Fig. 8

10 min. soak time PEELED

Lactic acid %	Appearance	Odor	Flavor	Texture
0%	2	2	2–3	2
1.5%	2	2–3	2	3
3.0%	2–3	4–5	5	5–6
6.0%	4–5	4–5	6	5–6

2–Like 4–Neutral 6–Dislike

Fig. 9

120 min. soak time SHELL–ON

Lactic acid %	Appearance	Odor	Flavor	Texture
0%	2–3	3	2–3	2
1.5%	2–3	4	4–5	4–5
3.0%	5–6	4–5	4	5
6.0%	5–6	6	7	6

2–Like 4–Neutral 6–Dislike

Fig. 10

120 min. soak time PEELED

Lactic acid %	Appearance	Odor	Flavor	Texture
0%	2	2	3	3
1.5%	4–5	4	5	6–7
3.0%	6–7	4–5	5–6	6–7
6.0%	7	4–5	7	7

2–Like 4–Neutral 6–Dislike

One hundred-twenty minute exposure soak results of shell-on and peeled shrimp (Fig's. 9,10) were quite different from those of the 10 minute soak. Only shell-on shrimp at 1.5 percent lactic acid was acceptable of all the evaluation catagories. Problems noted during the 10 minute exposure soaks became modified at the 120 exposures. With increased exposure time and lactic acid concentrations the appearance of the shrimp meat became more translucent, odor became more pronounced with an acidic smell, flavor took on a potent vinegary taste, and meat texture hardened even more where the shrimp heads had been removed while the body began to take on a pulpy feel.

After reviewing the data from the sensory test and noting that a 1.5 percent lactic acid concentration with a 10 minute exposure soak did not significantly alter end product appearance, odor, flavor, or texture, it was this reason that lactic acid concentration and time exposure of 1.5 percent and 10 minutes were chosen to treat the L. monocytogenes inoculated samples.

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Ozone Depuration of Vibrio vulnificus From the Southern Quahog Clam.

by

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INTRODUCTION

Recent awareness by the shellfish industry of Vibrio infections, especially V. vulnificus transmitted by raw shellfish consumption has prompted a re-examination of the depuration process. Various methods of depuration exist and are currently used worldwide (Blogoslawski and Stewart, 1983). One method that is of interest because of its powerful ability to inactivate microorganisms (Blogoslawski and Monasterio, 1982) and possibly certain marine toxins (Blogoslawski and Stewart, 1977) is ozone. Ozone is currently used in Europe as a method of depurating shellfish (Fauvel, 1963). For these reasons, it was of interest to evaluate the antimicrobial effectiveness of ozone against V. vulnificus in seawater and the southern quahog, Mercenaria campechiensis.

MATERIALS AND METHODS

Mercenaria campechiensis

Southern quahog clams, Mercenaria campechiensis, of approximately the same shell length (8-12 cm) were collected from the Indian River shellfish relay lease and the intercoastal waterway at Melborne, Florida. The clams were transferred to coolers containing water from the collection sites. Upon arrival, the specimens were then move to 210 liter holding tanks. The holding tanks contained dechlorinated tap water prepared to the same salinity as that of the collection sites. Clams were maintained in the holding tanks for a period 1-2 weeks to allow for adequate acclimatization to the laboratory environment. The clams were fed a mixed culture of algae consisting of Chlorella and Chlamydomonas. The clams were fed on alternate days until three days before inoculation with V. vulnificus. Algal cultures were added by slow drip infusion at a rate of 50 ml per minute.

Vibrio vulnificus

Vibrio vulnificus for both disinfection and artificial inoculation studies was prepared by streaking an isolated colony on a fresh alkaline peptone agar plate and incubating for 18-24 hrs at 37°C. Isolated colonies were inoculated into 1 liter of tryptic soy broth (TSB) containing 2.5% NaCl and incubated for 10 hours at 37°C. The culture density was determined by MPN (Standard Methods, 1985).

Artificial infection was performed in 210 liter aquaria. Clams were infected with V. vulnificus 8 hours prior to depuration studies. One hundred ml of V. vulnificus culture at a density of 2.4×10^9/ml were mixed with one liter of algal culture at a density of 1×10^6/ml. Background levels of Vibrio in shellfish were determined before inoculation. Immediately preceding depuration experiments, samples were taken to determine the extent of Vibrio uptake and to establish the precision of the uptake rate.

Four specimens of M. campechiensis (100g or greater of shellfish meat) were used in each shellfish assay for V. vulnificus. Specimens were scrubbed under running tap water then opened with an alcohol flamed oyster knife. The meat, including the liquid inside the shell, was placed in a sterile Waring blender jar. Samples were homogenized in an equal w/v of peptone water containing 2.5% NaCl. The homogenate was serially diluted with peptone water containing 2.5% NaCl and inoculated into 3 tube replicates of alkaline peptone broth containing 2.5% NaCl to determine the MPN.

Glassware Preparation

Oxidant demand free glassware was used in the disinfection experiments and in the quantification of residual oxidant levels. The glassware was placed in a tank containing 20 liters of artificial seawater which was subjected to ozonation for a period of 1 hour. Glassware remained in the ozonated seawater for 24 hours, at which time it was removed and placed in a 103°C drying oven. The glassware was covered with aluminum foil until needed.

Production of Ozone

Ozone gas was generated by corona discharge (Annox OPT Portable Ozone Generator Model HFC-1000) supplied with medical grade oxygen. Ozone gas flow was set at 1.2 liters per minute controlled by a teflon flow meter. Ozone was bubbled into artificial seawater via crystalline alumina gas diffusing stones.

Measurements were made by extracting five milliliters of a test solution and adding them to a neutral potassium iodide (KI) solution at room temperature. This was allowed to react for 30 minutes in the dark (Shechter, 1973). At the end of this time the absorbance of triiodide produced was measured using a 1 cm path length at a wavelength of 352 nm. The remaining 500 ml portion of the sample was titrated using the iodometric method number 408A (Standard Methods, 1985) for determining residual chlorine. The method was modified for bromine residuals that are present in

ozonated seawater. Values were expressed in parts per million (ppm) of bromine residual. A standard curve was prepared relating triiodide absorbance at 352 nm to oxidant concentration as measured by the iodometric titration method.

Pilot-Scale Systems

The experimental tank used in pilot-scale studies was of wooden construction (see Figure 1). All seams were sealed with silicon, and the entire tank was painted with two coats of coal tar epoxy paint. An epoxy paint was used to ensure a water tight system and to resist oxidation from ozonated seawater. The tank was divided into two identical but separate sections. The dimensions of each section was 240 x 45 x 25 cm and held approximately 300 liters of seawater. Each section was equipped with a 1710 liter per hour (LPH) impeller type pump. Each section was equipped with an in-line spiral wound cellulose 20 micrometer pore size cartridge filter to remove particulate matter. Flow rates were controlled by a PVC and stainless steel valve, and measured with a 0-38 LPH float-type flow meter.

Disinfection Studies

Each disinfection experiment included of sampling at eight or more predetermined time periods. In some experiments sampling times were varied to assess oxidant demand of the inoculum. Five ml aliquots were withdrawn and placed into sterile 15 ml test tubes containing 0.1 ml of 10% $Na_2S_2O_3$ to destroy residual oxidant and cease bactericidal effects. Concurrently, samples were removed for residual oxidant determination.

The reaction vessel consisted of an oxidant demand free flask containing a magnetic stirring bar and 500 ml of sterile artificial seawater. The contents of each flask were subjected to 2 1/2 minutes of ozonation. The inoculum consisted of 7 ml of washed V. vulnificus cells. The titer of V. vulnificus inoculum was 2.4 x 10^8/ml in the reaction vessel. Ten milliliter samples were removed at 5, 15, 30, 60, 120, 180 and 240 seconds for bacterial and residual oxidant analyses.

Depuration Studies

Each experimental trial consisted of recirculating ozonated or aerated seawater through the depuration system (Figure 1). Each section contained approximately 40 clams. Four specimens (100g or greater of shellfish meat) were collected at timed intervals for enumeration of Vibrio organisms. The microbial levels in control specimens allowed for an evaluation of any natural microbial die-off. Samples of overlying seawater and shellfish meat were tested for Vibrio organisms to distinguish between depuration and disinfection. Oxidant levels were monitored throughout the trials in the experimental section.

Depuration runs were comprised of five sampling periods at 0, 2, 6, 12, and 24 hours. At each sampling period four specimens of M. campechiensis were collected. Seawater samples were also collected to monitor changes in Vibrio levels using the MPN

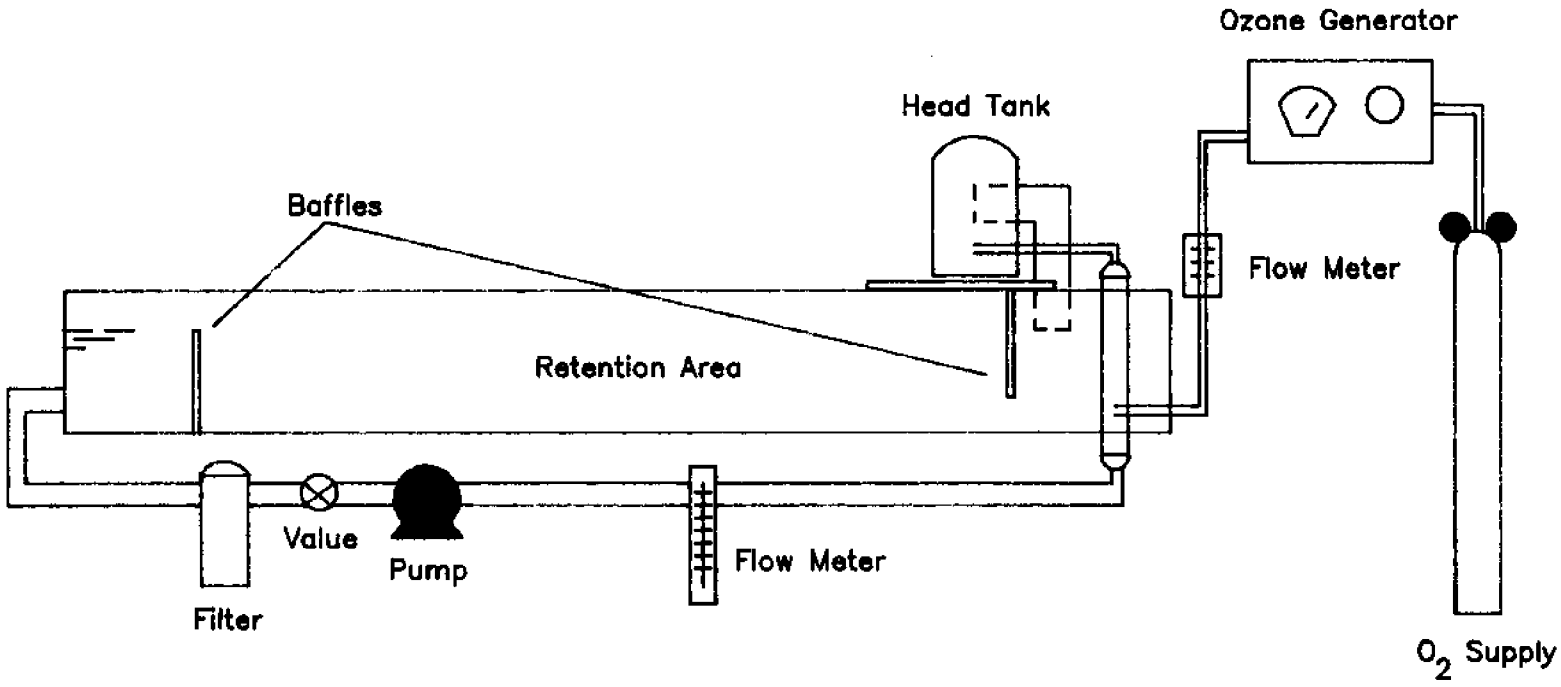

Figure 1: Schematic of the depuration system used for the pilot-scale experiments.

technique as previously described.

RESULTS

Inactivation of V. vulnificus, as seen in Table 1 was performed in 500 ml of ozonated seawater at a salinity of 23 ppt, pH 8.2, and a temperature of 19°C±1. The average initial bacterial concentration was 1.9×10^9 cells/100 ml. After four minutes, 8.1 (>99.99%) logs of kill was observed. The initial oxidant concentration was approximately 4.5 mg/L which fell to 3.24 mg/L. The inactivation rate constant for the average of the three trials is 0.45 s^{-1}. No inactivation was observed in any of the three control trials.

Table 1. The effects of ozone on the viability of V. vulnificus in seawater.

Treatment	Length of Ozone Exposure (seconds) 3-5	15	30	60	120	180	240
Without Ozone	1.9×10^9	1.9×10^9	1.9×10^9	1.1×10^9	1.1×10^9	1.1×10^9	1.1×10^9
With Ozone	8.6×10^6	6.7×10^4	1.2×10^3	2.9×10^2	<91	<34	<15
% Reduction of V. vulnificus	99.54	99.99	>99.99	>99.99	>99.99	>99.99	>99.99

All values are expressed as most probable number (MPN) of V. vulnificus per 100 milliliters of seawater. Each value represents a mean of at least three determination.

Salinity of the seawater water 23 ppt @ 19°C, pH 8.2, with an oxidant concentration of between 4.50 and 3.24 mg/liter. No oxidant was recorded for control.

The pilot-scale studies were conducted in approximately 300 liters of recirculated seawater at a flow rate of 8 liters/min. Table 2 shows the effect of ozonated seawater on V. vulnificus numbers in the overlying seawater and in artificially inoculated clams (Mercenaria campechiensis).

During a representative depuration trial, the initial oxidant concentration was 3.1 mg/L. The ozone generator was activated periodically during the experiment, with the lowest oxidant concentration reaching 0.6 mg/L. Low numbers of bacteria were initially recovered in seawater, in the experimental section. After 12 hours bacterial titers increased to 240 cells/100 ml. At this time it was noted that clams were pumping. The bacterial titers subsequently decreased over the next 12 hours to 9 cells/100 ml. The number of bacteria in the control section remained fairly constant at an average of 171 cells/100 ml (see Table 2).

Table 2. Depuration of V. vulnificus form Mercenaria campechiensis.

Treatment	Depuration Time (hours) 0	2	6	12	24
Without Ozone	$1.2x10^5$	$2.3x10^4$	$1.2x10^4$	$2.2x10^4$	$5.5x10^4$
With Ozone	$1.2x10^5$	$1.8x10^4$	$2.3x10^4$	$8.4x10^3$	$2.4x10^2$
%Reduction of V. vulnificus	0.00	85.0	80.8	93.0	99.8

Values presented represent on representative trial. V. vulnificus recovered from clam meat are expressed of CFU per gram of meat.

Salinity of the seawater was 23 ppt @ 19°C, pH 8.2, with an oxidant concentration of between 3.1 and 0.6 mg/liter. No oxidant was recorded for the control.

V. vulnificus numbers in clam meat for both the experimental and control were similar for the first 12 hours, with each experiencing approximately 1.0 log unit of inactivation. After 24 hours, ozone treated clams showed a 2.7 log unit decrease in bacterial titers. The control section showed an increase in recovered bacteria and had a final reduction of 0.3 log units.

DISCUSSION

Blogoslawski et al. (1979) reported that in depuration experiments with the softshell clam, pumping activity was severely reduced, or in some cases halted by oxidant concentrations in excess of 4.5 mg/L. Therefore, it was necessary to establish the rates of oxidant formation and dissipation to ensure a bactericidal environment that is conducive to pumping. From this work and other data obtained in previous experiments, the time of ozonation for all subsequent experiments were set as not to exceed these levels.

Chick (1912) reported that disinfection experiments where oxidant (or other bactericidal agent) was unlimited, the reaction proceeded in much the same way a first-order chemical reaction takes place. The inactivated bacterium is analogous to the compound being formed and the disinfectant, which in most cases is in great excess compared with the inoculum, remains unchanged. Under such conditions, the disinfection rate can be expressed as the inactivation rate and is independent of the initial inoculum titer, but is proportional to the oxidant concentration. When survival is plotted on a log scale versus time and produces a straight line plot, first-order kinetics are being followed. The reaction is then referred to as pseudo first-order. Table 2, the inactivation of V. vulnificus, appears to follow pseudo first-order kinetics.

Blogoslawski et al. (1976) reported that bromine in ozonated seawater is in fact the source of the disinfectant. A similar experiment using bromine to generate oxidant in seawater was performed.

The success of depuration depends on the ability to maintain an oxidant residual and pumping shellfish. In each of the depuration trials, the time of pumping coincided with the major reduction in bacterium recovered in shellfish meats. Table 2 illustrates a depuration trial with artificially infected Mercenaria campechiensis. Pumping was observed 12 hours into the experiment. At that time the oxidant demand increased as indicated by the decrease in measured concentration from approximately 3.0 to 1.0 mg/L. A 2 log unit increase in V. vulnificus was noted the overlying water in the experimental section in the time period when pumping began. In the first 12 hours the experimental section had only a 0.5 log unit difference in bacteria recovered in clam meat as opposed to the control section. In the second 12 hours after pumping was initiated, the difference in bacteria recovered in clam meat increased to 2.5 log units.

In summary the process of ozone assisted depuration proved effective in reducing the number of recoverable V. vulnificus. Further experimentation is needed dealing with the effectiveness and economics of ozone depuration in a commercial setting.

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USE OF FOUR AGAR MEDIA FOR EARLY DETECTION OF PROLIFIC HISTAMINE PRODUCING BACTERIA IN TUNA SAMPLES

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INTRODUCTION

Scombroid poisoning is caused from the consumption of scombroid fish containing substantial amounts of histamine in the muscle tissue (Arnold and Brown, 1978; Behling and Taylor, 1982). Histamine is produced by microbial decarboxylation of the free histidine in the fish tissue due to improper handling or storage of the fish (Arnold and Brown, 1978; Eitenmiller et al., 1981). *Klebsiella pneumoniae* (Kawabata et al., 1956; Sakabe, 1973), *Proteus morganii* (Taylor et al., 1979) and *Hafnia alvei* (Havelka, 1967) have been implicated as causative microorganisms in the formation of toxicologically significant levels of histamine in fish.

Histamine quantification is usually performed to confirm the etiological role of the fish samples that have been consumed and suspected to be responsible for scombroid poisoning. Limited effort has been made to develop an agar medium that can be used to detect and quantify histamine producing bacteria on scombroid fish. Moeller (1954) used a pH–based differential medium to examine the distribution of amino acid decarboxylase activity in the *Enterobacteriaceae*. Niven et al. (1981) modified Moeller's medium and developed a differential agar medium for quantitative and detection of histamine producing bacteria. In both cases, differentiation is based on the color change of bromocresol purple due to pH shift. Because a 36 – 72 hr incubation period is needed using the pour plate method (Niven et al., 1981), Niven's medium is impractical for commercial screening applications of suspect scombroid fish samples before they are served in restaurants. The development of a fast assay system to detect suspect fish samples is therefore needed.

Several factors such as incubation temperature (Eitenmiller et al., 1981), medium pH (Ienistea, 1971; Koessler et al. 1928), and the concentrations of histidine and carbohydrates (Ando, 1960; Arnold and Brown, 1978; Edmunds and Eitenmiller, 1975; Ferenick, 1970), are known to affect bacterial growth and the activity of histidine decarboxylase, the enzyme involved in the conversion of histidine to histamine. In the present study, we compared Niven's medium to three other media that contained different amounts of histidine and glucose to determine their effectiveness in detecting histamine producing bacteria in tuna samples.

MATERIALS AND METHODS

Bacterial cultures

Five histamine producing strains, *Klebsiella pneumoniae* 111–5 and T2, *Proteus morganii* 110SC–2 and JM, and *Hafnia alvei* T8, were provided by Dr. S. L. Taylor (Department of Food Science and Technology, University of Nebraska, Lincoln, NE). The cultures were maintained on trypticase soy–histidine (2%) agar slants and stored at refrigeration temperatures.

Culture media

Trypticase soy broth–histidine medium (TSBH) (Taylor et al., 1979) was used as a preinoculum broth for bacterial activation. The final pH of this medium was adjusted to 6.8 before autoclaving for 15 min at 121°C. Tuna fish infusion broth (TFIB) was prepared following the methods of Taylor et al. (1979). The compositions of Niven's medium (Niven et al., 1981) and the three modified differential media are shown in Table 1. The media were autoclaved for 10 min at 121°C.

Bacterial colony color changes and size measurement

The activated cultures of the five strains in TSBH were individually streaked onto the four differential agar plates and incubated at 35°C. At 6, 12, 18, 24 and 48 hr, the culture plates were examined for any color change of the bacterial colonies. A purple–blue color appearing in the vicinity of the colonies was considered a positive reaction for media A and B, while a red zone around the colonies was considered positive for media C and D.

The five activated cultures were also serially diluted with Butterfield's phosphate buffer and surface inoculated onto the four agar media. The size of 100 individual colonies was measured for each bacterial strain on each agar medium after 6, 12, 18, 24 and 48 hr.

Recovery of histamine–producing bacteria from tuna sample presumptive test

Tuna samples obtained from a local retail seafood store were divided into two groups and stored in 18 oz sterile whirl–pak bags. The tuna samples were cut in rectangular shapes each weighing about 200 g. One group was abused at room temperature for 2 days while the other was stored at 4°C for 2 days before being spiked with 1 mL TSBH culture containing about 10^8 mixed bacterial cells. The bacterial mixture was prepared by adding 0.2 mL of each of the five activated cultures into a sterile test tube and the bacterial number adjusted to 10^8 cells/mL with buffer.

A 50 g tuna sample was homogenized with 450 mL of Butterfield's phosphate buffer, serially diluted and plated onto the four differential agar plates. Based on the color change surrounding the colonies after incubation at 35°C for 24 hr, about

100 presumptive positive and negative colonies were randomly isolated from each agar medium. These were then inoculated into TSBH and incubated at 37°C for 24 hr, then re–streaked onto nutrient agar plates to ensure the purity of the cultures. Pure cultures were maintained on trypticase soy–histidine agar slants.

Confirmation test

Aliquots (0.5 mL) of the presumptive positive and negative bacterial isolates, after cultivation overnight in TSBH, were transferred into 5 mL TFIB and incubated at 35°C for 24 hr in a waterbath shaker (100 rpm). After 6% perchloric acid (PCA) was added to stop the reaction, histamine was extracted, and the quantity measured by high performance liquid chromatography (HPLC).

Bacterial isolates that produced histamine equal to or greater than 0.1 μmol/mL TFIB were considered confirmed histamine producers. Thus, the numbers of true histamine producers, false producers, true non–producers and false non–producers could be determined. The recovery rates of the differential agar media in detecting histamine production were expressed as the ratio of the total positive producers and non–producers to the total isolates obtained from the differential agar plate.

Histamine quantitation

The modified ion–moderated partition HPLC method (Gill and Thompson, 1984) using an HP 1090 liquid chromatograph (Hewlett Packard) equipped with an HP 85B personal computer, an HP 9121 multichannel integrator and an HP thinkjet printer was applied. The Aminex HPX–72S organic base analysis column (BioRad) with a guard columnn was used. Typical chromatographic conditions were: flow rate, 0.4–0.7 mL/min; oven temperature, 50°C; peakwidth, 0.05 nm; chartspeed, 0.3 cm/min; wavelength, 210 nm; and injection volume, 20 μL. The mobile phase was 5% acetonitrile in 0.25 M ammonium sulfate. Histamine standard solutions (1, 2, 4, 8 and 10 mg%) were prepared from the stock solution (100 mg%) by diluting with 6% PCA–30% KOH solution (pH 7).

The PCA extracts of the TFIB were brought to either 10 or 25 mL with 6% PCA followed by adjustment to pH 7.0 with a 30% KOH solution for HPLC analysis. Final histamine concentrations were calculated according to the formula:

$$C2 = \frac{C1 \times (V1 + V2) \div V1 \times 10^6 \div 0.93}{Mw}$$

where C1 was the concentration of histamine derived from the standard curve (mg/mL); C2 was the final concentration of histamine (nmole/mL); V1 was the volume of PCA extract neutralized (mL); V2 was the volume of 30% KOH added; 0.93 was the extraction recovery rate and Mw is the molecular weight of histamine (111.55). Each sample was analyzed in duplicate by HPLC.

Table 1. Composition[a] of the four histidine decarboxylase assay agar media (%)

	Medium A[b]	Medium B	Medium C	Medium D
Bacto–tryptone	0.5	0.5	0.5	0.5
yeast extract	0.5	0.5	0.5	0.5
L–histidine monohydrochloride	2.7	1	1	1
NaCl	0.5	0.5	0.5	0.5
$CaCO_3$	0.1	0.1	0.1	0.1
glucose	–	0.05	–	0.05
bromocresol purple	0.006	0.006	–	–
phenol red	–	–	0.0018	0.0018
pH	5.3	5.3	6.0	6.0
Bacto–agar	2	2	2	2

[a]All chemicals used were analytical reagent grade.

[b]Medium A is Niven's differential agar medium (Niven et al., 1981).

Table 2. Time–related colony color change[a] of histamine producing bacteria grown on four test media

	Medium A			Medium B				Medium C/Medium D		
Bacteria	18	24	48hr	12	18	24	48hr	18	24	48hr
K. pneumoniae 111–5	–	+	+	–	+	+	+	–	–	+
K. pneumoniae T_2	–	+	+	–	+	+	+	–	+	+
P. morganii 110SC–2	–	+	+	–	+	+	+	–	–	–
P. morganii JM	–	+	+	–	+	+	+	–	+	+
H. alvei T_8	–	–	–	–	–	–	+	–	–	+

[a]Colonies with a deep–blue purple color on agar media A and B, and those with bright red color on media C and D are considered as positive histamine producers.

RESULTS AND DISCUSSION

The time–related color changes of the colonies on the four media are shown in Table 2. The two *K. pneumoniae* and two *P. morganii* strains showed deep–blue to purple colonies following incubation for 24 or 18 hr on medium A or B, respectively. *Hafnia* failed to show a positive color change on medium A at 48 hr. However, they showed the color change on medium B at this time.

The five test strains showed similar results for time–related color change on medium C or D (Table 2). Only *K. pneumoniae* T2 and *P. morganii* JM showed positive results at 24 hr. *K. pneumoniae* 111–5 and *Hafnia* showed a positive reaction after 48 hr, while *P. morganii* 110SC–2 was the only one which failed to show any color change at 48 hr.

All the five histamine producers had a size range of 1.5 to 2.5 mm after 24 hr of incubation on the four agar media. By 48 hr, the colony size increased to 3.5–5.5 mm (data not shown) and the colonies began to coalesce; this often interfered with colony counting and thus the accurate enumeration of the total colony numbers. Therefore, a 24 hr incubation time was selected for identifying prolific histamine producers in this study, even though *Hafnia* did not show any positive color change on the four media at this time.

The results of the study to determine the efficiency of the four agar media in detecting prolific histamine producers isolated from temperature–abused tuna samples indicate that medium A is the best among the four (recovery rate 95.8%, Table 3). The recovery rates range from 64 to 71% for media B, C and D. High false–positive results are detected with these media. Similar results are found in bacteria–spiked tuna samples (Table 4). A 93.9% recovery rate is obtained for medium A, while 83.7%, 68.7% and 77.6% recovery rates for media B, C and D, respectively. High false–positive results are again found for media B, C and D, and high false–negative results for media C and D.

Thus, Niven's differential agar (medium A) was more effective than the other three media for early detection of prolific histamine producing bacteria from temperature–abused and bacteria–spiked tuna samples. By using the surface spread technique and an incubation temperature of 35°C, the time required to recognize histamine producers was reduced to 24 hr. Although *Hafnia*, a weak histamine producer (Behling and Taylor, 1982), failed to show positive results at 24 hr on this medium, the strong producers including *K. pneumoniae* and *P. morganii* strains (Lerke et al., 1978, Taylor et al., 1978 and 1979) were effectively detected. The slow growth and histamine production by *Hafnia* in liquid medium (Wei et al., 1988) could account for its low detectability on medium A after 24 hr.

Niven et al. (1981) suggested that the low pH of medium A could result in the exclusion of some histamine producers. Bacterial counts determined on this medium were found to be approximately one order of magnitude lower than on plate count agar (Niven et al., 1981). The present study demonstrating the delayed growth of *H. alvei* T8 on agar medium A supports their findings. Therefore, it is possible that some histidine decarboxylase–containing bacteria did not grow well enough to produce histamine at the low pH.

False–positive results still occur with medium A because initial differentiation is based solely on the color change of bromocresol purple. High false–positive results were obtained with the other three media. The use of tryptone as a nitrogen source by bacteria to produce alkaline metabolites in these media

Table 3. Efficiency of the four differential test media in detecting histamine producing bacteria from temperature–abused tuna

Medium	TI[a]	P(+)[b]	P(–)[c]	C(+)[d]	C(–)[e]	F(+)[f]	F(–)[g]	Accuracy[h]
A	96	61	35	60	31	1	4	95.8%
B	97	73	24	48	21	25	3	71.1%
C	81	47	34	30	22	17	12	64.2%
D	95	58	37	37	25	21	12	65.3%

[a]Total bacterial isolates obtained from abused tuna samples on each agar medium.

[b]Number of presumptive positive colonies based on colony color change.

[c]Number of presumptive negative colonies.

[d]Number of confirmed positive colonies that produced histamine in TFIB. Histamine production was determined by HPLC.

[e]Number of confirmed negative colonies that failed to produce histamine.

[f]Number of false positive colonies as determined by HPLC analysis of histamine produced in TFIB.

[g]Number of false negative colonies.

[h]The recovery rate was calculated by dividing the total confirmed positive and negative colonies to the total bacterial isolates.

Table 4. Efficiency of the four differential agar media in detecting histamine producing bacteria from bacteria–spiked tuna

Medium	TI[a]	P(+)[b]	P(−)[c]	C(+)[d]	C(−)[e]	F(+)[f]	F(−)[g]	Accuracy[h]
A	99	66	33	62	31	4	2	93.9%
B	98	61	37	46	36	15	1	83.7%
C	99	56	43	35	33	21	10	68.7%
D	98	54	44	42	34	12	12	77.6%

[a]Total bacterial isolates obtained from abused tuna samples on each agar medium.

[b]Number of presumptive positive colonies based on colony color change.

[c]Number of presumptive negative colonies.

[d]Number of confirmed positive colonies that produced histamine in TFIB. Histamine production was determined by HPLC.

[e]Number of confirmed negative colonies that failed to produce histamine.

[f]Number of false positive colonies as determined by HPLC analysis of histamine produced in TFIB.

[g]Number of false negative colonies.

[h]The recovery rate was calculated by dividing the total confirmed positive and negative colonies to the total bacterial isolates.

might have contributed to these results. The false–negative results found in agar media C and D were probably associated with the relatively short incubation time (24 hr) used.

Histidine concentration in the medium can affect bacterial conversion of histidine to histamine. Histidine could act either as a substrate or an inducer of histidine decarboxylase. Ferencik (1970) suggested that a minimum histidine concentration of 100–200 mg% was required for histidine decarboxylase activity. In the present study, two histidine levels of 1 and 2.7% (equivalent to 752.8 and 2032.5 mg%) were used. The 2.7% level appeared to be distinctly superior for the conversion of histidine to histamine.

CONCLUSION

The results indicated that Niven's medium (medium A) was superior to the other three test media for early detection of prolific histamine–producing bacteria including *K. pneumoniae* and *P. morganii* strains from bacteria–spiked and temperature–abused tuna samples. However, this medium is not satisfactory for the detection of *H. alvei* with a 24 hr incubation period. Since the supplementation of glucose may enhance histidine conversion in a culture medium, further studies are needed to investigate their effects on bacterial enzyme activity of these slow histamine–producers.

ACKNOWLEDGEMENT

This work was supported by the Departemnt of Commerce grant NA 86–WC–H–06106.

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EXTENSION OF SHELF-LIFE OF FRESH CHANNEL CATFISH FILLETS USING MODIFIED ATMOSPHERIC PACKAGING AND LOW DOSE IRRADIATION

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INTRODUCTION

The popularity of seafoods has sharply increased the demand for a fresh quality product in the past few years. Catfish, especially commercially cultured channel catfish (Ictalurus punctatus), is rapidly becoming a desirable seafood commodity as the catfish industry expands its markets. With the development of aquaculture methods, catfish production has increased by a factor of 10 from 22 million pounds (10 million Kg) in 1977 to a projected 250 million pounds (114 million kg) in 1987 (Rice, 1987). As the catfish industry becomes more competitive with other established seafood industries, inexpensive alternative processing methods to prolong shelf-life compared with expensive frozen storage will be needed. Two alternatives, modified atmosphere packaging (MAP) and irradiation, singly or together may be the economical methods needed.

Modified atmosphere packaging, packaging of a perishable product in an artificially altered atmosphere, may retard spoilage. Pioneer researchers such as Killeffer (1930) reported that meat and fish stored in 100% CO_2 kept fresh up to three times longer than air-stored controls, and Coyne (1933) reported an optimal inhibition of spoilage microorganism growth with 40-60% CO_2.

Early irradiation research used sterilizing doses, which resulted in poor sensorial quality. More recently, research emphasis has shifted towards low dose irradiation with refrigerated storage (Niven, 1958; Nickerson et al., 1983). Haddock irradiated with 0.6 to 0.7 Mrad could

be stored up to 6 weeks at refrigeration temperatures (Nickerson et al. 1954), and the shelf-life of cod irradiated with 0.46 Mrad was tripled (Miyauchi 1960).

Use of modified atmosphere packaging after low dose irradiation extended the shelf-life of haddock fillets (vacuum-packed, 150 Mrad; Conners and Steinberg, 1966) and cod fillets (60% CO_2, 100 krad; Licciardello et al., 1984) more than either process did alone. This study was to determine the effects of low dose irradiation and elevated CO_2 packaging on shelf-life extension.

MATERIALS AND METHODS

Preparation and Processing of Samples

Skinned, freshly processed catfish fillets, obtained from a local catfish processor were divided into approximately 50 g portions, placed in 12.7 X 17.8 cm polyethylene bags, and packaged using the Multivac Vacuum sealer (Model A300/22). Tescom proportional gas mixer (Model 299-027) was used to obtain 100% air, 100% CO_2, and 80%:20% CO_2:air mixtures. The fillets were then irradiated using a Co-60 radioactive source emitting approximately 4.2 krad per min at dosages of 0, 50, and 100 krad. The samples were stored on ice at approximately 0°C. Six replicated samples of each treatment were analyzed at 0, 10, 20, and 30 days.

Microbiological Analysis

Bacterial analyses followed procedures recommended in the Compendium of Methods for the Microbiological Examination of Foods (Speck, 1984). A 50 g sample was weighed into a blender jar, 450 ml of 0.1% peptone water was added to make a 1:10 dilution, and blended for 2 min. Aerobic plate counts were determined by serial dilutions in peptone water blanks from 1:10 to $1:10^6$. The pour plate method with Tryptone Glucose Extract (TGE) agar was used. Inverted plates were incubated at 35°C for 48$\pm$ 2 hr. Psychrotrophic plate counts used TGE agar for the same dilutions. The incubation temperature used here was 20°C for 4 days (ICMSF, 1986). Anaerobic plate counts used plates with a double pouring of Liver Veal agar incubated with inocula from the same serial dilutions. Inverted plates were incubated anaerobically at 35°C for 48$\pm$ 2 hr in a Gaspak system.

Color Analysis

Color characteristics of the catfish fillets were determined with the HunterLab Color Difference Meter, Model D-25M, equipped with a single processor (Model D-25-0) and an optical sensor (Model D-25). Samples, placed in a petri dish, were rotated 120° three times. These three readings were averaged with the readings from the center of the plate. Three values were determined: "L" or lightness value, "a" values (red +, green -), and "b" values (yellow +, blue -).

2-Thiobarbituric Acid

The distillation method of Sinnhuber and Yu (1958) as modified by Tarladgis et al. (1960) was used with triplicate 10 g samples. Absorbance was read at 532 nm using a Gilford Response UV-VIS Spectrophotometer. The TBA number was calculated as mg of malonaldehyde per kg of sample. A standard curve was prepared using TEP reagent (1,1,3,3-tetraethoxypropane) for each replication.

Statistical Design

Analysis of data utilized a randomized complete block design (RCBD) with a 3X3 factorial split plot with a subunit of storage. The main plot was irradiation and packaging atmosphere. The split plot was used in order to increase the scope of the experiment and gain greater precision for comparisons (Steel and Torrie, 1980). Analysis of variance used Statistical Analysis System (SAS, Cary, NC) program including the Duncan's Multiple Range test for comparison of the means.

RESULTS AND DISCUSSION

Catfish packaged in modified atmospheres combined with irradiation were subjected to microbiological, physical (color), and chemical (TBA) tests during a 30 day storage period to determine the shelf-life of an acceptable product.

Microbiology

Total plate counts, psychrotrophic plate counts, and anaerobic plate counts were used to evaluate the flora that can survive and propagate during processing and storage treatments. In the subsequent discussions, the modified atmosphere treatment consisting of 80% CO_2 and 20% air, shall be referred to as the "mixture". Acceptable bacterial levels, using the total aerobic plate count, range between 10^6 and 10^7 organisms per gram (Andrews et al., 1977).

In contrast with findings of other researchers (Banks et al., 1980; Parkin et al., 1981; Mokhele et al., 1983; Wang and Ogrydziak, 1986), who reported that CO_2 atmospheres in packages helped maintain lower bacterial counts, the non-irradiated catfish fillets at each time period had similar aerobic, anaerobic, and psychrotrophic plate counts regardless of the atmosphere used for packaging ($P>0.05$; Table 1). Furthermore, regardless of the atmosphere used for packaging, catfish irradiated with 50 krad or 100 krad doses showed no significant difference ($P>0.05$) in anaerobic plate counts nor, was there a significant difference in aerobic or psychrotrophic counts between those packaged in 100% air and those in 100% CO_2. However, significant difference ($P<0.05$) was observed between microbial counts of catfish packaged in 100% air and those in the "mixture", even though no significant differences were observed between catfish packaged in 100%

Table 1. Effect of storage and treatment on mean microbial counts[1].

Storage (days)	Irrad. (krad)	Atm.[2] (%CO_2)	APC[3] x 10^3	AnPC[4] x 10^3	PPC[5] x 10^3
0	0	0	3.5^{d}	0.97^{f}	5.6^{g}
		80	6.3^{cd}	0.70^{f}	8.8^{g}
		100	7.3^{d}	0.71	10.0^{g}
	50	0	0.38^{ae}	0.13^{d}	0.54^{c}
		80	0.35^{acf}	0.11^{d}	0.71^{d}
		100	0.16^{aef}	0.13^{d}	0.32^{cd}
	100	0	0.13^{b}	0.055^{be}	0.24^{e}
		80	0.12^{bcg}	0.032^{be}	0.26^{f}
		100	0.16^{bg}	0.071^{be}	0.40^{ef}
10	0	0	840^{d}	520^{f}	2580^{g}
		80	120^{cd}	71^{f}	460^{g}
		100	97^{d}	280^{f}	820^{g}
	50	0	13^{ae}	6^{d}	220^{c}
		80	0.33^{acf}	0.37^{d}	6.9^{d}
		100	3.1^{afe}	3.3^{d}	28^{cd}
	100	0	0.38^{b}	0.25^{be}	21^{e}
		80	0.19^{bcg}	0.11^{be}	0.62^{f}
		100	0.25^{bg}	0.073^{be}	4.0^{ef}
20	0	0	1100^{d}	1400^{af}	4900^{ag}
		80	650^{d}	780^{af}	3900^{ag}
		100	520^{d}	620^{af}	1600^{ag}
	50	0	440^{e}	550^{d}	4200^{c}
		80	150^{f}	340^{d}	1200^{d}
		100	300^{fe}	400^{d}	1600^{cd}
	100	0	42	42^{e}	1700^{e}
		80	6.5^{g}	120^{e}	380^{f}
		100	10^{g}	11^{e}	460^{ef}
30	0	0	2200^{d}	1700^{af}	4900^{ag}
		80	1700^{d}	1800^{af}	5400^{ag}
		100	1200^{d}	1400^{af}	2500^{ag}
	50	0	1400^{e}	1800^{d}	4600^{c}
		80	1200^{f}	1200^{d}	4100^{d}
		100	1300^{fe}	1500^{d}	3100^{cd}
	100	0	2000	2600^{e}	5600^{e}
		80	540^{g}	480^{e}	3400^{f}
		100	340^{g}	620^{e}	2600^{ef}

[1]Mean values are of triplicate samples of duplicate experiments for each test procedure. Mean values with the same letter within each column are not significantly different at alpha=0.05, as tested by the Duncan Multiple Range test (a-c indicate no significant differences over time, d-g indicate no significant differences between treatments).
[2]The balance of the atmosphere is compressed air.
[3]Aerobic plate count.
[4]Anaerobic plate count.
[5]Psychrotrophic plate count.

CO_2 and those in the "mixture". The effectiveness of the "mixture" atmosphere was first reported by Coyne (1933), who reported that 40-60% CO_2 atmospheres were superior to 100% CO_2 in inhibiting microbial growth.

Catfish packaged in the "mixture" maintained the initial microbial load at day 10, but the aerobic plate count significantly ($P<0.05$) increased by day 20 and again by day 30. The samples were still within the acceptable limits (Andrews, et al., 1977) at day 10. However, differences were seen in anaerobic and psychrotrophic plate counts for the entire 30 days. The "mixture" atmosphere maintained low aerobic plate counts during the initial 10 days of storage, which agreed with findings of Richter and Banwart (1983), who reported a lag phase in bacterial growth of 7 days for 80% CO_2-packaged sea trout fillets. The 100% CO_2-packaged fish showed significant growth of aerobic, anaerobic, and psychrotrophic organisms throughout the storage period. Thus, the combination of irradiation and the "mixture" maintained lower aerobic and psychrotrophic counts.

The dose of radiation affected the microbial load independent of the atmospheric composition effect of packaged catfish. Samples treated with 100 krad had lower bacterial counts than those treated with 50 krad, which were significantly ($P<0.05$) lower than the non-irradiated sample counts. These findings agree with other studies using low levels of gamma irradiation (MacLean and Welander, 1960; Emerson et al., 1966; Angel et al., 1986). Non-irradiated control samples packaged in air were unacceptable by day 10 (Andrews et al., 1977) and significantly deteriorated even further at days 20 and 30.

Aerobic bacterial counts increased significantly ($P<0.05$) from 0 to 10 to 20 to 30 days in the non-irradiated control catfish samples, while the anaerobic and psychrotrophic counts increased during the first 20 days, but did not significantly ($P>0.05$) increase from day 20 to 30. An extended lag phase and an overall reduced growth rate for psychrotrophic organisms, reported by Gray et al. (1983), was not evident.

For the 50 krad irradiated catfish, the aerobic plate count did not increase ($P>0.05$) during the first 10 days, but increased significantly ($P<0.05$) after 10 days. The anaerobic and psychrotrophic counts increased significantly ($P<0.05$) over the entire storage period.

The aerobic and anaerobic counts for the 100 krad irradiated samples were not significantly ($P>0.05$) different from day 0 to 10, but increased significantly ($P<0.05$) after day 10. Psychrotrophs significantly increased ($P<0.05$) throughout the storage period. The combination of the "mixture" and 100 krad of irradiation was effective

in restricting growth of aerobic bacteria for at least 10 days, but not psychrotrophic organisms. However, it should be noted that low dose irradiation of catfish, both the 50 and 100 krad doses, maintained the bacterial counts below the guidelines for unacceptability (Andrews et al., 1977) at day 20.

Color

As fish undergo deteriorative changes during spoilage, flesh color may darken due to browning or may bleach or change color due to breakdown of pigments. Preservation treatments may affect the color of fish flesh as well. Thus, color analysis gives an indication of quality and affects consumer acceptance of the product. In general, lightness or Hunter "L" values were not significantly different ($P>0.05$) for all combinations of treatments, nor did they vary significantly with respect to time. However, in the 50 krad-irradiated samples, the 100% CO_2-treated samples had a significantly higher ($P<0.05$) "L" value than had the 80:20-treated sample. The only significant change in "L" values over time was the decrease observed between day 20 and day 30 in the 100 krad treated fish and in the "mixture" treated fish. This may be due to a slight bleaching effect of the elevated CO_2 in the "mixture", as was first noted by Coyne (1933), who found that 100% CO_2 packaged haddock were noticeably bleached compared to 50% CO_2 packaged fish.

The Hunter "a" values (red/green) were significantly lower ($P<0.05$) (less red/more green) in non-irradiated samples packaged in air than those in CO_2 (Table 2). The 50 and 100 krad treated samples in various atmospheres showed no significant ($P>0.05$) variation in "a" values. The "a" values for air treated samples increased significantly ($P<0.05$) as irradiation dose increased from 0 to 100 krad, (Table 2).

In the "mixture" and the 100% CO_2 stored samples, no significant differences ($P>0.05$) were noted between 0 and 50 krad treated samples, although the non-irradiated samples had significantly lower ($P<0.05$) "a" values than 100 krad-treated samples. The 50 krad treated fillets also had a lower ($P>0.05$) "a" value than the 100 krad treated samples. Thus, the 100 krad radiation treatment prevented a greening or browning effect. Although high levels of CO_2 may promote a color change in more highly pigmented fishes from red to brown due to the oxidation of oxymyoglobin to methemoglobin (Coyne, 1933; Brown et al., 1980), this did not occur.

The Hunter "b values measure the spectrum from yellow to blue. Table 2 shows the changes in "b" values due to atmosphere. No significant differences ($P>0.05$) were seen between the various atmospheres for non-irradiated fish. In the 50 krad and 100 krad treated samples, there were no significant differences ($P>0.05$) between 100% CO_2 and

100% air-packaged samples, but the fish packaged in the "mixture" atmosphere had significantly higher ($P<0.05$) "b" values (more yellow/-less blue) than the others. However, the dose of radiation had no significant effect on the "b" value of color for samples packaged in any of the atmospheres.

Table 2. Effect of storage and treatment on mean color and 2-thiobarbituric acid values in catfish samples[1].

Indice	Irradiation Dose (krad)	Modified Atmosphere 100% air	CO_2/air (80:20)	100% CO_2
Hunter L	0	54.43^{ac}	54.83^{ac}	55.19^{ac}
	50	53.87^{abc}	53.49^{bc}	54.98^{ac}
	100	53.44^{ac}	54.32^{ac}	54.59^{ac}
Hunter a	0	0.91^{bc}	1.46^{ac}	1.51^{ac}
	50	1.56^{ad}	1.72^{ac}	1.74^{ac}
	100	2.15^{ae}	2.26^{ad}	2.36^{ad}
Hunter b	0	6.82^{ac}	7.16^{ac}	6.82^{ac}
	50	6.59^{bc}	7.24^{ac}	6.60^{bc}
	100	6.74^{ac}	7.29^{bc}	6.75^{ac}
TBA[2]	0	0.118^{f}	0.093^{f}	0.070^{f}
	50	0.098^{f}	0.164^{f}	0.212^{g}
	100	0.320^{g}	0.396^{g}	0.208^{g}

[1]Mean values are of triplicate samples of duplicate experiments for each test procedure. Mean values with the same letter are not significantly different at alpha = 0.05, as tested by the Duncan Multiple Range test. Superscripts a and b used samples pooled for storage period and irradiation treatment, and superscripts c, d, and e used samples pooled for storage period and modified atmosphere treatment.

[2]TBRS is defined as thiobarbituric reactive substance.

2-Thiobarbituric Acid

In non-irradiated, 50 krad and 100 krad samples, no significant differences in TBA values were noted between the various atmospheres. This corresponds with findings of Brown et al. (1980), who found no effect on TBA values with modified atmosphere packaged rockfish and salmon and with results of Parkin and Brown (1983) in Dungeness crab. Thus, the presence of CO_2 seems to have little effect on rancidity in catfish fillets.

Both the air and the "mixture" atmosphere packaged samples showed no significant differences ($P>0.05$) between the non-irradiated and the 50 krad-irradiated samples, as shown in Table 2. A significantly lower TBA value was seen for the non-irradiated samples than for the 100 krad dosed samples in both atmospheres; 50 krad treated samples were also

significantly lower than the 100 krad samples. The increase in TBA values with irradiation dose paralleled the increase in HunterLab"a" value, showing that lipid oxidation was indeed occurring.

In the 100% CO_2 atmosphere, the non-irradiated samples had a significantly lower ($P<0.05$) TBA value than did the irradiated samples Table 2). Therefore, higher doses of radiation indicate higher incidence of lipid oxidation occurring in fish. This could be attributed to the formation of free radicals by the passage of the gamma rays through the food. Picinni et al. (1986) found significantly higher TBA values in tuna and hake irradiated with 220 krad than non-irradiated control fish. Similar findings in tuna loins were also reported by Quaranta, et al. (1984).

Changes in TBA values during storage did not correlate with changes in treatment. This may be attributed to the low TBA values found in all fish samples compared with the values commonly reported in red meats. There was a trend of increasing TBA values with increasing doses of radiation; this may also relate to the increase in Hunter "a" values as radiation dose increased.

SUMMARY

The optimum treatment for extension of shelf-life while maintaining a good quality product would be the combination of the 80:20 CO_2/air with 100 krad of radiation. With this combination of treatments, low aerobic and psychrotrophic counts were maintained, and shelf-life was stable for 20 days. The radiation treatments had a more pronounced effect on sample preservation and shelf-life extension than did the alteration of the atmosphere itself. Since the average shelf-life for catfish is approximately 5 to 7 days, the extension of shelf-life by 13 days would be extremely desirable and would offset the increased cost due to the treatments.

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EFFECTS OF ONBOARD HANDLING METHODS ON THE QUALITY OF GULF BUTTERFISH

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INTRODUCTION

Butterfish (Peprilus triacanthus) have been popular food fish in New England and the middle Atlantic states for many years. However, the Gulf butterfish (P. burti) has been a truly latent resource until recent years. Cooperative cruises with Japan in the north central Gulf of Mexico in the fall of 1984 and the spring of 1985 demonstrated that commercial catches of Gulf butterfish could be made. Additional butterfish resource surveys have been made by National Marine Fisheries Service (NMFS) scientists aboard the National Oceanic and Atmospheric Administration (NOAA) research vessels Oregon II and Chapman, and by marine scientists from Gulf coastal states aboard the RV Tommy Munro of the Gulf Coast Research Laboratory. The maximum sustainable yield (MSY) has been estimated to be 47,000 metric tons for Gulf butterfish, more than four times the annual harvest of Atlantic butterfish (Anonymous, 1986).

The commercial harvest of Gulf butterfish was initiated in May 1986 by five New England butterfish trawlers and three converted Gulf shrimp boats (Fee, 1986). In less than three years, Gulf butterfish have developed into a multi-million dollar industry. Most of the catch is being frozen onboard the New England trawlers for export to Japan. The development of domestic markets is desirable, and growth of the fishery has resulted in questions about onboard handling and holding methods.

MATERIALS AND METHODS

Samples of Gulf butterfish were collected and onboard tests were made during a resource survey cruise of the NOAA research vessel Chapman in April 1988. The Chapman is a 127-ft stern trawler with the capability to operate high opening bottom trawls in deep water. The vessel was modified two years ago and now contains a plate freezer below decks on the port side and a 500-gal insulated fiberglass refrigerated seawater (RSW) storage tank on the starboard side.

The objectives of this study were to evaluate onboard storage of butterfish on ice or in RSW, to evaluate brine freezing versus plate freezing, and to bring back samples of Gulf butterfish and other latent species for proximate and fatty acid analyses. Fish were collected from a series of survey stations from 50 to 80 miles off the Louisiana coast, west of the Mississippi delta. Station coordinates and depths plus butterfish average weights are listed in Table 1.

Table 1. Stations from which Gulf butterfish samples were collected on cruise no. 88-03 of the RV Chapman.

Station No.	Date	Latitude North	Longitude West	Depth, fm	Avg. Wt., gm	Use
6	3/31	28°23'	89°59'	62	130	Canned
10	4/4	28°9'	90°16'	84	127	Iced
13	4/5	28°23'	90°14'	33	79	RSW
14	4/5	28°15'	90°29'	38	99	Proximates
15	4/5	28°9'	90°30'	57	109	Control[1/]
26	4/8	28°19'	90°44'	30	69	Proximates
27	4/8	28°11'	90°46'	52	111	Brine[1/]
35	4/10	28°10'	91°13'	52	123	Proximates

[1/] Butterfish from Station 27 were brine frozen on-board. A plate-frozen control from Station 15 was compared with the No. 27 fish and with fish that were brine-frozen by the commercial shrimper, Captain B-4, in early May off the Mississippi coast.

Fish collected for onboard handling and storage studies were from stations with significant catches of butterfish. Control samples were frozen immediately in waxed cartons (10-lb nominal size) in the Dole[1] plate freezer below deck on the Chapman. An Omega Model 650 electronic digital thermometer with type K thermocouples was used to monitor cooling and/or freezing rates in the different tests.

Iced Storage - Approximately 15 lbs of butterfish were placed on ice in a styrofoam cooler and covered with flaked ice. Thermocouples were inserted into several individual fish. Liquid was drained and fresh ice was added twice daily. One-half of the fish were removed after one day, packed in a waxed carton, and plate frozen. The remaining iced fish were plate frozen after 3 days. After they were frozen solid, the boxes of fish were transferred to the ship's storage freezer at -20°C.

[1]The use of trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

RSW Storage - Two large mesh bags, each containing about 20 lbs of freshly caught butterfish, were suspended in the RSW tank, pre-chilled and held at 30°F (-1°C). One bag was removed after 2 days and the second bag after 3 days of RSW storage. Butterfish samples were rinsed with fresh water to remove rust that was present in the RSW, and then boxed and plate frozen.

Brine Freezing - A brine/sugar solution was prepared to approximate the mixture normally used for the freezing of shrimp onboard shrimp boats in the Gulf of Mexico. Approximately 1.6 lbs of salt and 0.2 lbs of sugar were dissolved in 10 lbs of water. Waxed cartons were half filled with the brine and placed into the plate freezer. After 3 hours the boxes, containing slush ice at about -23°C, were removed. The fish were added and agitated and the brine boxes were returned to the plate freezer for a total brine freezing time of 30 minutes. The fish, at an internal temperature of 28°F (-2°C), were rinsed off and packed into a new box which was held in the plate freezer for two hours before it was transferred to the storage freezer. A sample of butterfish brine-frozen on a commercial shrimp boat, the Captain B-4, was later obtained and compared with our experimental sample and the plate-frozen control (Table 4).

Transportation - The butterfish samples remained in the storage freezer until the Chapman returned to home port at Pascagoula, MS. They were then packed in insulated containers with blocks of dry ice and shipped to Charleston by overnight express service. They were stored again at -20°C until they were evaluated. The brine-frozen sample was evaluated 6 weeks after harvest and processing. The iced and the RSW stored samples were tested 7 weeks after they were collected.

Sensory Evaluations - Experimental samples were thawed overnight in a chilled room, filleted, skinned, and cooked to an internal temperature of 70°C by a standard boil bag procedure in a water bath at 71°C. The cooked, skinless fillets were served in glass petri dishes to a trained sensory panel. The panel evaluated sample characteristics on a form composed of open 10 cm intensity scales. Color intensity, four textural characteristics, five flavor characteristics (as listed in Table 2), and relative overall quality were rated. Since only five trained panel members were available, they evaluated each set of experimental samples on two occasions.

Proximate Analysis - Samples of fillets with skin were homogenized in a food processor and frozen for later analysis. Several whole samples were also analyzed, in which case frozen chunks were passed through a meat chopper prior to the food processor. Moisture, protein and ash were analyzed by AOAC procedures (AOAC, 1985) and fat was analyzed by a modification of the Bligh and Dyer chloroform-methanol extraction method (Smith, et al., 1964). Salt contents were also analyzed by the AOAC method and thiobarbaturic acid (TBA) values, as a measure of lipid oxidation, were determined by the distillation method of Tarladgis et al. (1960).

RESULTS AND DISCUSSION

Iced Storage - The sensory results for the iced samples and plate frozen control are listed in Table 2. All sensory data are expressed as percent of full scale on the open line, 10 cm intensity scale (mean and standard deviation). Results were analyzed by a SAS (Statistical Analysis System, Inc., Cary, NC) one way analysis of variance. When the F test was found to be significant, differences between treatment means were determined using Tukey's studentized range test. No rancidity was detected in any of the iced samples. There was a slight downward trend in average intensity ratings for sweetness and buttery flavor during 3 days of iced storage. The plate frozen control was significantly

Table 2. Iced storage of Gulf butterfish, sensory ratings (percent of full scale), mean and standard deviation. Zero (none) to 100 (extremely intense).

Characteristic	Control (Plate Frozen)	Iced, 1 day	Iced, 3 days
Color	47.6 ± 17.5	52.4 ± 11.2	50.7 ± 11.0
Hardness	41.7 ± 13.9	45.1 ± 14.3	51.2 ± 10.1
Flakiness	49.0 ± 15.3	48.8 ± 15.3	47.7 ± 16.8
Moistness	44.0 ± 10.4	43.4 ± 11.4	39.0 ± 12.0
Oily Mouth Coat	12.6 ± 8.9	14.0 ± 9.5	14.6 ± 11.9
Saltiness	4.4 ± 5.1	3.5 ± 3.9	4.3 ± 5.2
Sweetness	15.9 ± 11.9	10.4 ± 9.2	9.3 ± 6.9
Rancidity	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Buttery	13.8 ± 9.6	11:3 ± 9.6	8.9 ± 5.9
TIF[1/]	41.9 ± 12.8	41.5 ± 10.5	45.6 ± 11.4
Relative Quality	56.7 ± 13.9	54.4 ± 11.7	43.5 ± 12.0

1/ Total Intensity of Flavor

sweeter than the 3-day sample at the 0.05 level, but buttery flavor ratings were not significantly different. The 3-day sample was lower in overall quality than the control at the 10% level, but not at the 5% level of significance.

RSW Storage - Sensory results for the RSW-stored samples are listed in Table 3. Differences between the 2-day and 3-day stored samples were not significant. There were, however, significant differences at the 0.05 level between the RSW-stored samples and the plate frozen control in terms of saltiness, total intensity of flavor (TIF), and relative overall quality.

The color of the cooked flesh of the RSW-stored samples was rated a bit darker than the plate frozen control, and the difference between the control and the 3-day sample was significant at the 10% level. The external color and appearance of the whole fish samples were not evaluated, but no color washout or other appearance differences were noticed.

Differences in TIF and quality may have been caused in part by the excessive amount of rust in the RSW tank as a result of corrosion of the chiller tubes. Rancidity may also have been affected, but the differences between samples were not statistically significant.

Table 3. Refrigerated seawater (RSW) storage of Gulf butterfish, sensory ratings (percent of full scale), mean and standard deviation. Zero (none) to 100 (extremely intense).

Characteristic	Control (Plate Frozen)	RSW, 2 days	RSW, 3 days
Color	41.7 ± 10.0	49.3 ± 8.9	52.9 ± 9.4
Hardness	41.0 ± 9.0	43.3 ± 11.6	47.8 ± 10.3
Flakiness	52.2 ± 8.1	55.1 ± 13.3	53.9 ± 13.4
Moistness	37.3 ± 11.4	43.3 ± 9.4	44.9 ± 11.1
Oily Mouth Coat	6.3 ± 5.6	8.3 ± 7.5	7.6 ± 8.8
Saltiness	10.9 ± 9.2	58.9 ± 13.4	65.8 ± 8.0
Sweetness	13.3 ± 9.5	11.1 ± 8.7	9.7 ± 7.1
Rancidity	0.0 ± 0.0	3.5 ± 8.2	12.5 ± 18.7
Buttery	12.3 ± 9.4	12.1 ± 8.7	9.0 ± 6.7
TIF[1/]	40.9 ± 9.2	51.9 ± 8.8	60.5 ± 10.6
Relative Quality	58.4 ± 9.1	44.4 ± 13.8	32.2 ± 15.5

[1/] Total Intensity of Flavor

Brine Freezing - Sensory results are presented in Table 4. Both brine-frozen samples were saltier than the plate frozen control at the 0.05 level of significance. The sample that was frozen on the commercial shrimp boat was moderately rancid and was significantly different from the other two samples in terms of rancidity. The boxes containing the shrimper's frozen butterfish were only partially filled and the fish may have been exposed to more oxygen during storage, contributing to the higher rancidity rating. These butterfish had a significantly stronger flavor (TIF) and were lighter than the control at the 10% level. Again, external color differences were not noticeable.

Table 4. Brine-frozen Gulf butterfish, sensory ratings (percent of full scale), mean and standard deviation. Zero (none) to 100 (extremely intense).

		Brine-Frozen	
Characteristic	Plate Frozen	Chapman	Shrimp Boat
Color	52.7 ± 8.2	54.2 ± 11.4	36.9 ± 11.6
Hardness	50.6 ± 9.5	43.7 ± 8.6	40.4 ± 12.8
Flakiness	46.9 ± 6.7	56.3 ± 9.9	57.4 ± 9.9
Moistness	34.6 ± 12.3	44.7 ± 8.8	43.1 ± 7.0
Oily Mouth Coat	12.8 ± 10.2	14.1 ± 10.8	15.5 ± 11.9
Saltiness	20.0 ± 9.9	58.0 ± 23.6	66.1 ± 13.3
Sweetness	19.2 ± 13.2	16.7 ± 9.0	15.2 ± 7.6
Rancidity	0.0 ± 0.0	1.8 ± 3.4	8.1 ± 11.0
Buttery	17.9 ± 17.7	14.2 ± 10.3	11.7 ± 8.4
TIF[1/]	35.9 ± 11.1	43.0 ± 11.5	51.8 ± 8.3
Relative Quality	54.7 ± 9.3	54.4 ± 13.7	43.5 ± 15.6

1/ Total Intensity of Flavor

Chemical Tests - Chemical determinations are compared with sensory ratings for saltiness and rancidity in Table 5. The sensory ratings are from zero to 100 on the open line intensity scale. Salt contents measured for the RSW-stored and brine-frozen samples are not particularly high. The higher salt contents were objectionable, however,

for these samples cooked in boiling pouches without any added ingredients. The salt content may be acceptable for more normal cooking methods, but this was not determined. Some researchers have proposed a TBA value of 18 µ-moles/kg (1.3 mg/kg) as the threshold for rancidity. Only one of these samples exceeded that level.

Table 5. Sensory ratings and analytical measurements for saltiness and for rancidity in experimentally stored and processed Gulf butterfish.

	Salty (Sensory)	Analyt. % Salt	Rancid (sensory)	TBA Value µmol/kg
Plate Frozen	4	0.23	0	5.3
Iced 1 day	4	n.d.[1/]	0	1.6
Iced 3 days	4	0.23	0	3.0
Plate Frozen	11	0.27	0	6.9
RSW, 2 days	59	0.92	4	11.1
RSW, 3 days	66	1.18	13	11.7
Plate Frozen	20	0.32	0	3.9
Brine Frozen, (Chapman)	58	0.87	2	10.9
Brine Frozen, (Shrimper)	66	0.99	8	20.5

[1/] Not determined.

Parasites - A perceived problem with Gulf butterfish is the fairly common occurrence of small cestode (tapeworm) cysts in the flesh along the backbone. They are visible if the fish are filleted, but they are not a health hazard and whether the Gulf butterfish are more heavily infested than the Atlantic species has not yet been resolved. The cysts have also been documented as being common in the Atlantic butterfish (Hoffman and Sindermann, 1962). The semi-objective parasite ratings listed in Table 6 are averages of the numerical ratings: 0 - none, 1 - slight, 2 - moderate, and 3 - heavy infestation. Samples from 6 stations of the Chapman cruise plus the sample from the "Captain B-4" were evaluated. The degree of infestation was generally slight to moderate and the parasite rating was more or less proportional to the size of the fish.

Table 6. Relative occurrence of parasites (cestode cysts) in butterfish samples.

Station No.	No. Fish Examined	Avg. Wt., gm	Relative Infestation				
			None	Slight	Moderate	Heavy	Rating[1/]
6	32	130	1	21	7	3	1.38
10	17	127	0	14	3	0	1.18
13	24	79	6	14	4	0	0.92
15	6	109	0	4	2	0	1.33
26	7	69	2	4	1	0	0.86
35	8	123	1	4	3	0	1.25
B-4[2/]	10	69	6	3	1	0	0.50

[1/] Weighted average, 3 for heavy, 2 for moderate, 1 for slight: Rating = (1S+2M+3H)÷ TF (total fish).

[2/] Sample B-4 was caught off the Mississippi coast and brine-frozen onboard the commercial shrimper, the Captain B-4.

Proximate Composition - Proximate chemical compositions for nine samples of Gulf butterfish are shown in Table 7. For five of the collected samples both fillets (with skin) and whole butterfish were analyzed. These data indicate that for Gulf butterfish weighing 100 grams or more the fat content of whole fish and of fillet plus skin samples are about the same. Oddly, the two samples of smallest fish had the highest total fat content.

Table 7. Proximate chemical compositions of Gulf butterfish samples from Chapman cruise 88-03 and a commercial shrimp boat.

Station No.	Form[1/]	No. of Fish	Avg. Wt.,gm	% Mstr.	% Protein	% Fat	% Ash
6	FS	5	132	75.8	21.7	3.70	1.41
6	W	4	115	75.9	20.4	4.13	3.00
10	FS	6	127	75.7	20.0	3.73	1.41
13	FS	8	81	74.9	19.3	3.81	1.48
14	FS	8	100	74.0	21.2	6.55	1.43
14	W	6	98	74.4	18.3	6.54	2.59
15	FS	6	109	74.3	19.3	4.09	1.41
26	FS	7	69	74.3	19.5	6.90	1.37
26	W	5	61	72.4	17.6	8.52	2.37
27	FS	3	107	75.3	18.8	3.26	1.78
35	FS	8	123	74.3	20.1	6.74	1.39
35	W	5	123	73.2	17.4	6.45	2.94
B-4[2/]	FS	10	69	71.7	19.1	5.53	1.98
B-4	W	4	61	68.6	16.7	9.87	3.30

1/ FS = fillet with skin; W = whole.

2/ Brine-frozen sample from the shrimp boat, Captain B-4.

CONCLUSIONS

1. Iced butterfish maintained very good quality. The 3-day iced sample was not as sweet as the plate-frozen control, but there were no other significant sensory differences.

2. After three days there were significant differences between the RSW-stored samples and the control in terms of saltiness, flavor intensity, and relative overall quality. Rust in the RSW tank may have contributed to the differences, however.

3. The brine-frozen butterfish were rated significantly saltier than the control by the sensory panel. The salt concentrations measured analytically were not particularly high, however, for these or for the RSW-stored samples.

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OVERVIEW OF WASTEWATER TREATMENT OPTIONS FOR LOUISIANA SEAFOOD PROCESSORS

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INTRODUCTION

The coastal waters of Louisiana are becoming increasingly impacted by growing population and development. Thus, preservation of suitable water quality for the State's valuable commercial and sport fishery has become a focus in Louisiana's economic, public health, and stewardship obligations. Balancing these goals requires careful management of the pollution loads permitted for discharge into coastal waters. Seafood processors, economically important to the state with annual estimated revenues of $254 million (1), are often located in environmentally sensitive areas. The permits section of the Louisiana Department of Environmental Quality (LADEQ) has identified the need for technological support to develop wastewater treatment guidelines for Louisiana's seafood processing industries.

Seafood processing wastewaters consist primarily of biodegradable, nontoxic wastes containing high concentrations of soluble organics (biological oxygen demand (BOD_5)), total suspended solids (TSS), and fecal coliform bacteria. Wastes are generated from on-shore processing facilities which support both in-shore and near-shore fisheries for popular finfish, crustacean, and molluscan products. The wastes are produced on a seasonal basis by a large number of small facilities located in rural areas. Direct discharge of partially treated or untreated wastewater adversely impacts coastal sport fisheries, commercial oyster beds, and local drinking water supplies.

The United States Environmental Protection Agency (USEPA) has developed national technology-based treatment guidelines for the seafood processing industry (1). These standards dictate the daily 30-day maximum allowable values for BOD_5, TSS, oil and grease (O/G), and pH for several subcategories of seafood processors. Future regulatory policies may move from technology-based effluent limitations to more stringent water quality-based limits, requiring more advanced wastewater treatment. Therefore, economically achievable treatment options and pollutant limits providing for the protection of natural waters and the environment must be identified.

The objectives of this paper are to review the environmental regulatory requirements for Louisiana seafood processors by state and

federal agencies, and present treatment options for nontoxic, biodegradable wastes produced by seafood processing facilities.

PERMITTING PROCESSES

Seafood processors in Louisiana are required to file for four separate permits depending upon the location and type of operation. These permits include:

(1) National Pollution Discharge Elimination System (NPDES) permit issued by the USEPA.

(2) Five year Louisiana Water Discharge Permit System (LWDPS) permit issued by the LADEQ

(3) Sanitary Permit issued by the Louisiana Department of Health and Human Resources (DHHR), Office of Preventive and Public Health Services.

(4) Transportation permit issued by the Louisiana State Department of Transportation and Development (DOTD).

Of these four required permits, only the USEPA and LADEQ permits pertain to environmental regulation of seafood processors.

USEPA Permit

The USEPA permits wastewater discharge and other waste emissions, such as nonprocess water, under the Consolidated Permits Program. The discharge of pollutants into United States' waters requires a National Pollutant Discharge Elimination System (NPDES) permit (2), required by the Clean Water Act of 1977 (33 U.S.C. 1251).

The application requires: (1) the anticipated start-up date if not already in operation, (2) the location of each outfall by latitude and longitude, (3) the name of the receiving waters, (4) a list of operations contributing wastewater to the effluent, with average flow contributed by each operation and any wastewater treatment, (5) a line drawing showing flow through the facility, (6) a description of intermittent flows, and (7) the level of production with affected outfalls (3,4).

The applicant must report effluent pollutant information including: conventional pollutant analyses--BOD_5, chemical oxygen demand (COD), total organic carbon (TOC), ammonia (as N), flow, temperature (winter and summer), and pH; and identification of listed toxic pollutants and hazardous substances in any expected discharge. If a toxic or hazardous substance is present, the applicant must report any analytical data in his possession and give reasons for its

presence. New facility applicants must list names of existing facilities which, to the best of their knowledge, resemble the one for permitting(4).

The deadline for filing an NPDES permit application is 180 days before a present permit expires or 180 days prior to start-up of a new facility. If a new effluent source is constructed under the NPDES program, construction of the new source may not begin before the issuance of a permit under the applicable program (2). USEPA does not require a permit application fee.

LADEQ Permit

The LADEQ issues a five year LWDPS permit to dischargers meeting effluent limitations and monitoring requirements. The applicant should also apply for permits with the DHHR, and the DOTD (if applicable) (5).

Most of the data required in the LADEQ permit application is included in the USEPA permit. Non-redundant requirements in the LADEQ permit include total solids (TS), total dissolved solids (TDS), turbidity, how the waste reaches the "State water" (map), disposal methods and facilities, and treatment methods. For each significant source at the facility, the same data is required excluding the flow diagram, toxicity data, and how the waste reaches State water. The applicant must present and evaluate alternatives concerning the avoidance of potential and adverse environmental effects, cost-benefit analysis, alternative projects, sites, and mitigating measures that would offer more environmental protection.

After receiving the application, LADEQ issues a draft permit. The draft does not grant authorization to discharge; it describes the requirements for the final permit. Proof of public notification must be sent to the LADEQ office before final permit issuance. LADEQ will consider comments submitted in writing within 30 days of the notice date.

The permit fee is 20% of the annual maintenance and surveillance fee, or not less than $227.50 (5), payable prior to issuance of the final permit. The permittee is required to submit Discharge Monitoring Reports (DMR) defining sample type, frequency and parameters.

The facility may be liable for damages to private property as well as to State waters regardless of permit compliance. If set loading levels are exceeded for a specific waste component for a specific period of time, the processor is in non-compliance with the permit.

EFFLUENT STANDARDS

Several different effluent limits apply to seafood processors depending upon the receiving body, whether the facility is new or existing, and the intent to discharge to a POTW or directly to the environment. The flow diagram in Figure 1 indicates all the LADEQ effluent limit assignation procedures that apply to seafood processors. Waste load allocation covers all dischargers and may supersede all other effluent regulations.

Waste Load Allocation

The waste load allocation (WLA) process determines effluent limitations for point source discharges based upon the assimilative capacity of the receiving water. Technology-based effluent limits may apply to the seafood processors discharging into water bodies with high assimilative capacities. The Atchafalaya, Red, and Mississippi Rivers fall into this category. Effluent limits for point source discharges to systems with limited assimilative capacity are based on the results obtained from a water quality model. The model identifies the least stringent effluent limit allowing the in-stream DO to be maintained at or above 5.0 mg/l (4.0 mg/l in coastal waters), determining the degree of treatment; secondary effluent limits (30 mg/l BOD_5 / 30 mg/l TSS), nutrient limits, or no discharge of any pollutants.

Existing Point Source Performance Standards

These effluent limitation guidelines promulgated by USEPA represent the effluent reduction attainable by industrial wastewater treatment facilities through the application of the best practicable control technology currently available (BPT). These technology based standards are applied in developing effluent limits for TSS, O/G, and pH discharged from existing point sources. Primary treatment methods removing suspended and floating material from the waste stream are required to meet these effluent standards.

Existing Source Pretreatment Standards

These effluent limitation guidelines issued by USEPA apply to seafood processors that discharge to a POTW. Pretreatment for TSS, oil and grease (O/G), and pH may be required before the wastewater can be discharged to a POTW. The program regulating these dischargers is normally administered through local authorities in cooperation with LADEQ.

New Point Source Performance Standards

The BCT or best conventional technology standards required by USEPA apply to new seafood processing facilities or proposed additions

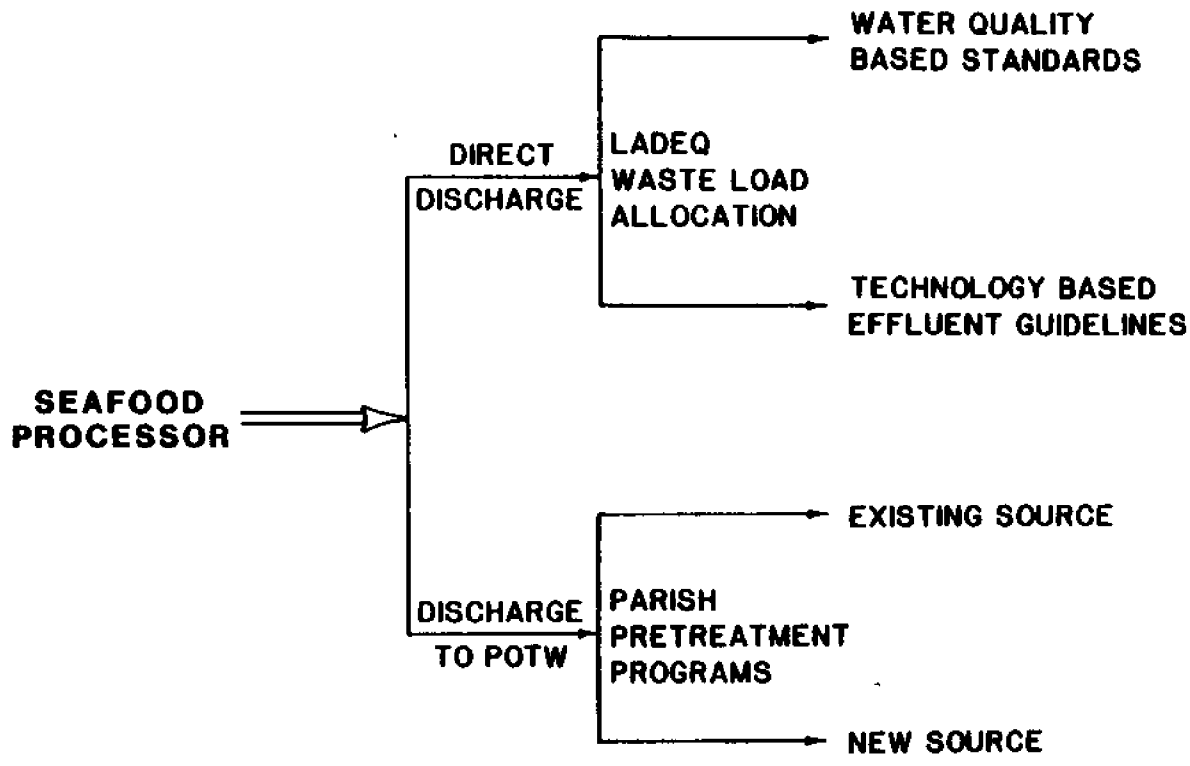

Figure 1. LADEQ effluent limitation procedures applicable to seafood processors.

to existing facilities. The waste components covered by these standards are BOD_5, TSS, O/G, and pH.

New Source Pretreatment Performance Standards

These standards promulgated by USEPA apply to new facilities that discharge to a local POTW. These standards cover BOD_5, TSS, O/G, and pH. The program regulating these dischargers is normally administered through local parish or city authorities in cooperation with LADEQ.

TREATMENT METHODS

Effluent treatment options for seafood processors vary considerably depending upon the size and location of the facility. Traditional "end-of-pipe" wastewater technologies such as activated sludge or trickling filters can be applied to almost any wastewater, but capital and operational costs may be high. In-plant water and solid waste reduction practices or "waste minimization" can offer a more economical approach to lowering overall wastewater treatment costs.

Waste Minimization

Waste minimization reduces wastewater flow and strength through management practices emphasizing washwater reduction, separation of solids and reuse of process water. The waste minimization practices for plant processes and wastewater treatment are shown in Figure 2. Washwater reduction can be achieved by simple in-plant management, such as turning off washwater to processes when not needed, or by installing simple devices such as foot-operated pressure plates, timers or metered flowmeters.

The reuse of process water in the seafood industry is regulated by the Federal Drug Administration (FDA) and, in Louisiana, by DHHR. Water reuse may reduce wastewater generation from some facilities. Any modifications used at a facility must: (1) be approved by DHHR and FDA for coliform bacteria standards, and (2) be relatively free of solids to avoid clogging of equipment.

Wastewater Treatment Processes

Wastewater treatment processes can be divided into four categories: primary, roughing, secondary, and polishing. The sequence of these wastewater treatment practices is shown in Figure 3. Table 1 compares treatment efficiencies of respective treatment methods. Table 2 lists possible treatment trains for various process wastes to achieve secondary effluent limits (30 mg/l BOD_5/30 mg/l TSS). Primary treatment is a physical or chemical process intended to remove coarse TSS and some BOD_5 from the raw wastewater. Roughing treatment can be physical, chemical or biological reducing high strength wastewaters to

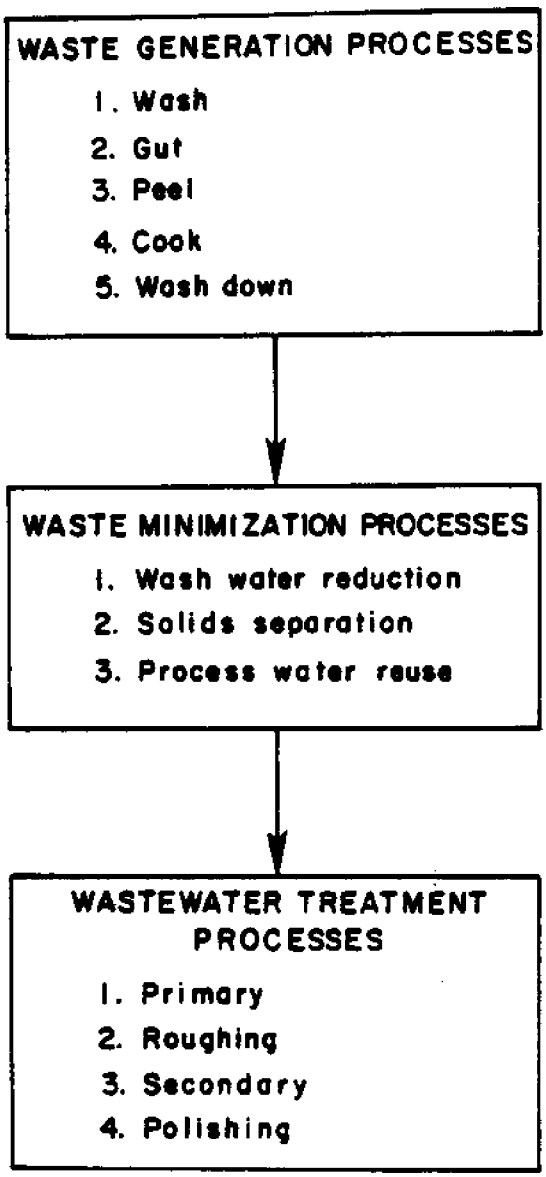

Figure 2. Relationship of in-plant waste generation processes to waste minimization and wastewater treatment practices.

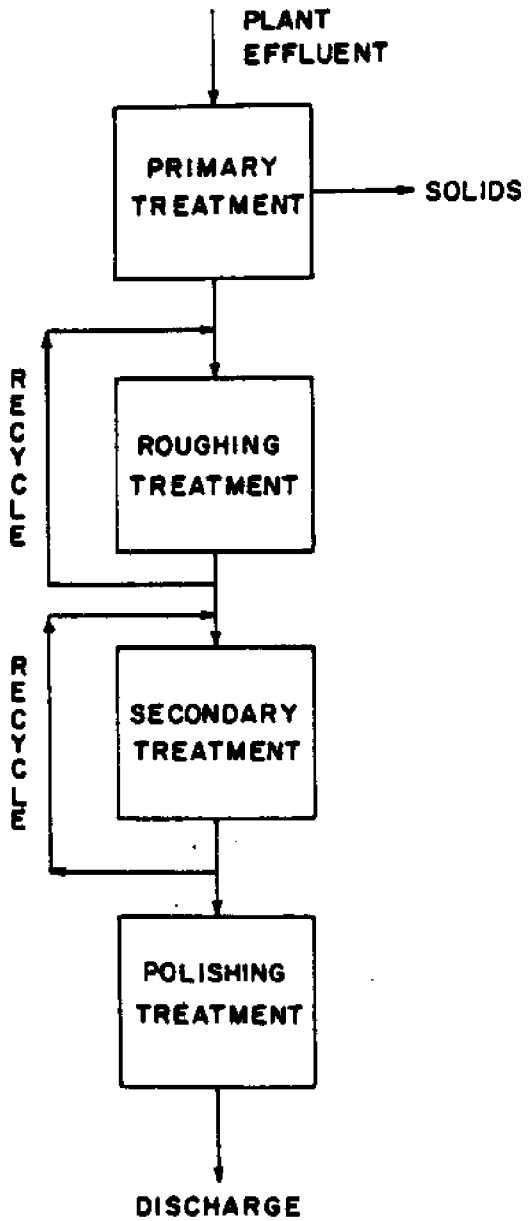

Figure 3. Sequence of wastewater treatment practices for seafood processors.

Table 1. Comparison of Treatment Efficiencies for Various Treatment Methods (7).

Treatment	Waste Parameters Removed	Expected Removal Efficiency, % of Influent
Primary	TSS	50 - 65
	BOD_5	24 - 45
Roughing	TSS	40 - 60
	BOD_5	40 - 60
Secondary	TSS	85 - 95
	BOD_5	75 - 95
Polishing	TSS	80 - 99
	BOD_5	65 - 98
	Phosphorus	70 - 80
	Nitrogen	85 - 98

Table 2. Possible wastewater treatment trains for specified processors based upon 30(mg/l BOD_5) / 30(mg/l TSS) effluent guidelines.

Processor	Waste Minimization	Primary	Roughing	Secondary	Polishing
Shrimp Peelers	--	--	Aerated Lagoon	Facultative Lagoon	Rock-reed Filter
	Solids Separation	Sedimentation	Trickling Filter	Extended Air Activated Sludge	--
Hand Pick Shrimp Processors	Solids Separation	Simple Screens	--	Septic Tanks	Rock-reed Filter
	--	--	--	Facultative	Rock-reed Filter
	--	Sedimentation	--	Extended Air Activated Sludge	--
Oyster Processors	Solids Reduction	--	--	Facultative Lagoon	Rock-reed Filter
	Solids Reduction	Sedimentation	--	Conventional Activated Sludge	--
Hand Pick Crab Processors	Solids Separation	Simple Screens	--	Aerobic Lagoon	Rock-reed Filter
	Solids Separation	Sedimentation	--	Extended Air Activated Sludge	Rock-reed Filter
	Solids Separation	Sedimentation	--	DAF	Rock-reed Filter
Crawfish Processors	--	--	--	Facultative Lagoon	Rock-reed Filter
	Solids Separation	Sedimentation	--	Conventional Activated Sludge	--
Edible Finfish Processors	Solids Separation and Washwater Reduction	--	--	Aerobic Lagoons	Rock-reed Filter
	Solids Separation and Washwater Reduction	Sedimentation	--	Extended Air Activated Sludge	--

-- Treatment is unnecessary.

acceptable levels for subsequent secondary treatment methods. Secondary treatment is physical-chemical or biological, removing BOD_5 and fine suspended solids to specified levels. The remaining BOD_5 and TSS, as well as nutrients such as ammonia, are removed using polishing treatment.

Primary Treatment: Primary treatment can be used alone to meet effluent standards for TSS, but is primarily used to reduce wastewater strength to levels acceptable for secondary treatment processes. Screening, sedimentation, and flow equalization are the major primary treatment methods used by seafood processors.

The screen types used most frequently in the seafood processing industry are the static, rotating, vibrating, tangential, and moving screens (6). The USEPA recommends a 20-mesh (number of openings per inch) screen as the minimum treatment for most seafood processing effluents (6). Screens are relatively inexpensive, easily retrofitted to sumps and discharge weirs, easy to maintain, and can be designed for automated operation. Screens may be inadequate for small particle removal, or providing sufficient treatment for secondary or polishing treatment technologies.

Sedimentation is the gravitational settling of suspended particles (TSS) which are heavier than water (7). The removal of TSS in the sedimentation process may be enhanced by adding flocculating chemicals such as calcium carbonate, ferric chloride, polymers or other wastewater streams. These materials chemically react with both dissolved and suspended compounds, creating heavier, more settleable sludges. Capital costs are generally greater for sedimentation units than screens, but effluent quality suitable for subsequent secondary treatment is achieved.

Flow equalization, another primary treatment option, may be incorporated into the sedimentation process. A flow equalization system consists of a holding tank and pumping equipment designed to reduce flow fluctuations and shock loadings. The system maintains a constant effluent flowrate, regardless of the influent flowrate.

Secondary Treatment: The main objective of secondary treatment is to remove fine suspended solids and soluble BOD_5. Secondary treatment is generally, but not exclusively, biological.[5] The majority of currently used biological processes are aerobic systems, requiring oxygen from mechanical aeration devices to support waste-consuming microorganisms. If oxygen is not supplied, the system is anaerobic, producing troublesome waste products, requiring further treatment. Anaerobic treatment systems are used in some industries; however, odors and lower treatment efficiencies are a problem.

Septic tanks are the most widely used treatment system for small wastewater flows (8). A well designed septic tank system consists of

two components: (1) a tank designed to settle and anaerobically degrade TSS, and retain floatable grease and scum, and (2) a soil absorption field or polishing treatment to further degrade soluble BOD_5, and remove TSS and nutrients.

The soil absorption field is critical to proper operation of these systems, using a septic tank alone does not provide adequate wastewater treatment. For many seafood processors located along bayous or streams, septic tank treatment systems can be severely limited by shallow water tables or inadequate land availability for the soil absorption components.

Activated sludge is a biological wastewater treatment process in which a mixture of wastewater and biological sludge is agitated and aerated (7). Conventional activated sludge consists of a primary clarifier or sedimentation basin followed by the aeration basin and secondary clarifier. Biological sludge is produced in the aeration basin and separated in the secondary clarifier. Some of the sludge is recombined with the influent wastewater, the rest is wasted. Aeration and mixing is provided by diffusers or mechanical mixers. Expected removal of BOD_5 and TSS can range from 85 to 93 percent and 80 to 90 percent, respectively.

Wastewater stabilization lagoons are another option for secondary treatment. The three major types of lagoons include: facultative, aerated, and anaerobic. However, effective lagoon systems require large land areas.

Facultative lagoons are shallow and have three layers or zones. The upper layer is 100% aerobic due to wind-induced oxygen transfer or algae photosynthesis. The middle layer contains facultative bacteria which can function in either aerobic or anaerobic conditions. The bottom layer is anaerobic and actively decomposes accumulated solids. Plant upsets may cause facultative lagoons to become anaerobic, incurring odor problems and lowered treatment efficiency.

Aerated lagoons are 100 percent aerobic, with oxygen supplied mechanically by surface aerators or submerged diffusers. Greater, more reliable oxygen availability allows treatment of a higher daily volume of wastewater. Lower land requirements and costs of construction make aerated lagoons advantageous to use.

Anaerobic lagoons provide no mechanism for oxygen transfer. Incomplete degradation of organic wastes and TSS is performed by anaerobic bacteria, therefore this treatment must be followed by an oxidation pond or aerated lagoon to "polish" the effluent. Odor problems are commonly associated with anaerobic lagoons.

Attached growth biological contactors include processes such as trickling filters, rotating biological contactors (RBC), and fluidized

beds (FB). The media is covered with aerobic microorganisms that degrade organic matter in wastewater. Bacterial biomass builds up on the rocks or media and eventually sloughs off from the surfaces. A second clarifier must be provided to remove these solids from the effluent. Trickling filters must be preceded by primary treatment or the bed will clog with solids (8).

Rotating biological contactors (RBC's) are series of rotating disks (biological filters) placed equidistantly, partially submerged in a wastewater tank. The rotation provides aeration. RBC's require less space for operating than trickling filters.

In biological fluidized beds, microorganisms are attached to a media as described for trickling filters and RBC's; however, the media is a sand grain that is continuously suspended in an aerated flow of wastewater. Because of the large surface area provided by the sand, fluidized beds are more efficient than trickling filters, activated sludge, or RBC's, and usually require less space. However, pumping costs associated with the process can be prohibitive.

Dissolved air flotation (DAF) is a physical treatment method to remove dissolved material and O/G from wastewater with the aid of very tiny air bubbles (10). Air is dissolved through pressurization into the wastewater. The wastewater is held in a flotation tank to allow the dissolved air to escape and attach to suspended particles, which float to the top for skimming.

Roughing Treatment: Roughing treatment is designed to reduce BOD_5 and TSS concentrations to levels acceptable for secondary treatment (50 to 500 mg/l BOD_5). Roughing treatment pre-treats high waste loads minimizing shock loading effects. Roughing treatments are designed to treat a higher concentration of wastewater. Stabilization lagoons, RBC's, and trickling filters are commonly used for roughing because of tolerance to shock loading, resistance to clogging, and low operating costs.

Polishing Treatment: Polishing treatment is usually employed as a final treatment step to reduce TSS, BOD_5, and nutrients to meet more stringent effluent limitations. Polishing treatments for BOD_5 and TSS removal include: sand filters, rock-reed filters, and wetlands treatment. Polishing treatments for nutrient removal include: fluidized beds, RBC's, and chemical precipitation.

Sand filters are effective for the removal of BOD_5 and TSS down to low concentrations. Two types available to seafood processors include rapid and slow sand filters. In rapid sand filters, wastewater flows into the system and downward through the filter bed until flow is restricted due to clogging. The filter is backwashed (cleaned) by expanding the bed countercurrently with clean water. The backwash water must be discharged to a sewer or to a slow sand filter

for solids collection. The rapid sand filter is effective for high flowrate systems, though package units are available for almost any sized facility.

Slow sand filters are passive filters that have a graded sand media with no provision for backwashing. Wastewater flow is distributed over the bed and flows downward through the media where solids collect in the upper two inches of the bed. When the filter begins to clog, the flow is diverted and the wastewater is allowed to slowly seep into the bed until the bed is drained. The bed is cleaned by scraping the upper one to two inches of sand, which should contain most of the filtered suspended solids. This material is disposed of as a solid waste in a dumpster or landfill.

Wetlands naturally remove TSS and BOD_5 through a variety of mechanisms: (1) Plants and bottom surfaces provide sites for microbial activity and the lowered water velocity effects sedimentation (9), (2) plant uptake of nutrients may effect nutrient removal, and (3) long hydraulic detention times and shallow water provide re-aeration conditions that will degrade soluble BOD_5. The use of natural wetlands for wastewater treatment is experimental and direct application for seafood processors is limited. Discharge regulations perceive wetlands as receiving water bodies, not treatment units. Further study is needed for widespread use of this approach, but it does show promise as a low-cost treatment method for seafood processors located near wetlands.

Constructed artificial wetlands, or rock-reed filters, consist of shallow lined or unlined basins filled with two to three feet of rock media supporting the growth of emergent plants and attached microorganisms. Wastewater flows into the system via a submerged inlet. The plants uptake some nutrients, however, microorganisms attached to plant roots and rock media provide the bulk of the treatment. Suspended solids are removed by simple entrapment in the rock and roots. Water levels are maintained just below the surface of the rock media to minimize odor and insect problems and to hinder the growth of algae. Rock-reed filters are very effective for treating low strength wastewaters.

SUMMARY

The United States Environmental Protection Agency and the Louisiana Department of Environmental Quality issue permits to regulate discharges from seafood processors. These permits require the processor to supply detailed information about process flows and waste generation. All information supplied becomes public record. In general, seafood processors will interact more frequently with the state than the federal agency.

Most states presently use technology based effluent limitations to determine treatment requirements. Waste Load Allocation procedures may be used in the near future for coastal areas or sluggish streams with low reaeration potential. These procedures will almost certainly result in more stringent effluent regulations.

Use of waste minimization techniques may offer the most cost-effective alternative for reducing wastewater flow and strength. Success of these techniques will be dependent upon the type of processor. Support utilities to pick up by-products such as solids must be inplace before solids separation will be effective. Further studies are needed to investigate actual wastewater reductions that can be achieved through specific technology and management practices. In addition, FDA regulations that apply to reuse and recycle of process water must be fully addressed to identify limitations.

Conventional wastewater treatment technologies can be applied to seafood processing wastewaters, but land availability can be limiting. Specific treatment components to be included depend upon permit restrictions, site constraints, and overall cost considerations. Further studies are needed to examine the integration of compact wastewater treatment methods with waste minimization practices.

ACKNOWLEDGEMENTS

This work was supported, in part, by a grant from the Louisiana Department of Environmental Quality and the Louisiana Sea Grant Program. The authors would like to thank Renee Thomasson for her editorial investigative assistance for this project and to Susan Sartwell for typing the manuscript.

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THE USE OF HYDROCYCLONES TO TREAT MECHANICAL SHRIMP PEELER EFFLUENT

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INTRODUCTION

Disposal of seafood processing effluents is a major problem of Gulf of Mexico shrimp processors. Most large shrimp processing plants use Laitram Model A mechanical peelers capable of peeling up to 1100 pounds (499 Kg) of head-on shrimp per hour. The peelers require an average of 8.6 gallons (32.5 L) of water to peel each pound of head-on shrimp. Based on those parameters, each mechanical peeler can generate 9,460 gallons (35,808 L) of effluent per hour or 75,680 gallons (286,464 L) per 8-hour workday. Some Gulf Coast shrimp processors operate as many as eight shrimp peelers simultaneously.

Shrimp processing plants are located throughout the Gulf region, either singularly or clustered together in seafood ports. Singular, remote shrimp processing plants may discharge their processing effluents directly to receiving waterways according to the stipulations of their National Pollution Discharge Elimination System (NPDES) permits. Clustered shrimp processing plants generally discharge their effluents to municipal sewage treatment systems. The sewage treatment plants' effluents must also comply with NPDES permit limitations.

NPDES permits are maintained by either state environmental regulatory authorities or the U. S. Environmental Protection Agency (USEPA) depending on locality. Nonbreaded shrimp processing NPDES permit limitations generally include permissible pH, biochemical oxygen demand (BOD), and total suspended solids (TSS) levels. In recent years, state and federal environmental regulators have stringently enforced seafood processing plant NPDES permits. Many remote processors and municipal sewage treatment plants which serve clusters of processors have had difficulty meeting their NPDES permit limitations. As a result, state and federal environmental regulators levied fines and entered lawsuits in courts against processors and municipalities.

Mauldin and Szabo (1974) indicated that mechanical shrimp peeler wastewater contained 3.2 -4.7 lb (or Kg) of BOD per 100 lb (or 100 Kg) of shrimp processed. They also noted TSS in the range of 1.6 - 5.5 lb (or Kg) per 100 lb (or 100 Kg) of shrimp processed. Assessing the effectiveness of dissolved air flotation (DAF) for removal of pollutants from screened shrimp peeler wastewater, the investigators found that DAF reduced effluent BOD, TSS, turbidity and protein by 65.1%, 65.6%, 83.0% and 52.5%, respectively.

Perkins (1977) found that screened raw shrimp processing wastewater contained 0.2 - 1.0 lb (or Kg) of BOD per 100 lb (or 100 Kg) of shrimp processed. Protein content ranged from 0.2 - 2.6 g per gallon (0.053 - 0.687 g/L) of screened raw shrimp processing wastewater. The study also showed that isoelectric precipitation and centrifugation reduced effluent BOD and protein by 61.0% and 57.9%, respectively.

More recently Johnson and Lindley (1982) illustrated that a three-stage hydrocyclone system could remove 77.5% of TSS and 72% of the turbidity from screened shrimp processing wastewater. In a further refinement, Johnson and Gallanger (1984) showed that adding the coagulant chitosan to screened shrimp processing wastewater (after passage through the three-stage hydrocyclone system) removed 98% of TSS and 96% of the turbidity.

Perkins and Harper (1988) conducted a study to determine the quantity and quality of proteinaceous solids recoverable from mechanical shrimp peeler effluent. Solids were recovered by HCl precipitation and centrifugation. Recovery of solids from untreated effluent was 1% - 2% by weight and was predictable by turbidity. The precipitation/centrifugation process reduced supernatant total organic nitrogen, biochemical oxygen demand and turbidity by 49.8%, 50.0% and 92.4%, respectively, compared with untreated effluent. Total aerobic plate counts (APC) of bacteria recovered from unprocessed shrimp and precipitated solids were 10^5 - 10^6 CFU/g, approximately 1.5 log units greater than from peeled shrimp or untreated effluent. Total APC of bacteria recovered from clarified effluent was 3.2×10^1 CFU/ml.

MATERIALS AND METHODS

Byproduct Recovery Apparatus

Water containing dissolved and suspended fragments of shrimp meat, shrimp shell, and shrimp protein was collected in a stainless steel tank following its separation from the primary product (peeled shrimp tail meat). The recovery system was installed in-line following the peeled shrimp/peeler effluent separation step.

Peeler effluent was piped from the collection tank to the recovery system with 3-inch diameter Schedule-80 PVC pipe, and peeler effluent flow controlled by a 3-inch gate valve. The 3-inch PVC pipe was connected to the inlet of a Gould's Model 3196-ST stainless steel food pump (rated at 100 GPM at 70 psi) driven by a 5 hp electric motor. The outlet of the pump was connected to the inlet of the first hydrocyclone with 2-inch PVC pipe. The first hydrocyclone was a 6-inch Dorr-Oliver Model 6-NZ-C Dorrclone Hydrocyclone, fitted with a 1-inch vortex finder and 3/8-inch apex valve. The overflow from the 6-inch hydrocyclone was connected to the inlet of the second hydrocyclone with 1 1/2-inch PVC pipe. The second hydrocyclone was a 3-inch Dorr-Oliver Model 3-NZ-C Dorrclone Hydrocyclone, fitted with a 5/8-inch vortex finder and a 1/4-inch apex valve. The overflow from the 3-inch hydrocyclone was piped to a floor drain with 1-inch PVC pipe.

Pilot Plant Operation and Sample Collection

Eleven collections of mechanical shrimp peeler effluent were made from July through September 1988. The pickup gate valve was opened, and the pilot system electric motor started. Peeler effluent was forced through the pilot system at a rate of 100 GPM and 70 psi. The system was allowed to run for a minimum of 2 minutes to allow liquid to exit both underflows and the overflow.

Untreated effluent samples were collected from the stainless steel tank before entry into the recovery system. Underflow 1 and Underflow 2 samples were collected following exit from the 6-inch hydrocyclone and 3-inch hydrocyclone, respectively. Overflow samples were collected at end of pipe, immediately prior to entry in the floor drain.

All samples were collected in sterile 1-quart plastic whirl-paks. Duplicate 1-quart samples were collected on each sample date at each sample point. All samples were placed on ice in an insulated plastic cooler for transport to the laboratory thirty miles away.

Sample collections usually took 1 hour, and transport from the processing plant to the laboratory never took more than 1 hour. Sample analyses were begun no later than 1 hour after arrival at the laboratory. Therefore, no samples were more than 3 hours old at the time analyses were begun.

Physical and Chemical Determinations

Turbidity measurements of all four sample types were taken on all eleven sample dates. Turbidity measurements were made with a Hach Model 2100A turbidimeter calibrated with standard tubes.

Total Kjeldahl Nitrogen (TKN) and ammonia nitrogen (NH4) determinations of all four sample types were made on all eleven sample dates according to standard methods (AOAC, 1980). Five-day biochemical oxygen demand (BOD5) analyses were performed according to the standard Winkler Azide Modification (EPA, 1983).

Microbiological Determinations

Total aerobic plate counts (APC) of all four sample types were performed on ten collection dates. Fecal coliform counts for all four sample types were conducted on seven collection dates. All handling and preparation, total aerobic plate count, and fecal coliform methods conformed to methods prescribed in the FDA Bacteriological Analytical Manual (AOAC, 1984).

RESULTS AND DISCUSSION

Mean turbidity values for untreated effluent, underflow 1, underflow 2, and overflow are presented in Table 1. The results show that the 6-inch hydrocyclone concentrated the turbidity of the untreated effluent by 94%. The 6-inch hydrocyclone performed as hypothesized, and nearly doubled the concentration of dissolved and suspended organic particles found in the untreated effluent.

TABLE 1. SHRIMP PEELER WASTEWATER TURBIDITY.

Sample Type	No. of Trials	Turbidity, FTU (Mean ± SD)
Untreated Effluent	11	49 ± 29
Underflow 1	11	95 ± 57
Underflow 2	11	81 ± 50
Overflow	11	71 ± 33

However, underflow 2 turbidity was concentrated to a mean of only 81 Fluorometric Turbidity Units (FTU), or only 65% more turbid than the untreated effluent. Apparently, the 3-inch hydrocyclone was unable to additionally concentrate effluent turbidity. And, the mean overflow turbidity was 71 FTU, or 45% more turbid than the untreated effluent. The overflow turbidity should have been less than the turbidity of the untreated effluent. Apparently, the 3-inch hydrocyclone's inability to sufficiently concentrate turbidity in the underflow caused the unexpectedly high reading.

The rather large standard deviations shown in Table 1 indicate the extreme variability of the effluent samples encountered throughout the course of the study. From sample type to sample type, and from collection to collection, no definite turbidity recovery pattern could be determined.

Mean Total Kjeldahl Nitrogen (TKN), ammonia nitrogen (NH4), and total organic nitrogen (TON = TKN - NH4) values for untreated effluent, underflow 1, underflow 2, and overflow are presented in Table 2. The 6-inch hydrocyclone concentrated the TON of the untreated effluent by 109%, or 15% more than the turbidity was concentrated at the same point.

TABLE 2. SHRIMP PEELER WASTEWATER NITROGEN.

Sample Type	No. of Trials	TKN, mg/L (Mean ± SD)	NH4, mg/L (Mean ± SD)	TON, mg/L (Mean ± SD)
Untreated Eff	11	100 ± 75	4.5 ± 1.5	95 ± 75
Underflow 1	11	209 ± 144	10 ± 21	199 ± 146
Underflow 2	11	168 ± 122	10 ± 20	158 ± 122
Overflow	11	170 ± 64	6.2 ± 7.3	164 ± 64

However, underflow 2 TON was concentrated to a mean of only 158 mg/L, or only 66% more concentrated than the untreated effluent. (That percentage increase in TON concentration was almost equivalent to the percentage increase in concentration of untreated effluent turbidity at the same point.) And, the mean overflow TON was 164 mg/L, or 72% greater than the untreated effluent. The overflow TON should have been less than the TON of the untreated effluent. Apparently, the 3-inch hydrocyclone's inability to sufficiently concentrate TON in the underflow caused the unexpectedly high reading. The rather large standard deviations shown in Table 2 indicate the extreme variability of the effluent samples encountered throughout the course of the study.

Mean 5-day biochemical oxygen demand (BOD5) values for untreated effluent, underflow 1, underflow 2, and overflow are presented in Table 3. The 6-inch hydrocyclone concentrated the BOD5 of the untreated effluent by 135%. The 3-inch hydrocyclone concentrated the BOD5 of the untreated effluent by 136%, or slightly more than did the 6-inch hydrocyclone. These results were consistent with the project's hypothesis that each hydrocyclone in the pilot system would provide additional concentration of organic materials from the untreated effluent.

TABLE 3. SHRIMP PEELER WASTEWATER BIOCHEMICAL OXYGEN DEMAND.

Sample Type	No. of Trials	BOD5, mg/L (Mean ± SD)
Untreated Effluent	7	591 ± 342
Underflow 1	7	1387 ± 1272
Underflow 2	7	1395 ± 1594
Overflow	7	1038 ± 647

However, the mean overflow BOD5 was 1038 mg/L, or 75% greater than untreated effluent BOD5. (That percentage increase in BOD5 concentration was nearly equivalent to the percentage increase in concentration of untreated effluent TON at the same point.) The overflow BOD5 should have been less than the BOD5 of the untreated effluent. Apparently, the 3-inch hydrocyclone's minimal additional concentration of effluent BOD5 in the underflow caused the high reading. The rather large standard deviations shown in Table 3 indicate the extreme variability of the effluent samples encountered throughout the course of the study. From sample type to sample type, and from collection to collection, no definite BOD5 recovery pattern could be determined.

The results of total aerobic plate count (APC) determinations (expresses as geometric means) for all four sample types are summarized in Table 4. The mean Log10 APC of underflow 1 was elevated 1.4 log units above the mean Log10 APC of the untreated effluent. The mean Log10 APC of underflow 2 was elevated an additional 0.17 log unit. Those results were consistent with the project's hypothesis.

TABLE 4. SHRIMP PEELER WASTEWATER, LOG10 APC.

Sample Type	No. of Trials	Log10 APC/ml (Mean ± SD)
Untreated Effluent	10	3.72 ± 0.67
Underflow 1	10	5.13 ± 0.96
Underflow 2	10	5.30 ± 1.09
Overflow	10	4.78 ± 0.57

However, the mean Log10 APC of the overflow remained 1.06 log units greater than the mean Log10 APC of the untreated effluent. Apparently, the 3-inch hydrocyclone's limited ability to additionally concentrate microorganisms caused a greater number to report to the overflow.

The smaller standard deviations associated with the aerobic plate count organisms indicated that the recovery of miroorganisms was much less variable (and therefore more reproducible) than the recoveries of turbidity, TON, and BOD5. The microbiological recoveries noted in the present study were also closely aligned with the microbiological recoveries shown in the previous bench-scale phase of the study (Perkins and Harper, 1988).

The results of fecal coliform determinations (expressed as arithmetic means) for all four sample types are shown in Table 5. The number of fecal coliforms in underflow 1 was reduced to a level which was 44% lower than the number of fecal coliforms noted in the untreated effluent. However, the number of fecal coliforms in underflow 2 was increased by 142% above the number of fecal coliforms found in the untreated effluent. And, the number of fecal coliforms in the overflow was increased by 277% above t the number of fecal coliforms in the untreated effluent. Given the large standard deviations shown in Table 5, and the unpredictable recoveries of fecal coliforms, the pilot system apparently exhibited little control on fecal coliforms.

TABLE 5. SHRIMP PEELER WASTEWATER FECAL COLIFORMS.

Sample Type	No. of Trials	Fecal Coliforms/100 ml (Mean $\pm$ SD)
Untreated Effluent	7	22.8 $\pm$ 14.9
Underflow 1	7	12.8 $\pm$ 23.9
Underflow 2	7	55.3 $\pm$ 41.0
Overflow	7	85.9 $\pm$ 15.5

In summary, the mean values of all parameters (turbidity, TON, BOD5, Log10 APC, and fecal coliforms) were concentrated above untreated effluent base levels in all underflow 1, underflow 2, and overflow samples except one. Concentration of the chemical and microbiological parameters was expected in underflow 1 and underflow 2. The concentration noted in the overflow was not consistent with either the system design or the project hypothesis.

The authors recommend that the shrimp byproduct recovery technologies analyzed in years 1 and 2 be combined. Specifically, hydrochloric acid should be injected into the system to assist with coagulation of dissolved and suspended solids. Analyses of system input and output flows should be conducted to calculate material balances. A third 1 1/2-inch hydrocyclone should be installed to allow for additional pilot plant test loop configurations. It is hypothesized that shrimp peeler effluent treatment efficiency will be increased, yielding more complete dewatering of solids and final effluent clarification.

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ACKNOWLEDGMENTS

The contributions of the following organizations and individuals are gratefully acknowledged:

- Alabama Department of Economic and Community Affairs for providing project funding.

- Auburn University/Alabama Cooperative Extension Service for providing matching funds.

- Dr. Burke Brown, University of South Alabama, for providing laboratory facilities.

- The cooperating shrimp processor for allowing access into the plant for sample collection, and for assisting with byproduct recovery system installation and equipment and supply purchase and installation.

EVALUATION OF PROTEOLYTIC ENZYMES FROM THE HEPATOPANCREAS OF CRAWFISH (PROCAMBARUS CLARKII).

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INTRODUCTION

It has been found that crawfish develop a "mushy" texture during cold storage. This textural defect has been presumed to be due to degradation of structural proteins and is most prominent in the first section of the abdomen (tail). The cause of this mushiness may be due to proteinases from the hepatopancreas, muscle tissue, or a combination of both.

Several proteinases have been found in the internal organs of fish. Trypsins have been found in the pyloric caeca of mackerel (Kim and Pyeun, 1986) and sardine (Murakami and Noda, 1981), in the gut of capelin (Hjelmeland and Raa, 1982), in the pancreas of catfish (Yoshinaka et al., 1983a) and in the digestive tract of various crustacea (Lee et al., 1980; Kimoto et al., 1983; Zwilling and Neurath, 1981). Chymotrypsins were found in the pancreas of dogfish (Ramakrishna et al., 1987) and catfish (Yoshinaka et al., 1981), in the pyloric caeca of sardine (Murakami and Noda, 1981) and mackerel (Kim and Pyeun, 1986), and in the digestive tract of krill and various shrimps (Kimoto et al., 1985; Tsai et al., 1986; Zwilling and Neurath, 1981). Pepsin-like proteinases, aminopeptidases and carboxypeptidases have also been isolated from various internal organs of sardine (Noda and Murakami, 1981; Vo et al., 1983) and other fish species (Osnes and Mohr, 1986; Osnes et al., 1986; Osnes and Mohr, 1985; Kimoto and Murakami, 1984). Collagenolytic proteinases were found in the catfish pancreas (Yoshinaka et al., 1986a; 1987) and in the hepatopancreas of the fiddler crab (Welgus and Grant, 1983; Welgus et al., 1982; Eisen et al., 1973). Also, elastases were detected in the pancreas of various sharks (Yoshinaka et al., 1986b), catfish (Yoshinaka et al., 1984; 1983b; 1982) and several species of fish (Yoshinaka et al, 1985a: 1985b).

Because of the wide range of proteinases that could be present in the hepatopancreas of crawfish, it is difficult to study the effect of enzymes on textural quality or the effect of processing on their activity. Therefore the long-range purpose of this research is to determine the type of enzymes responsible for mushiness in crawfish during refrigerated storage (4°C) and to develop appropriate processing techniques to preserve the quality of refrigerated crawfish. The objective of this initial study was to isolate and characterize proteinases that could be responsible for the degradation of the tail meat.

MATERIALS AND METHODS

The hepatopancreas of live crawfish was collected, frozen immediately in a liquid nitrogen cabinet freezer and stored at -85°C until use in crude enzyme extraction.

Four hundred ml of 1% sodium chloride solution containing 1 mM Na_2EDTA was added to 100 g of the hepatopancreas and homogenized in a Waring blender for 30 sec. The extract was held at 30°C for 4 hrs to autoactivate zymogens. The activated extract was centrifuged at 12,000xg for 20 min at 4°C and the supernatant was filtered through Whatman No.1 filter paper. The supernatant was dialyzed against distilled water overnight and centrifuged at 12,000xg for 20 min. The supernatant was considered the crude enzyme solution.

Caseinolytic activity of the crude preparation was measured by a modification of the method of Rick and Fritch (1974). The assay mixture was composed of 0.75 ml of buffer (using a pH range from 2.0-11.0), 0.25 ml of 4% casein solution and 0.05 ml of enzyme solution. The reaction mixture was incubated for 30 min at various temperature between 20 and 70°C. The reaction was stopped by addition of 1.25 ml of 5% (w/v) trichloroacetic acid (TCA) solution. After standing for 30 min at room temperature, the solution was centrifuged at 3,000xg for 15 min. To 0.5 ml of supernatant was added 1.25 ml of 0.55 M sodium carbonate solution and 0.5 ml of 3-fold diluted solution of phenol reagent. The mixture was incubated at 30°C for 20 min and proteinase activity was determined by absorbance at 660 nm. One unit of proteinase activity was defined as the absorbance equivalent of 1 umole tyrosine produced per min under experimental condition.

Purification of proteinase from crude enzyme solution was undertaken by first employing ammonium sulfate fractionation with 30-70% saturation. The suspension was dialyzed against 0.01 M Tris-HCl buffer, pH 6.8 and applied to a DEAE-Sephadex column (2.6x40 cm) equilibrated with the same buffer solution. The column was eluted with a 2000 ml linear gradient ranging from 0 to 1.0 M sodium

chloride. The high proteolytic activity fraction (DEAE#1) obtained by this chromatography was concentrated and dialyzed against the same buffer, pH 6.8. The DEAE#1 fraction was rechromatographed using a DEAE-Sephadex A-50 column (2.6x40 cm), equilibrated with the same eluent and the high proteolytic activity fraction (DEAE#2) was concentrated and dialyzed against 0.002 M phosphate buffer, pH 6.8. The DEAE#2 fraction was applied to a HTP-hydroxylapatite column (1.5x7 cm) equilibraed with the same phosphate buffer, pH 6.8. The elution was performed with a linear gradient from 2 to 200 mM phosphate buffer, pH 6.8. The major peak (HTP) with caseinolytic activity was concentrated and dialyzed against 0.01 M Tris-HCl buffer, pH 6.8, containing 0.1 M NaCl. The dialyzed solution was applied to a Sephacryl S-300 column (2.5x90 cm) equilibrated with the same buffer, pH 6.8. The fraction (Sephacryl) with high caseinolytic activity was concentrated with ultrafiltration and stored at -85^{o}C until use in subsequent characterization studies.

Protein concentration was determined by the method of Lowry et al (1951) using bovine serum albumin as a standard protein. Electrophoresis was carried out according to the method of Davis (1964). Molecular weight of the purified proteinase was determined by Sephaacryl S-200 gel filtration according to the method of Andrews (1964).

A preliminary evaluation of enzyme identity was carried out using various inhibitors. The classes of inhibitors used included: serine enzyme inhibitors, diisopropylfluorophosphate and soybean trypsin inhibitor; the trypsin inhibitor, tosyl-lysine-chloro-ketone; the chymotrypsin inhibitor, tosyl-phenylalanine-chloro-ketone; metalloenzyme inhibitors, ethylenediaminetetraacetate (EDTA) and o-phenanthroline; the thiolenzyme inhibitor, p-chloromercuribenzoate; and the acid protease inhibitor, iodoacetate.

RESULTS AND DISCUSSION

The optimum temperature for activity of the crude enzyme extract from crawfish hepatopancreas was found to be 50^{o}C, with total loss of enzyme activity occurring between 70 and 75^{o}C (Figure 1.). The optimum pH was found to range from 5.8 to 9.0 (Figure 2.). The pH results established that one or more enzymes existed in the crude extract with activity in the neutral to alkaline pH range, but activity in the acid range was low. Therefore, it was decided to concentrate on the enzyme or enzymes with activity in the neutral to alkaline pH range.

The first DEAE-sephadex liquid chromatographic analysis revealed only one fraction with significant proteolytic activity. This fraction was purified through a

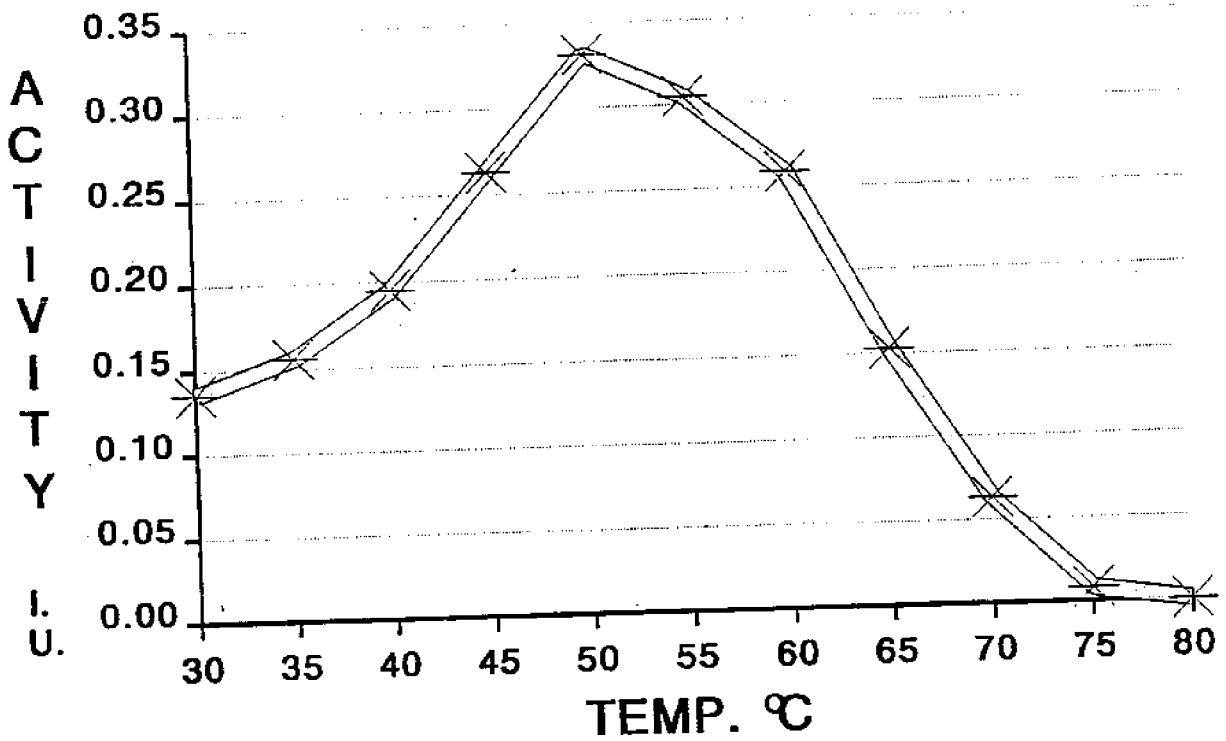

Figure 1. Effect of temperature on the enzyme activity of crude extract.

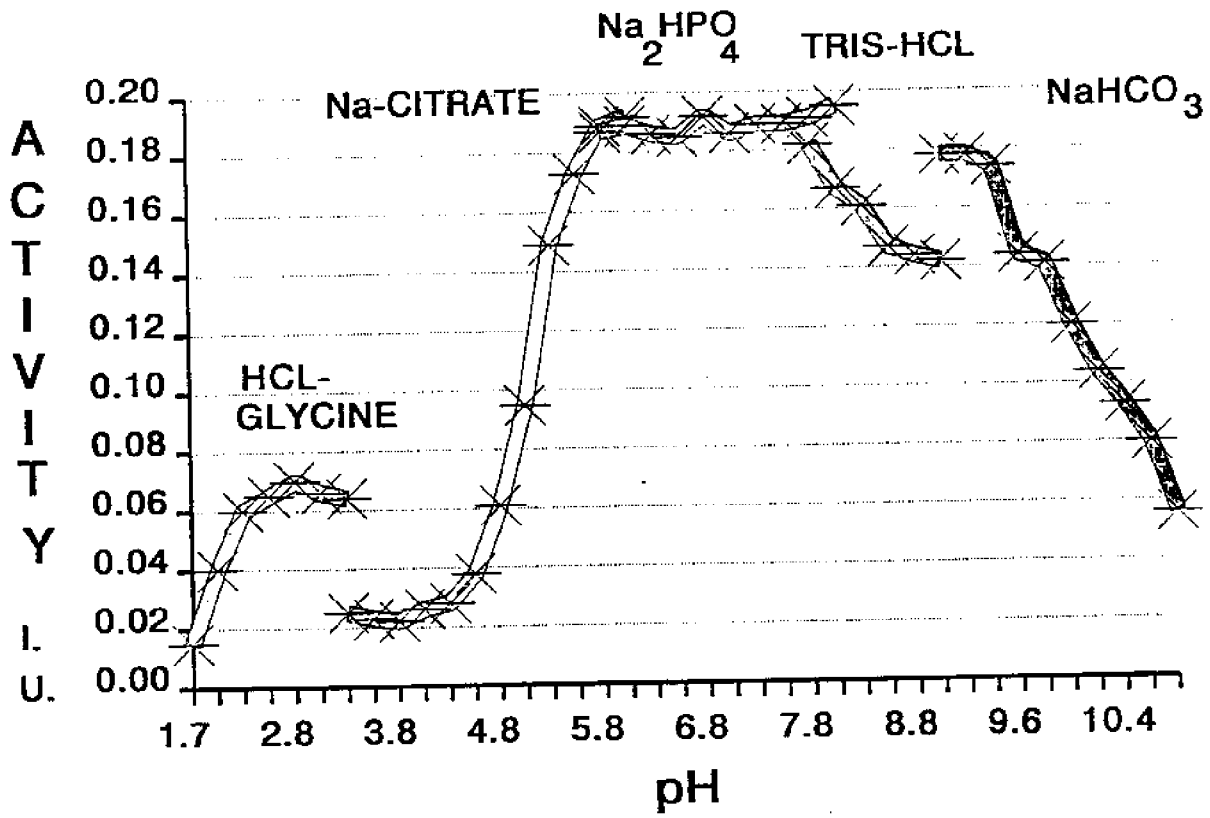

Figure 2. Effect of pH on enzyme activity using a crude extract.

series of susequent chromatographic separations (Table 1.). A purified enzyme extract was obtained with a 75 fold increase in activity. The overall yield was approximately 12 %.

Using standard protein markers with gel filtration analysis, it was established that the approximate molecular weight of the purified proteinase was 17,000 D (Figure 3.). Inhibitor analysis established that the enzyme was most probably a trypsin-like serine protease (Table 2).

This preliminary study established the existence of a major protease with optimum activity in the alkaline pH range. Preliminary identification as a serine proteinase will enable a more extensive study of its properties. Also, a purification scheme has been established, which will allow further study of processing effects on purified enzymes and possibly allow large scale recovery procedures.

Table 1. Summary of purification for isolation of protease from crawfish hepatopancreas.

Fraction	Volume (ml)	Protein (mg/ml)	Specific Activity (I.U.)	Relative Purity	Yield %
Crude	200	1.25	0.20	1.0	100
Salted	20	2.40	0.68	3.5	67
DEAE#1	8	0.36	4.96	25.4	48
DEAE#2	5	0.32	6.99	35.8	23
HTP	5	0.36	8.75	44.9	16
Sephacryl	3	0.34	14.60	74.9	12

DEAE-cellulose (DEAE)
Hydroxyapatite (HTP)

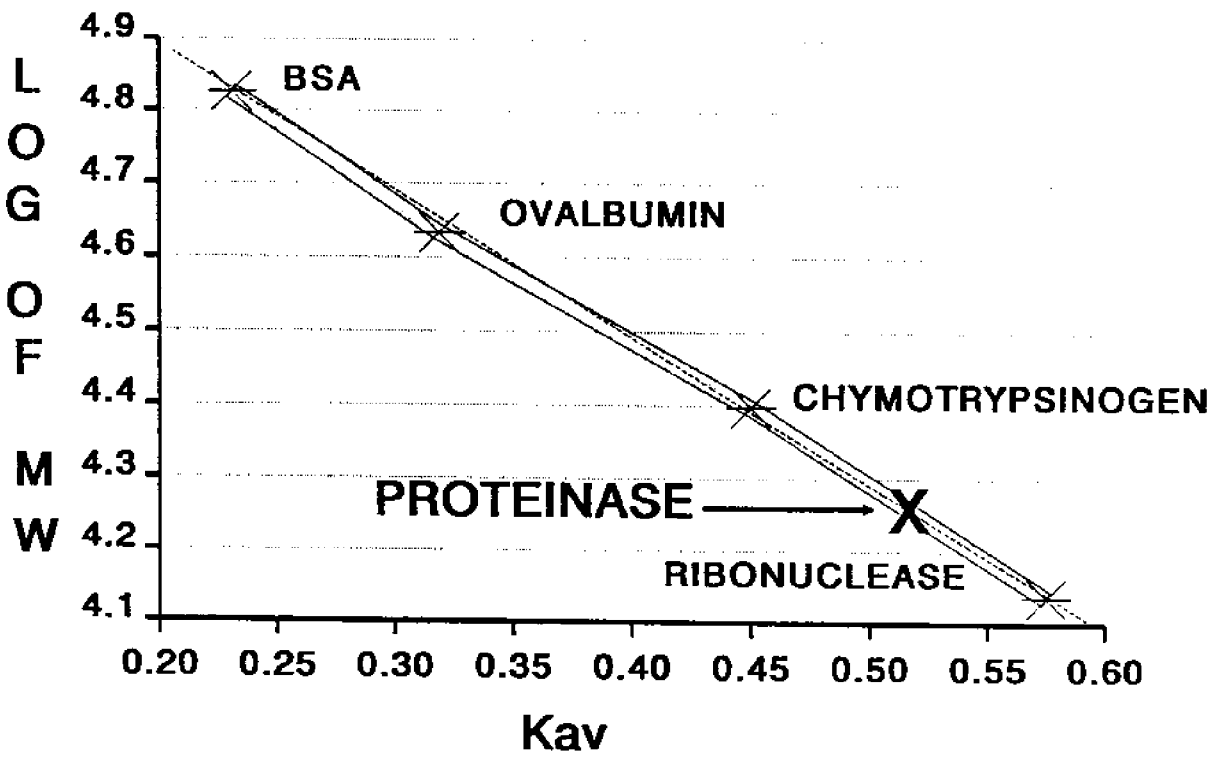

Figure 3. Estimation of molecular weight of purified enzyme.

Table 2. Effects of enzyme inhibitors on activity of purified protease.

Inhibitor	Type	Concentration	Relative Activity %
Diisopropyl-fluorophosphate	Serine	1 mM	11
Soybean trypsin inhibitor	Serine	1 mg/ml	7
TLCK[1]	Trypsin	1 mM	62
TPCK[2]	Chymotrypsin	0.34 mM	100
EDTA	Metallo	1 mM	63
o-Phenanth-roline	Metallo	1 mM	91
p-Chloromer-curibenzoate	Thiol	1 mM	95
Iodoacetate	Acid	1 mM	106

[1]Tosyl-lysine-chloro-ketone (TLCK)

[2]Tosyl-phenylalanine-chloro-ketone (TPCK)

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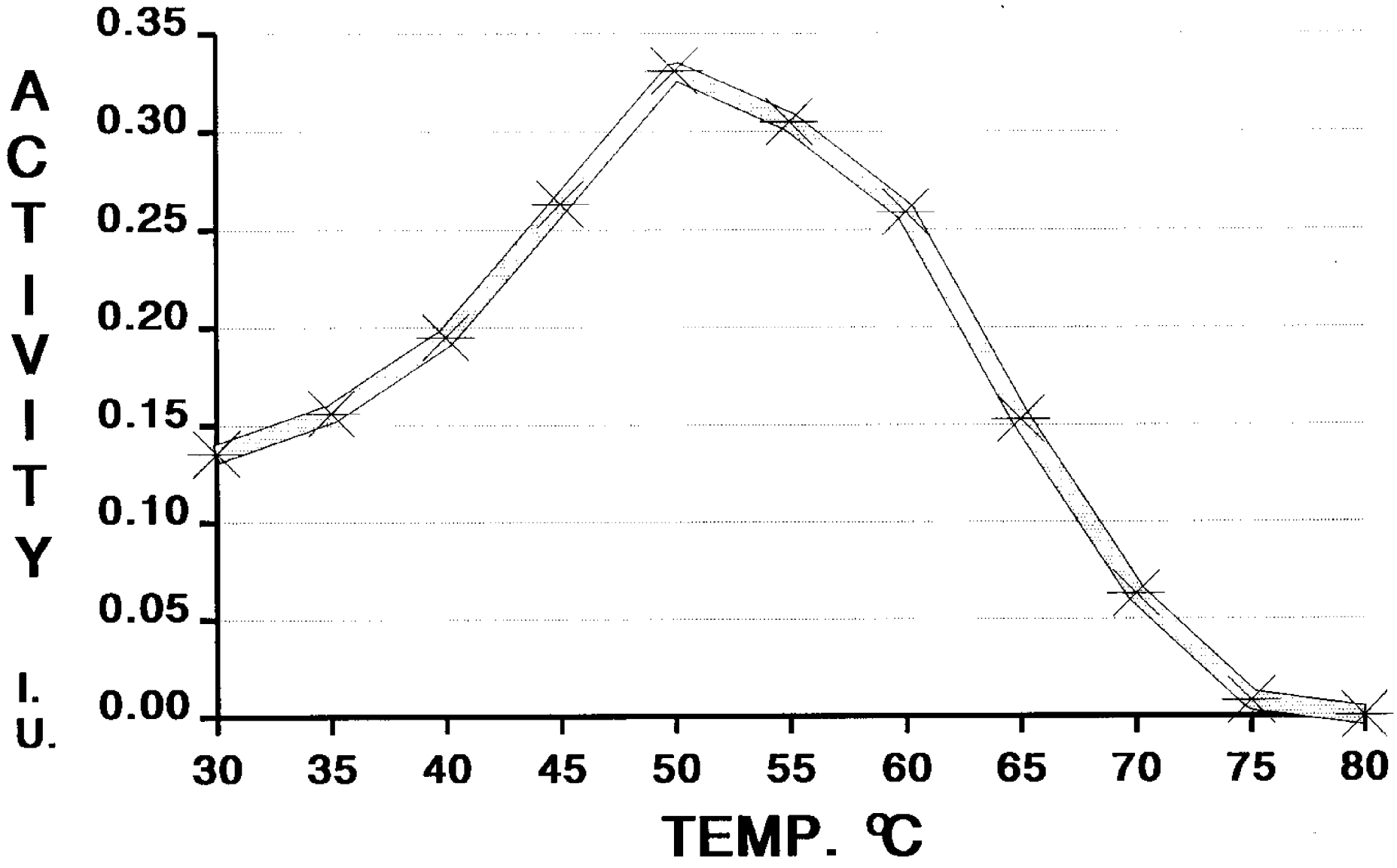

Figure 1. Effect of temperature on the enzyme activity of crude extract.

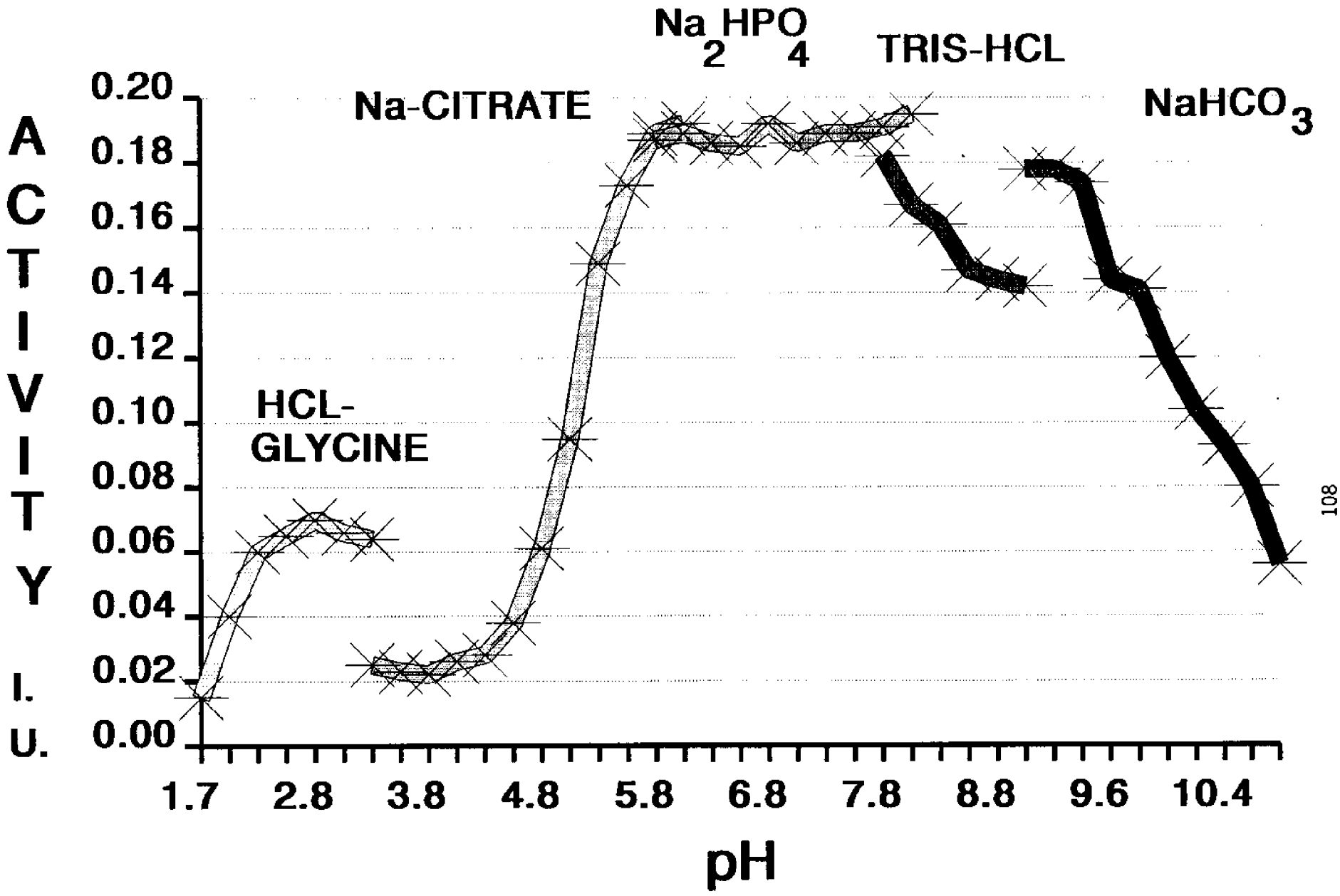

Figure 2. Effect of pH on enzyme activity using a crude extract.

Table 1. Summary of purification for isolation of protease from crawfish hepatopancreas.

Fraction	Volume (ml)	Protein (mg/ml)	Specific Activity (I.U.)	Relative Purity	Yield %
Crude	200	1.25	0.20	1.0	100
Salted	20	2.40	0.68	3.5	67
DEAE#1	8	0.36	4.96	25.4	48
DEAE#2	5	0.32	6.99	35.8	23
HTP	5	0.36	8.75	44.9	16
Sephacryl	3	0.34	14.60	74.9	12

DEAE-cellulose (DEAE)
Hydroxyapatite (HTP)

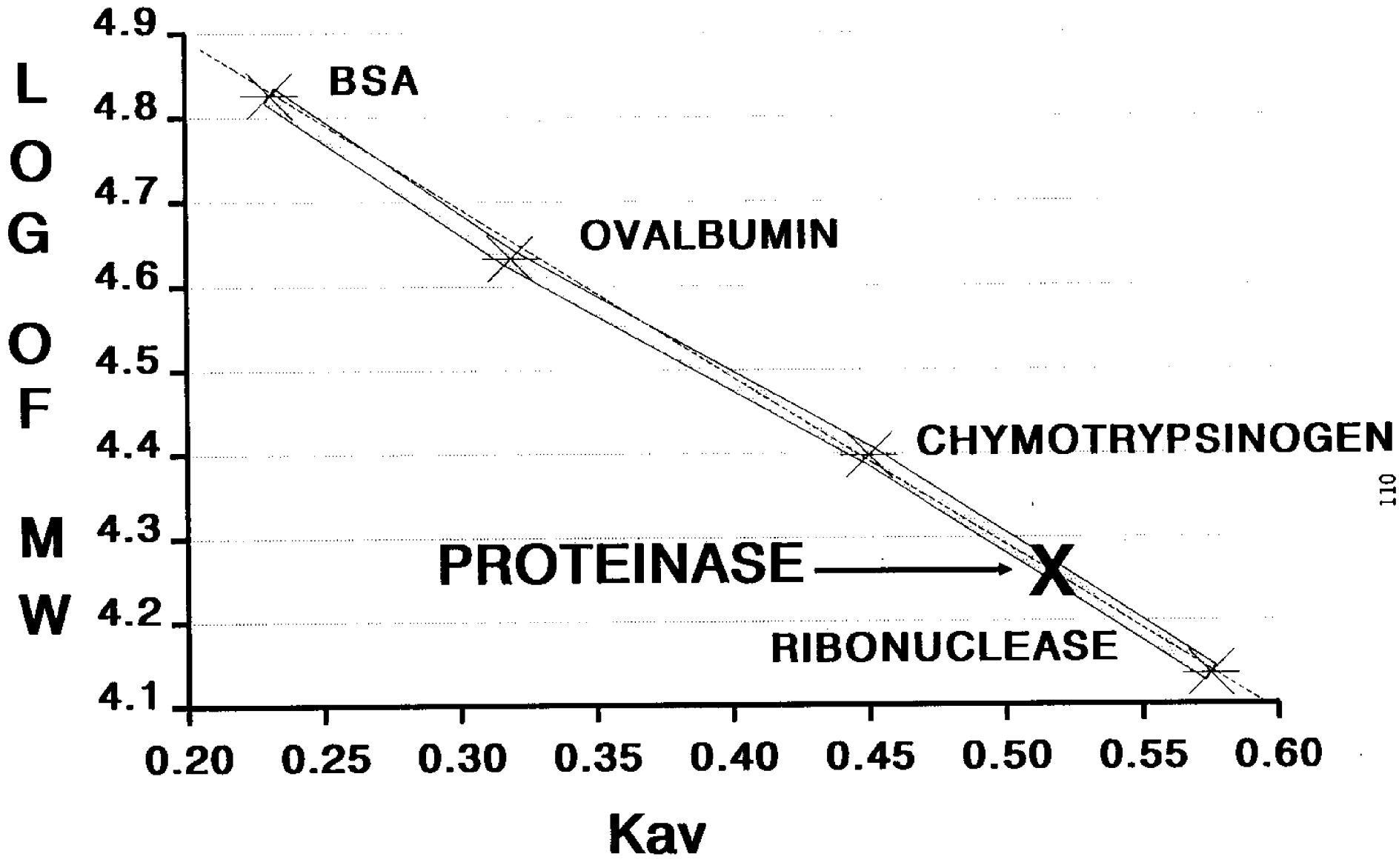

Figure 3. Estimation of molecular weight of purified enzyme.

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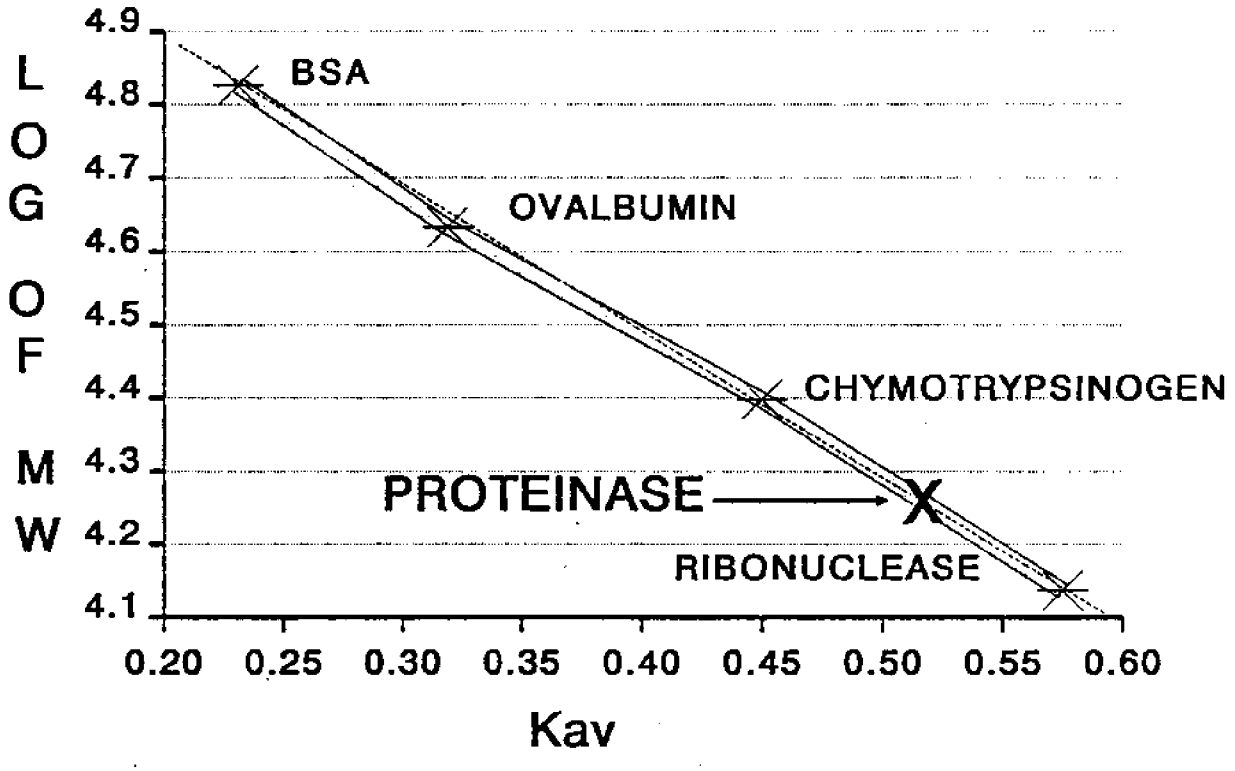

Figure 3. Estimation of molecular weight of purified enzyme.

OCCURRENCE OF BLACK SPOTS IN SHRIMPS UNDER PHYSIOLOGICAL CONDITIONS

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INTRODUCTION

Shrimp constitutes one of the principal marine products exported by Brazil mainly from its region of North and North-East. The country exports about 16,000 metric tons of frozen shrimps to U.S.A. and Japan. In the importing country, the frozen shrimps are usually thawed and refrozen in new packaging material which induces the occurrence of melanosis due to reduction in sulfite levels.

Melanosis in shrimps manifests as black spots localized principally in cephalothorax, and in junctions and bases of segments. This phenomenon constitutes a great problem for shrimp industry as in order to improve the quality of the crustacean, peeling is recommended which consequently results in an increase in labor cost and reduction in yields. Though there is no statistical data available on the incidence of melanosis in shrimps, yet it is estimated to be about 2% by the processing industries in Brazil.

The enzymic reaction responsible for black spots formation has been investigated mainly from biochemical aspects. Many studies on isolation, purification and characterization of phenoloxidases from crustacean species have been performed (Bailey et al., 1960; Savagaon and Sreenivasan, 1978; Marshall et al., 1984; Simpson et al., 1987). Cobb (1977) reported that there exists possibly a relation between molt cycle and melanosis in shrimps.

Ogawa et al. (1983) studied the mechanism of black discoloration in spiny lobster tails from the physiological

viewpoint. He reported that melanosis is induced by traumatisms occurred in live lobsters and associated the phenomenon with physiological factors such as molt stage, sex and other conditions at the time of death of crustacean. The present work is an attempt to investigate the black spots formation in shrimps from the viewpoint of physiological conditions.

MATERIALS AND METHODS

Fresh white shrimps (Penaeus schmitti) were acquired live and transported to a small aquarium in the "Laboratório de Ciências do Mar", Fortaleza, Brazil.

Reproduction of Melanosis:

Treatments:

After adaptation for a minimum period of 1 day in aquarium, the live shrimps were submitted to the following treatments:

Treatment-I: About 200 forced movements of flexion in tail for 90 seconds were applied.

Treatment-II: The junctions of the segments in the dorsal part were initially cut to the size of about 3-5 mm in length and 2 mm in depth, and forced movements as described in treatment-I were later applied. The shrimps still alive were left in sea water for 90 min at ambient temperature (28±2°C) in the proportion of 10 shrimps per 5 L of water.

Treatment-III: After confining the shrimps for 3 hours in a plastic bucket containing sea water, the shrimps - dead or almost dead - were later injured in the manner described in treatment-II.

After termination of each treatment, shrimps were individually analyzed for parameters such as weight, length, stage of the molt cycle and sex. After deheading, the tails were preserved in ice maintained in perforated containers which were kept in a refrigerator at 5±2°C for a period varying from 7 to 10 days.

Methods of Analysis:

The stages of the molt cycle were designated according to

the following physiological attributes (Drach and Tchernigovtzeff, 1967):

Stage A: Immediately after the molt, the exoskeleton is entirely deformable under the application of small pressure.
Stage B: The dorsal part is no more deformable but the rest remains flexible in certain regions; the ventral portion is still soft.
Stage C: The exoskeleton possesses a hard texture.
Stage D: The formation of pigmented layer of cuticula and reabsorption of old exoskeleton occurs (Period of preparation for the molt).

The intensity of black spots in shrimps was visually evaluated at junctions and bases of segments. At junctions, a scale varying from 0 (zero) to 5 scores was attributed wherein score 0 stands for absence of any black spots and score 5 designates that all junctions were dark. A scale between 0 (zero) representing absence of any black spots and 10 representing complete darkening, was used to evaluate the discoloration at bases of segments.

The statistical evaluation of data consisted of analysis of variance and comparison between the means (Steel and Torrie, 1980).

Melanin Indicator:

Shrimps used for this study were already dead and did not possess any signs of melanosis. After initial determination of weight, length, sex and molt-cycle of shrimps, a test procedure to indicate melanosis was developed. About 3 ml of catechol solutions of varying concentrations (0.2; 0.4; 0.6; 0.8; 1.0; 1.5; 2.0 M) prepared in Sorenson phosphate buffer at 6.8 pH were injected by applying 2 ml at five junctions of segments and 1 ml at two sides of cephalothorax, and later the time necessary for the start of enzymic reaction was noted. Initiation of enzymic reaction was considered at the instant when darkening was observed for the first time. Three shrimps were tested for each concentration of catechol.

The tendency for the appearance of black spots was analyzed in function to catechol concentration and reaction time. Regression equation and coefficient of correlation were calculated (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Reproduction of melanosis:

The distribution of shrimps utilized in the present study according to length and sex is presented in Table 1.

Table 1. Classification according to the length of shrimps utilized for reproduction of melanosis.

Range of length (cm)	Number of samples		
	Male	Female	Total
8 - 10	1	2	3
10 - 12	10	20	30
12 - 14	25	22	47
14 - 16	4	29	33
16 - 18	-	12	12
Total	40	85	125

Results on the incidence of black spots in shrimps submitted to three treatments are presented in Table 2. Highest incidence of black spots was observed in treatment-II (93.47%), which happened to be the most severe treatment with respect to traumatism suffered by shrimps. Treatment-I resulted in 78.6% of shrimps possessing black spots while treatment-III had incidence in 73.33% of shrimps. The average intensity of black spots was also found to be the highest (3.53 score) in treatment-II as compared to treatment-I (1.47 score) and treatment-III (0.98 score). Junctions were found to be more susceptible for darkening as compared to bases of segments. The results in Table 2 also reveal that in general, female subjects were more sensitive to darkening as compared to male subjects. However, the data (100%) for black spots formation for male subjects at junctions of segments in treatment-III should be ignored as the number of samples in this case had been minimum. Ogawa et al. (1983) observed the phenomenon of melanosis reproduction in lobsters to 100% level when injuries such as making small holes in cuticle were practiced.

Data on incidence of black spots in meat and shell of tails when examined after peeling, are presented in Table 3.

Table 2. Effect of different treatments on the appearance of black spots in shrimp tails.

Characteristics	Treatments		
	I	II	III
No. of samples:			
Male	21	19	1
Female	44	27	46
Total	65	46	15
Shrimps possessing black spots (%)	78.6	93.47	73.33
Average of the scores of black spots	1.47	3.53	0.98
Shrimps having black spots (%) at:			
Junctions:			
Male	16.9	100.0	100.0
Female	67.7	100.0	71.4
Bases:			
Male	6.1	13.0	0
Female	10.7	21.7	26.7

Appearance of black spots at junctions was found to be more predominant in superficial meat as compared to carapace. Highest incidence (73%) of black spots was observed in the meat portion of junctions of shrimps subjected to treatment-II, followed by treatment-III (40.4%) and treatment-I (26.1%). This difference led us to suppose that more severe injuries such as cuts at junctions in the case of treatments II and III provoked occurrence of black spots to a greater extent, attaining even the superficial meat. The fact that the incidence has been higher for treatment-II than treatment-III, is related to higher vitality (treatment-II) of animals with regard to suffering which had distinct difference between the treatments. On the other hand, for bases of segments, the predominance of black spots was in the shell. Incidence of black spots was higher for treatment-I (41.5%) than treatment-II (24.4%).

In treatment-II, shrimps remained alive for some time

Table 3. Incidence of black spots in meat and exoskeleton of shrimps submitted to different treatments.

Treat.	Location of black spots	Incidence (%) in		Absence of black spots (%)
		Shell	Meat	
I	Junctions	16.9	26.1	57.0
	Bases	41.5	6.2	52.3
II	Junctions	4.3	73.0	22.7
	Bases	24.4	2.2	73.4
III	Junctions	13.3	40.4	46.7
	Bases	6.7	6.7	86.6

after suffering injuries. During this period, it is possible that there is an accumulation of hemolinf in traumatized portions which serves to repair the injured cells. This may consequently result in higher concentration of activated enzymes and substrates involved in browning reaction. Thus, in our understanding, the mechanism of melanosis in shrimps is a consequence of the conditions at the time of death of animal in relation to practice or absence of injuries. Furthermore, lack or delay in supply of ice enhances the oxyenzymic reaction which would further be facilitated by

Table 4. Analysis of variance and comparison between means for male and female shrimps submitted to treatment-II.

Sex	Male	Female
No. of shrimps	19	27
	Junctions of segments	
X	3.44	3.59
s	1.63	1.36
Fo	1.44 (NS)	
	Bases of segments	
X	2.47	1.74
s	3.75	2.90
Fo	1.66 (NS)	

higher ambient temperature, exposition to air, etc.

The data was analyzed to find any significative difference in black spots formation between males and females (Table 4), and between molt cycles C and D (Table 5). No evidence of any significative difference between these parameters was observed. Cobb (1977) associated melanosis in shrimps to the stages of molt cycle and reported occurrence of this phenomenon as one of the technological effects of stages between inter and pre-molt.

Table 5. Analysis of variance and comparison between means for different molt stages of shrimps submitted to treatment-II.

Molt stage	C	D
No. of shrimps	31	15
	Junctions of segments	
X	3.6	3.3
s	1.35	1.69
Fo	1.07 (NS)	
	Bases of segments	
X	1.77	2.6
s	2.98	3.81
Fo	1.63 (NS)	

Melanin Indicator:

The individuals used in these experiments belonged to the stage C of molt cycle. According to Cobb (1977), there is an accumulation of N-acetyldopamine (a precursor of melanin) in shrimps in the period of intermolt, and during the premolt stage. This compound gets activated to form new epicuticle whereby the individuals become more susceptible for the appearance of black spots. Ogawa et al. (1984) also observed this vulnerability in lobsters.

Results on the initiation of darkening in shrimps injected with catechol solutions at different concentrations are presented in Table 6. It can be observed that the reaction time for the appearance of black spots decreases with the increase in catechol concentration. The relationship

Table 6. Initiation of darkening in shrimps (C-stage molt) submitted to catechol injections at different concentrations.

Shrimps				Conc. of catechol (M)	Initiation time for darkening (min)
No.	Wt. (g)	Length (cm)	Sex		
1	13.0	12.0	F	0.2	28
2	8.0	10.5	F	0.2	28
3	5.5	9.5	M	0.2	28
4	6.5	10.5	M	0.4	26
5	6.0	10.0	M	0.4	26
6	5.0	10.5	M	0.4	26
7	6.0	10.5	F	0.6	23
8	23.0	14.0	F	0.6	23
9	10.5	11.5	F	0.6	23
10	20.0	14.0	F	0.8	18
11	6.0	10.5	F	0.8	18
12	6.0	9.5	M	0.8	18
13	9.5	12.0	F	1.0	15
14	6.0	11.0	M	1.0	15
15	6.0	10.5	F	1.0	15
16	6.0	10.0	M	1.5	11
17	5.0	9.5	M	1.5	11
18	10.0	11.0	F	1.5	11
19	22.0	15.0	F	2.0	8
20	10.0	11.0	F	2.0	8
21	19.5	14.0	F	2.0	8

Regression equation: $Y = 33.3\ e^{-0.729X}$

Coefficient of correlation (r) = -0.99

between time for the change in color (Y) versus catechol concentration (X) could be expressed by the regression equation:
$Y = 33.30\ e^{-0.729X}$ whose correlation coefficient (-0.99) was found to be very significant.

All the concentrations of catechol tried had their effect on black spots formation. However, concentrations higher than 1 M could result in protein denaturation from the breakage of carotene-protein complex. Due to the denaturation of proteins, white clots mainly at the bases of segments were observed which could be due to the accumulation of hemolinf in this area. Melanosis was not noticed at the places where denaturation occurred. When catechol was injected to a live shrimp, there was an appearance of white milky fluid which demonstrates a circulation of hemolinf at bases and junctions of segments.

Based on results presented in Table 6, it is possible to formulate a test for melanin indicator in shrimps. This test would serve to analyze the immunity or susceptibility of shrimps to enzymic reaction. Catechol at a concentration of 1 M could be used for performing the test in frozen shrimp industry. However, large scale application trials and storage studies need to be undertaken.

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ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support received from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

REDUCTION OF SULFITING AGENTS IN PREVENTING SHRIMP MELANOSIS

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INTRODUCTION

Regulatory and consumer concern for sulfiting agents used as preservatives on foods continues to demand alternative or reduced treatments. Although sulfite treated shrimp have caused limited incidences of adverse sulfite aggravated reactions and human feeding trials have demonstrated that sulfited shrimp are not a culprit for initiating hyper-(sulfite)sensitive asthmatic responses (1), sulfited shrimp are still considered problematic. This concern is enhanced by occasional regulatory actions for raw shrimp exceeding the allowable sulfite residual level of 100 ppm measured as SO_2 on the edible portion (2). Thus further work was necessary to find controls that prevent shrimp melanosis ('blackspot') and replace or reduce sulfite residuals.

Materials and Methods

Prior work (3) had demonstrated that controls beyond typical icing and washing were necessary to prevent the on-set of shrimp melanosis which was obvious within 3 days and severe within 7 days post-harvest on ice. The test species was pink shrimp (Penaeus duorarum) harvested near Key West, Florida. The selection of test species and procurement of fresh untreated samples are critical to interpretation of any treatment results.

The basic established test procedure used fresh (within 24 hour post-harvest) heads-on pink shrimp. The head-on shrimp is more prone to develop obvious melanosis. All shrimp were routinely washed on-board and temporarily stored in ice. The basic experimental procedure was to rinse 400-600 grams of shrimp in 2.5 liters of variable dip compositions and concentrations for 1 minute, then drain and package in plastic bags to be stored in ice. The bags were considered necessary to eliminate the variable influence of melting ice. Three bags or replications were prepared per treatment. Iced containers with packaged shrimp were stored in 35°F (1.7°C) refrigeration, and reicing every other day.

Development of melanosis was scored and photographed routinely during 2 weeks storage. The bags of shrimp had been numbered such that the investigator could not distinguish amongst the various treatments. One experienced investigator did all scoring relative to a 10 point melanosis scale (Table 1). The scale was accompanied by pre-developed color prints depicting common examples of the advancing stages for melanosis. The intent was to screen for obvious differences between treatments.

The various dips or chemical treatments included controls (no treatment), customary sodium bisulfite used in varying concentrations, and a variety of single compounds and/or mixtures prepared in varying concentrations (Table 2.). Three field trials (Oct. 1987 and June 12 and 18, 1988) were necessary to accommodate all the variable treatments. A control was run for each trial to account for the variable expression of melanosis as could be influenced by the post-harvest condition of the shrimp and the particular stage of molt.

Prior Results

Previous studies (3) attempting to find alternatives for sulfiting agents to control shrimp melanosis have used similar procedures with various dips containing; sodium bicarbonate, potassium bromate, calcium chloride, erythrobate, ascorbic acid, boric acid, citric acid, phosphoric acid, sodium tripolyphosphate, disodium phosphate, sodium hexametaphosphate, EDTA, glycine, taurine, hydrogen peroxide and a commercial blend, BL7. Comparative results suggest dips containing mixtures of bisulfite plus citric acid, erythrobate, and/or EDTA could only offer moderate prevention of melanosis.

Sulfited residuals expressed as SO_2 ppm of edible meat were determined for select samples run in triplicate by the standard A.O.A.C procedure (4).

Results and Discussion

The onset of melanosis in the controls was typically scored between 4 to 5 within the first seven days of storage, advancing to scores over 8 after seven days. This adverse reaction demonstrates the necessity for treatments to prevent melanosis. The use of 1.25% sodium bisulfite dips provided adequate prevention of melanosis more so than the 0.5% sodium bisulfite dip. Also, the variable expression of melanosis per trial demonstrates the necessity for untreated controls and customary sodium bisulfite treatments per trial (Tables 3-5). These results are consistent with prior work (3).

The less successful treatments are listed in Tables 3-5. In the October trials (Table 3) the commercial preparation of HQ Bacterol F at 1.5% seem to provide adequate control, yet this commercial blend contains 67% sulfites thus providing a treatment equivalent to 1.2% sodium bisulfite alone. The treatments with gluconic acid and various concentrations of glucose oxidase/catalase offer only moderate to no prevention of melanosis.

Table 1. Scale used to describe and rate the occurrence of melanosis (blackspot) on pink shrimp.

Melanosis Scale	
0	Absent
2	Slight, noticeable on some shrimp
4	Slight, noticeable on most shrimp
6	Moderate, noticeable on most shrimp
8	Heavy, noticeable on most shrimp
10	Heavy, totally unacceptable

Table 2. Compounds used individually and in mixtures to prepare dips for treating pink shrimp to control melanosis.

Compounds	Comments
Sodium bisulfite	Reducing agent with legal sanction (2)
Commercial blends	
BL-ACT-S	Shimakyu Chemical Co., Ltd Osaka, Japan
HQ Bacterol F	Hispano Quimica S.A. Barcelona, Spain
Carrageenan blends (RE 9247 & 9387)	FMC Corp., Marine Colloids Div. Rockland, ME
Flavor Guard I	Antioxidant, Oscar Mayer Ingredients Co., Madison, WI
Gluconic Acid (GDL-glucono-delta-lactone)	Acidulent, Finnsugar Biochem., Inc.
Glucose oxidase/Catalase (GO/CAT. or Ovazyme; HP=high purity)	Acidulent and reducing agent via enzymes, Finnsugar Biochem., Inc.
Keltrol T	
Lactic acid	Acidulent, PURAC, Inc. Arlington Heights, IL
Proteinases (Corolase, C-PN & C-7018)	Rohm Tech., Inc. Malden, MA

Table 3. Average melanosis ratings for the October, 1987 treatment trials to prevent 'blackspot' on pink shrimp.

Treatment	Dip (1 min.)	Days Storage 3	7	10	12
Controls	No Dip	5	8	9	--
BIS	0.5%	3	4	5	7
	1.25%	1	3	4	6
HQ Bactrol F	0.5%	2	7	9	--
	1.0%	1	3	8	--
	1.5%	1	2	3	5
Gluconic Acid	0.25%	5	8	--	--
	0.75%	4	7	8	--
	1.25%	4	9	--	--
GO/CAT	1 unit/oz.	1	5	8	--
	10 unit/oz.	1	3	7	--
	100 unit/oz.	2	3	8	--
GO/CAT (HP)	1 unit/oz.	5	9	--	--
	10 unit/oz.	5	8	--	--
	100 unit/oz.	6	10	--	--
GO/CAT + + BIS (0.5%)	1 unit/oz.	1	5	7	8
	10 unit/oz.	1	3	7	8
	100 unit/oz.	2	3	8	8
GO/CAT (HP) + Bis (0.5%)	1 unit/oz.	1	1	4	4
	10 unit/oz.	1	3	5	7
	100 unit/oz.	1	5	5	7

GO/CAT - Glucose oxidase/Catalase; HP - high purity; Bis - sodium bisulfite

The June 12 trial (Table 4) suggested a 0.1% Keltrol T plus 0.5% sodium bisulfite effectively retarded melanosis, yet the long term results (beyond 7 days) were less effective than the customary industry practice of a 1.25% sodium bisulfite dip.

Likewise, the June 18 trial (Table 5) did not yield any treatments more effective than the sodium bisulfite treatments alone. Effectiveness of the Flavor Guard was enhanced by the addition of 0.5% bisulfite, but the combined long term results (beyond 7 days) were equivalent to use of 0.5% sodium bisulfite alone. Again the apparently effective commercial blend, BL-ACT-S contained an equivalent portion of sulfiting agents.

The most effective treatments appeared to be dips with various carrageenans or lactic acid plus reduced concentrations of sodium bisulfite (Figures 1 and 2). The carrageenans were initially (Oct. 1987) mixed with 0.5% sodium bisulfite to provide dip treatments that were as effective or better melanosis controls than the customary 1.25% sodium bisulfite dip alone (Fig. 1A). Use of carrageenans alone did not retard melanosis. Subsequent field trials (June, 1988) verified these initial observations (Figs. 1B and C). The inclusion of organic acids (citrate and lactate) and antioxidants (BHT) did not alter the protective influence of the carrageenan plus sodium bisulfite dips.

Although the carrageenans had been prepared by the manufacturer to allow for easier mixing, the carrageenan blends required more mixing time as for other ingredients to assure complete dissolution. The resulting dips were slightly more viscous and imparted a noticeable surface film on the shrimp. The film was slightly opaque, but not objectionable and could be easily removed with subsequent washing in tap water. This 'coating' phenomenon was thought to play a role in preventing melanosis by decreasing the surface exposure to oxygen, retaining sulfite, and possibly evolving the sulfite residuals in the carrageenan molecules. Likewise, this 'coating' could serve as an helpful glazing material for frozen shrimp.

The trials with lactic acid and sodium bisulfite treatments indicated the use of this acidulent enhanced the protective influence of the sulfiting agent (Fig. 2). With a lactic acid concentration of 0.5% the protective influence increased with higher sulfite concentrations (Fig. 2A). The 0.5% lactic acid plus 0.75% sodium bisulfite dip was equivalent to the standard treatment of 1.25% sodium bisulfite alone. With a higher concentration of lactic acid (1.0%) the protective influence for all sulfite concentrations was similar and equivalent to the customary 1.25% sodium bisulfite treatment.

Note, the experimental procedure and melanosis rating scale did not account for the clean, glossy appearance for shrimp treated with 1.25% bisulfite at any lactic acid concentrations. This 'polished' appearance was distinct from shrimp dipped in 1.25% sodium bisulfite alone, and was not objectionable.

Table 4. Average melanosis ratings for the June 12, 1988 treatment trials to prevent 'blackspot' on pink shrimp.

Treatment	Dip (1 min.)	Days Storage 3	5	8	10
Control	No Dip	2	4.5	5	9
BIS	0.5%	0	1	3.5	7
	1.25%	0	0	2	3.5
Keltrol T + BIS	0.075% + 0%	3	7	7	9
	0.075% + 0.5%	0	1.5	3	4.5
	0.010% + 0.5%	1	1	4	5.5

BIS - sodium bisulfite

Table 5. Average melanosis ratings for the June 18, 1988 treatment trials for prevent 'blackspot' on pink shrimp.

Treatment	Dip (1 min.)	Days Storage 3	5	7	11
Control	No Dip	3	9	--	--
BIS	0.5%	0	3	4.5	6
	1.25%	0	1	2.5	3
BL-ACT-S	0.5%	1	4	6	6.5
	1.0%	0	2.5	4	4
Flavor Guard	0.05%	1	7	10	--
	0.1%	1	6	10	--
	0.5%	1	7	10	--
Flavor Guard + BIS	0.05% + 0.5%	0	1	3	3
	0.1% + 0.5%	0	1.5	4	6
	0.5% + 0.5%	0	2	4.5	5
Proteinases					
C-PN	0.1%	1	6.5	10	--
	0.5%	2.5	8	10	--
C-7018	0.5%	1	8	10	--
	1.0%	1	7	10	--
	2.0%	1	7	10	--

BIS - sodium bisulfite

Although these lactic acid dip treatment did not impart any detectable change in color, flavor or cooked taste, the dip solutions, particularly when initial mixed, emit a strong sulfur dioxide vapor. The vapor can irritate the eyes and respiratory tract and be lethal if not adequately vented.

Thus, continuing work to develop sulfite alternatives for prevention of shrimp melanosis (blackspot) indicates dip treatments containing combinations of particular carrageenans or lactic acid reduce the effective concentrations of sodium bisulfite. Carrageenans or lactic acid combinations with 0.5% bisulfite were comparable to the standard one minute dip in 1.25% sodium bisulfite. These alternative treatments substantially reduced the residual sulfite concentrations (expressed as SO_2) on the raw edible portions of the treated shrimp. Residual concentrations for shrimp dipped in 1.25% sodium bisulfite for 1 minute ranged from 50 to 197 ppm (5). Residuals for the 1.0% lactic acid + 0.5% sodium bisulfite dip ranged from 20 to 60 ppm, while that for the carrageenans + 0.5% sodium bisulfite ranged from 35 to 90 ppm. The wide range in residuals per treatment is due to the variable condition of the shrimp prior to treatment and inherit variability in the standard SO_2 analytical procedure.

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AN IMPROVED PHOSPHATE FORMULATION FOR SEAFOOD PRODUCTS

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INTRODUCTION

Long-term storage of seafood products, particularly fatty fish, presents a problem regarding fat rancidity, freezer burn, and general product deterioration.

Sodium tripolyphosphate has been used in many seafood products for prevention of drip loss and freezer burn (Mahon, 1962). Researchers agree that phosphate and phosphate and salt solutions improve shelf life and flavor of seafood products (Sjostrom, 1948; Sen, 1964; Tarr, 1969; Watts, 1961).

Metallic ions actually trigger or enhance the development of oxidative rancidity (Batra, 1965). Research also indicates that both hexametaphosphate and citric acid chelate or react with metallic ions and help retard rancidity (Hunt, 1955; Burhans, 1942; Madrazo, 1964; Rose, 1962). These two compounds in combination have a synergistic effect. Research has also shown that ascorbic acid and its isomer, sodium erythorbate serve as an anti oxidant (Grossmann, 1962).

Industry practices have shown that hexametaphosphate solutions tend to decrease the glassy appearance of seafood products and also have a lower pH.

Materials and Methods

An instantized highly soluble mixture of sodium tripolyphosphate and hexametaphosphate was used as one phosphate source (Brifisol 512, BK—Ladenburg). While the other phosphate was sodium tripolyphosphate containing lemon solids (Lemophos, Stauffer Chemical Co).

Two lots of catfish (5 oz. fillets) were injected with a Townsend injector. One lot of fillets was injected with a five percent solution containing 97.40 percent Brifisol 512 (B K — Ladenburg), 2.5 percent citric acid and 0.10 percent sodium erythorbate. The other lot of fillets was injected with a five percent solution of Lemophos (Stauffer Chemical Company) containing sodium tripolyphosphate and lemon solids. A non-injected control was used for comparison. Both lots along with control were frozen on a spiral freezer to a core temperature of twelve degrees to fifteen degrees F and placed in a blast freezer at minus 40°F overnight. After 24 hours, both lots, along with the control, were held at 0°F throughout the 90-day period of shelf life examination.

TBA (thiobarbituric acid) numbers were performed on both lots of fish and the control. On the first, 45th, and 90th day of frozen storage, the TBA analyses were performed. Moisture, fat, and free fatty acids analyses were performed initially on all three lots.

Results and Discussion

Fat analyses showed the three lots of fish to be very similar in fat levels. Both of the injected lots of fish contained approximately three percent higher moisture levels than the control. The free-fatty acid levels were approximately the same for all lots (See Figure 1).

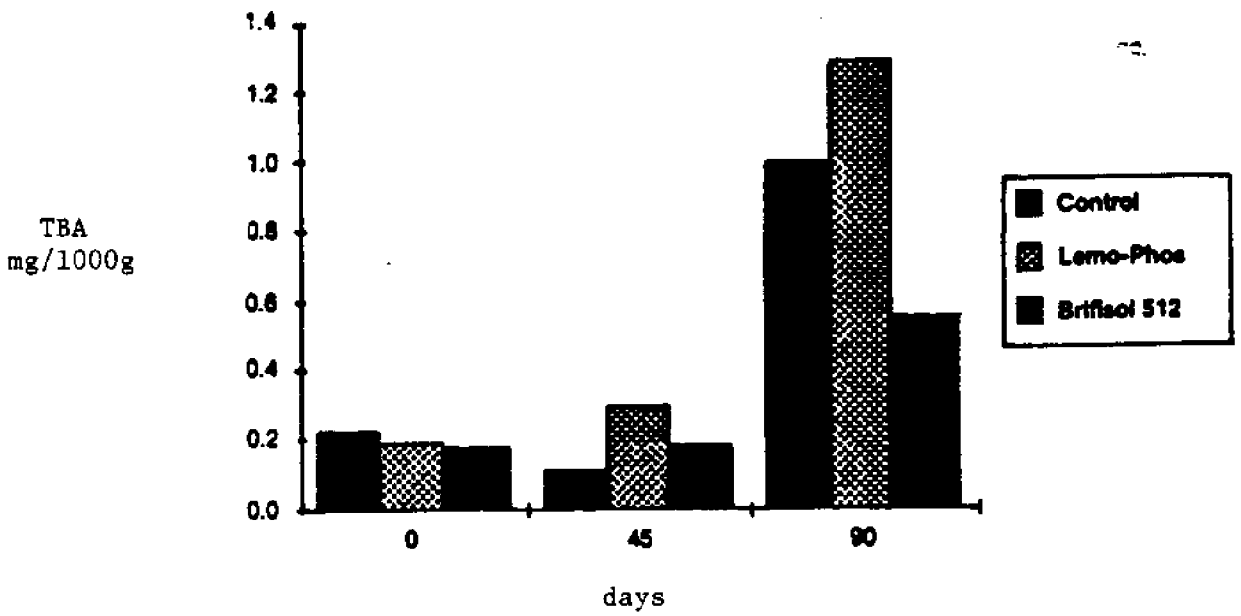
Figure 1

Sample	Control	BK-Ladenburg	Lemo-Phos
Moisture	76.3%	79.27%	79.91%
Fat (As received)	5.78%	4.42%	4.48%
Free Fatty Acid (as Oleic)			
As Received	0.061%	0.047%	0.046%
Oil basis	0.25%	0.22%	0.21%

TBA numbers for all three lots were very similar on the beginning date (see Figure 2). On the 45th day, both the control and the sample marked BK-Ladenburg remained unchanged while the Lemophos sample showed an increase. After the 90th day, the control increased from 0.22 to 1.01. The Lemophos increased from 0.19 to 1.29 and the sample marked BK-Ladenburg increased from 0.20 to 0.55. The level of increase in the control or uninjected sample has been noted on research done by other researchers. The level of TBA increase in the Lemophos sample is somewhat higher than results from other researchers. The low level of increase in TBA's in the BK-Ladenburg sample is noteworthy after 90 days of 0°F storage. The low level of TBA increase in the BK-Ladenburg sample would indicate a superior catfish product after 90 days.

Figure 2

Anti-Oxidant Comparison



One can only assume that the antioxidant effect of the sodium erythorbate in conjunction with the citric acid (which prevents oxidation by chelating metallic ions) had an overall positive effect in prevention of oxidative rancidity.

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Development of a Qualified Nutrient Database for Southeastern Species

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INTRODUCTION

As more Americans recognize seafood's potential health benefits, the need for accurate, up-to-date compositional data has become quite apparent. Seafood nutrition information is vital to a wide range of user groups including health professionals, home economists, marketing specialists, restaurants, seafood technologists, and the seafood industry as a whole.

Currently available references on the nutrient composition of seafoods come from a variety of sources. Both the National Marine Fisheries Service and the United States Department of Agriculture have been key contributors in generating and compiling compositional data on seafoods (1-13). Likewise, other researchers have published hundreds of scientific papers on the nutritional value of various species, while groups such as trade associations, research foundations, marketing specialists and the food industry have produced numerous seafood composition tables (12-14). Unfortunately, much of this information on seafood composition is scattered, inconspicuous and difficult to obtain. In addition, written handbooks require periodic updates and modifications as new research is published.

Also, the information in these references or the format in which it is presented is sometimes incompatible with certain users' needs. For example, while Sidwell's 1981 publication (7) offers a descriptive and detailed listing of all available analytical results for 1500 species, it is somewhat inconvenient for practical users such as clinical dietitians and home economists. The volume is large and cumbersome and the data for one particular species can appear in up to twenty two different tables within the reference.

By the same token, other composition tables are too general for researchers interested in obtaining all the available data for a particular species.

Lastly, many of these references contain mislabeled tables, inappropriate nomenclature, obvious mistakes in assigning units of measure and questionable data. Unfortunately, this erroneous information is often found in the same handbooks that users commonly rely on for seafood composition data.

In view of these limitations, we have developed a qualified nutritional database for 260 Southeastern species with the following objectives in mind:

1. Produce a more unified, complete and accurate review of compositional data for Southeastern seafood species (Texas to North Carolina).

2. Computerize the database, allowing future analytical work to be easily accommodated.

3. Provide the data in various formats (both printed and computerized) that would meet the needs of different user groups and be easily accessible.

4. Enhance the utility of the database by providing nomenclature listings, retention factors for cooked seafoods, and a complete list of screened analytical references.

METHODS

1. Species Selection - Our work focused on marine and fresh-water seafood species from the Southeast. Species selection was based on three criteria:

    - a marine and/or freshwater species common to waters in the southeastern sector (Texas through North Carolina)

    - a species with current or future commercial or recreational interest

    - an edible species

2. Literature Review - More than 1500 references were screened for content and methods relative to providing compositional data on selected southeastern species. Each reference was designated as a primary, background or nonacceptable reference, based on the following criteria:

> **primary references** contained original data and provided proper species identification (based on the FDA and NMFS current "Fish List"), acceptable sample procurement and treatment (only data for raw muscle was used) and appropriate analytical methods.
>
> **background references** contained questionable species identification, questionable analytical methods, unacceptable sampling techniques or sample preparation, and/or results that substantially deviated from the average (more than 3 standard deviations away from the mean of values reported in other references). Also, review publications were considered background references.
>
> **nonacceptable references** did not offer any useful data. For instance, some references applied only to cooked or processed seafoods, while others contained data for nonregional species.

(Note: Any exceptions to the above criteria were documented.)

As each new primary or background reference was obtained, it was sequentially numbered and added to the seafood nutrition reference file. A reference list was simultaneously prepared.

3. Construction of species/reference table - As a way to catalog the data found in each reference, we developed a species/reference table. Each primary and background reference was tabulated according to the species that were analyzed and nutrient values that were reported for each species.

4. Nutritional analysis of selected southeastern species - The initial literature review prompted laboratory analysis of twenty-five southeastern seafood species for which there was little or no data available in the literature.

5. Construction of nutrient data spreadsheets - Using only primary references, we transferred nutrient data onto spreadsheets (one set of spreadsheets per species). The data from each reference was recorded separately, along with the author's name, publication date, and any special notes pertaining to the data in the reference. If a reference provided more than one value for a particular nutrient (for example, three lipid values for striped mullet obtained at three different times of the year), we recorded the mean and range of the values given. Also, we made the necessary calculations and conversions for data presented on a dry-weight basis or in non-acceptable units.

6. Review and verification - Each reference and spreadsheet, as well as the species/reference table, were reviewed to verify that all of the following were correct: reference designation, species identification and methods, tabulation of references in species/reference table, data transfer onto spreadsheets and calculations and conversions of weight units. Also, we checked for proper identification of outliers (values greater than three standard deviations away from the means of other values) and identification of overlapping data (identical data reported in more than one reference).

7. Summary of nutrient data - For each species, we determined a series of **average nutrient values**, based on data from primary references only. Also, for each average nutrient value, we recorded the **number of references** used to determine that average, plus an **overall range** (the highest and lowest observed values reported in the primary references for that particular nutrient and species).

RESULTS

The database for Southeastern seafood species contains the following components:

1. **Reference File and Reference Lists:** From approximately 1500 references that were reviewed, we identified 91 primary references and 86 background references for the reference file. All others were designated as non-acceptable; however, we did include 77 of the non-acceptable references in the file because they were nonregional publications useful for comparison with data of similar southeastern species. Two versions of the reference list are available: one in alphabetical order and one in numerical order.

2. **Species/Reference Table:** As shown in table 1, the species/reference table catalogs each primary and background reference. Thus a user can easily identify the sources of data for any particular species. For example, the table indicates that six references provide proximate data for swordfish: **247**, **250**, 7,8,68 and 138 (these numbers correlate with the numbers used in the reference file). Numbers printed in bold type (**246** and **250**) indicate primary references, while the others are background references. With this information, a user could then go back to the reference list and obtain citations for all six references. Also, the table indicates instances where no data is available (i.e. goldface and sand tilefish).

3. **Spreadsheets:** For each species, the spreadsheet(s) displays all the available data from primary references. Each spreadsheet provides columns for thirty-three nutrients. At the bottom of each nutrient column appear three summary values: an average nutrient value, the number of references used to determine each average nutrient value, and an overall range. As new primary references are added to the reference file, the data will be transferred onto the appropriate spreadsheets and the summary information at the bottom of the spreadsheets will be adjusted accordingly.

Table 1. Excerpt from Species/Reference Table

Common Name	Proximates	Lipid/ Fatty Acids	Cholesterol	Amino Acids
Swordfish	**247,250,7, 8,68,138**	**12,247,250, 1,8,10**	**250,1,7,8**	**250**
Tilapia(F),				
Blue				
Mozambique	7			7
Tilefish,	8	8	8	
Blackline	**67**	**67**		
Blueline	**246**	**247,255**	**255**	
Goldface				
Sand				
Tilefish	**98,247, 250,7**	**247,250,255**	**255**	**250**

4. **Vernacular and Nomenclature Lists:** To help users properly identify species, we included a limited vernacular reference which cross-lists vernacular and common names. One version of the list is alphabetized by common names, another is alphabetized by the vernaculars. Likewise, a nomenclature list is included which indicates common, scientific and 'proposed' market names for each species. With these lists available, a user can determine any form of the name that is needed (the spreadsheets and data are arranged according to common names).

   Both vernacular and 'proposed' market names are based on "The Fish List - FDA Guide to Acceptable Market Names for Food Fish Sold in INTERSTATE COMMERCE, 1988" (by Mary Snyder, FDA Division of Regulatory

Guidance; preprinted version). The common and scientific names were taken directly from the "official" list published by the American Fisheries Society (special publication no. 12, "Common and Scientific Names of Fisheries from the United States and Canada" Fourth Edition, 1980).

5. **Footnotes:** Although we attempted to follow the standard methods that were outlined, special circumstances related to a species or reference often dictated that we deviate from those methods. Any exceptions were documented and recorded as footnotes. For example, while reviewing the data for Eastern oysters, we discovered that reference 213 presented values for samples taken from Louisiana, Alabama and Maryland (17). In this case, we retained the publication as a primary reference, although we excluded the data from Maryland since it is not representative of oysters harvested in the Southeast. This decision was documented as a footnote.

DISCUSSION

In order to develop a qualified database, we had to make certain decisions and accommodations for data that was variable, erroneous and insufficient.

Variable data - Many references have demonstrated that natural and environmental factors (maturity, sex, size, season, location, migration, etc.) are partially responsible for the variability found in seafood composition data. Also, it is very likely that lab variation, both within and between labs, accounts for a portion of variance found in the literature. Hollman and Katan (18) found that major laboratories that were sent identical food samples produced highly variable analytical results, even when typical sources of error had already been reduced or eliminated. Thus, the variability of seafood composition data most likely results from a combination of factors, both natural and analytical.

To accommodate the variability in the data, yet still offer an average value for each nutrient, we chose to provide an average nutrient value, the number of references used to determine that value, and an overall range. The range should help users appreciate the limitation of the single average value.

Erroneous data - There was a surprising amount of erroneous data in the literature, most of which was related to mislabeled tables and misidentified species. When we were unable to contact the investigators and did not have enough evidence to make a reasonable assumption, we designated the reference as 'background'. On the other hand, when mistakes were fairly obvious, when we were very familiar with an author's work, or when we were able to contact the investigator and obtain the correct information, we retained the publication as a primary reference and made an appropriate footnote.

Insufficient data - Oftentimes, a reference supplied only part of the necessary information. For example, most fatty acid data is reported as weight percent of fatty acids. Kinsella et al. (19) point out that this is useful for comparative purposes, however, for dietary evaluation, it is important to know actual quantities of fatty acid in the food (grams of fatty acid per 100 grams of tissue). To make this conversion, it is necessary to know total lipid content, as well as fatty acid content of the lipid, a crucial piece of information that researchers often omit. Thus, Weihrauch et al. (20) derived conversion factors for seafoods and other foods, which calculate the weight of fatty acids present in 1 gram of fat when only total fat is given. Although this conversion factor only estimates the actual fatty acid content, it is a consistent and effective way to utilize fatty acid data which otherwise would have only limited applications.

In other instances, references provided data on a dry weight basis without providing moisture values. In that case, we used the average moisture value for that species as determined from other primary references. In a similar example, a reference presented sterols as a percentage of total lipid but did not provide a value for total lipid. Again, we made the necessary calculations using the average lipid value determined from other primary references.

Insufficient data also refers to the general lack of data in the literature for certain species and nutrients. For example, limited analytical work has been done on underutilized species that have just recently generated commercial interest, such as rock shrimp and sandbar shark. Likewise, researchers have mostly focused on the proximate and fatty acid content of seafoods, so that much less attention has been given to other nutrients, especially

vitamins, trace minerals, amino acids and carbohydrates (which are thought to be present in limited amounts in shellfish, although very few studies have actually analyzed carbohydrate in shellfish). When no data was available, we simply left the spreadsheet blank. Hopefully documentation of this limited information will encourage more basic analytical research on seafoods. In the meantime, users can estimate missing values by looking at data for a similar species, provided they indicate that they have done so.

CONCLUSION

Through a systematic process of screening the literature, cataloging the information, and combining the data into a comprehensive and useful form, we have produced a qualified database for Southeastern seafood species. Recommended publications are summarized for quick and convenient reference, while the species/reference table allows users to refer to background information and original articles. The continued plan of work involves completing published and computerized versions of data collected thus far.

Recognizing the database will be continually supplemented with future publications, this initial phase of the project focused on establishing a basic foundation which could receive future additions, yet always remain accessible and useful for the intended audiences. Likewise, this regional effort was designed for direct integration with similar national data bases maintained by the U.S. Department of Agriculture's Human Commerce's National Marine Fisheries Service. Thus, this project represents a benchmark in assembling and organizing the nutrient composition database for Southeastern seafoods.

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FATTY ACID NUTRITIONAL PROFILES IN GULF OF MEXICO FISHES

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INTRODUCTION

The fatty acids, linoleic, linolenic and arachidonic, cannot be synthesized by the body therefore are considered to be essential components in our diet. They are needed for maintaining the funtion and integrity of membrane structure, for fat metabolism, for growth and for synthesis of certain prostaglandins. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both omega-3 fatty acids, are currently considered essential fatty acids for all marine organisms. Fish obtain omega-3 fatty acids from the phytoplankton or algae in their diet. There is evidence that omega-3 polyunsaturated fatty acids (n-3 PUFA) found in fish oils provide many potential health benefits when added to the human diet. Research reports have indicated that cold water fish species are rich sources of omega-3 fatty acids. The implications have been to the general public that warm water fishes may not contain omega-3 fatty acids or that they do not contain enough to be utilized as a source of omega-3 fatty acids. Current research reported in this manuscript is funded by the National Marine Fisheries Service and has as study objectives: to determine the fatty acid content, particularly the omega-3 acids, as well as cholesterol and the proximates of over 40 Gulf fishes, most of which have no state-of-the-art fatty acid identification; to provide this information in the format that can be utilized by the consumer; to promote consumption of a broad spectrum of seafood especially highlighting the lesser known species; and to format the data in such a way that it can be used for nutritional labeling. The species that were chosen for the first year of this study are shown in Table 1.

Diets extremely rich in land plant foods and oils and animals and poultry fed on grain products provide almost exclusively omega-6 fatty acids. Omega refers to any of 12 families of naturally occurring acids having more than one double bond where unsaturation is entirely methylene-interrupted and of cis (Z) configuration. Each family is designated by the number of carbon atoms from the last double bond to the terminal methyl group. The fatty acids most often found in foods belong either to the omega-6 or to the omega-3 families, the omega-3 fatty acids found almost exclusively in seafood. The letter n has now replaced the term omega and will be used in this manuscript. Most land plants and vegetable oils contain large quantities of linoleic (C18:2) acid which can be quickly metabolized through elongation and desaturation to form arachidonic acid, an n-6 fatty acid which is further metabolized to form various potent hormone-like derivatives called prostaglandins and leukotrienes sometimes referred to as eicosanoids. Some of these hormones are necessary for certain body functions while others play a specific role which leads to a variety of diseases including thrombosis, arthritis, asthma and other immune-related diseases. Two events led to the significant interest and intense medical research in n-3 fatty acids: the epidemiological investigations of Bang and Dyerberg (1972) and later the discovery by Needleman (1979) that prostaglandins derived from n-3 fatty acids had differnt biological properties than those derived from arachidonic acid, an n-6 fatty acid. Research data supports the evidence that n-3 fatty acids both suppress eicosanoid production and antagonize the biosynthesis of arachidonic acid from linoleic acid. Therefore, by eating

Table 1. Gulf of Mexico Fishes Examined for Nutritional Content in 1988.

Scientific Name	Common Name
Cynoscion arenarius*	sand seatrout
Pogonias cromis	black drum
Anchoa mitchilli	bay anchovy
Anchoa hepsetus	striped anchovy
Carcharhinus limbatus	blacktip shark
Arius felis	hardhead catfish
Dasyatis sabina	Atlantic stingray
Lagodon rhomboides	pinfish
Orthopristis chrysoptera	pigfish
Mugil cephalus	striped mullet
Leiostomus xanthurus	spot
Rachycentron canadum	cobia
Euthynnus alletteratus	little tunny
Caranx crysos	blue runner
Bagre marinus	gafftopsail catfish
Thunnus atlanticus	blackfin tuna
Morone saxatilis x Morone chrysops	striped bass, hybrid
Callinectes sapidus	blue crab

*names from American Fisheries Society (1970).

more seafood rich in n-3 acids, the body would synthesize fewer eicosanoids, and the risk for getting these diseases would be lessened (Lands, 1986).

METHODS

Sample design. There are a number of factors that can lead to variations in the fatty acid content of fish. Seasonal changes, state of reproductive maturity, feeding habits, spawning versus non-spawning and geographic differences are but a few of the conditions that may affect content and distribution of these fatty acids (Exler and Weihrauch, 1976; Exler et al., 1975; Jangaard et al., 1967; Joseph, 1985; Stansby, 1973). A collection scheme was devised that would permit a measure of the variability associated with most of these factors. Each species was collected during four seasons in sufficient quantitites to yield information on seasonal variability of fatty acid concentration and distribution for each of the species.

One species was selected in 1988, spot (Leiostomus xanthurus), for an in-depth examination of other factors leading to fatty acid variability. Collections of this fish were made from several locations to examine geographic variability; both juvenile and adult specimens were collected to measure the effect of maturity on fatty acid profiles.

Though previous investigators have recognized the importance of many of the factors leading to variability in fatty acid content of fish, few have expressed awareness of a very important source of variability, individual variability. Stansby (1981) has stressed the need to look at individual specimen

variability in the design of a proper sampling program. Without knowledge of specimen to specimen variability, it is impossible to assess with what reliability reported values of fatty acids in fish estimate the true mean content for any particular species. Furthermore, this measure of individual variability is essential in establishing upper and lower limits of the mean which are necessary for establishing nutritional guidelines for fish.

The task of establishing a sample size that would lead to a statistically valid sampling program was discussed with Al Rainosek, biostatistician of the National Marine Fisheries Service, Pascagoula Laboratory. Most means of setting proper sample size (i.e. No. of individual specimens) are based upon some knowledge of expected individual variability. With no previous information available on the individual variability of fatty acids in Gulf fishes, it was felt necessary that some background information needed to be generated. Striped mullet (Mugil cephalus) and spot (Leiostomus xanthurus) were chosen as representative of Gulf fishes included in this study. Ten individual fish of both species were collected and analyzed separately. After careful statistical evaluation of this data, it was decided that collections each season would consist of at least nine separate fish of each species taken from one location at one time. These fish were grouped into three subgroups of three fish each and a composite of fish tissue prepared within each subgroup. If following analyses of the three subgroups, lower limits of means proved to be unacceptable, additional composites were prepared and analyzed as needed until an acceptable level of statistical reliability was achieved.

Sample preparation. Immediately after delivery of fresh fish to the laboratory, the fish were measured, weighed, sexed and state of health recorded. The fish were filleted and given a unique identification number, placed in plastic containers, flushed with nitrogen to remove oxygen and then frozen at -40°C. Before compositing any of the fish, an examination was made of the entire collection of each species made during the previous season. As many unique characteristics (e.g. size, sex, location) of each species as possible were used in sorting the individuals into the three subgroups. Identical quantities of tissue from each of the fish in the subgroups was used in the composites so that no bias was placed upon size of fish. Tissues were taken from the entire longitudinal cross section of fillet so that the sample was representative of all of the edible tissue of each fish. All tissue from individual fish not used for compositing was archived.

Analytical procedures. Samples for compositing were thawed and thoroughly homogenized before 0.5 g aliquots were placed into 30 ml centrifuge tubes for saponification using 1 ml 50% KOH and 4 ml 95% ethanol. Duplicate analyses were made on all composite samples. After saponification and separation of nonsaponifiable material, pH was adjusted to 1 and extraction was made of the saponifiable fraction containing the fatty acid salts. Fatty acids were converted to methyl esters using 7% BF_3-MeOH by the method of Metcalfe and Schmitz (1966). A nitrogen atmosphere was maintained throughout all procedures to minimize oxidation. Gas-liquid chromatography (GLC) of methyl esters was carried out using a Perkin-Elmer Model Sigma 2000 gas chromatograph with an all-glass split injection system onto a 30 m x 0.25 mm i.d. fused silica capillary column coated with 0.25 micron film thickness of Dura Bond WAX (J & W Scientific). Helium was used as the carrier gas. The data was quantified by internal standardization using a Perkin-Elmer Sigma 10 data system. Tentative identification of peaks was by comparison of retention time with those of standards.

RESULTS AND DISCUSSION

During the first half of 1988, most analytical attention was directed towards the individual variability study used in setting sample size and towards performance evaluation studies of analytical techniques. Only data from two seasonal collections of fishes from the Gulf will be discussed in this report. Sufficient information is available from these analyses to make a number of valuable observations.

Consumers have been erroneously led to believe that cold water fishes are the only sources of n-3 acids. The overall results indicate that the levels of n-3 fatty acids in Gulf fishes listed in Table 1 fall within 0.25 to 1.0 g per 100 g portion of fish fillet. This compares favorably with the 0.1 to 3.0 g per 100 g portion concentrations reported for cold water fishes (Nettleton, 1985; USDA, 1987). Warm water species examined in 1988 do contain somewhat less absolute quantities of n-3 acids than those cold water fishes such as mackerel and salmon continually mentioned in nutrional news releases. However, most of the Gulf species examined thus far have a fairly low fat content as compared to some of the fattier cold water fishes. Because the American diet is high in fat (Kinsella, 1988), larger portions of leaner fish could be consumed to obtain the same amount of n-3 fatty acids as the cold water fishes but with perhaps fewer fat calories.

In order to assess the fatty acids in seafood products, we looked at several quality indicators. The total polyunsaturated fatty acids (PUFA) are compared to total saturated fatty acids since an increase in saturated fat intake as a percent of total fat intake is associated with an increase in total plasma cholesterol, and appears to raise equally low-density lipoprotein (LDL) and high-density lipoprotein (LHL) cholesterol (Grundy, 1986). Biochemical and physiological findings show that when n-3 fatty acids are a significant fraction of total fat intake, plasma triglycerides are lowered with little effect on LDL and HDL (Parks and Bullock, 1987), therefore we include data on both the total n-3 fatty acid concentration and the total concentration of EPA and DHA, the two primary n-3 fatty acids in seafood, per 100 g fish serving. Lands (1986) has stressed the importance of balancing our dietary n-3 and n-6 PUFAs. We have provided the ratio of the concentrations of the two families of acids in Gulf fishes for the consumer who is interested in increasing intake of these n-3 dietary acids.

Fatty acid quality parameters computed for four representative species of Gulf fishes are shown in Table 2. The greatest distinction among these fishes is seen in the quantity of EPA and DHA expressed as percent of total fatty acids. Thirty-nine percent of the fatty acids of bay anchovy (Anchoa mitchilli) are made up of EPA and DHA, the highest value among these four fishes. These two acids comprise but 0.22% of the tissue weight, therefore, this fish contains both high levels of n-3 fatty acids as well as a low quantity of total fatty acids. The n-3/n-6 ratio for this fish is also the highest of the four fishes. Based strickly upon percentage of EPA and DHA in total fatty acid and n-3/n-6 ratios, bay anchvoy would be the clear choice, however, the other three fishes are distinctive in other fatty acid quality parameters. Cobia (Rachycentron canadum), a popular game fish, has a high concentration of PUFA. Striped mullet also has high PUFA concentrations as well as fatty acids enriched in n-3 acids. Black drum (Pogonias cromis) has moderately high values in all of the quality parameters.

In Table 3a-c are the fatty acid quality parameters computed for subgroups of cobia, black drum and hardhead catfish (Arius felis). The unique features

Table 2. Fatty Acids in Gulf Finfish.

	Striped Mullet	Bay Anchovy	Black Drum	Cobia
PUFA, wt%[1]	0.46	0.26	0.39	0.51
(EPA + DHA), wt%	0.31	0.22	0.22	0.26
(EPA + DHA)/ total fatty acids, %	20	39	13	10
n-3/n-6	5.8	9.7	3.6	2.1
Unsat/sat	2.4	2.3	2.2	1.7

[1]Fatty acid quality parameters tabulated as follows: PUFA, wt% -- total polyunsaturated fatty acids expressed as wt% of wet tissue weight; (EPA+DHA), wt% -- combined wt. of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as % of wet tissue wt; (EPA+DHA)/total fatty acids, % -- EPA+DHA as % of total fatty acid fraction; ratio of total n-3 acids to total n-6 acids; unsat/sat -- ratio of total unsaturated acids to total saturated acids.

Table 3a. Fatty Acids, Cobia[1]

	10-21 lbs	30-31 lbs	34-50 lbs
PUFA, wt%	0.28	0.35	0.91
(EPA + DHA), wt%	0.15	0.20	0.42
(EPA + DHA)/ total fatty acids, %	13	11	9.3
n-3/n-6	2.2	2.1	2.0
Unsat/sat	1.9	1.6	1.7

Table 3b. Fatty Acids, Black Drum[1]

	9-10 lbs	11-12 lbs	13-15 lbs
PUFA, wt%	0.43	0.43	0.30
(EPA + DHA), wt%	0.23	0.28	0.16
(EPA + DHA)/ total fatty acids, %	10	16	14
n-3/n-6	3.1	4.2	3.5
Unsat/sat	2.1	2.2	2.4

[1]See Table 2 for parameter explanation.

Table 3c. Fatty Acids, Hardhead Catfish[1]

	212-432 g (M & F)	465-549 g (F)	612-675 g (F)
PUFA, wt%	0.21	0.29	0.19
(EPA + DHA), wt%	0.12	0.16	0.12
(EPA + DHA)/ total fatty acids, %	17	16	21
n-3/n-6	2.6	2.3	3.4
Unsat/sat	2.4	2.2	2.2

[1]See Table 2 for parameter explanation.

of these subgroups were weights. In addition the hardhead catfish was subdivided by sex. For both the hardhead catfish and blackdrum, no clear trends with weights were observed for the fatty acid quality parameters. For cobia there was a direct relationship of fatty acids and size of fish. In larger fish, PUFAs comprise a higher concentration in the fish tissue. EPA and DHA concentrations increase in larger fish expressed as percent of fish tissue but shrink as a percent of the total fatty acids.

Fatty acid quality parameters in juvenile and adult spot are shown in Table 4. The trend seen with size in the cobia was also observed for the spot with a dramatic increase in PUFAs going from juvenile to adult stages as well as large increases in EPA and DHA expressed as percent of tissue. Note that EPA and DHA comprise 36% of the total fatty acids in the juvenile stage with a decline to 8.6% in the adult.

Table 4. Spot Fatty Acids

	Juvenile	Adult
PUFA, wt%	0.15	0.78
(EPA + DHA), wt%	0.10	0.46
(EPA + DHA)/ total fatty acids, %	36	8.6
n-3/n-6	3.4	4.4
Unsat/sat	2.9	3.6

[1]See Table 2 for parameter explanation.

A listing of the individual fatty acid composition of black drum are shown in Table 5 which contains data from two seasonal collections of this fish. Fatty acid data in this study are computed both as absolute weight concentrations in tissue, shown here, and as percent of the total fatty acids. The latter form of data presentation is more often seen in the literature but is also of less use in evaluating nutritional information. Also included in Table 5 are proximate data, detailing other nutritional characteristics of black drum. The

TABLE 5. Fatty Acids in Black Drum (Pogonias cromis)

	Winter (January 16, 1988)				Spring (May 10, 1988)			
CONCENTRATION, ug/g	A	B	C	seasonal mean	A	B	C	seasonal mean
Saturated								
14:0	449.52	342.50	222.05	338.02	312.01	323.98	382.46	339.48
16:0	5167.99	3872.34	2331.76	3790.69	2822.19	3997.15	4000.44	3606.59
18:0	1573.98	1123.93	839.82	1179.24	960.49	1188.70	1256.51	1135.24
20:0	99.44	90.72	38.84	76.33	45.62	46.00	57.17	49.59
22:0	64.43	49.59	21.70	45.24	28.82	18.33	26.89	24.66
23:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Monounsaturated								
16:1	5265.08	3934.78	2289.61	3829.82	3403.46	4073.06	3678.77	3718.43
18:1	5175.42	3468.83	2388.82	3677.69	2493.25	3713.29	3591.41	3265.99
20:1	902.09	457.35	390.80	550.08	446.00	435.67	531.98	471.21
22:1	121.10	72.32	81.82	91.75	0.00	0.00	0.00	0.00
Polyunsaturated (PUFA)								
18:2n-6	259.31	154.81	89.55	167.89	103.94	116.64	131.70	117.43
18:3n-3	137.23	68.66	30.30	78.73	46.28	61.00	65.97	57.75
20:2n-6	120.52	120.49	74.62	105.21	82.62	100.49	109.40	97.50
20:3n-3	115.29	77.12	66.57	86.33	64.19	67.90	84.27	72.12
20:4n-6	642.79	524.52	496.83	554.71	449.91	461.87	524.51	478.77
20:5n-3 (EPA)	1041.81	854.64	625.47	840.64	707.72	563.82	784.72	685.42
22:2n-6	20.29	19.32	0.00	13.20	30.21	31.94	30.60	30.92
22:3n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:5n-3	699.82	572.43	609.70	627.32	487.10	451.63	578.51	505.74
22:6n-3 (DHA)	1258.24	1903.46	999.93	1387.21	1288.21	1345.80	1426.97	1353.66
Profile variables								
total fatty acids	23014.35	17707.80	11598.21	17440.12	13772.03	16997.25	17262.26	16010.51
total PUFA	4295.31	4295.45	2992.99	3861.25	3260.19	3201.08	3736.64	3399.30
total unsat/total sat	2.13	2.23	2.36	2.24	2.30	2.05	2.02	2.12
total n-6	1042.91	819.14	661.01	841.02	666.69	710.94	796.20	724.61
total n-3	3252.40	3476.31	2331.98	3020.23	2593.50	2490.15	2940.44	2674.69
(EPA+DHA)/n-3,%	70.81	79.34	69.70	73.29	76.96	76.70	75.21	76.29
total n-3/total n-6	3.13	4.24	3.53	3.63	3.89	3.50	3.69	3.70
PROXIMATES								
% moisture	76.30	77.56	78.28	77.38	79.80	79.67	78.88	79.45
% ash	1.67	1.24	1.50	1.40	1.24	1.80	1.47	1.50
% Kjeldahl N	3.13	2.96	3.55	3.21	3.08	2.78	2.74	2.87
% protein	19.54	18.48	22.19	20.07	19.25	17.38	17.12	17.91
cholesterol, ppm	687.56	741.70	612.17	680.48	399.42	387.83	494.86	407.37
cholesterol, mg/100g	68.76	74.17	61.22	68.05	39.94	38.78	43.49	40.74
% lipid	ERR	ERR	ERR	ERR	ERR	ERR	ERR	ERR

Notes: For each season, three subgroups of fish were analyzed, A, B & C. The data for each subgroup represents means for duplicate analyses. Each fatty acid is denoted by Carbon chain length: No. of double bonds. For explanation of entries under profile variables, see note in Table 2.

seasonal means for all the fatty acids give the impression that there is a slight decline in the concentration of most of the fatty acids going from the winter to spring season though the decline is generally less than 10%. This decline in fatty acid concentration is accompanied by somewhat lower protein and cholesterol also. One should note, however, that the relative concentrations change little between these two seasons. The profile variables which reflect relative contributions of various groupings of fatty acids such as n-3/n-6, unsat/sat, and (EPA+DHA)/n-3,% show no significant differences between the seasons. The data within the three subgroups comprising each seasonal analysis show variations among subgroups that are much larger than the variations between seasonal means. Therefore most likely the variation between the two seasonal collections is more a reflection of individual to individual variation than true seasonal variation.

More comprehensive information on seasonal, biological (age, size, sex) and geographic variations on the 20 species of fishes chosen for study in 1988 will be presented in future reports.

ACKNOWLEDGMENTS

This research was supported by NOAA, National Marine Fisheries Service, U.S. Department of Commerce, Grant Number NA88AA-H-SK018 and by the Gulf Coast Research Laboratory (GCRL) and the State of Mississippi. We acknowledge Constancia Ramos and Faye Mallette for their technical assistance. We are grateful for the fish supplied by Clark Seafood, Pascagoula, MS; Jim Franks, Ocean Springs, MS; and the Parasitology Section, GCRL.

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Characterization of Volatile Odor Components in Crude Fish Oils from Gulf and Atlantic Menhaden (Brevoortia spp.)

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INTRODUCTION

The world's total fish catch in 1986 was 85 million metric tons, 30% of which was processed into fish oil and fish meal. In the U. S., fish landing in 1986 totaled 2.7 million metric tons and a larger percentage, 44%, was reduced to fish oil and fish meal. Ninety-nine percent of this oil was manufactured from menhaden, and most was produced in the Gulf states. In the same year, 90% of the U. S. fish oil was exported to Europe and refined and partially hydrogenated to make margarine and as ingredients for use in the bakery industry (Bimbo, 1987). Recently, there has been a large increase in the public's interest in fish oils and omega-3 fatty acids due to potential benefits in the reduction of cardiovascular risks. Fish oils are rich in polyunsaturated fatty acids including omega-3 acids such as eicosapentaenoic acid and docosahexaenoic acid. Since refined American fish oil is not yet available, the capsules containing refined and deodorized fish oil have been imported from Japan and Europe. It is estimated that as much as 300 million dollars will be spent on these imported capsules in 1988. In 1986, the National Fish Meal and Oil Association filed a petition to FDA to affirm GRAS status of menhaden oil as a direct human food ingredient. If menhaden oil is approved as GRAS, a tremendous economic potential exists for Louisiana, which is the largest fish oil producing state in the U. S.

However, there is a major problem with fish oils, namely, formation of fish oil odors due to degradation of lipids, proteins, and amino acids by microbial spoilage, autoxidation and other reactions. Nutritional and health benefits can not be realized if fish oils are unacceptable due to their objectionable odors.

The overall objectives of this project were (1) to identify the odorous components in the U.S. fish oils, (2) to determine the efficiency and to monitor the reduction of odorous components in refining and processing leading to food grade oil, and (3) to study the flavor stability of food grade oil during storage. In this paper, we report results obtained from both the Gulf and Atlantic menhaden oils to achieve the first objective.

MATERIALS AND METHODS

Zapata Haynie Corp. provided crude oils from Gulf menhaden (*Brevoortia tyrannus*) and Atlantic menhaden (*Brevoortia patronus*). Volatile compounds were collected from the samples by a Tekmar dynamic headspace sampling system (Cincinnati, OH) using helium as a purging gas and Tenax TA (Chrompack, Inc., Raritan, NJ) as a trapping sorbent. Figure 1 shows a schematic diagram of the dynamic headspace sampling system. The trapped volatiles were then flash-desorbed into a cryogenically focussed high resolution capillary column (Supelcowax 10, 60 m x 0.25 mm i.d. x 0.25 μm film thickness) for gas chromatography. The separated volatile compounds were identified by electron ionization mass spectrometry and computer mass spectral matching using a Hewlett Packard Mass Selective Detector. Authentic compounds were used for confirmation of the identification under comparable experimental conditions. The odors of the components were also perceived as they were eluted from the column. Details of this combined analytical procedure have been described elsewhere (Hsieh et al, 1989).

RESULTS AND DISCUSSION

Figure 2 shows the total ion chromatograms, or the "finger print profiles", of the volatiles from the crude oils of Gulf and Atlantic menhaden. Both samples were 1988 oils. The compounds identified and the odor perceived are labelled at the appropriate chromatographic retention times. Many of the compounds noted derive from

lipid oxidation, and contribute negatively to the flavor quality of the fish oils. It also was observed that Gulf crude oil contained more volatiles than did the Atlantic oil.

The analytical procedure used did not involve high temperature heating for a prolonged period or organic solvent extraction with potential impurity contamination. It has decreased possibility of artifact formation in comparison with conventional analytical techniques. This procedure will be used to determine the efficiency of refining and deodorization of the crude menhaden oils leading to food grade oil with acceptable flaovr quality as well as the flavor stability of refined and deodorized oil in storage. This procedure may also be used to characterize the critical volatile components that affect flavor quality in other oils

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ACKNOWLEDGMENTS

Manuscript No. 88-21-2783 of the Louisiana Agricultural Experiment Station. This study was supported, in part, by a grant from the Louisiana Sea Grant College Program, an element of NOAA, Office of Sea Grant, U. S. Department of Commerce. The authors wish to thank S. S. Williams and W. Vejaphan for their assistance in the earlier part of this project, and S. P. Meyers for helpful discussions.

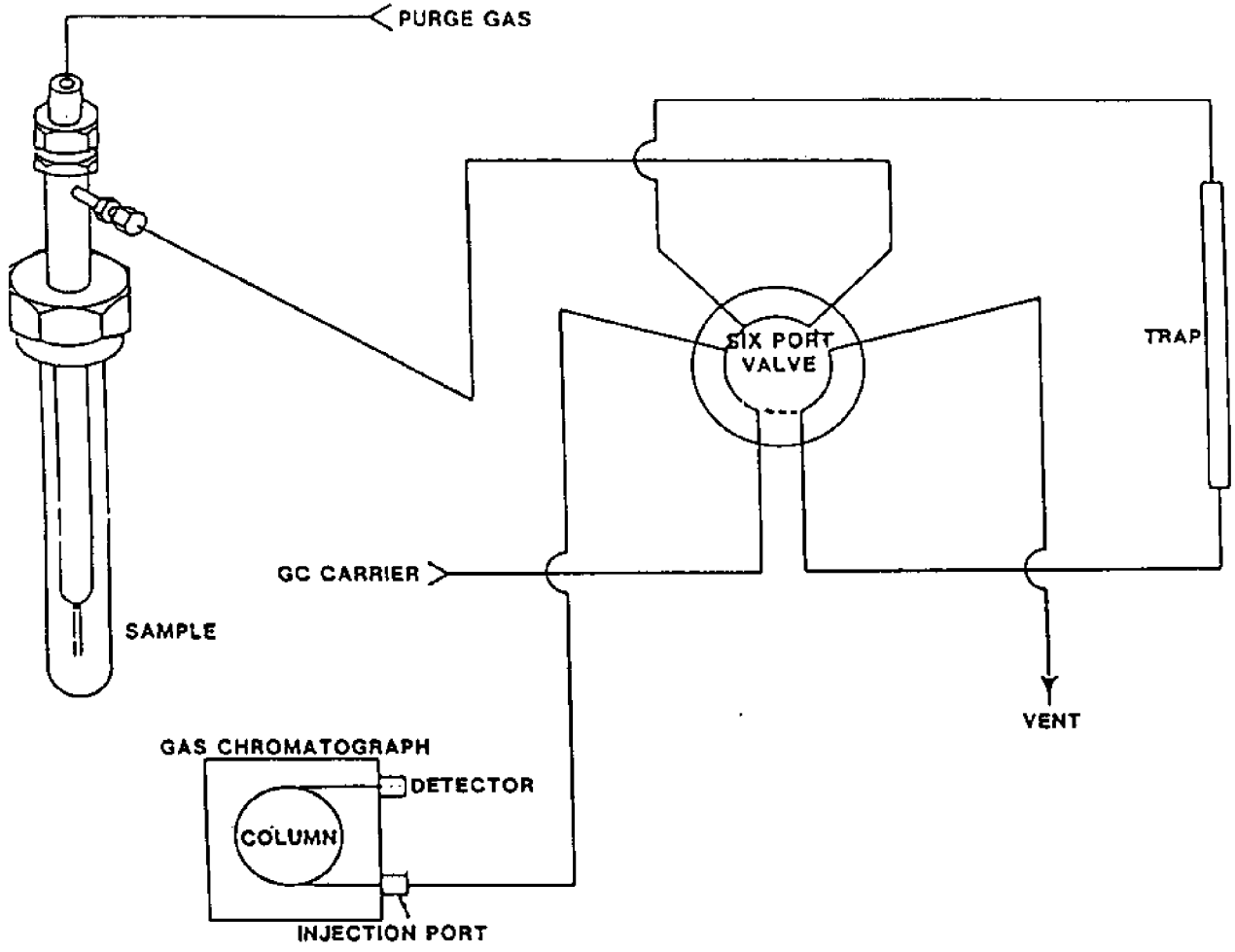


Figure 1. Schematic Diagram of Tekmar Dynamic Headspace Sampler Coupled to a Gas Chromatograph

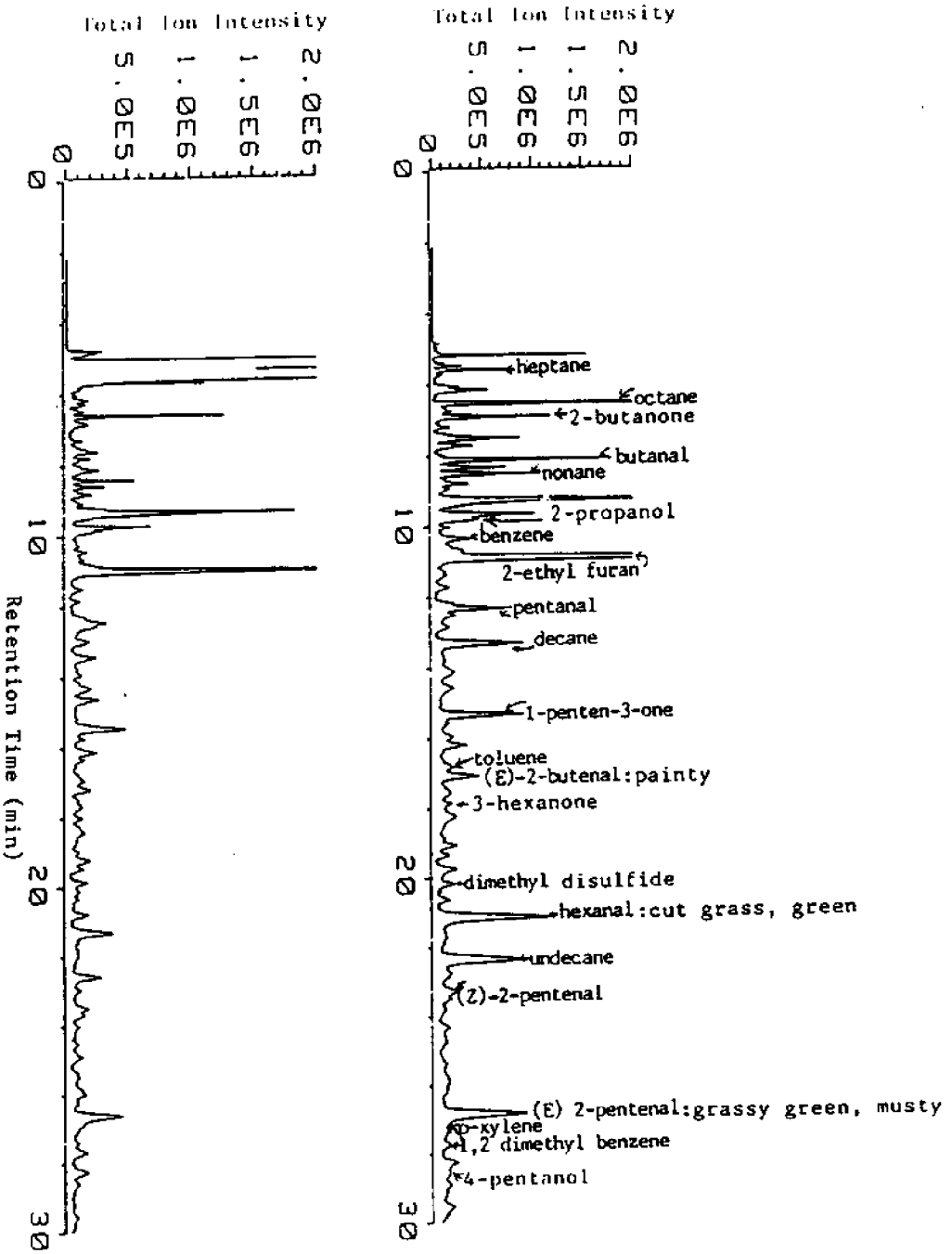


Figure 2. Total Ion Chromatograms of Dynamic Headspace Volatiles from Gulf (Top Panel) and Atlantic (Bottom Panel) Crude Menhaden Oils: Compound Identification and Odor Perception.

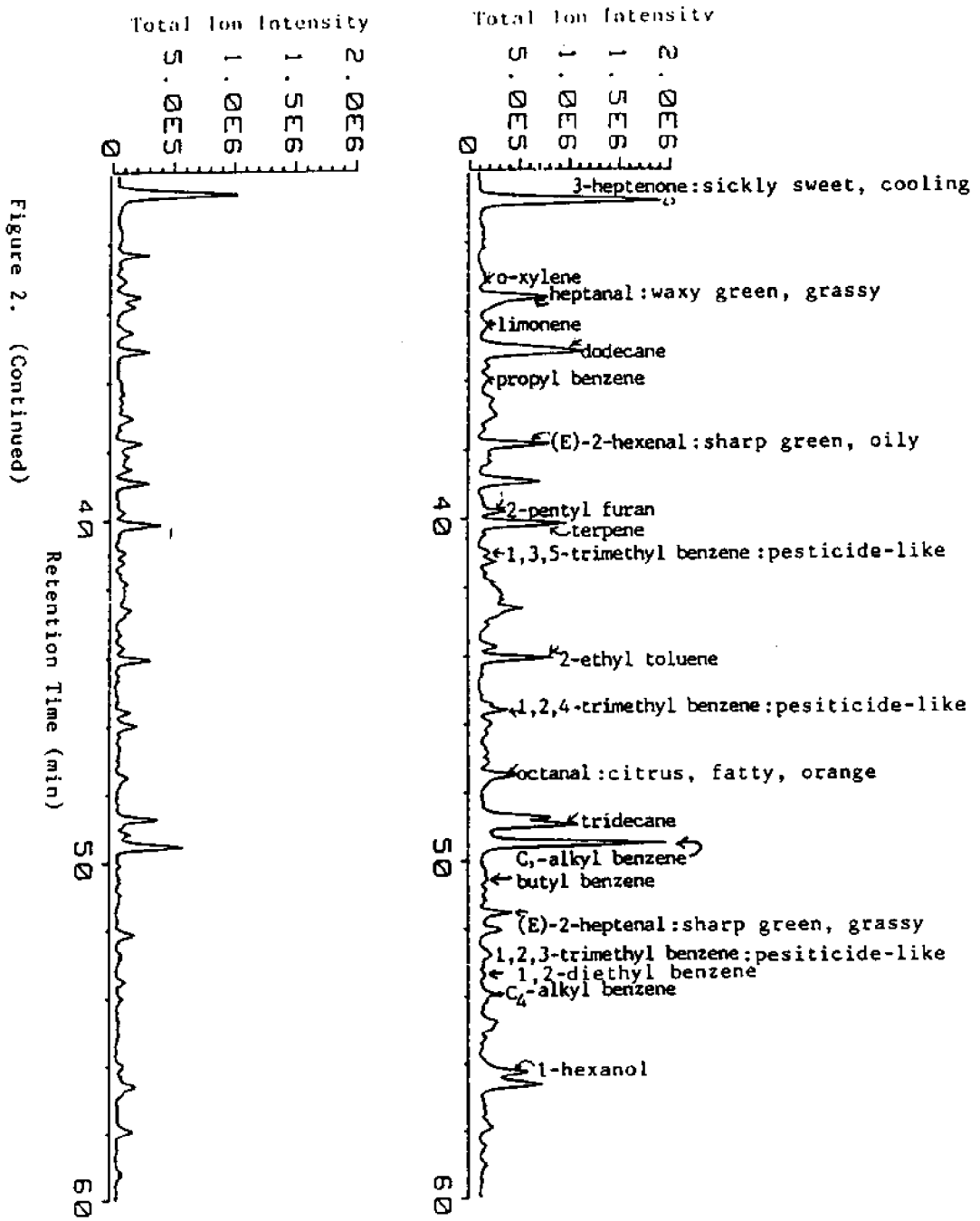
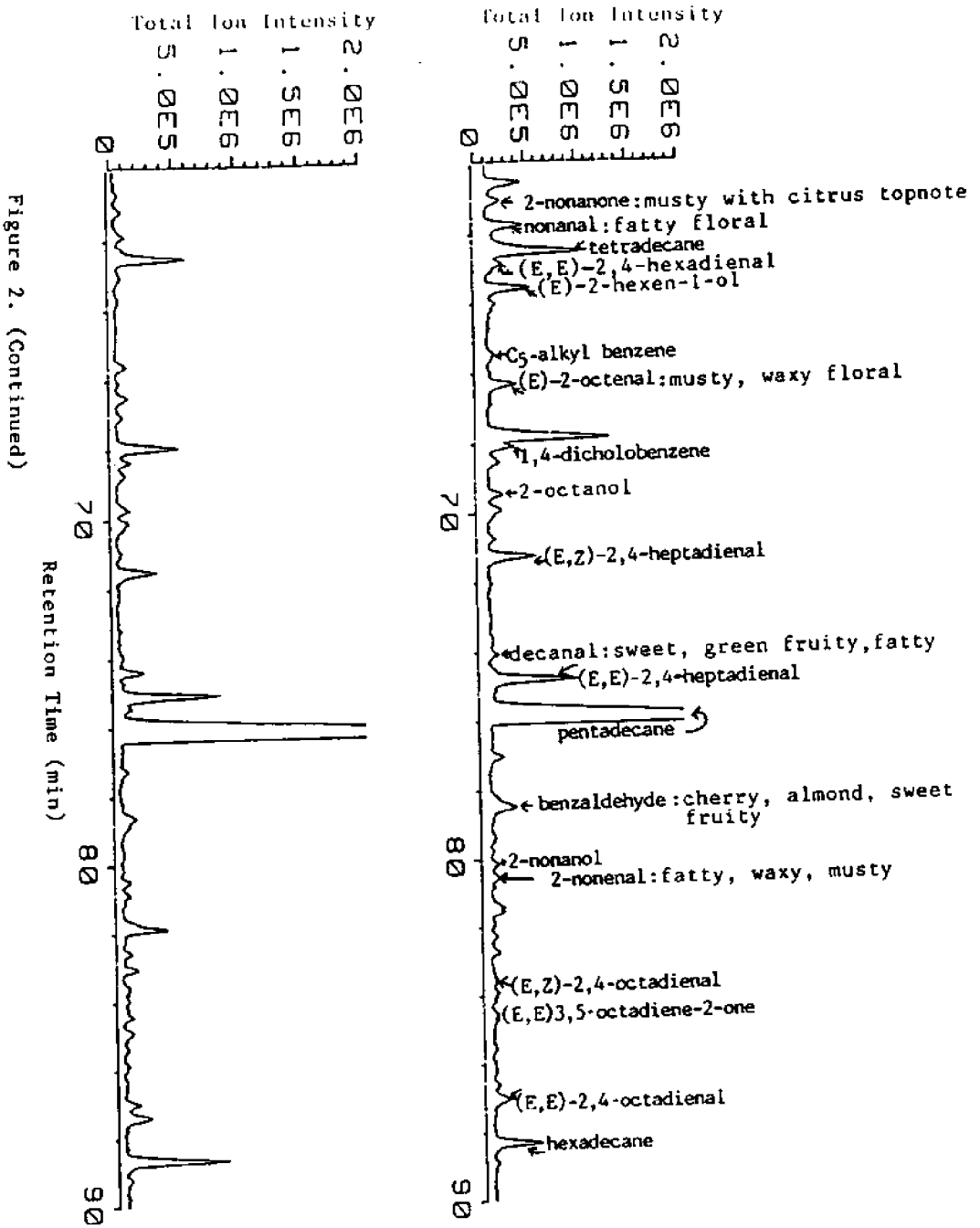


Figure 2. (Continued)



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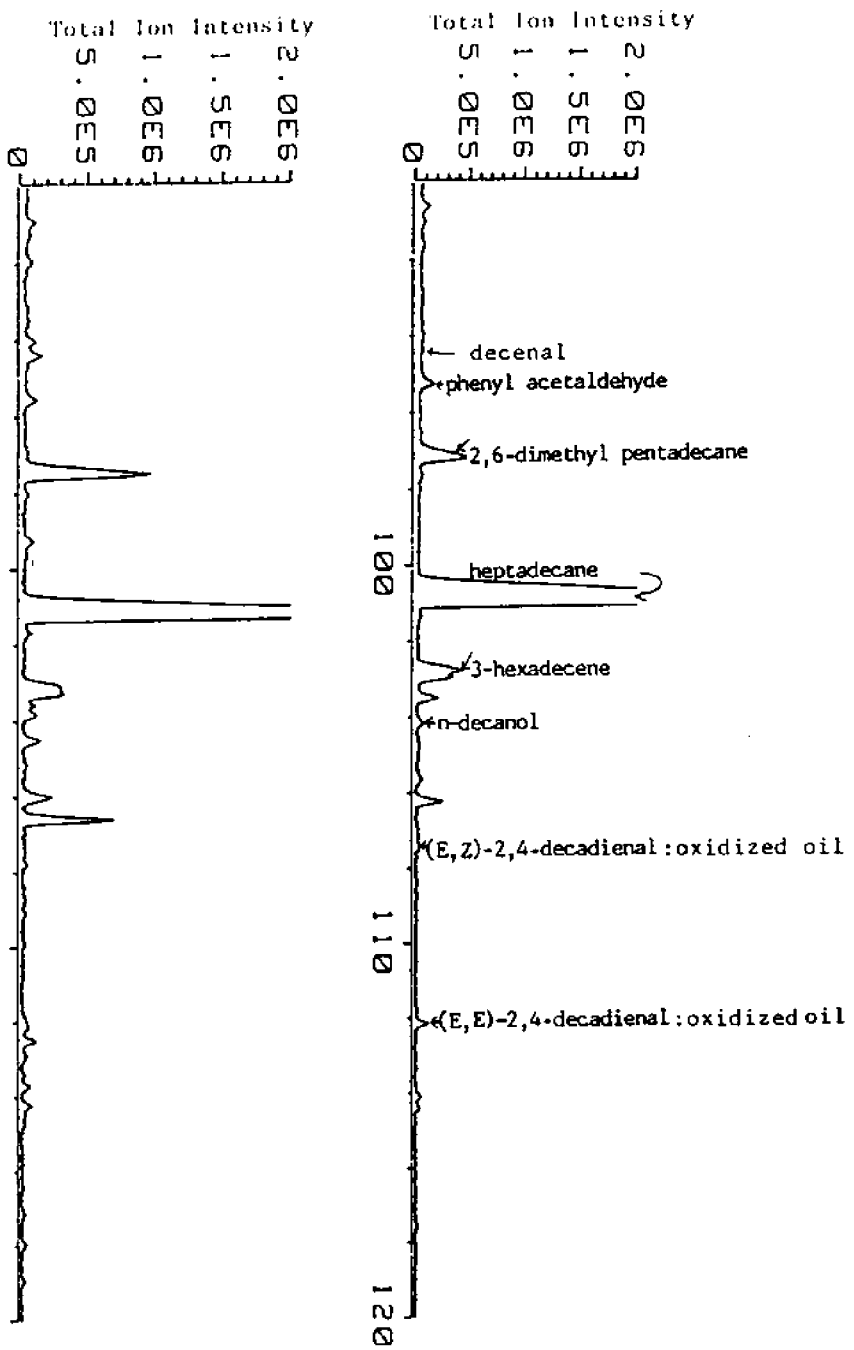

Figure 2. (Continued)

SHRIMP SPECIES IDENTIFICATION USING UREA GEL ISOELECTRIC FOCUSING SYSTEM

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INTRODUCTION

Isoelectric focusing (IEF) has been extensively used for seafood species identification because it provides reliable and reproducible protein patterns for differentiating closely related species. Using thin layer polyacrylamide gel IEF, various fish species have been readily identified (Hamilton, 1982; Lundstrom, 1981 and 1983).

Identification of cooked or processed seafood has not been extensively studied using this methodology. Most difficulties encountered are due primarily to alterations of protein molecules by heat treatment. The extractability of heat denatured proteins can be increased by the use of sodium dodecyl sulfate (SDS) or urea. Krzynowek and Wiggin (1979 and 1981), using urea to extract crab meat proteins and IEF, identified with 98% accuracy the genus of cooked or frozen crab meat among 79 samples. Recently An et al. (1988a) used 8M urea or 1% SDS to extract boiled shrimp proteins and they properly identified the genus of cooked shrimp using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).

Urea has been incorporated in electrophoretic gels to enhance protein separation and resolution (Boulikas, 1985; Keung et al., 1985; O'Farrell, 1975; Tuszynsky et al., 1978). By utilizing a 9.2 M urea IEF gel for the first dimension, O'Farrell (1975) successfully separated an *Escherichia coli* lysate into over 1,000 components by two–dimensional gel electrophoresis. Therefore the objectives of this study were (1) to investigate if the addition of urea in IEF polyacrylamide gels would enhance the resolution of protein patterns for shrimp species identification, (2) to optimize urea IEF conditions for speciation of raw and cooked shrimp, and (3) to examine the species–specificity of shrimp protein banding patterns by combining the protein extraction systems with urea gel IEF.

MATERIALS AND METHODS

Sample preparation

Three shrimp species, pink (*Penaeus duorarum*, Key West, FL), white (*Penaeus setiferus*, Jacksonville, FL), and rock shrimp (*Sicyonia brevirostris*, Port Canaveral, FL), were obtained and stored at –33°C until needed. Individual shrimp were thawed under tap water, peeled, and deveined. For the cooking treatment, shrimp were placed in boiling water for 5 min.

Water and 1% SDS (w/v) were used to extract proteins from raw or cooked shrimp, respectively (An et al., 1988a). The solvents contained 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM EDTA and 0.01% (w/v) sodium

azide to inhibit proteases and microbial growth. Both raw and cooked shrimp samples were chopped and each combined in separate beakers with the individual solvents at a ratio of 1:3 (w/v). Samples were homogenized at room temperature for 1 min using a Polytron (Brinkmann Instrument), and then centrifuged for 20 min at 26,900 x g (20°C). The supernatants were collected and the protein concentration determined (Lowry et al., 1951), and 100 μg of protein was applied on the IEF gel. For mixture samples, shrimp extracts were mixed in a 1:1 protein ratio (w/w) between pink and white, pink and rock, or white and rock and a 1:1:1 ratio between all three species. The mixtures were processed as above and subjected to IEF analysis.

Optimization of IEF conditions

The IEF method of O'Farrell (1975) was modified for a slab gel system. Various conditions were compared to establish the best conditions for species identification using banding patterns of water extracts from raw pink shrimp and protein standards (Broad pI kit, pH 3–10, Pharmacia). The protein standards contained: trypsinogen, pI 9.30; lentil lectin–basic band, pI 8.65, –middle band, pI 8.45, –acidic band, pI 8.15; horse myoglobin–basic band, pI 7.35, –acidic band, pI 6.85; human carbonic anhydrase B, pI 6.55; bovine carbonic anhydrase, pI 5.85; beta–lactoglobulin A, pI 5.20; soybean trypsin inhibitor, pI 4.55; and amyloglucosidase, pI 3.50.

Urea concentration: A non–denaturing (without urea) and a denaturing gel system with 6 or 9.2 M urea were compared on a polyacrylamide gel containing 6.2% (v/v) ampholytes (Pharmalytes pH 3–10, Pharmacia). Proteins were focused at room temperature for 17 hr at 400 V with a circulating tap water coolant system.

Ampholytes concentration: Banding patterns of the water extract of raw pink shrimp and protein standards were compared using a 9.2 M urea polyacrylamide gel containing 5.0 or 6.2% (v/v) ampholytes. The gel was focused for 17 hr.

Focusing time: Two focusing times, 10 and 17 hr, were compared using a 9.2 M urea gel containing 6.2% (v/v) ampholytes.

Electrolytes: Two different electrolyte systems, 20 mM sodium hydroxide–10 mM phosphoric acid (O'Farrell, 1975) and 10 mM histidine–10 mM glutamic acid (Anonymous, 1988) were compared for narrow pH range gel containing 100% ampholytes of pH 4–6.5 (Pharmalytes 4–6.5).

Ampholytes mixtures: Ampholytes were mixed to focus the bands at the anodic side of the pH 3–10 gel. Ampholytes 4–6.5 (Pharmalytes 4–6.5) at 100, 80, and 60% were mixed with 0, 20, and 40% ampholytes of pH 3–10 (Pharmalytes 3–10); the mixture was added into the urea gel at the final concentration of 6.2% (v/v).

Isoelectric focusing

A gel mixture containing 4% (w/v) acrylamide, 2% (w/v) Triton X–100 and 9.2 M urea was heated to 37°C with shaking for five min to dissolve urea. Ampholytes at a final concentration of 6.2% (v/v) was added, the gel mixture was degassed for 5 min, then combined with fresh persulfate solution and TEMED (final

concentration: 0.02% (v/v) and 0.14% (v/v), respectively) and poured into 16 x 20 cm slab gel plates (0.75 mm thick) assembled with a comb. The gel was allowed to polymerize for 1 hr. Lysis buffer containing 9.5 M urea, 2% (w/v) Triton X–100 and 2% (v/v) ampholytes was overlayed on the gel following the removal of the comb. The gel was allowed to sit for another hour. After the lysis buffer was replaced with a fresh one, prefocusing of the gel was done at 200 V for 15 min, 300 V for 30 min and then 400 V for 30 min in a Protean II electrophoresis slab gel unit (BioRad) using a 10 mM phosphoric acid as the anode electrolyte solution and a 20 mM sodium hydroxide as the cathode solution.

Following prefocusing, protein samples were applied on the gel, and an aqueous solution containing 2% (w/v) Triton X–100 and 2% (v/v) ampholytes was overlayed atop the protein samples. The gel plate was reassembled in the electrophoresis unit and fresh cathode solution was added to the chamber. Proteins were focused at room temperature for 17 hrs at 400 V with circulating tap water. After the IEF run, the gel was stained with Coomassie blue R–250 and destained (Anonymous, 1986). Positive image was developed from the stained gel using EDP paper (Electrophoresis Duplicating Paper, Eastman Kodak). Protein profiles of the IEF gel was scanned using the video densitometer (Model 620, BioRad) with the developed pictures on EDP paper. The densitometer was set at the reflectance mode.

pH Measurement of the gel and determination of the apparent pI values of shrimp protein bands

Apparent pI values of shrimp proteins were determined indirectly by comparing their R_f values on the gel with those of the protein standards. A pH profile of the whole gel was determined at room temperature (25 + 2°C) before protein fixation using a micro–surface pH electrode (Ingold Electrodes Inc.). A linear relationship of gel pH and the R_f values of protein standards enabled determination of the apparent pI values for the corresponding standards. From the apparent pI and the R_f values of the protein standards and the R_f values of the shrimp proteins on the gel, the apparent pI values of shrimp proteins were determined.

RESULTS AND DISCUSSION

Optimization of IEF gel conditions

Urea concentration: The presence of urea in the gel greatly increased protein resolution. Among the three different urea concentrations used (0, 6 and 9.2 M), the 9.2 M gel provided the best protein banding pattern for raw pink shrimp and standards (Fig. 1). Proteins were well focused at the anode side and the bands were highly resolved. Good protein separation also occurred with the 6 M urea gel, but the resolution was not as good as the 9.2 M gel. Four major bands were found with shrimp samples run on gels containing no urea. In addition, the banding patterns and the pI values of the protein standards were different in the gels with or without urea.

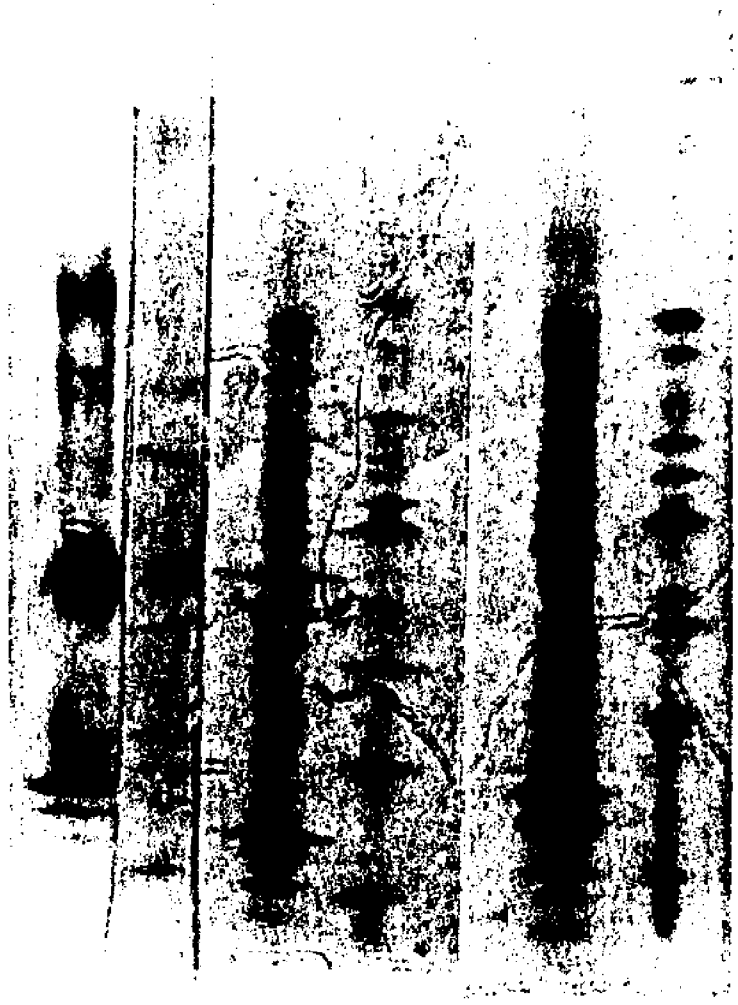


Figure 1 (Left). Protein banding patterns of the water extract of raw pink shrimp (P) and protein standards (Std) focused on polyacrylamide gel containing 0, 6, or 9.2 M urea (cathode on top). The numerical designations indicate apparent pI values of the protein bands.

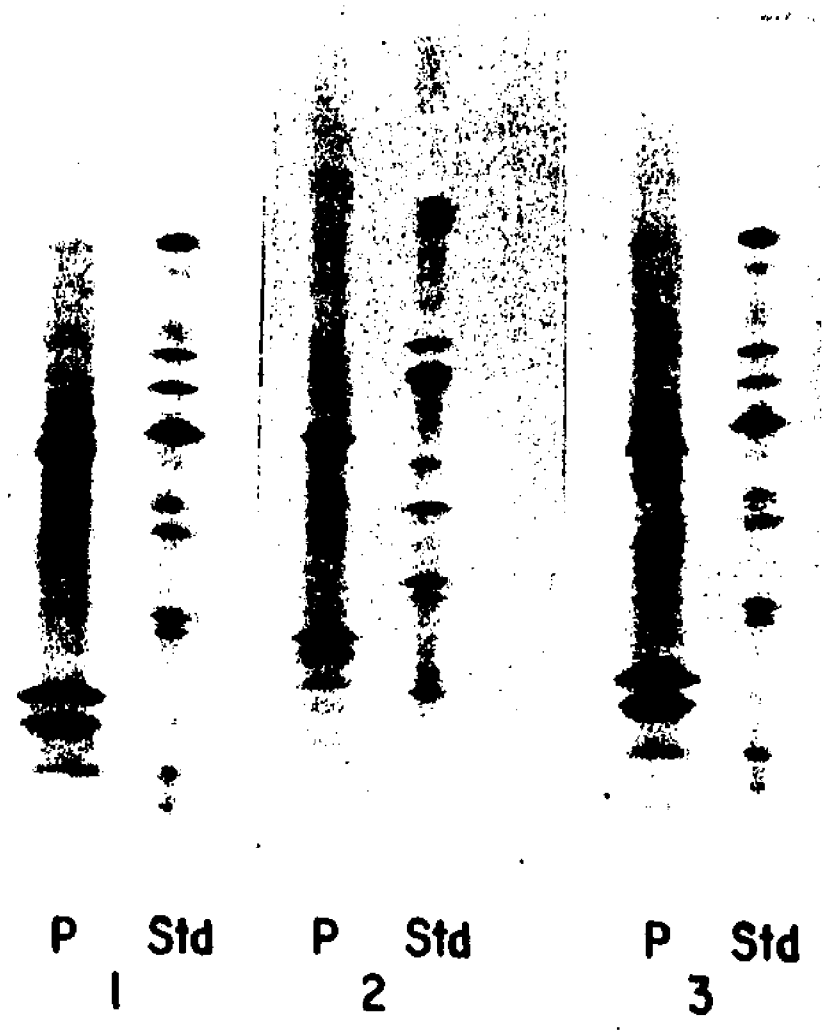


Figure 2 (Right). IEF patterns of the water extract of raw pink shrimp (P) and protein standards (Std) focused on polyacrylamide gel containing (1) 5% ampholytes for 17 hr, (2) 6.2% ampholytes for 10 hr, and (3) 6.2% ampholytes for 17 hr (cathode on top).

Ampholytes concentration: More stable protein banding patterns were obtained on gels containing 6.2% ampholytes (Fig. 2). Compared to the bands on the 5% ampholytes gel, protein bands on the 6.2% gel migrated less from the cathodic end where the sample was applied. Protein bands were more evenly distributed on the 6.2% ampholytes gel, while they were primarily focused near the anode side in the gel containing 5% ampholytes. In addition diffusion of protein bands occurred on the gel containing 5% ampholytes.

Focusing time: Focusing for 17 hr provided better band resolution than did a 10 hr period (Fig. 2). Proteins focused for only 10 hr were not completely resolved. This observation supported the results of Duncan and Hershey (1984) who showed that most proteins were completely localized by 6,400 volt–hour.

IEF patterns of raw shrimp for pH 3–10

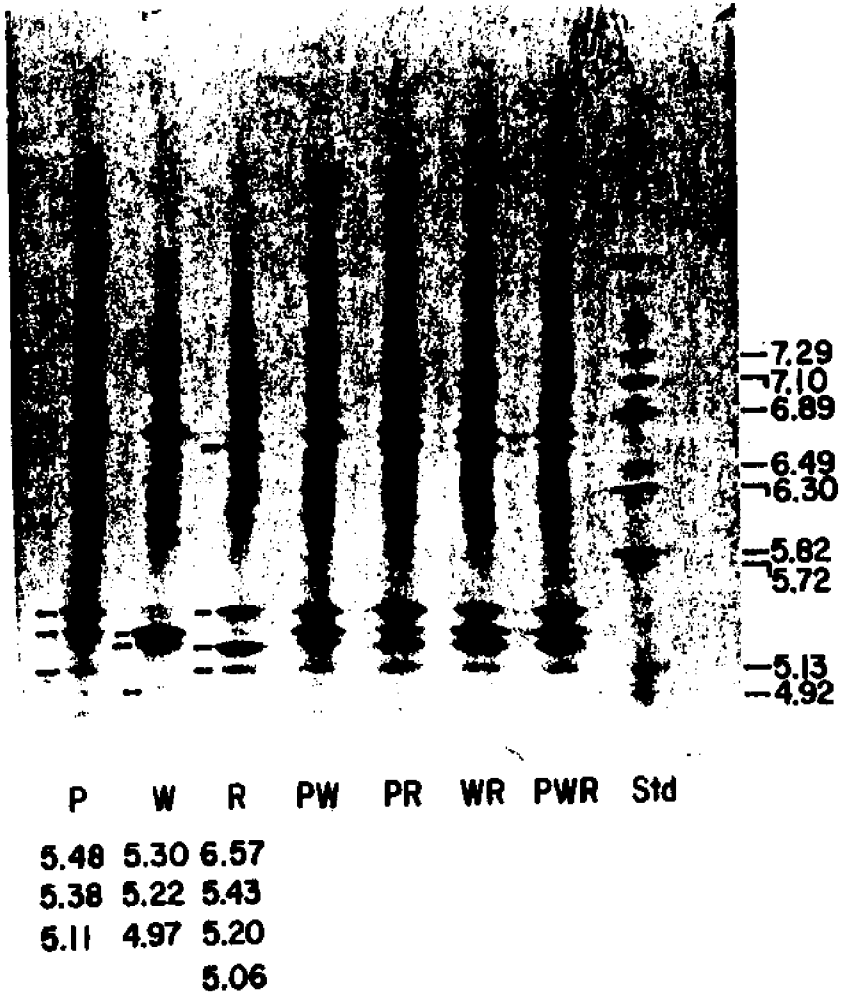
IEF patterns of water extracts of raw pink, white and rock shrimp were compared. Water extracts gave rise to excellent banding patterns for species identification (Fig. 3). The most diverse and species–characteristic bands occurred at the low pI region. Bands with pI values of 5.48, 5.38, and 5.11 were specific for pink; 5.30, 5.22, and 4.97 for white; and 5.43, 5.20, and 5.06 for rock shrimp. Rock shrimp also had an additional specific band with a pI of 6.57.

Although most of the major bands at the anodic side of the gel were resolved and could be used effectively for species comparison, the minor protein bands at the lower pI region of the gel were clustered together due to the use of the pH 3–10 ampholytes. Since these minor protein bands may also provide useful information to further aid species identification, anodic region of the gel was expanded by incorporating into the gel a narrower range (pH 4–6.5) ampholytes.

Optimization of IEF gel conditions for narrow pH range

Electrolytes: The histidine–glutamic acid electrolyte systems recommended by the manufacturer interfered with proteins focusing at the narrower pH range of 4–6.5. There was no stable pH gradient formation in the gel nor was protein separation (data not shown). The interference still occurred even when proteins were focused at 4°C on the gel containing no urea. It was speculated that the low pH of histidine might cause protein precipitation on the gel and thus interfering their entering into the gel. The precipitated proteins might also prevent electolytes from moving continuously. This problem was alleviated by the use of the sodium hydroxide–phosphoric acid electrolyte system; a stable pH gradient and a reproducible banding patterns were obtained. Therefore the sodium hydroxide–phosphoric acid system was used for future studies.

Ampholytes mixtures: Different ampholytes mixture affected the pH profile of the gel at the cathodic region. Incorporation of 40% pH 3–10 ampholytes with 60% pH 4–6.5 ampholytes caused the increase of about one pH unit at the very end of the cathodic region as compared to the gel that contained only pH 4–6 ampholytes (Fig. 4). Thus the actual pH of the gel containing only pH 4–6.5 ampholytes was determined to range from 4.3 to 7.2; from pH 4.6 to 7.7 for the one containing 80% pH 4–6.5 ampholytes; and from pH 4.3 to 8.3 for containing only 60% pH 4–6.5 ampholytes.



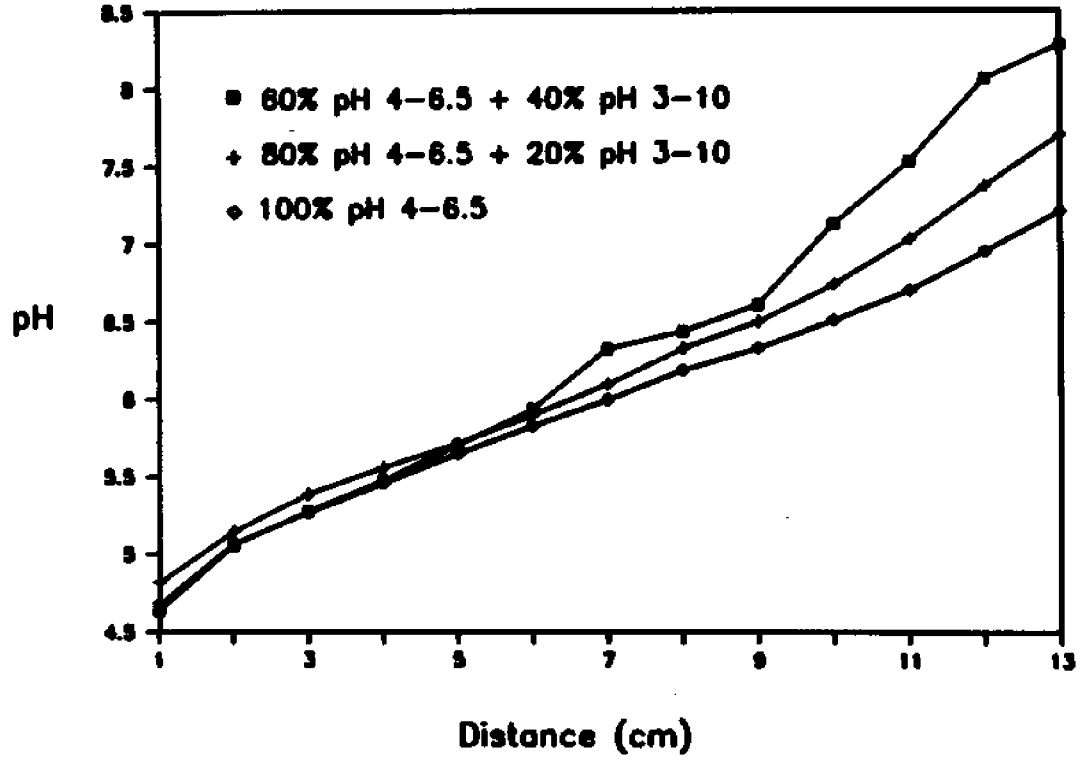


Figure 3 (Left). IEF patterns of water extracts of raw pink (P), white (W), and rock (R) shrimp, and their mixtures (cathode on top). Protein standards (Std) are also included. The numerical designations indicate apparent pI values of the protein bands.

Figure 4 (Right). pH profiles of IEF gel containing three different ratios of wide and narrow pH range of ampholytes.

The change of the gel pH range also affected the resolution of raw pink shrimp proteins and protein standards. The distance between two specific protein bands was increased on the gel containing higher percentages of the pH 4–6.5 ampholytes. Among the three different mixtures, the 80% mix (of pH 4–6.5 ampholytes) was found to efficiently separate the raw shrimp proteins. Most of the protein bands useful for shrimp species identification, including those bands with low pI values to provide most information for species identification were well resolved and distributed throughout the gel (Fig. 5). The use of only the narrow pH range ampholytes (4–6.5) showed a good protein band separation; but some protein bands at the cathodic end were excluded from the gel due to the narrower gel pH range. Although most of the protein bands found on the gel containing only pH 3–10 ampholytes were present on the gel containing 40% pH 3–10 and 60% pH 4–6.5 ampholytes mixture, the protein bands were not separated as clearly as those on the gel containing 20% pH 3–10 and 80% pH 4–6.5 ampholytes mixture. Therefore the latter ampholytes mixture was used to separate shrimp proteins for further studies.

IEF patterns of raw shrimp for narrow pH range

Banding patterns of the water extracts of raw pink, white and rock shrimp and their mixtures were compared for species specificity (Fig. 6). As reported previously using only the pH 3–10 ampholytes (An et al., 1988b), species identification could be achieved by checking the presence of the major species–specific protein bands that were present at the anodic side. These included the protein bands with pI values of 6.97, 6.79, 5.52 and 5.45 for pink shrimp, the 6.87, 5.45 and 5.42 bands for white shrimp, and the 5.54 and 5.31 bands for rock shrimp.

Species identification could also be achieved by comparing among the species patterns of the minor bands that were well separated. The bands with pI values of 7.41, 7.28 and 7.26 for pink shrimp, the 7.41, 7.29, 7.02, 6.77, 6.66 and 5.96 bands for white shrimp, as well as the 7.16, 6.78, 6.75, 6.33, 6.24 and 6.20 bands for rock shrimp were shown to be specific for each species. The presence of these bands together with the major bands could be used effectively in identifying the species as well as in detecting the presence of a specific shrimp species in the mixture. For example, the presence of rock shrimp in the pink + rock, white + rock, or pink + white + rock shrimp mixture could be determined from the presence of the 7.16, 6.75, 6.33, 6.24 and 5.31 protein bands that were specific for rock shrimp.

The difference in protein banding patterns of the three shrimp species was well reflected by the diversity of their densitometric scanning profiles (Fig. 7). Since better protein separation occurred in these gels containing 20% pH 3–10 and 80% pH 4–6.5 ampholytes mixture, species–specific peaks were distributed throughout the profiles. Peaks at the 5–40 and 90–110 nm regions appeared to be more useful for species differentiation.

IEF patterns of cooked shrimp for narrow pH range

The water extracts of the cooked shrimp also showed different protein patterns (Fig. 8). Less shrimp proteins were extracted due to heat treatment. Thus many minor protein bands that were used for species identification in fresh sample

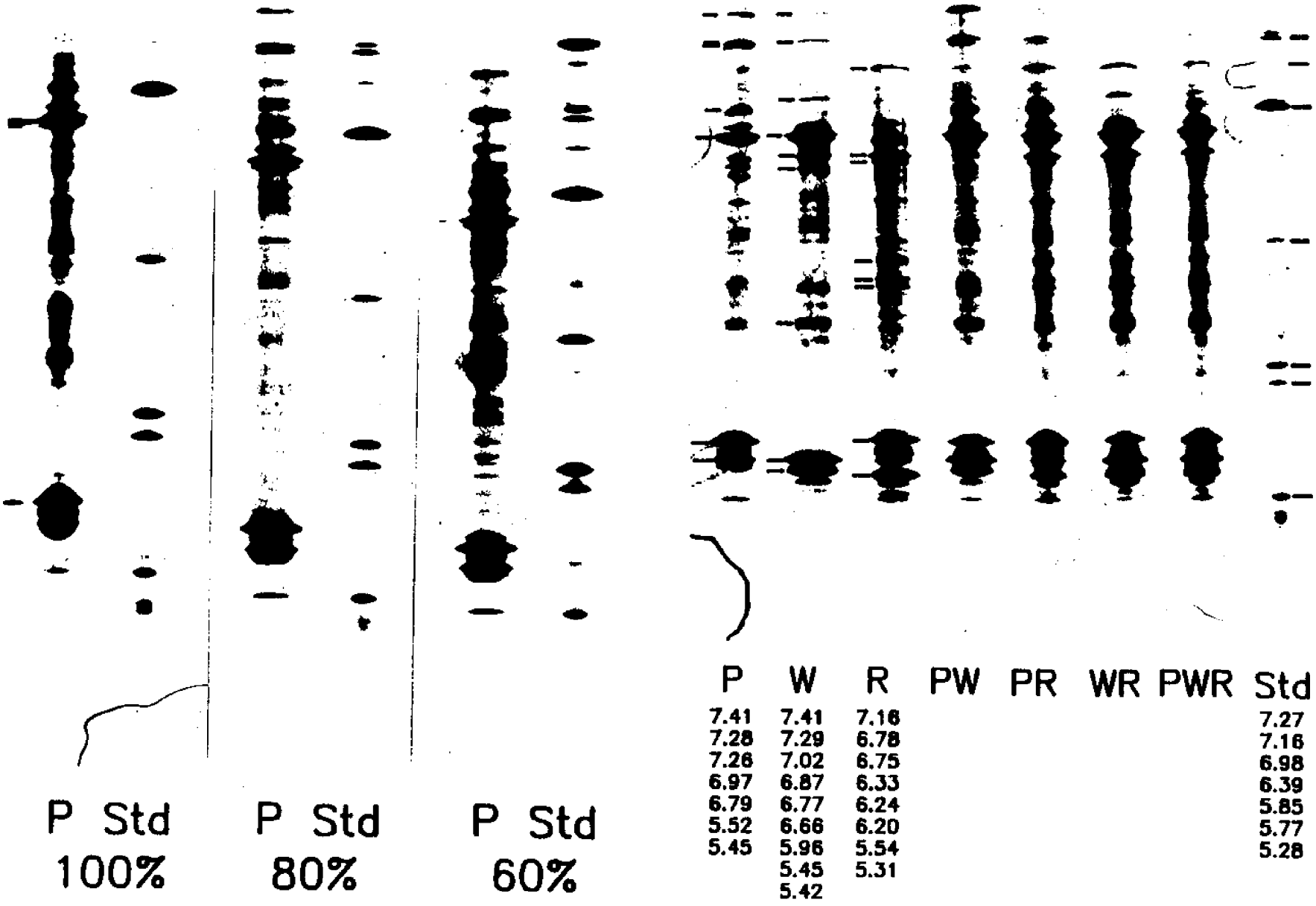


Figure 5 (Left). IEF patterns of water extracts of raw pink and protein standards focused on urea polyacrylamide gel containing 100, 800, and 60% of pH 4-6.5 ampholytes with addition of 0, 20, and 40% those of pH 3-10 (cathode on top).

Figure 6 (Right). IEF patterns of water extracts of raw pink (P), white (W), and rock (R) shrimp, and their mixtures (cathode on top). Protein standards (Std) are also included. The numerical values indicate apparent pI values of the protein bands.

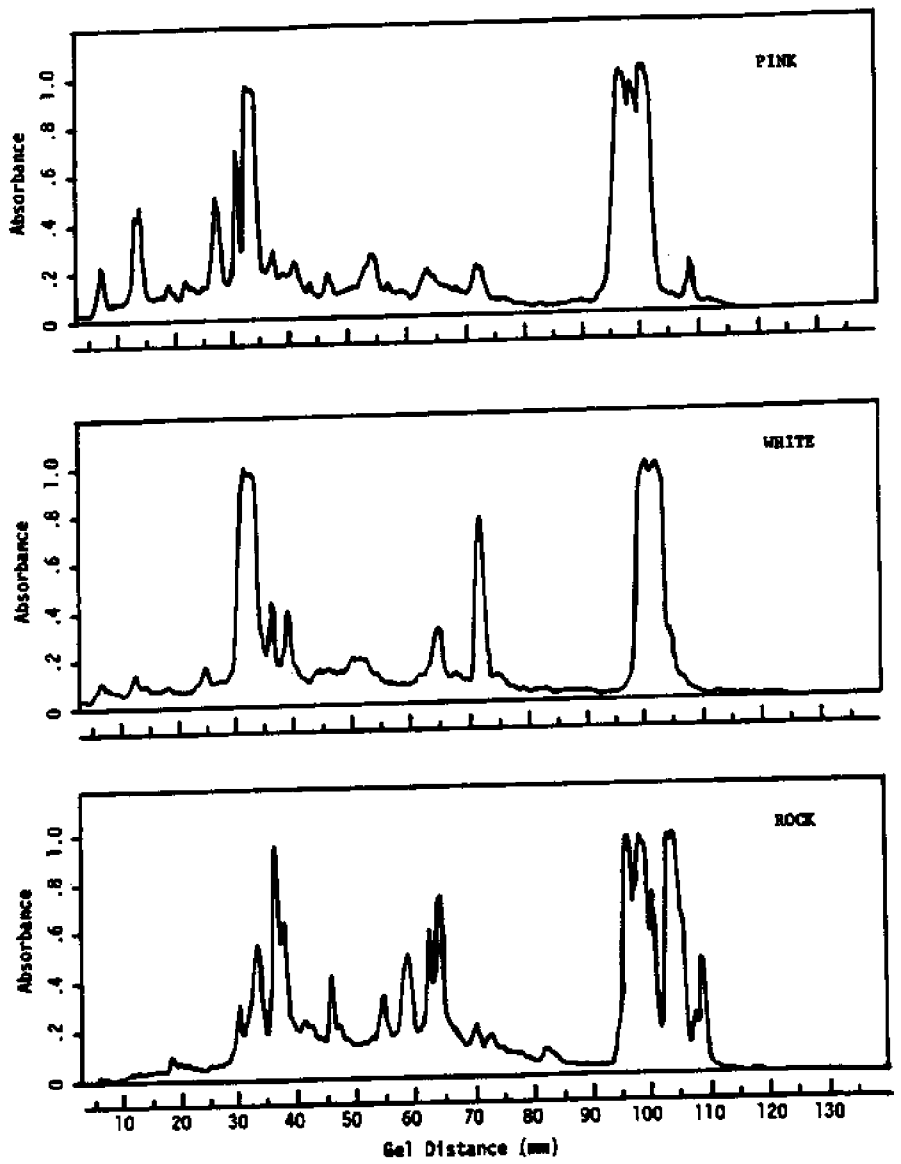


Figure 7. Densitometric profiles of water extracts of pink, white, and rock shrimp extracted with water.

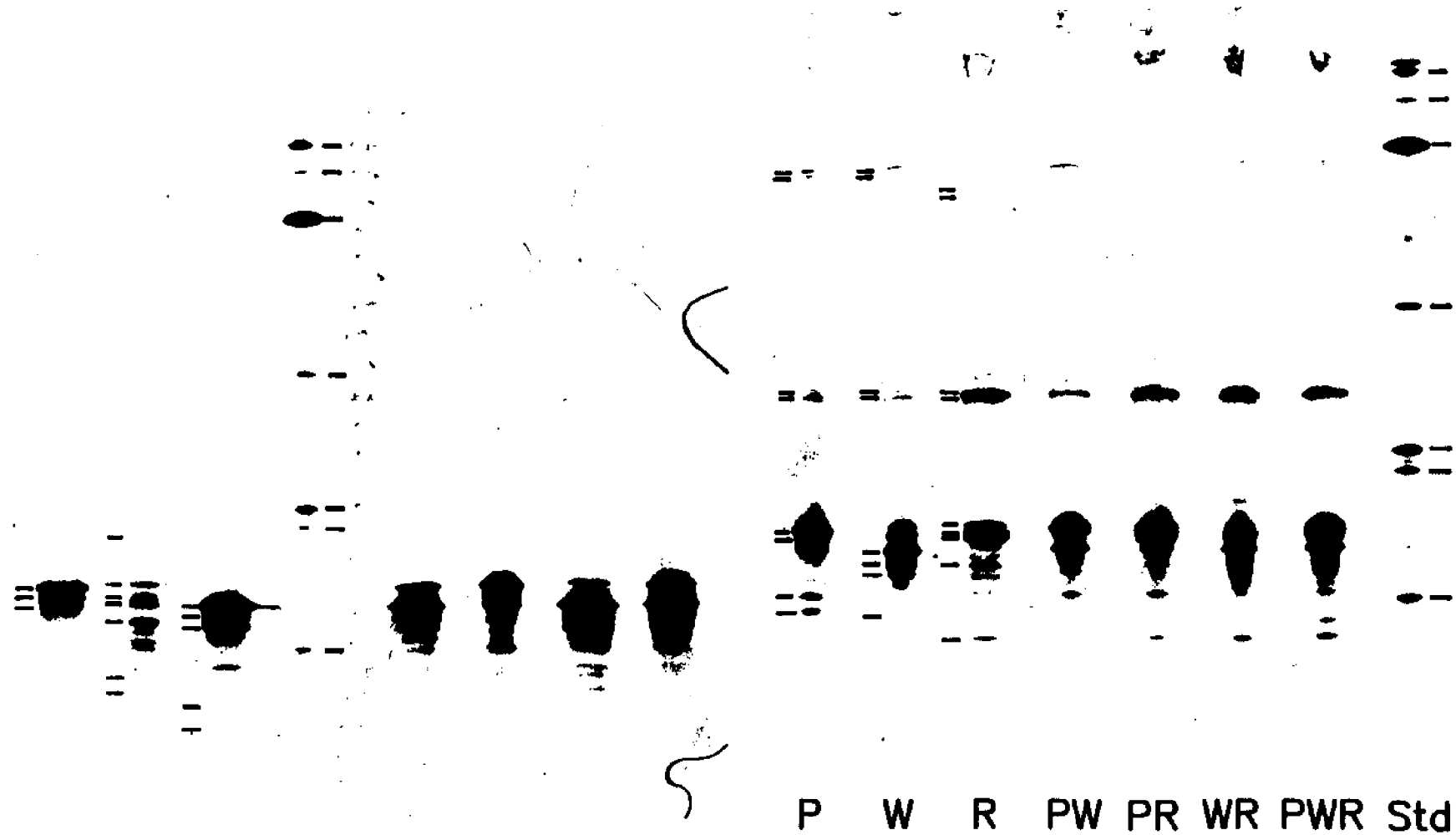


Figure 8 (Left). IEF patterns of water extracts of cooked pink (P), white (W), and rock (R) shrimp, and their mixtures (cathode on top). Protein standards (Std) are also included. The numerical values indicate apparent pI values of the protein bands.

Figure 9 (Right). IEF patterns of SDS extracts of cooked pink (P), white (W), and rock (R) shrimp, and their mixtures (cathode on top). Protein standards (Std) are also included. The numerical values indicate apparent

case were not present on the gel. The bands with low pI values, such as the 5.56, 5.51 and 5.48 for pink shrimp, the 5.48, 5.43 5.38, 5.07 and 4.98 for white shrimp, and the 5.76, 5.56, 5.51, 5.49 5.42, 5.20 and 5.14 for rock shrimp, were most useful for species identification. Identification of shrimp species in mixture samples, however, was complicated because of the similar patterns between mixture samples.

SDS enhanced protein extractibility and thus caused the increase of protein band numbers on the IEF gel (Fig. 9). The protein bands with pI values of 6.83, 6.79, 6.03 and 5.13 were specific for rock shrimp;,these rock shrimp bands could easily be distinguished even in mixture samples. Species could also be identified by checking the presence of the major protein bands with pI values of 5.33 and 5.26 for pink shrimp, and 5.40 and 5.23 for white shrimp. Due to the clustering of these bands, the identification of species specificity in mixture samples was difficult except for rock shrimp.

CONCLUSION

Incorporation of urea (9.2 M) in the gel greatly increased the protein separation and the band resolution. On the optimized condition using gels containing 9.2 M urea and 6.2% ampholytes and focused for 17 hrs, shrimp species were identified by the patterns of major protein bands.

Furthermore, the use of a 20% pH 3–10 and 80% pH 4–6.5 ampholytes mixture in IEF gels produced a narrower pH gel ranging from pH 4.7 to 7.7, and greatly enhanced protein separation as compared to gels containing only the pH 3–10 ampholytes. Not only the major bands but also the species–specific minor bands were well separated, so that species were easily differentiated. Presence of the species–related protein bands of raw samples was helpful to identify species present in a mixture samples. However the differentiation of the specific shrimp species in mixture of cooked samples was not effective due to the loss of some species–related protein bands by the heat treatment.

ACKNOWLEDGEMENT

This work is the result of research sponsored by NOAA, Office of Sea Grant, Department of Commerce, under Grant No. R/LR–Q–11–PD.

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IDENTIFICATION OF FISH SPECIES USED IN SURIMI PRODUCTS BY ELECTROPHORETIC TECHNIQUES

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INTRODUCTION

Surimi is a mechanically deboned, minced fish meat which has been washed to remove blood, fat, soluble pigments and other odoriferous substances, and mixed with cryoprotectants such as sugar and/or sorbitol (Lee, 1984 and 1986a). The washing process greatly improves the color and the odor of the minced fish and stabilizes the functional properties of surimi during frozen storage (Rasekh et al., 1980). Due to its light color, bland odor and unique gelling properties, surimi is used as a functional protein ingredient in the manufacture of a variety of fabricated seafood products such as crab legs, scallops, lobster and shrimp analogs (Lanier, 1986; Regenstein and Lanier, 1986).

To formulate the fabricated seafood products, surimi is combined with natural shellfish meat, shellfish flavoring agents, salt, water and starch, and/or egg white to modify the functionality and to enhance the flavor and texture. The finished products must be labeled properly to meet the Food and Drug Administration (FDA) guidelines which reflect the nature of the products. Martin (1986) has also stressed the need to establish proper nomenclature and labeling for newly developed seafood analogs. These must meet FDA requirements which have an impact on the two major ingredients in the fabricated seafood products: the fish species as the main ingredient and the other species, such as snow crab meat for crabmeat analog, as the additional ingredient. Problems exist regarding the labeling of the content of the specific seafood components. Products with claims of 35% crabmeat are widely sold when the use of over 10% crabmeat is known to show detrimental effect to the product (Regenstein and Lanier, 1986). Thus the establishment of useful methods to identify species origin, and, if possible, the content of surimi product is of great need for regulatory purposes and for protection of consumer's values.

Our previous studies using 1% sodium dodecyl sulfate (SDS) or 8M urea to extract proteins (An et al., 1988a and b) have shown that the species and genus of cooked shrimp could be successfully identified on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and urea gel isoelectric focusing (IEF). This study was carried out to determine if the combined application of extractants (water, 1% SDS or 8M urea) and the SDS–PAGE and IEF techniques could effectively differentiate the protein patterns of Alaska pollock and red hake and to identify these two species used in surimi.

MATERIALS AND METHODS

Alaska pollock (*Theragra chalcogramma*) and red hake (*Urophycis chuss*) were used. Red hake fillet, and surimi prepared at the Department of Food Science and Nutrition Department (Lee, 1986b) at the University of Rhode Island were shipped frozen to the Food Science and Human Nutrition Department at the University of Florida. Frozen Alaska pollock fillet was provided by Arctic Alaska Seafoods, Inc. (Seattle, WA), while surimi was manufactured by the Alaska Pacific Seafoods (Kodiak, AK). The samples were stored at –33°C until needed. Heat treatment of the samples was done by cooking in boiling water for 5 min. All experiments were repeated at least twice.

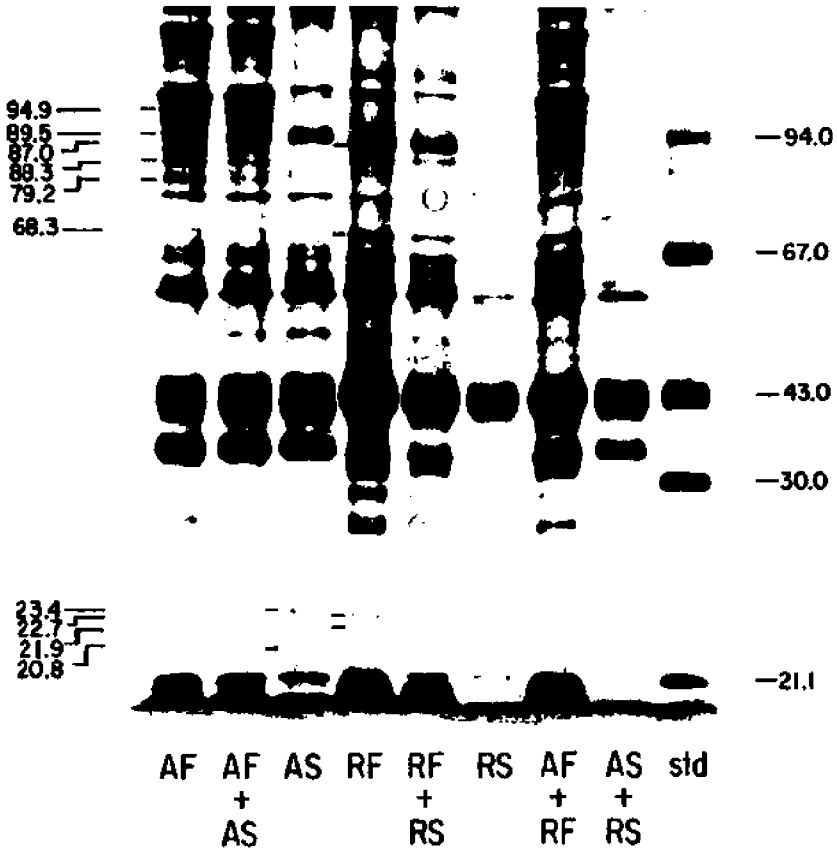
Protein extraction and sample preparation

Raw fish fillet and surimi samples were extracted with water only, while cooked samples were extracted with water, 1% SDS or 8 M urea. All solvents contained 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM EDTA and 0.01% sodium azide (An et al., 1988a). The chopped samples of the fish fillet or surimi were combined with each solvent at a ratio of 1:3, and homogenized at room temperature for 1 min using a Polytron (setting 5.5). The homogenates were centrifuged at 26,900*g* for 20 min at 20°C, the supernatants collected, and the protein contents determined (Lowry et al., 1951). For IEF, the protein content in the supernatant was adjusted with water to 5 μg/μL. Sucrose (3–4 granules) was added to each 100 μL extract, and 100 μg protein was applied on a IEF gel. For SDS–PAGE, 35 μg protein was loaded on the gel after the protein content of the supernatant was adjusted to 1.75 μg/μL with Tris–HCl buffer (pH 6.8) and SDS solution, heat–denatured, then finally sucrose and bromophenol blue were added (O'Farrell, 1975). Mixture samples were prepared by mixing the fish and surimi extracts in a 1:1 protein ratio.

Isoelectric focusing

A gel mixture containing 4% acrylamide, 2% Triton X–100 and 9.2 M urea was heated to 37°C. Ampholyte was added at a final concentration of 6.2%. After being combined with fresh persulfate solution and N,N,N',N'–tetramethyl–ethylene–diamine (TEMED) (final concentration: 0.02 and 0.14%, respectively), the mixture was poured into 16 x 20 cm slab gel plates (0.75 mm thick) assembled with a comb. Following polymerization, lysis buffer containing 9.5 M urea, 2% Triton X–100 and 2% ampholyte was overlayed on the gel following the removal of the comb. The gel was allowed to sit for another hour. Prefocusing of the gel was done, after the lysis buffer was replaced with a fresh buffer, at 200 V for 15 min, 300 V for 30 min, and then 400 V for 30 min in a Protean II electrophoresis slab gel unit (Bio–Rad) using 0.01 M phosphoric acid as the anode solution and 0.02 M sodium hydroxide as the cathode solution.

Both the lysis buffer and cathode solution were removed after prefocusing. Following the application of protein samples, an aqueous solution containing 2% Triton X–100 and 2% ampholyte was overlayed on the top of the protein samples. After the gel plate was reassembled in the electrophoresis unit, fresh cathode



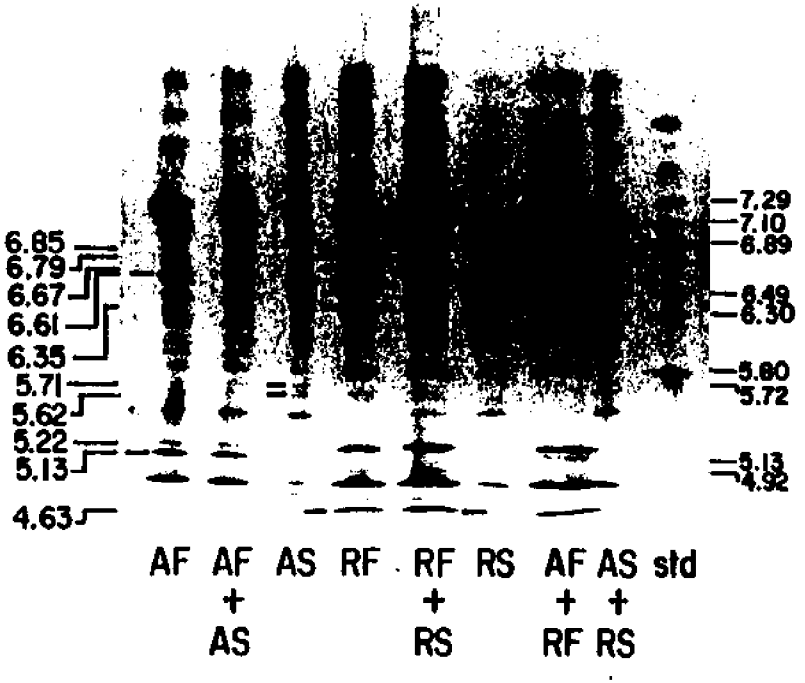


Figure 1 (Left). SDS-PAGE patterns of intact fillet and surimi of Alaska pollock and red hake extracted with water. Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate molecular weights (kD) of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.

Figure 2 (Right). IEF patterns of intact fillet and surimi of Alaska pollock and red hake extracted with water (cathode on top). Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate apparent pI values of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.

patterns can hardly be differentiated as being AS alone or as the combination of AS and RS. However, the use of the IEF patterns, as indicated in the later sections, will help the identification process.

The study with IEF showed that Alaska pollock had specific protein bands with pI values of 6.61 and 5.13 while the red hake had specific bands of 6.85, 6.79, 6.67, 6.35 and 4.63 (Fig.2). These specific bands were evident in the fish and fish–surimi mixtures. The 5.22 band of Alaska pollock and red hake, the 5.13 band of Alaska pollock, and the 4.63 band of red hake were missing in the surimi samples. Tow bands with pI values of 5.71 and 5.62 were found in Alaska pollock surimi. The reason for their presence in surimi and not in the corresponding fish is not known.

The identification of fish species used in surimi was made possible using a urea IEF system. The patterns of AS and RS could be differentiated from each other using the specific bands of 5.71 and 5.62 for AS and 6.85 and 4.63 for RS. These specific bands were also present in the AS+RS mixture; this makes the differentiation of AS, RS, and AS+RS possible.

Water extraction of cooked fish and surimi

Heat treatment of fish and/or surimi greatly reduced the number of water–extractable protein bands present on the SDS–PAGE and IEF gels (Figs. 3 and 4). Minor bands with MW's of 21.7 and 23.6 kD were specific for the cooked surimi of Alaska pollock and red hake, respectively (Fig. 3). Therefore they could be used for species identification of a surimi in mixture (AS+RS) containing Alaska pollock and red hake. The 19.9 kD band was specific for the cooked Alaska pollock fillet and could be used to indicate the presence of Alaska pollock in a AF+RF fish mixture.

The IEF study also indicated that a protein band with a pI of 5.03 was specific for Alaska pollock whereas the one at 4.56 was specific for red hake (Fig.4). The 5.09 band was detected only in the water extracts of the two cooked fish samples but not in the surimi, whereas the 4.70 band was detected only in cooked Alaska pollock surimi. The 4.82 minor band of the Alaska pollock fillet was not detected in the corresponding surimi sample. Differentiation of AS, RS and AS+RS mixture could be achieved from the presence of the 4.70 band for AS and the 4.82 band for RS.

SDS and urea extracts of cooked fish and surimi

The use of SDS and urea greatly enhanced protein extraction from cooked samples than that of water (An et al., 1988a and b). Fish and surimi also showed an increased number of protein bands on the electrophoretic gels after treating cooked samples with SDS and urea. In addition, the banding patterns of the SDS and urea extracts were very similar on the SDS–PAGE gels. The SDS–extractable bands with MW's 47.1, 45.9, 35.5, 23.8 and 21.2 kD were specific for Alaska pollock fillet and surimi, and the 25.3 and 20.3 kD bands for red hake fillet and surimi (Fig. 5). For urea extracts, the 46.1, 45.1, 23.6, and 20.7 kD bands, and the 58.2, 24.7 and 19.5 bands were specific for Alaska pollock and red hake, respectively (Fig. 6).

solution was added to the chamber. Proteins were focused at room temperature for 17 hrs at 400 V with circulating water. The gel was stained with Coomassie blue R–250 and destained.

The apparent pI values of the fish and surimi proteins were determined indirectly by comparing their R_f values on the gel with those of the protein standards following the previously described procedures (An et al., 1988b). The protein standards (Broad pI kit, pH 3–10, Pharmacia) contained: trypsinogen, pI 9.30; lentil lectin–basic band, pI 8.65, –middle band, pI 8.45, –acidic band, pI 8.15; horse myoglobin–basic band, pI 7.35, –acidic band, pI 6.85; human carbonic anhydrase B, pI 6.55; bovine carbonic anhydrase, pI 5.85; β–lactoglobulin A, pI 5.20; soybean trypsin inhibitor, pI 4.55; and amyloglucosidase, pI 3.50.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)

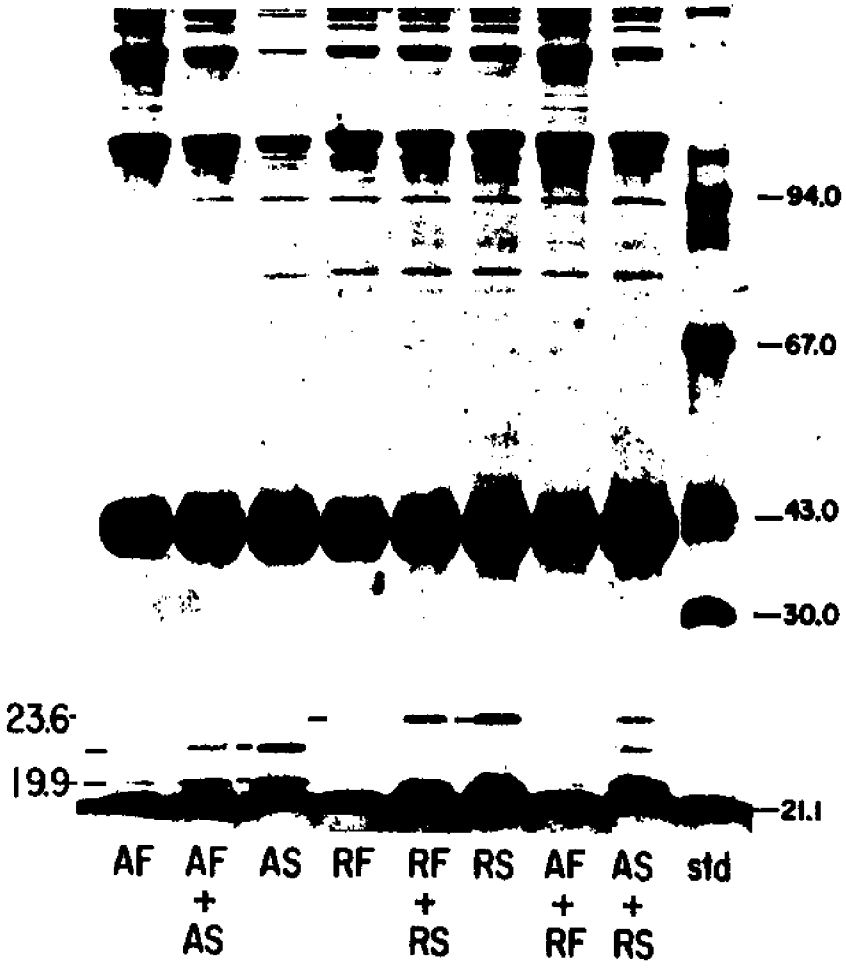
SDS–PAGE was performed according to the modified procedure of Laemmli (1970) and O'Farrell (1975) using a Protean II (vertical slab) unit. Slab gels consisted of a running gel (10.4%) and a stacking gel (3.1%). The protein samples were run initially at a constant current of 15 mA/slab and then increased to 30 mA/slab when the marker front reached the running gel. Following electrophoresis, the proteins were stained with Coomassie Brilliant Blue R–250 and destained. Electrophoretic patterns were recorded by developing the positive image using a Kodak Electrophoresis Duplicating Paper. Molecular weights of the protein bands were determined according to the method of Weber and Osborn (1969) and Weber et al. (1972) using a low molecular weight protein kit (Pharmacia) containing phosphorylase b (MW 94 kD), bovine serum albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), soybean trypsin inhibitor (21.1 kD) and α–lactalbumin (14.4 kD).

RESULTS AND DISCUSSION

Water extracts of intact fish and surimi

SDS–PAGE and IEF were shown to be effective in demonstrating species differences in protein patterns of the water extracts of raw fish and surimi samples (Figs. 1 and 2). Using SDS–PAGE, Alaska pollock (AF) showed characteristic bands with MW's of 94.9, 89.5, 88.3 and 79.2 kD while the red hake (RF) showed specific bands with MW's of 87.0, 68.3, 22.7 and 21.9 kD. All these species specific bands were found in the fish and fish–surimi mixtures (Fig. 1).

Generally, the surimi samples still showed the basic patterns as those of the fish fillet from which they were made. Some of the characteristic protein bands, such as the 94.9, 88.3 and 79.2 kD of Alaska pollock and the 68.3, 22.7 and 21.9 kD of red hake, were missing in the corresponding Alaska pollock and red hake surimi (AS and RS, respectively) samples possibly due to the washing process during surimi preparation. New bands with MW of 51.3, 23.4 and 20.8 kD were found in Alaska pollock surimi. These bands were also present in the AS+RS mixture and they can be used with reservation to differentiate surimi mixtures. Due to the similarities of the AS and RS banding patterns, the surimi mixture (AS+RS)



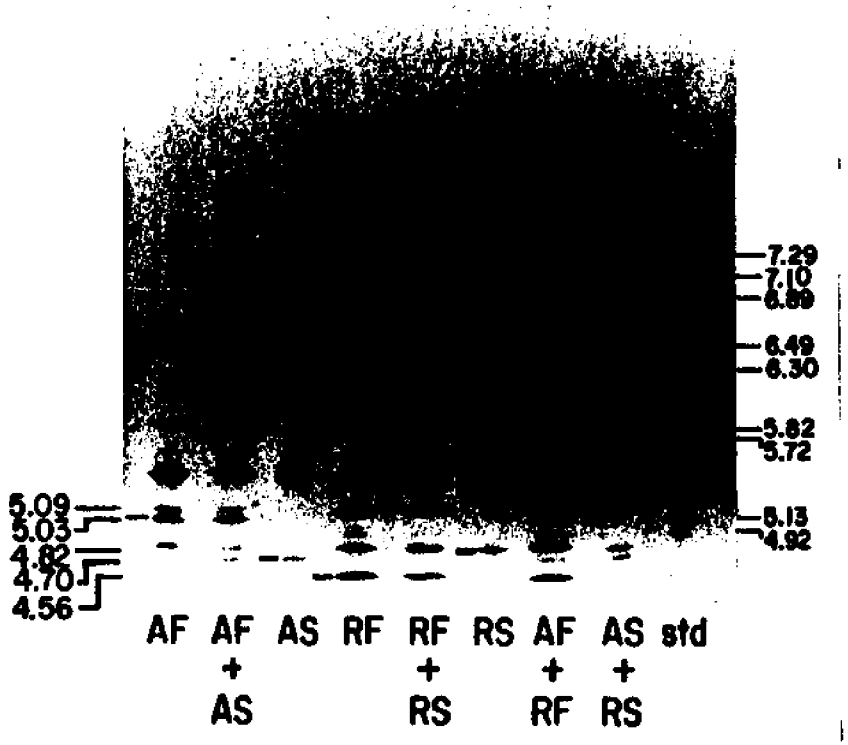


Figure 3 (Left). SDS-PAGE patterns of cooked fillet and/or surimi of Alaska pollock and red hake extracted with water. Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate molecular weights (kD) of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.

Figure 4 (Right). IEF patterns of cooked fillet and/or surimi of Alaska pollock and red hake extracted with water (cathode on top). Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate apparent pI values of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.

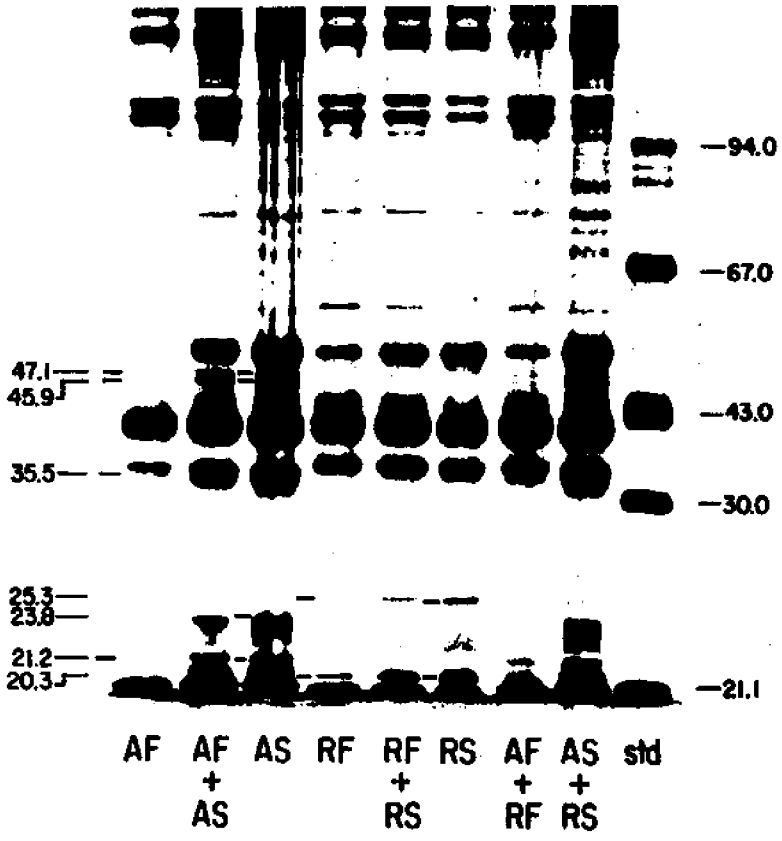


Figure 5 (Left). SDS-PAGE patterns of cooked fillet and/or surimi of Alaska pollock and red hake extracted with SDS. Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate molecular weights (kD) of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.

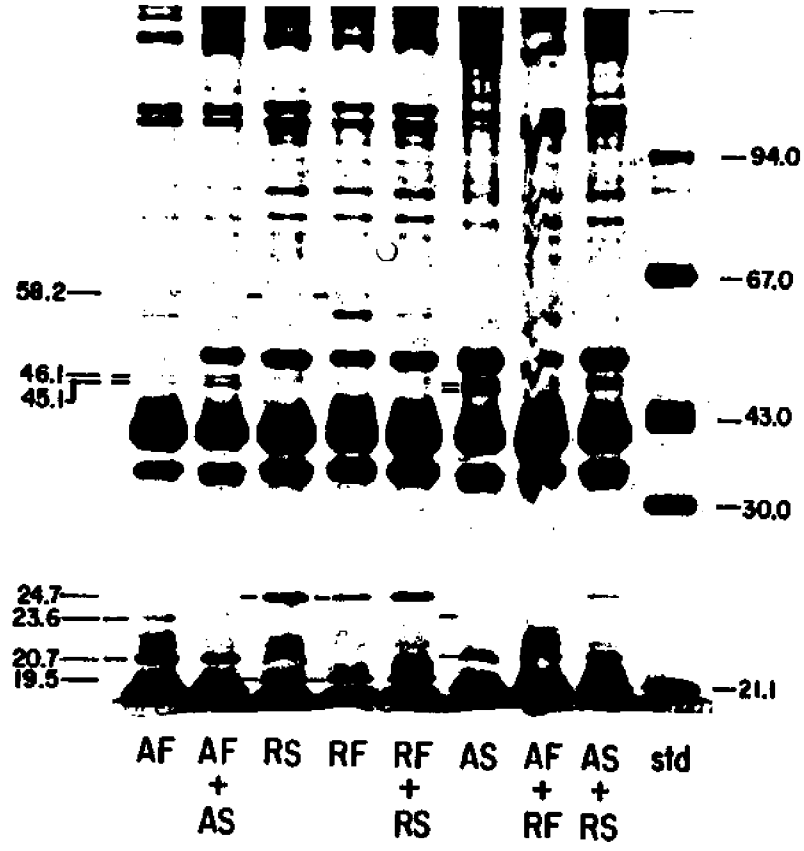
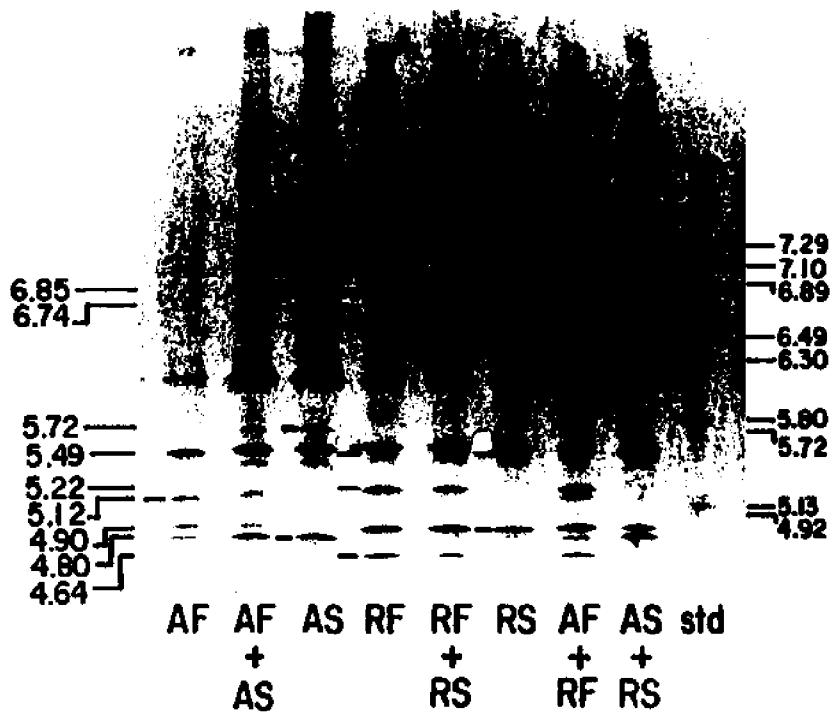


Figure 6 (Right). SDS-PAGE patterns of cooked fillet and/or surimi of Alaska pollock and red hake extracted with urea. Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate molecular weights (kD) of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.



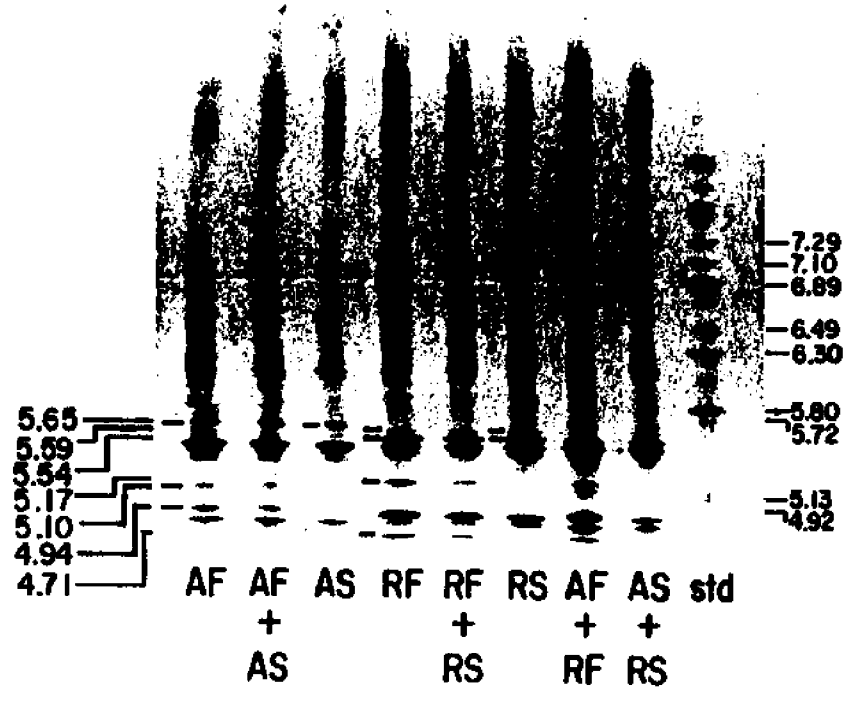


Figure 7 (Left). IEF patterns of cooked fillet and/or surimi of Alaska pollock and red hake extracted with SDS (cathode on top). Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate apparent pI values of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.

Figure 8 (Right). IEF patterns of cooked fillet and/or surimi of Alaska pollock and red hake extracted with urea (cathode on top). Sample mixtures were prepared as shown by the abbreviations. The numerical designations indicate apparent pI values of the protein bands. AF: Alaska pollock fillet, AS: Alaska pollock surimi, RF: red hake fillet, and RS: red hake surimi.

From the specific banding patterns and the presence of species–specific protein bands displayed on IEF gels, the species origin of the SDS and urea extracts of fish and/or surimi samples could be achieved (Figs. 7 and 8). More protein bands were extracted by urea from cooked samples than by SDS or water (Figs. 4, 7 and 8). For SDS extracts, the bands with pI values of 5.72, 5.12 or 4.80 were found specific for Alaska pollock surimi, fish fillet, or both, respectively; whereas the bands of 6.85, 6.74, 5.22 and 4.64 were specific for red hake fish sample but not the surimi. The 5.49 band of red hake was specific both for the fish and surimi. Differentiation of AS, RS and AS+RS mixture could be achieved from the presence of the 5.49 band of RS and 4.80 band of AS (Fig. 7). For urea extracts, the protein patterns on the IEF gels were similar between the fish and surimi samples even though some specific bands were missing in surimi samples. The 5.65 band was specific for Alaska pollock, whereas the 5.59 and 5.54 bands were specific for red hake (Fig. 8). The 5.10 and 4.94 bands of the Alaska pollock fish, the 5.17 and 4.71 bands of the Alaska pollock fish, and the 5.17 and 4.71 bands of red hake were missing in their respective surimi samples and therefore could be used for identification purposes in this instance.

CONCLUSION

The SDS–PAGE and IEF methods were shown in this study to be effective in distinguishing the species specificity between Alaska pollock and red hake of raw or cooked fish and surimi samples. Although water is a good protein extractant for raw samples, it does not provide the specificity needed for cooked samples. The use of SDS or urea, however, will increase the number of proteins extracted from cooked samples of fish or surimi and thus improve the effectiveness of species differentiation with SDS–PAGE or IEF. This method appears to have potential application for identifying raw and cooked fish species and surimi samples.

ACKNOWLEDGEMENT

This work is the result of research sponsored by NOAA, Office of Sea Grant, Department of Commerce, under Grant No. R/LR–Q–11–PD.

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DEVELOPMENT OF RECONSTITUTED SALTED AND DRIED FILLETS FROM TILAPIA

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INTRODUCTION

In the North-East region of Brazil, the production of tilapia do Nilo (Sarotherodon niloticus) has been increasing lately. In the year 1973, this fresh water fish was introduced in the region where it found favorable growth conditions for development, and soon attained a status of prominence (Superintendência de Desenvolvimento de Pesca, 1987). However, tilapia is considered to be of low commercial value as consumers object to its earthy odor.

Traditionally, people of the North-East of Brazil are accustomed to consuming salted and dried meat products which are sold under a generic name of "Carne de Sol". Such products have well established identity and are regular items, even in sophisticated food supermarkets. Thus, in order to cater to the local market, it was thought appropriate to utilize tilapia for the elaboration of a salted and dried product with a longer shelf life while maintaining the process economically feasible. Tilapia was considered promising not only due to its high productivity in the region but also for its low fat content of less than 2%.

A quick salting process for fish has been described previously (Del Valle and Nickerson, 1968; Del Valle and Gonzalez-Inigo, 1968; Del Valle et al., 1973; Anderson and Mendelsohn, 1973). The process involves grinding of fish flesh with simultaneous addition of salt, pressing of the ground salted meat to form cakes, and finally, sun drying of the cakes. However, in the process, no ingredients other than salt were added for preparation of the salted and dried cakes.

The objectives of the present study were (1) to standar-

dize the processing conditions for the elaboration of reconstituted salted and dried fillets from tilapia, (2) to evaluate addition of other ingredients such as wheat flour and egg, in the formulation of product, and (3) to determine rancidity index before and after frying of products stored up to 90 days at ambient, refrigeration and freezing temperatures.

MATERIALS AND METHODS

Materials:

The tilapia do Nilo (Sarotherodon niloticus) was obtained from the municipality of Cuité-PB., Brazil. Refined iodine salt was the brand "Merlin" manufactured by "Companhia Industrial do Rio Grande do Norte", Macau-RN, Brazil, while refined corn oil was the brand, "Mazola", made by "Companhia de Refinaçöes de Milho Brasil Ltda.", Säo Paulo-SP, Brazil. Large size grade A eggs were purchased locally and wheat flour was "Boa Sorte" manufactured by "Companhia Cabedelo Industria S.A"., Cabedelo-PB., Brazil.

Procedure for the preparation of reconstituted salted and dried fillets:

The fish were captured alive, washed with running water and transported to the processing plant under ice. After evisceration, deheading and cleaning, the fillets 18 cm (length) x 8 cm (maximum diameter) x 1 cm (thickness) were obtained manually. These were considered as control samples.

The flow-diagram for processing of reconstituted salted and dried fillets is shown in Figure 1. The ground fish (triturated meat) was divided for the two treatments. Treatment-I did not have the quick salting, pre-pressing and drying operations, and in this treatment, the salt concentration was only 2 or 3%. In treatment-I, addition of salt was done simultaneously with other ingredients, while in treatment-II, the salt was added at the level of 20 or 30% during the quick salting operation and the mixture was left for 30 min. The various formulations tried for elaboration of different products are presented in Table 1.

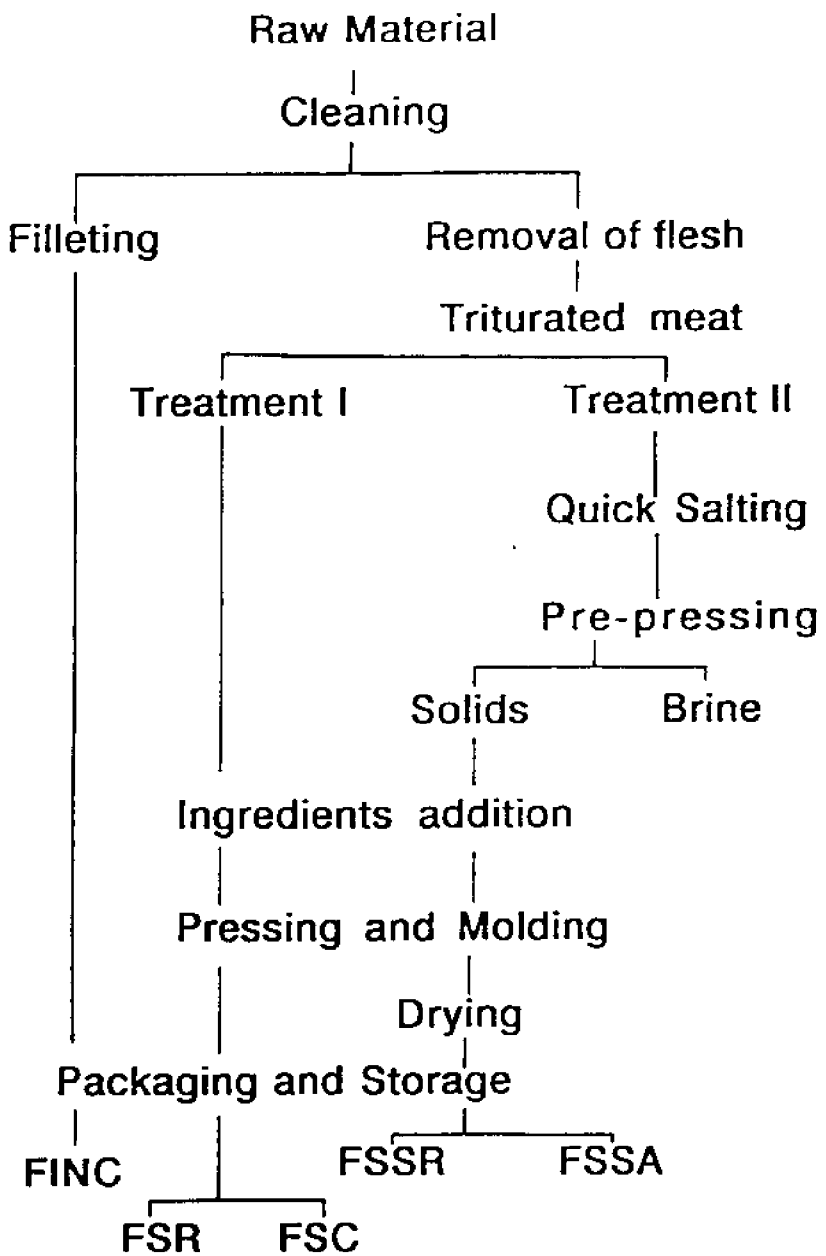

Figure 1. Flow-diagram for processing of reconstituted salted and dried fillets from tilapia.

Table 1. Composition (%) of ingredients for elaboration of different products.

Ingredients	Product designation							
	A	B	C	D	E	F	G	H
Wheat flour	10	20	20	20	20	20	20	20
Egg	10	10	10	-	-	-	-	-
Salt	30	30	2	2	3	30	20	20
p-hydroxyben-zoate (methyl)	-	-	-	-	-	-	-	0.025

Pre-pressing was done by putting the mixture (ground fish and salt) in a cheesecloth and exerting torsion at the extremities. The pressing and molding operation was performed in a modified press (Figure 2).

Drying of reconstituted fillets was done initially at a temperature of 45±2°C for different time periods. However, in the final experiments, drying was at a temperature of 50±2°C for 16 hours. The reconstituted fillets and the control

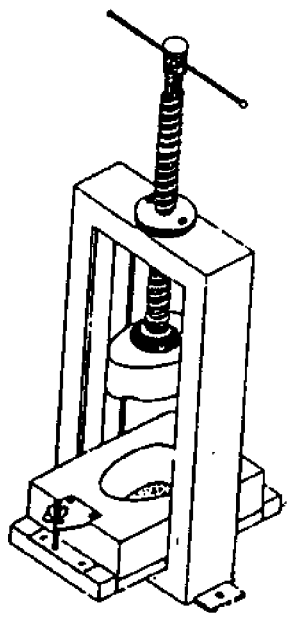

Figure 2. Modified press

sample were packed individually in medium density (0.93 g/cm^3) polyethylene bags under vacuum.

The following codes (Figure 1) were assigned for fillets stored at different conditions:

FINC: Fillets "in nature" stored at freezing temperature (-17 ± 1^oC) for 0, 30, 60 and 90 days.
FSR: Fillets salted, and stored at refrigeration temperature (8.5 ± 1.5^oC) for 0, 5 and 10 days.
FSC: Fillets salted, and stored at freezing temperature (-17 ± 1^oC) for 0, 30, 60 and 90 days.
FSSR: Fillets salted and dehydrated, and stored at refrigeration temperature (8.5 ± 1.5^oC) for 0, 30, 60 and 90 days.
FSSA: Fillets salted and dehydrated, and stored at ambient temperature (28 ± 2^oC) for 0, 30, 60 and 90 days.

Product Analysis:

Determinations of moisture, ash, fat, and salt content were according to methods described by A.O.A.C. (1982). Total protein and TBA analyses were according to methods described by Pearson (1975). Microbial counts were determined by methods described by the American Public Health Association (1976).

The desalting of reconstituted salted and dried fillets was carried out by leaching the products three times in boiling water. The product and water proportion was 1:20 with leaching for 10 min. The rehydration ratio was calculated by dividing the rehydrated weight by that of the dehydrated sample.

The sensorial evaluation was undertaken after desalting, rehydration, and frying of reconstituted fillets. Frying of fillets was done at a temperature of 200 ± 5^oC for 2 min. After frying, the reconstituted fillets were cut in cubes of 1 cm^3 approximately, codified, and presented for evaluation. A panel of 10 trained persons evaluated products B and F for the preference test between the products.

RESULTS AND DISCUSSION

Chemical and Microbiological Composition:

Chemical and microbiological composition of fresh tilapia do nilo based on analysis of 5 samples in duplicates is

presented in the Table 2. The values are in agreement with those reported by Freitas (1979) for the same species. It should be noted that the low fat content (1.7%) makes tilapia suitable for quick salting process since one of the principal problems for quality of salted fish products is the rancidity. Furthermore, its low TBA value (0.34) also makes it more appropriate for elaboration of salted and dried products.

Table 2. Chemical and microbiological composition of tilapia.

Characteristic	Maximum value	Minimum value	Average value
Water (%)	80.50	78.90	79.70
Protein (%)	17.86	17.75	17.80
Fat (%)	2.00	1.32	1.70
Ash (%)	0.81	0.79	0.80
pH	6.5	6.3	6.4
TBA No. (mg of malonaldehyde/1000 g)	0.35	0.33	0.34
Mesophilic count (CFU/g)	$7.9x10^5$	$5.6x10^5$	$6.7x10^5$
Psychrophilic Count (CFU/g)	$8.8x10^5$	$8.1x10^5$	$8.4x10^5$
Molds and yeasts (CFU/g)	$4.0x10^3$	$1.3x10^3$	$2.6x10^3$

Salting:

In treatment-II, the two salt concentrations tried for a 30 min quick salting operation were 20 and 30%, and the moisture contents of the ground fish after the treatment were 60.6 and 59.4% respectively (Figure 3). Del Valle and Nickerson (1968) reported a moisture content of 57.7% for products treated with 20% salt. Since in the elaboration of reconstituted salted and dried fillets, there would further be operations like pressing and drying, the salting treatment at lower level of 20% was standardized. It is known that higher quantity of salt utilized in the treatment will result in greater difficulty in its removal during desalting and rehydration processes. Furthermore, salting at higher concentrations results in increased costs as well as adversely affects organoleptic characteristics such as texture and flavor.

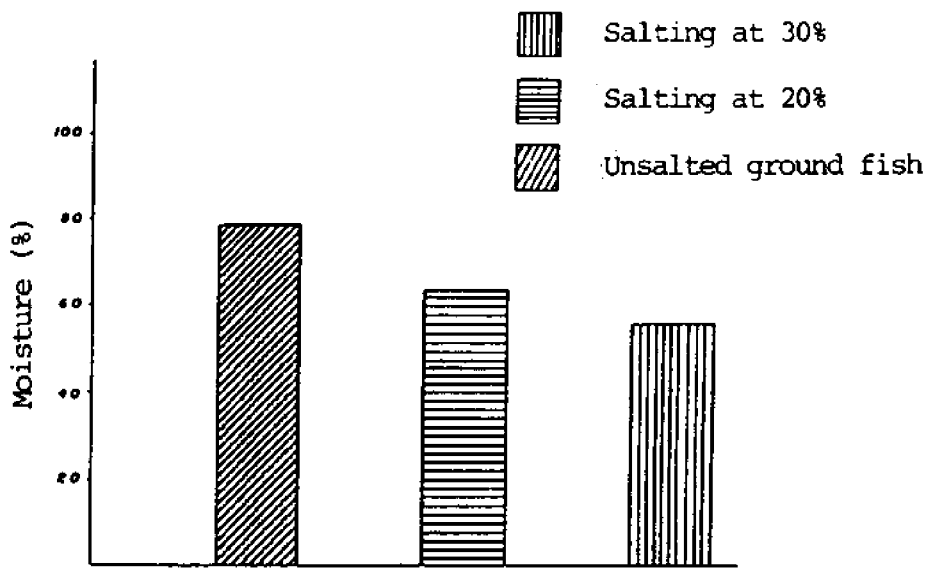

Figure 3. Effect of salt concentration on moisture of ground fish.

Addition of Ingredients:

The formulations presented in Table 1 were designed to select optimal concentrations of different ingredients to develop a high quality product suited for the local market. While comparing products A and B, in the product A (prepared with 10% wheat flour), a difficulty in the formation of cohesive blocks after pressing and thus maintaining its integrity, was observed. It was therefore decided to standardize the wheat flour concentration at 20% in the formulation.

Formulations with and without egg (Products C and D) were tested to verify the eggs property of agluttenization. These experiments were performed in treatment-I to minimize the effect of other variable parameters or operations as in treatment-II. No noticeable effect due to the addition of egg was observed, however, its addition presented difficulties during drying, and hence its elimination was standardized in the formulation.

The only difference in the formulations between products D and E was in the concentration of salt. Product D had 2% salt while product E had 3%. The formulation of 3% was standardized since products elaborated with this concentration in treatment-I resembled taste-wise to those desalted

and rehydrated products obtained from the reconstituted salted and dried fillets prepared in treatment-II.

Since wheat flour has been used as one of the ingredients, it was necessary to include a fungistat, methyl p-hydroxybenzoate, at a concentration of 250 ppm, in the formulation.

Pressing:

The objective of this operation was to give a form to the products, and at the same time, decrease its moisture content. A press designed for this purpose (Figure 2) was used. About 80 g sample was standardized for the final pressing which resulted into a triangular form of about 10 cm (length) x 6.5 cm (maximum diameter) x 1 cm (thickness).

Drying:

Initially, the reconstituted salted fillets were dehydrated in a forced air circulation cabinet dryer at 45±2°C for a period sufficient to attain about 20% moisture in the fillets. The drying time at 45±2°C was found to be 20 hours. To make the drying operation more efficient, the drying temperature was raised to 50±2°C and the drying time was optimized at 16 hours (Figure 4).

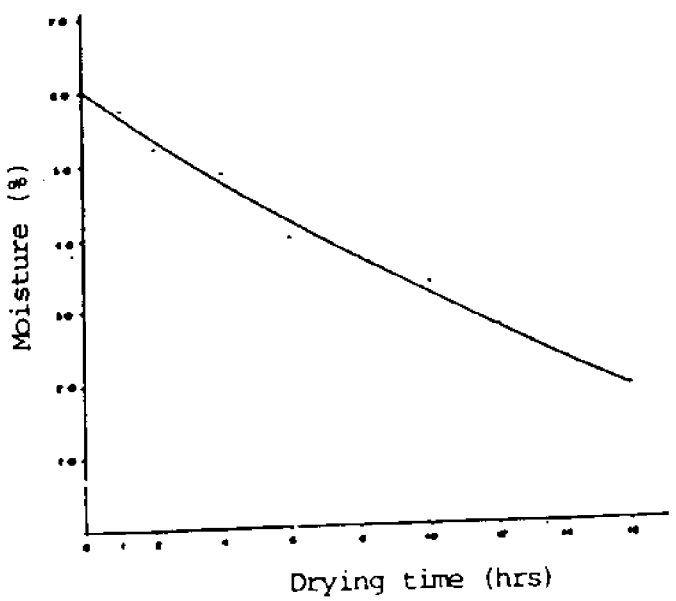

Figure 4. Effect of drying time at 50±2°C on moisture content of reconstituted fillets.

Desalting and Rehydration:

An evaluation of salt concentration in products B (elaborated with the addition of egg) and F (elaborated without egg) was undertaken after desalting and rehydration. Results (Table 3) demonstrate that there was practically no variation in the water retention capacity of the two products. Also no variation was evident in the weights of the two products after frying. The salt content after the third leaching in the two products was below 2%, which increased to about 3% after frying.

Preliminary sensory evaluation for the preference test between products B and F resulted in five members preference for product F, three members preference for product B, and two members did not find any difference between the products.

Table 3. Desalting, Rehydration and Salt Content of Reconstituted Fillets.

Product	Treatment	No. of leaching	Salt content (%)	Rehydration ratio
B	Desalting	1	3.8	1.23
		2	2.9	1.13
		3	1.8	1.10
	Salt content (%) after frying	-	2.8	-
F	Desalting	1	3.9	1.21
		2	3.0	1.15
		3	1.9	1.13
	Salt content (%) after frying	-	3.0	-

Rancidity Index:

The rancidity index was assessed by determining the TBA (mg of malonaldehyde per Kg of sample) values of reconstituted fillets stored for different time periods and also before and after frying. Results are presented in Figures 5 and 6 for reconstituted fillets before and after frying, respectively.

The TBA values of various products at the time of their

preparation varied between 0.86 and 0.90. These low values demonstrated the excellent state of the products in relation to the oxidation of lipids. The TBA values of all products decreased until 30 days of storage (Figures 5 and 6). Minimal variation in TBA values was observed with further increase in storage period (60 and 90 days). The TBA value for the product FSSA, on 30 days of storage before frying was found to be higher (0.52) in comparison with product FSSR (0.25). The TBA values decreased on frying which was observed in all products tested. Data demonstrate that the storage conditions do not promote rancification in reconstituted salted and dried fillets.

CONCLUSIONS

Principal processing conditions for the elaboration of reconstituted salted and dried fillets from tilapia do Nilo have been standardized as: (1) quick salting of 20% for 30 min, (2) mixing of 20% wheat flour and 250 ppm of methyl p-hydroxybenzoate to ground fish after salting and pre-pressing operations, (3) drying at 50°C for 16 hours.

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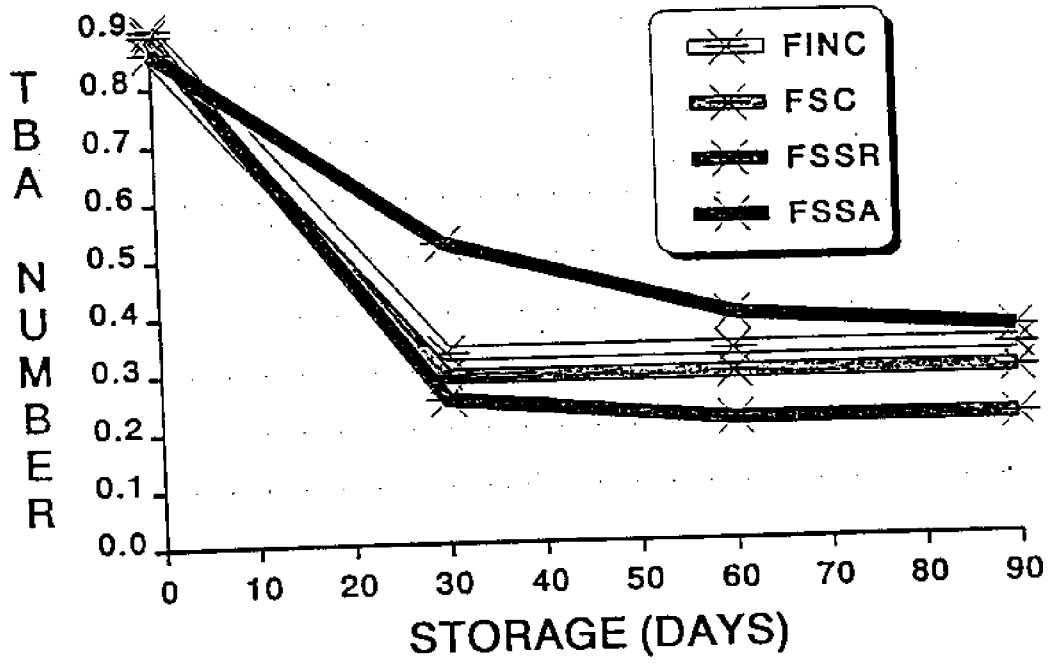

Figure 5. Effect of storage time on the rancidity of reconstituted fillets before frying.

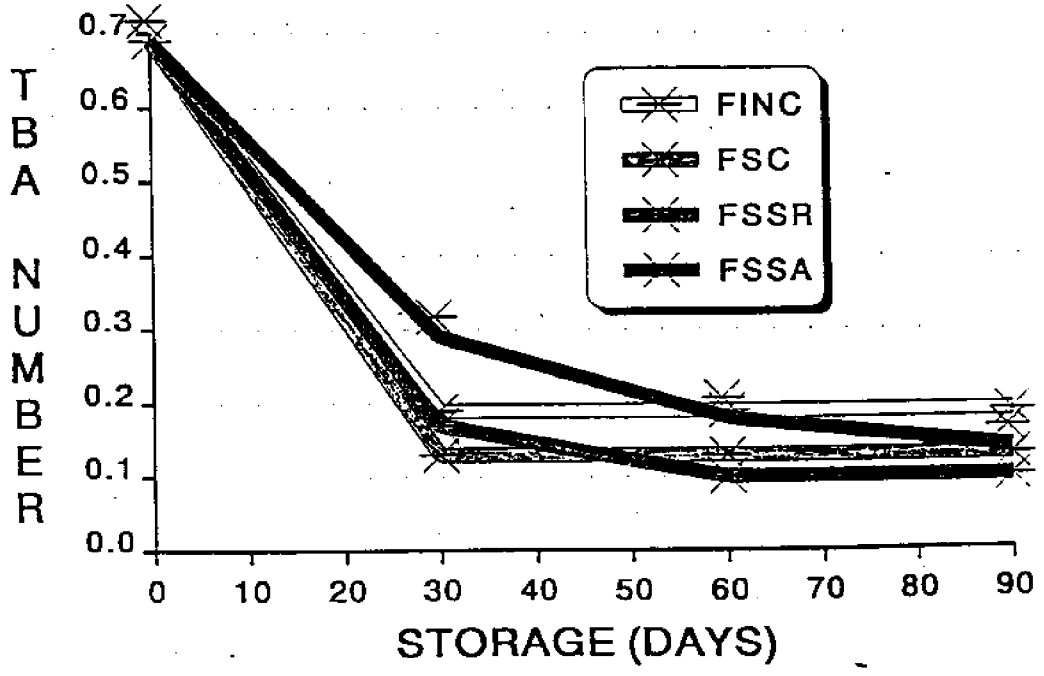

Figure 6. Effect of storage time on the rancidity of reconstituted fillets after frying.

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ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of the Núcleo de Pesquisa e Processamento de Alimentos (NUPPA) for the usage of pilot plant facilities at Mangabeira, João Pessoa, and for financial support to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

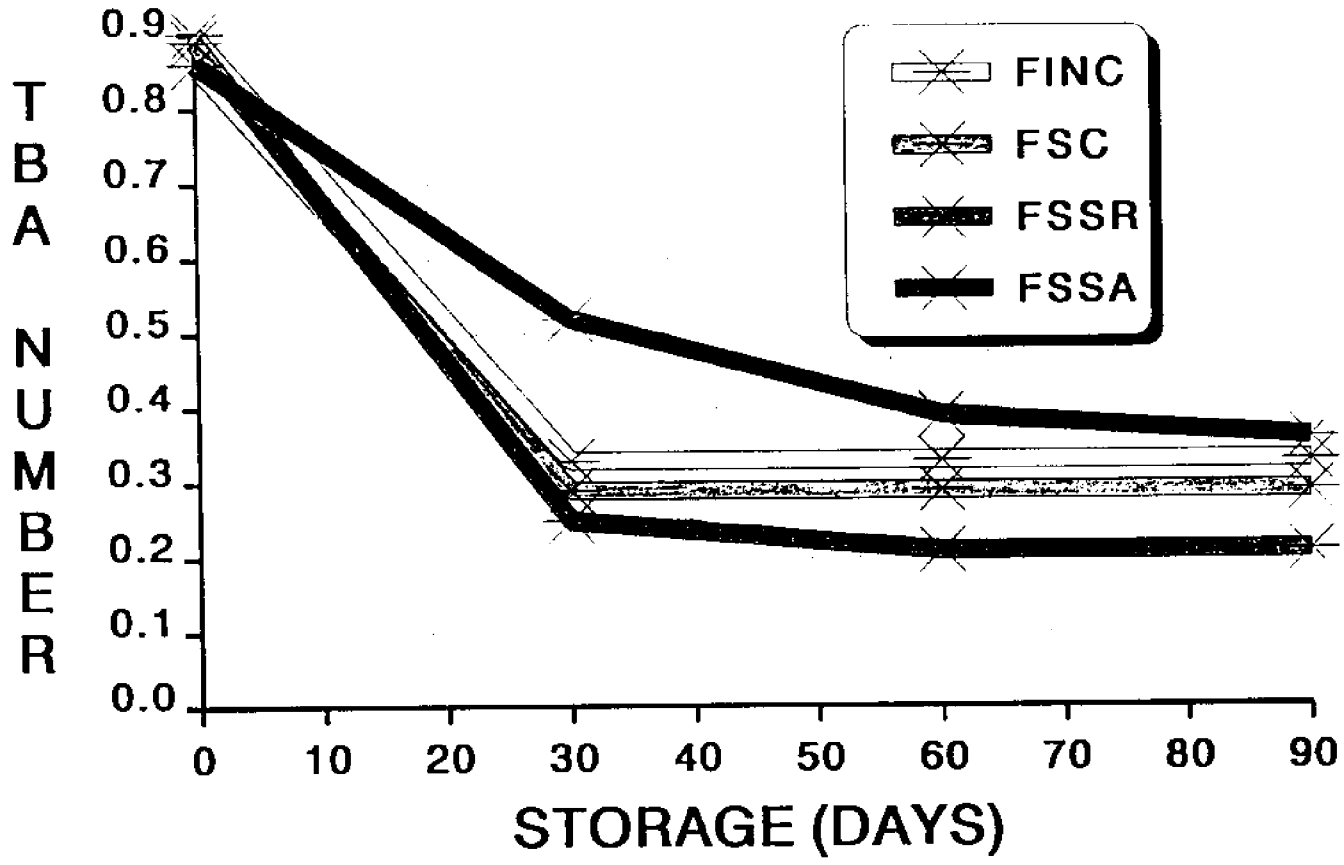

Figure 5. Effect of storage time on the rancidity of reconstituted fillets before frying.

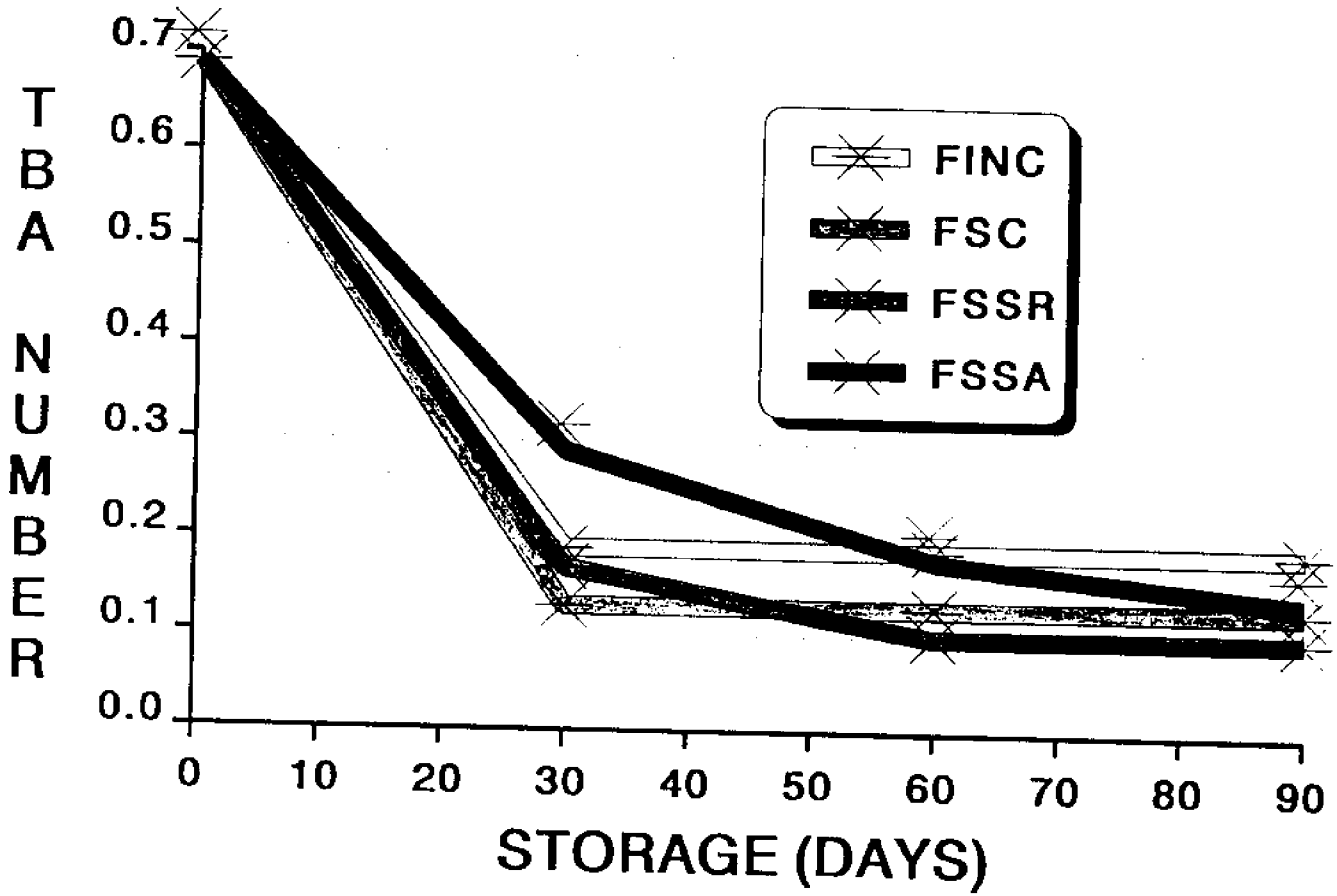

Figure 6. Effect of storage time on the rancidity of reconstituted fillets after frying.

TRENDS IN THE IMPORTATION OF SELECTED FRESH AND FROZEN SEAFOOD PRODUCTS INTO THE SOUTHEASTERN UNITED STATES

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INTRODUCTION

Imported seafoods are becoming an increasingly important source of product for America's seafood consumer. Imported seafoods have continuously represented over 50 percent of the total edible seafood supplies in the U.S. since 1966. From 1966 to 1987, imports increased at an average annual rate of approximately 5 percent, in contrast to the domestic landings rate of increase of about 2 percent. Yet the rate of increase in imports is becoming even greater. Since 1980, imports of edible seafood seafood products have increased at an average annual rate of over 6 percent, compared to a decline of about 2 percent for domestic landings. Imports of edible seafood products reached a record 6.6 billion pounds (round weight) in 1987, which accounted for 63 percent of the total U.S. seafood supplies.

Although imports are important to U.S. seafood suppliers in general, imported product is even more important in meeting the demand for certain key Southeastern species. Faced with a growing domestic demand for high-quality finfish and shellfish products and stable sources of domestic product, suppliers of key Southeastern species will need to become better informed of import product sources, product form, seasonal

availability and obtainable volumes. This information will be particularly important to seafood suppliers wishing to begin import activities or expand existing operations. In addition, regional fishery managers need this information to gain a better appreciation for how imported seafood is becoming an increasingly important element of the domestic seafood market.

The purpose of this paper is to describe general trends in imports for selected seafood products arriving at Southeastern U.S. ports of entry. These trends will be discussed in terms of volumes, seasonality, fresh versus frozen, product form, and country of origin. The major ports of entry will also be identified.

DATA

The paper presents import data collected by the National Marine Fisheries Service (NMFS). These data were originally reported in the New Orleans "Goldenrod" Market News Report. Imports of many shellfish and finfish products are reported each Wednesday in the New Orleans Market News Report by port of entry, species, fresh or frozen, product form (i.e. whole, fillet, loins, other), country of origin, and volume received. "Whole" refers to product received eviscerated and/or head off. Although seafood imports are reported on a Wednesday, a lag of several days between product actually passing U.S. Customs and being reported in the Market News Report may occur. Import weight presented is product weight (i.e. weight of items received by Customs regardless of product form -- not converted to whole weight). Where possible, import volumes are compared to regional NMFS landings data for each species. "Country of origin" refers to country where product was first landed and exported (not transshipped).

A primary objective of the study was to compile the data, which had never been databased, and examine trends in imports of species key to the Southeast U.S. region. For the purpose of the study, only marine tropical and subtropical species (i.e. freshwater and cold water marine species are not included), arriving from primarily Latin American countries of origin, and entering Southeastern ports of entry (i.e. Brownsville/Port Isabel, TX; New Orleans, LA; Tampa, FL; Port Everglades, FL; Miami, FL; West Palm Beach, FL; Savannah, GA; Charleston, SC) were utilized. In addition, only data from fresh and frozen product were analyzed (e.g. canned/cured products excluded). Imports of shrimp products were also not included in the study since these data are already comprehensively reported in the monthly NMFS report entitled "Shrimp Statistics".

NMFS Market News data for 1983-1987 were utilized which included eight ports of entry, 54 countries of origin, and 68 finfish and shellfish species (Table 1). For the sake of brevity, only 18 species are reported in this study.

For each species, the average monthly distribution import volumes are discussed. The term "availability" is used in each of these discussions. This term implies that the volumes of imported product arriving each month reflects the relative availability of the species in the original country of origin. The reader should note however, that this discussion does not account for volumes of a given species which may have been exported to other destinations not reported by NMFS Market News.

TABLE 1

FINFISH AND SHELLFISH SPECIES INCLUDED

Albacore
Amberjack
Sea Bass
Cobia
Conch[1]
Congrio
Corvina[1]
Stone Crab
Lobster[1]
Mahi Mahi[1]
Drum, Black[1]
Drum, Red[1]
Flounder
Grouper[1,2]
Grouper, Black
Grouper, Red
Grouper, Yellowedge
Grouper, Warsaw
Whiting
Kingclip[1]
Langostinos
Mackeral, King[1]
Mackeral, Spanish[1]
Marlin[1]
Mullet
Octopus
Pomfrets
Pompano[1]
Scallops[1]
Shark[1,2]
Shark, Mako
Shark, Thresher
Sheepshead
Snapper[1,2]
Snapper, Black
Snapper, Lane
Snapper, Mutton
Snapper, Mangrove
Snapper, Red
Snapper, Spotted
Snapper, Tomato
Snapper, Vermillion
Snapper, Yellowtail
Squid
Swordfish[1]
Tilefish[1]
Tongue
Triggerfish
Trout, Sea[1,2]
Trout, Sand
Trout, Spotted
Trumpeter
Tuna
Tuna, Big Eye
Tuna, Yellowfin
Wahoo

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[1] Species discussed in this study.

[2] Species discussed in aggregate, without details presented on individual species of grouper, snapper, or shark.

DISCUSSION

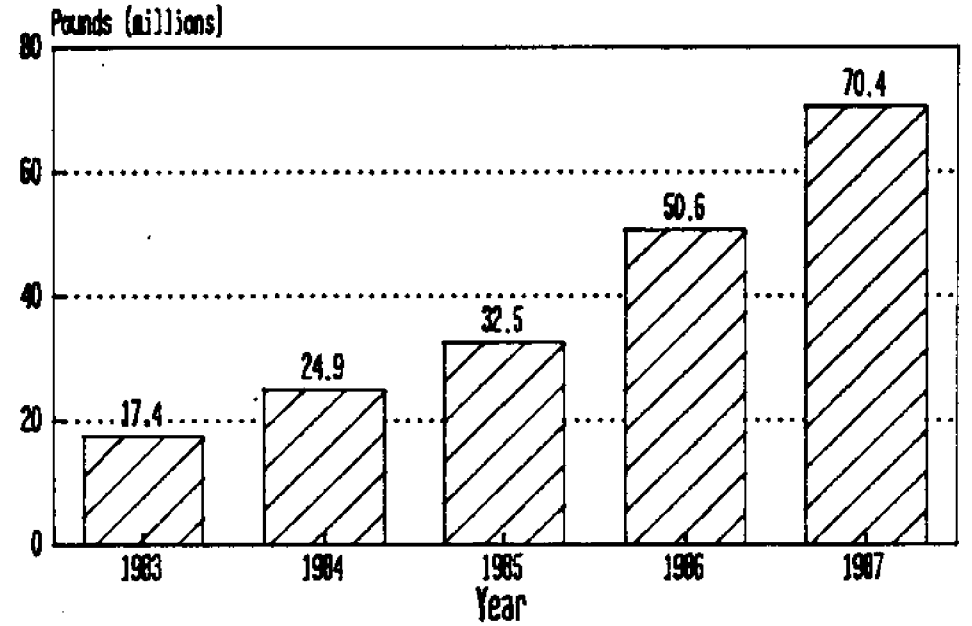
All Species

The total volume of imports for the 68 species arriving in the Southeast U.S. ports of entry increased from 17.4 million pounds in 1983 to 70.4 million pounds in 1987 (Figure 1). This represents an approximate fourfold increase or an annual percentage increase of 42 percent over the 5-year period. Monthly volumes varied considerably. Although the seasonal distribution of imported product varies by species, monthly 5-year averages indicate that import volumes for all species remain fairly consistent from month to month, with the late summer and fall months accounting for a slightly larger share of the volume for an average year.

As the total volumes of imports have increased, so have the numbers of species imported. Although snapper, grouper, seatrout, swordfish, and mackerel continue to be important mainstays, new species such as congrio, dorado, pomfrets, corvina, kingklip, mako shark, and others are being imported in increasing amounts. In 1983, a total of 32 species were being reported by Customs (Figure 2). By 1987, the number of species had risen to 60. The growing strength of the U.S. seafood market has provided inroads for some of these lesser known, "non-traditional" species. Development of markets for such species may become increasingly important if the domestic demand for seafood continues to grow at current paces.

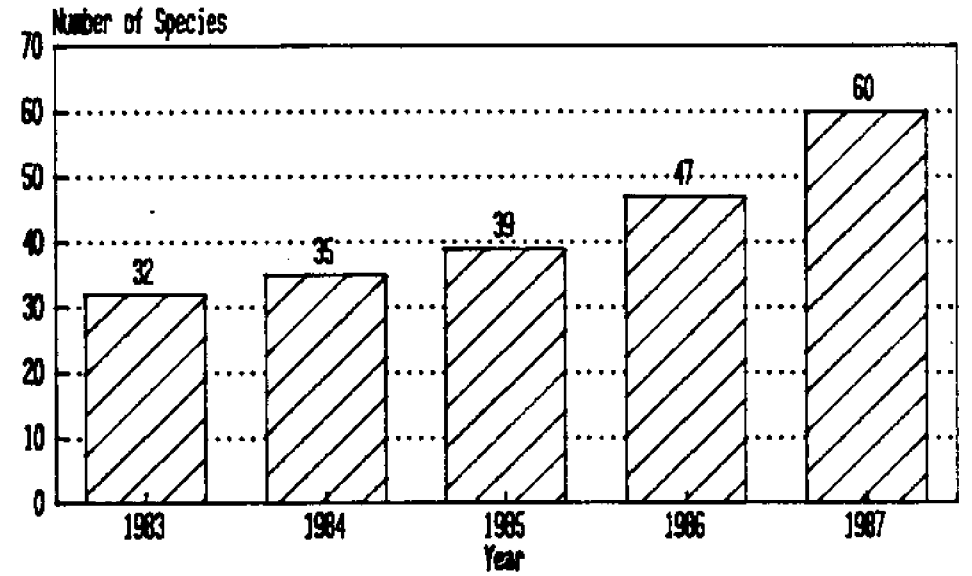
Prior to 1986, the volume of frozen seafood products imported into the Southeast U.S. exceeded that for fresh products. In 1983, the volume of frozen exceeded fresh product by nearly threefold (Figure 3). However, in 1986 and 1987, fresh imports exceeded frozen by approximately 25 percent.

FIGURE 1
IMPORTS OF KEY SPECIES INTO SOUTHEASTERN PORTS OF ENTRY: 1983-87



DATA SOURCE: NMFS Market News Reports

FIGURE 2
NUMBERS OF KEY SPECIES IMPORTED INTO SOUTHEASTERN PORTS OF ENTRY 1983-87

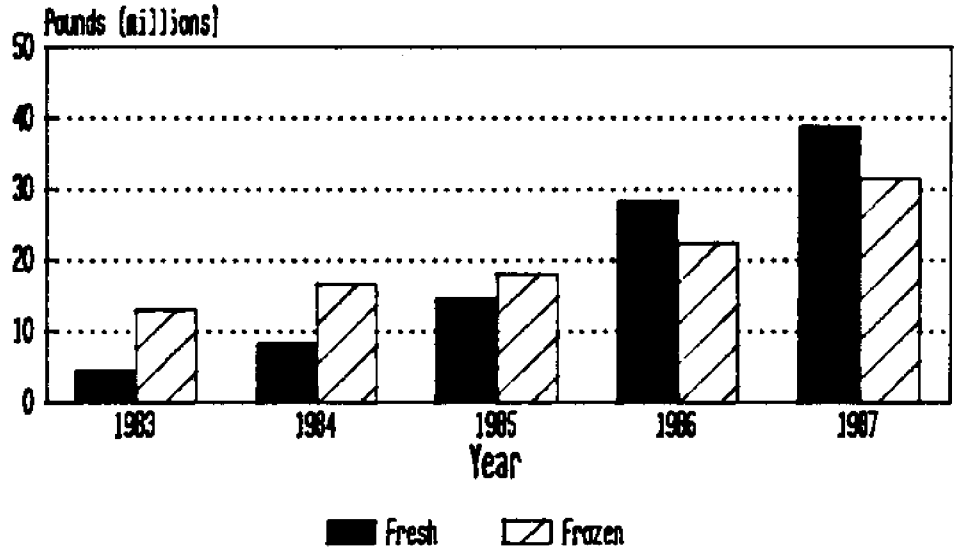


DATA SOURCE: NMFS Market News Reports

Seafood is imported in a variety of product forms. Brokerage reports indicate that product is received in whole form, fillets, loins, portions, and "other". The latter term refers primarily to shellfish products, such as crab meat, lobster tails, and scallop meats. The predominant product form for all species in general imported during the 1983-87 period was whole product. However, the importance of this product form declined following 1986 (Figure 4). For example, whole product represented 93 percent of the seafood imports in 1983, but declined to 58 percent in 1987. A rapid increase in the import volume of fillets (6.8 million pounds in 1986 to 15.5 million pounds in 1987) suggests an increase in demand for the more processed finfish products. Although the advent of reporting miscellaneous product forms in 1986 somewhat clouds the message statistically, the data suggest that the import market is responding to an increased market demand for prepared (i.e. filleted) finfish product.

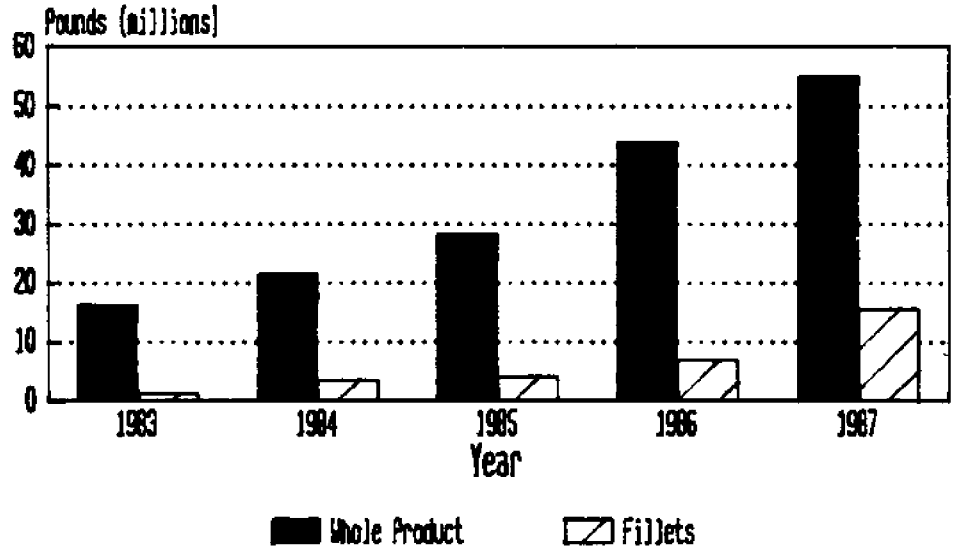
Major sources of imported product also changed over the 5-year period. In both 1983 and 1987, six countries provided at least 70 percent of the seafood import volume, while the remaining 30 percent was exported to the southeastern U.S. from a number of other countries. The leading six countries, however, changed following 1983 (Figure 5). In 1983, the major country of origin for imports was Mexico (31 percent), followed by Costa Rica, Peru, Honduras, Belize, and Bahamas. By 1987, three new countries had moved into the top six, with all six contributing a more equal share of the seafood export market to the U.S. Mexico was still the leading source in 1987, followed by Ecuador, Costa Rica, Panama, Peru, and Chile. Other countries exporting lesser volumes to the U.S., include Venezuela, Honduras, El Salvador, and Argentina.

FIGURE 3
IMPORTS OF KEY SPECIES INTO SOUTHEASTERN PORTS OF ENTRY: 1983-87
FRESH vs FROZEN



DATA SOURCE: NMFS Market News Reports

FIGURE 4
IMPORTS OF KEY SPECIES INTO SOUTHEASTERN PORTS OF ENTRY: 1983-87
WHOLE vs FILLETS



DATA SOURCE: NMFS Market News Reports

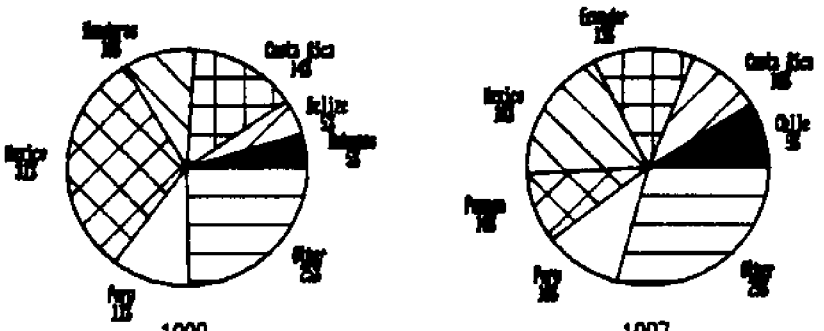
Snapper

Imports of all species of snapper into Southeastern U.S. ports of entry increased from 4.8 million pounds in 1983 to 14.0 million pounds in 1987 (Figure 6). This reflects an average annual percentage increase of 33 percent. Snapper imports nearly doubled from 1984 to 1985. The significance of these imported products to meeting domestic demand for snapper is suggested by comparing southeast snapper landings to imports. As imports increased during the 1983-87 period, landings of snapper in the southeast declined by annual average rate of 6.8 percent. Imports exceeded landings volume for the first time in 1985. Landings of snapper declined dramatically in 1987, as import volume continued to rise. The monthly distribution of snapper imports is somewhat variable, with peak 5-year averages occurring in April, July, and September (Figure 7). Imported snapper products are apparently not as readily available in the winter months.

Market News data identifies several species of snapper being imported into Southeastern ports of entry. Those include black, lane, mutton, red, spotted, vermillion, yellowtail, and "tomatoe" snapper. In addition, a large category of unclassified volume is reported. In 1987, the unclassified snapper imports represented 88 percent of the total volume, followed by red (9 percent), yellowtail (2 percent), and lane (1 percent). The remaining species represented only a small volume of the total.

Import volume of fresh snapper consistently exceeded that for frozen products. In 1983, fresh snapper imports represented 68 percent of the total snapper imports (Figure 8). By 1987, fresh snapper imports

FIGURE 5
IMPORTS OF KEY SPECIES BY COUNTRIES OF ORIGIN: 1983 and 1987

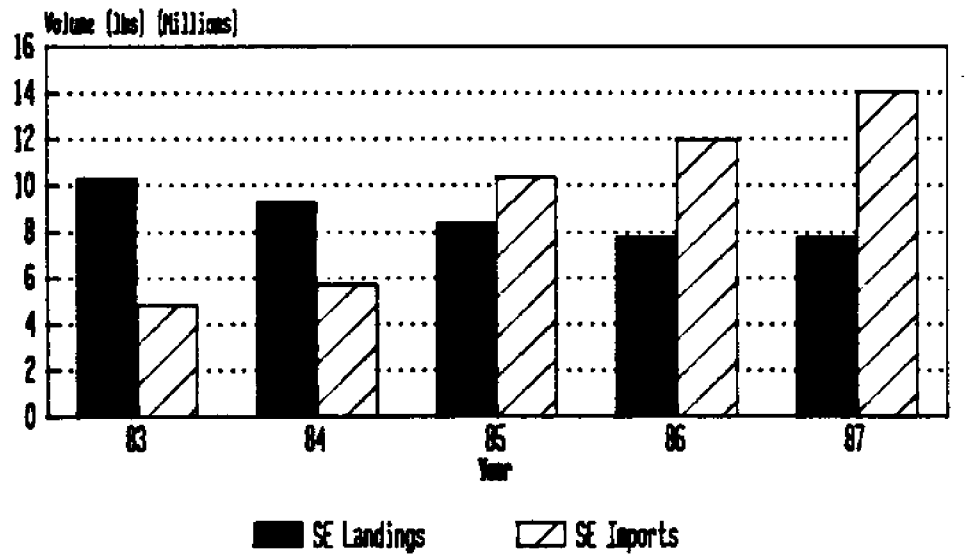


1983
17.4 million lbs

1987
70.4 million lbs

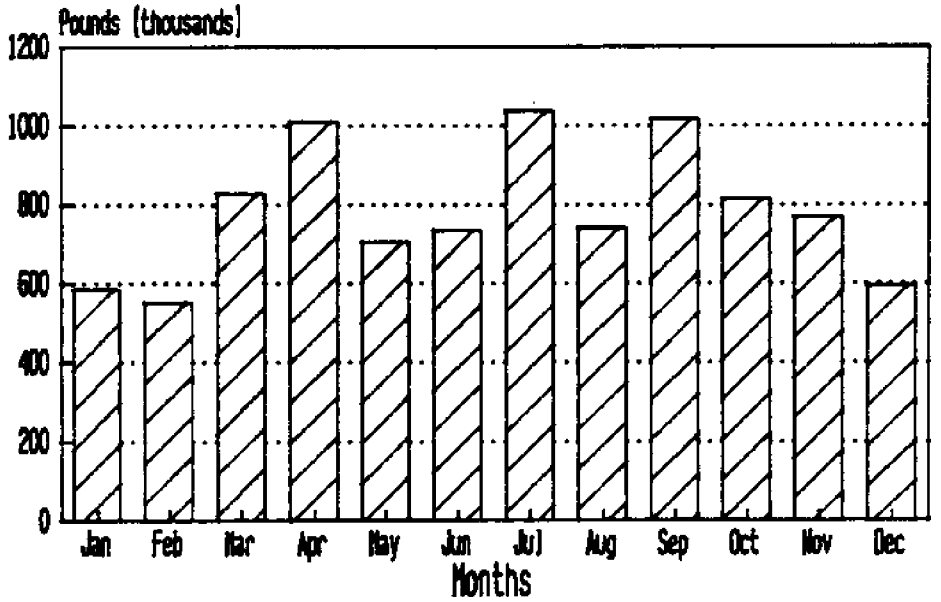
DATA SOURCE: NMFS Market News Reports

FIGURE 6
SOUTHEAST U.S. SNAPPER LANDINGS AND IMPORTS: 1983-87



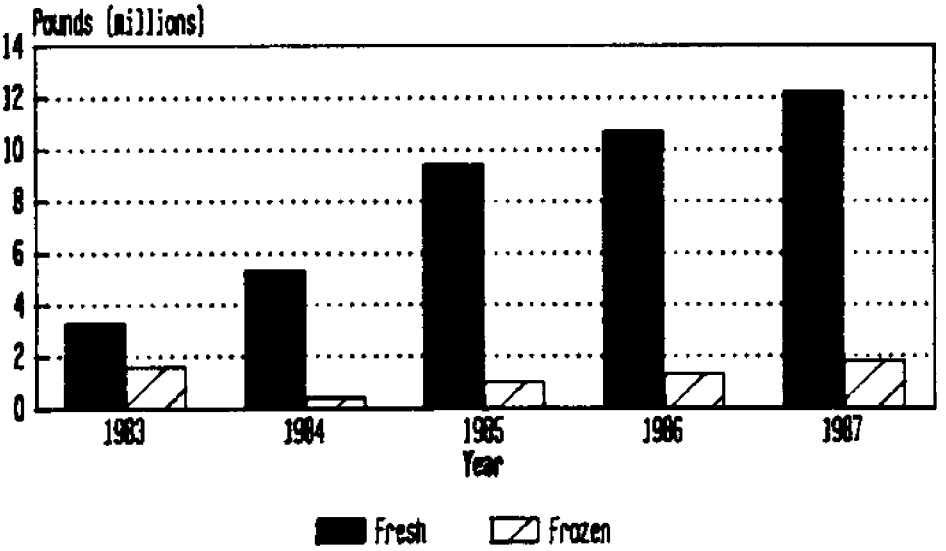
DATA SOURCE: NMFS data. Import product weight given. All species included.

FIGURE 7
FIVE-YEAR AVERAGE MONTHLY DISTRIBUTION
OF SOUTHEAST U.S. SNAPPER IMPORTS



DATA SOURCE: NMFS Market News Report Data. Data pertains to 1983-87.

FIGURE 8
IMPORTS OF SNAPPER INTO SOUTHEASTERN
PORTS OF ENTRY: 1983-87
FRESH vs FROZEN



DATA SOURCE: NMFS Market News Reports

accounted for 87 percent of the total. Frozen snapper imports in 1987 (1.8 million lbs.) were only slightly higher than reported for 1983 (1.6 million pounds).

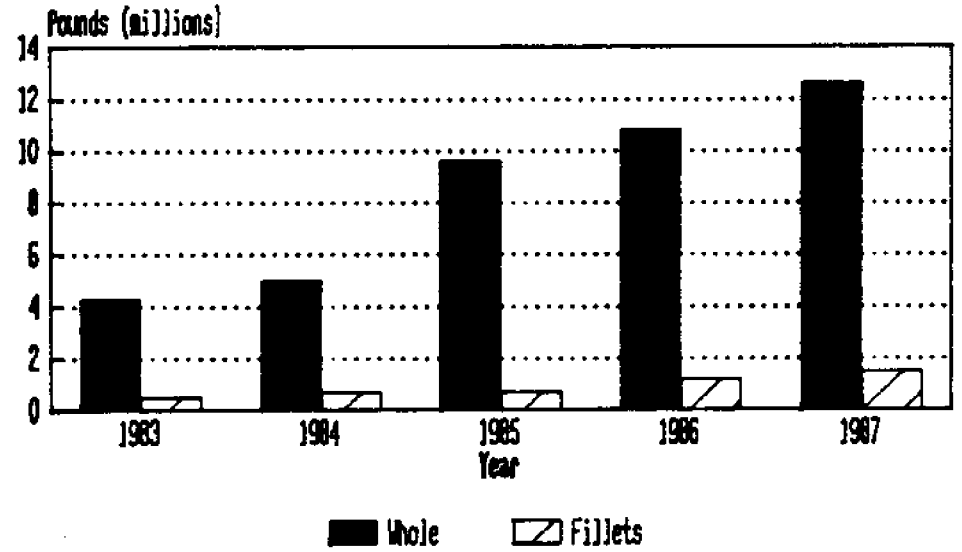
Snapper is primarily imported in whole form In 1987, 12.6 million pounds of whole snapper was imported to southeastern U.S. ports of entry, which represented 90 percent of the total snapper import volume (Figure 9). This percentage distribution between whole and filleted product has remained relatively constant during the 1983-87 period.

The major countries of origin for snapper products has remained relatively constant over the past five years. In 1983, Costa Rica and Mexico contributed 44 and 30 percent, respectively, of the total volume of snapper imports reported (Figure 10). Brazil and Venezuela were also major sources of product. By 1987, Mexico remained the most important single source of snapper, with Venezuela and Costa Rica each supplying 18 percent of the volume arriving at southeastern U.S. ports of entry. Panama and Guatemala provided 12 and 5 percent, respectively. The Central American region has, therefore, become the leading source of snapper products for the southeastern U.S. region. Approximately 86 percent of the total volume of snapper imports arrived through Miami. The remaining volume arrived through Brownsville and Port Everglades.

Grouper

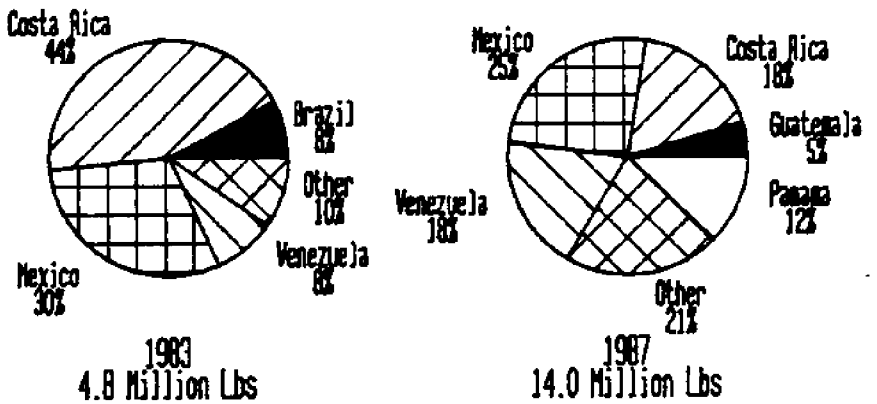
The volume of grouper imports into the southeastern U.S. increased dramatically during the 1983-87 period. Grouper imports increased from .5 million pounds in 1983 to 8.9 million pounds in 1987 (Figure 11). This represents an average annual increase of 122 percent over the five-year period! Grouper landings in the Southeast region remained stable through 1986, but decreased to 9.5 million pounds in 1987.

FIGURE 9
IMPORTS OF SNAPPER INTO SOUTHEASTERN PORTS OF ENTRY: 1983-87 PRODUCT FORMS



DATA SOURCE: NMFS Market News Reports

FIGURE 10
IMPORTS OF SNAPPER BY COUNTRY OF ORIGIN 1983 AND 1987



DATA SOURCE: NMFS Market News Reports

This represents an annual average decrease in grouper landings of 5.8 percent since 1983. Grouper imports are relatively mmore abundant in the fall months, with April also being an important month for grouper import arrivals (Figure 12).

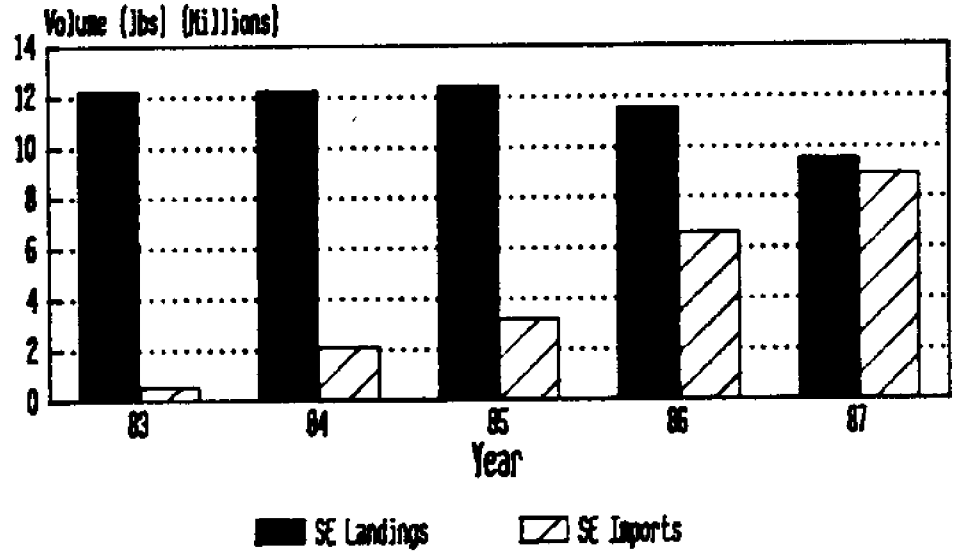
Several varieties of grouper are imported from Latin American sources. In 1987, the species reportedly imported were black, red, yellowedge, and warsaw grouper. As with snapper, the majority of grouper imported were unclassified by Customs. This unclassified category represented 96 percent of the total grouper imports in 1987. Yellowedge and red grouper represented 2 and 1 percent of the total, respectively.

Import volumes of fresh grouper have dominated the southeastern grouper import market since 1984 (Figure 13). However, the rate of increase in fresh imports declined dramatically from 1986 to 1987. During the same period, frozen grouper imports increased from 1.4 million pounds to 1986 to 3.3 million pounds in 1987, after having remained stable for 1983 to 1985. Currently unavailable data for 1988 will be needed to determine if the increased importance of frozen grouper will continue.

Grouper imported in whole form remained the most important product form during the 5-year period. However, filleted grouper accounted for 33 percent of total import volumes in 1987. Loins and portions were of less importance, representing only 2 percent of the total (Figure 14).

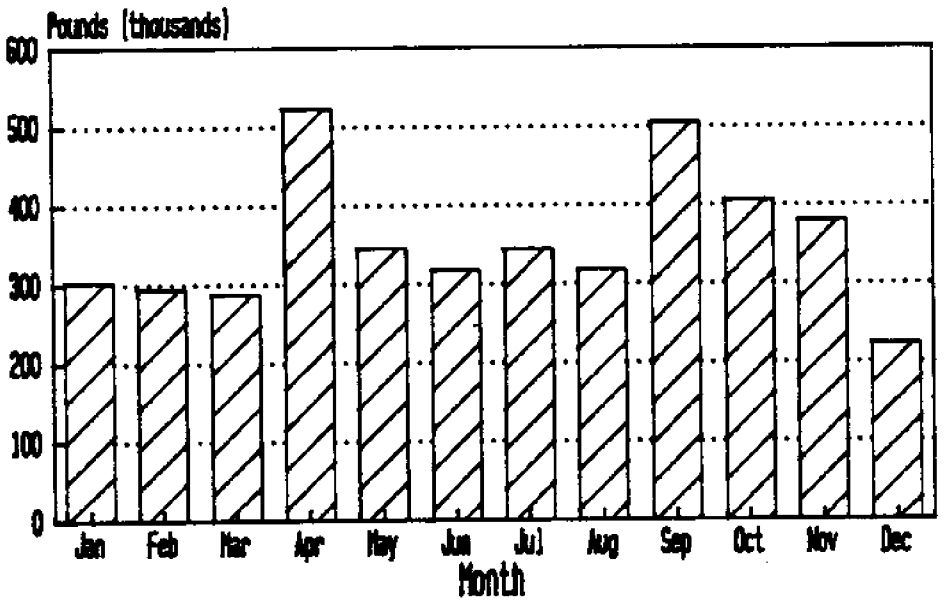
During the 1983-87 period, three countries provided over 80 percent of the imported grouper product arriving at southeastern U.S. ports of entry (Figure 15). Mexico was the most important source of grouper product from 1983 to 1987, providing approximately one half the total

FIGURE 11
SOUTHEAST U.S. GROUPER LANDINGS AND IMPORTS: 1983-87



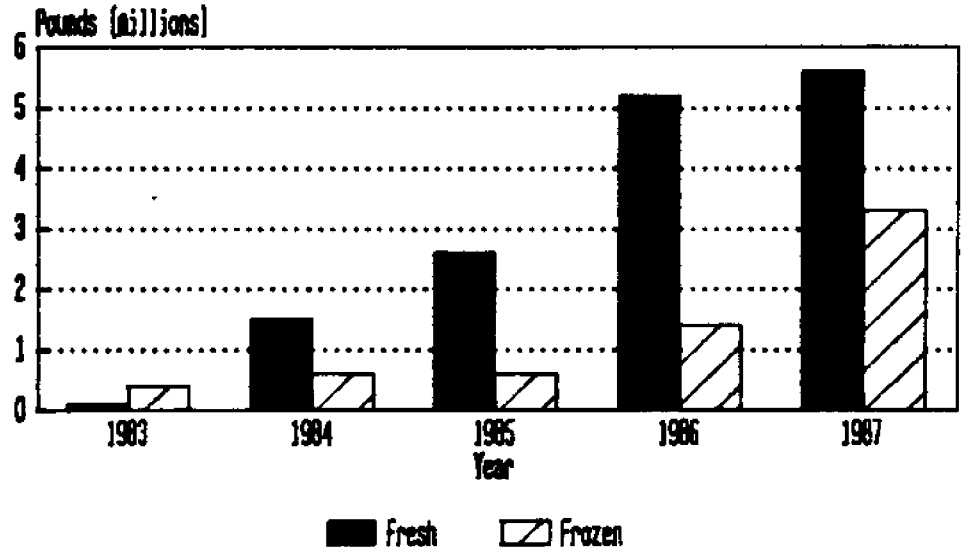
DATA SOURCE: NMFS data. Import product weight given. All species included.

FIGURE 12
FIVE-YEAR AVERAGE MONTHLY DISTRIBUTION OF SOUTHEAST U.S. GROUPER IMPORTS



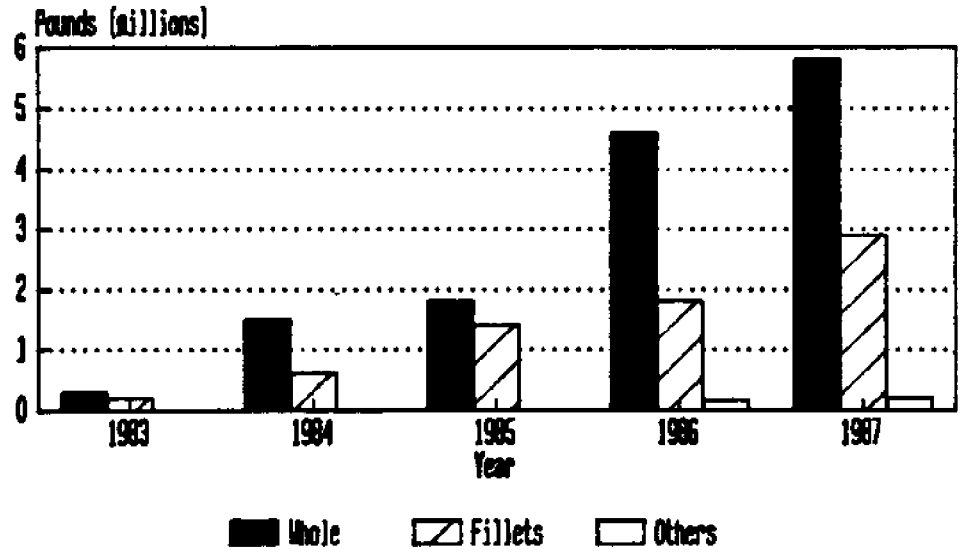
DATA SOURCE: NMFS Market News Report Data. Data pertains to 1983-87.

FIGURE 13
IMPORTS OF GROUPER INTO SOUTHEASTERN
PORTS OF ENTRY: 1983-87
FRESH vs FROZEN



DATA SOURCE: NMFS Market News Reports

FIGURE 14
IMPORTS OF GROUPER INTO SOUTHEASTERN
PORTS OF ENTRY: 1983-87
PRODUCT FORMS



DATA SOURCE: NMFS Market News Reports

supply. Costa Rica and Chile provided an additional 12 and 10 percent, respectively, in 1987. Ecuador also became an important source by 1987, providing 7 percent of the total volume exported to the southeastern U.S. The remaining 20 percent was supplied by Ecuador, Dominican Republic, Argentina, Panama, Guyana (in order of importance), and others. Miami served as the major port of entry for grouper products. Approximately 82 percent of the grouper imports arrived through Miami, with 18 percent arriving in Brownsville. Lesser volumes arrived in Savannah, Port Everglades, West Palm Beach and New Orleans.

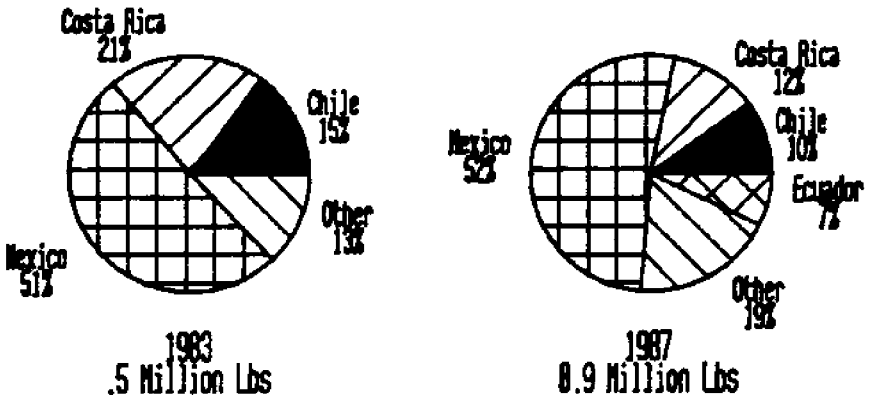
Mahi-Mahi (Dolphin)

Mahi-mahi imports remained below 1 million pounds through 1985, doubled in 1986, and increased dramatically to 7.4 million pounds in 1987 (Figure 16). Monthly distribution of imported product is fairly even, with peak 5-year average months being April, May, and June (Figure 17). Landings of mahi-mahi in the southeast region have been fairly stable, with production increasing from 318,000 pounds in 1983 to 507,000 pounds in 1986. Landings then increased to 645,000 pounds in 1987. Import volumes exceeded landings for the first time in 1985.

Prior to 1986, mahi-mahi was imported primarily as frozen product (Figure 18). In contrast to snapper and grouper, however, the majority of mahi-mahi imported into southeastern ports of entry since 1985 has been fresh product. In 1986, fresh product represented 74 percent of the total import volume. This increased to 83 percent in 1987.

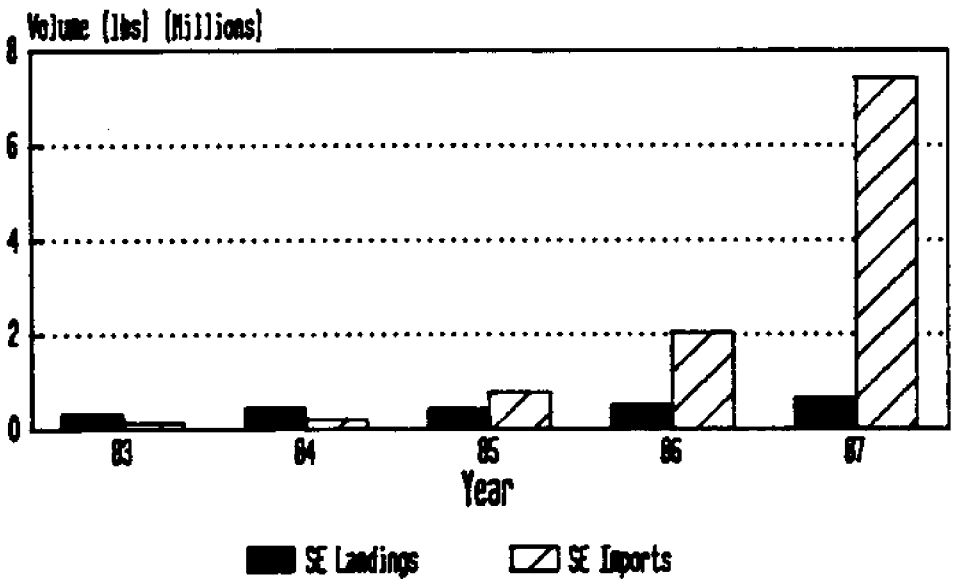
Prior to 1987, the composition of the total volume of mahi-mahi imports was not consistently dominated by either whole or filleted product. However, whole product accounted for 84 percent of the total volume in 1987, as compared to 74 percent in 1986 (Figure 19). The

FIGURE 15
IMPORTS OF GROUPER BY COUNTRY OF ORIGIN: 1983 AND 1987



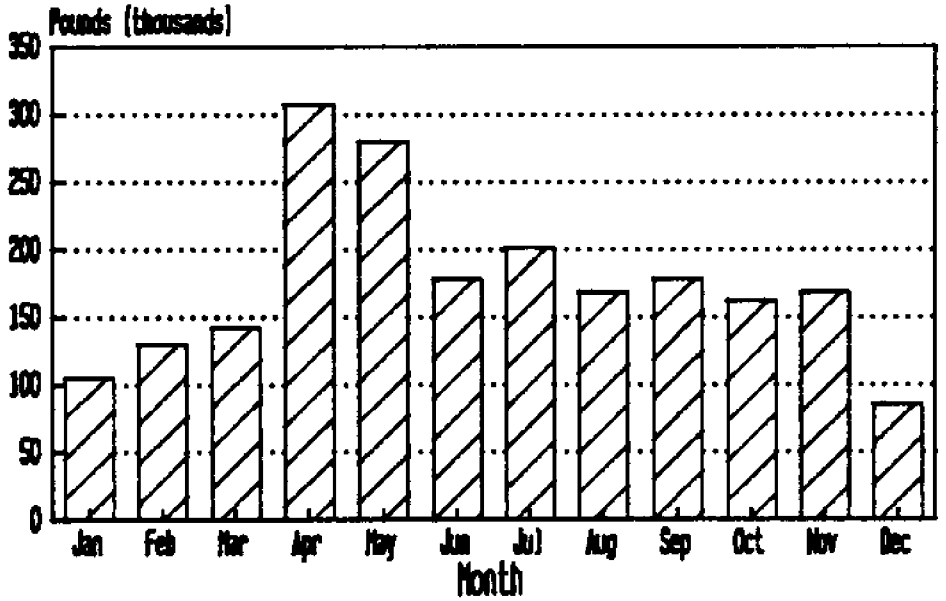
DATA SOURCE: NMFS Market News Reports

FIGURE 16
SOUTHEAST U.S. DOLPHIN (MAHI-MAHI) LANDINGS AND IMPORTS: 1983-87



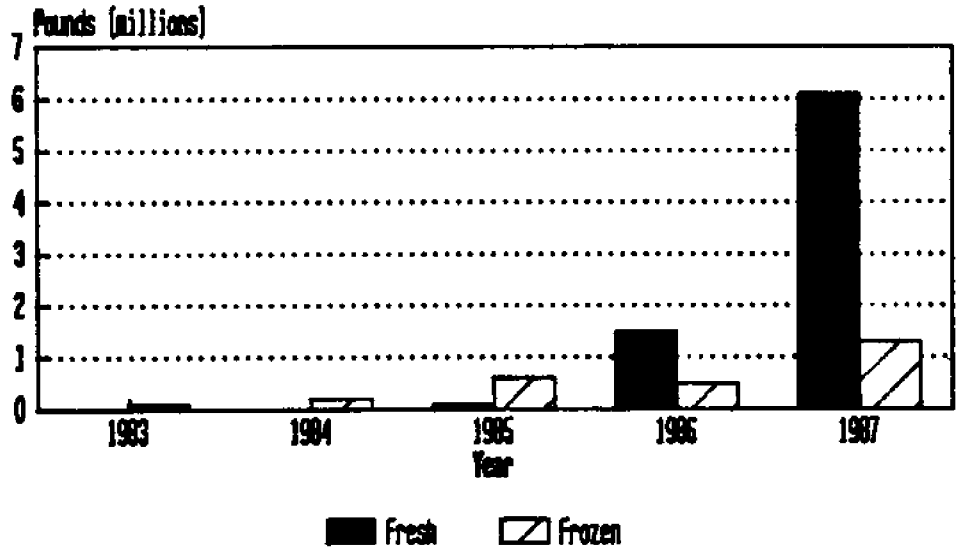
DATA SOURCE: NMFS Market News Data.
Import product weight given.

FIGURE 17
FIVE-YEAR AVERAGE MONTHLY DISTRIBUTION
OF SOUTHEAST U.S. MAHI-MAHI IMPORTS



DATA SOURCE: NMFS Market News data.
Data refers to 1983-87.

FIGURE 18
IMPORTS OF MAHI-MAHI INTO SOUTHEASTERN
PORTS OF ENTRY: 1983-87
FRESH vs FROZEN



DATA SOURCE: NMFS Market News Reports

dramatic increase in the volume of whole product may be due to the versatility demanded by a strengthening domestic restaurant market for mahi-mahi, although market data are not available to support this hypothesis.

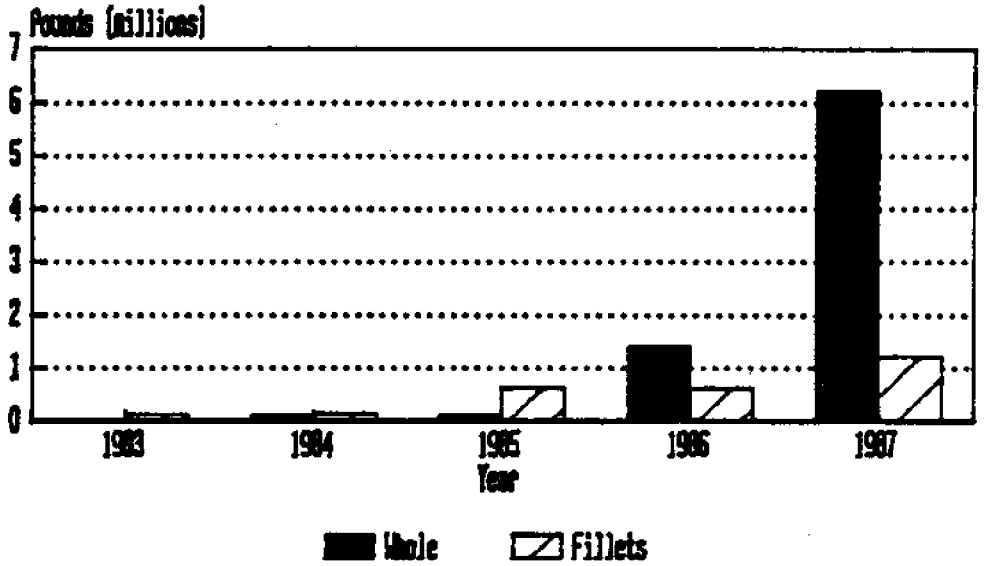
A shift in countries of origin has occurred for mahi-mahi during the 1983-87 period (Figure 20). Approximately 99 percent of the mahi-mahi exported to the southeastern U.S. ports of entry in 1983 originated from oriental countries. Taiwan and Japan provided 65 and 34 percent, respectively, of the total volume in 1983. By 1987, however, Central and South American sources were dominating the market. Ecuador and Costa Rica accounted for 51 and 40 percent, respectively, of the total volume of mahi-mahi import volume in 1987. The remaining 9 percent came primarily from Peru. All reported mahi-mahi imports arrived through Miami.

SUMMARY

As the demand for seafood in the U.S continues to strengthen, increased pressure will be exerted on domestic seafood suppliers to find alternative sources of seafood. The import market will likely continue to grow in importance in the near future. Specifically, an increased dependence on Latin American countries has developed for providing supplies of tropical/subtropical finfish and shellfish species to a growing U.S. seafood market.

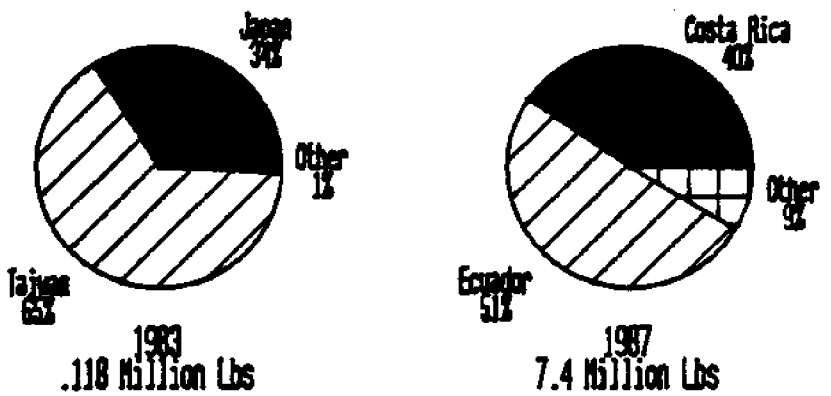
The volume of seafood imports arriving at Southeastern U.S. ports of entry has increased steadily since 1983. Along with this increase in volume has been an increase in the diversity of species. Many non-traditional species have become important components of the species

FIGURE 19
IMPORTS OF MAHI-MAHI INTO SOUTHEASTERN PORTS OF ENTRY: 1983-87
PRODUCT FORMS



DATA SOURCE: NMFS Market News Reports

FIGURE 20
IMPORTS OF MAHI-MAHI BY COUNTRIES OF ORIGIN: 1983 AND 1987



DATA SOURCE: NMFS Market News Reports

complement available to domestic seafood suppliers. The market continues to be dominated by fresh product, imported in whole form. However, this relationship may vary from species to species. In addition, the apparent monthly "availability" of imported finfish and shellfish varies by species. A knowledge of these and other aspects of the imported seafood market may be useful to domestic seafood suppliers wishing to enter the import market or expand existing seafood importing activities.

RESEARCH AND MARKETING DEVELOPMENTS FOR THE REX EEL, *OPHICHTHUS REX*

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INTRODUCTION

Research efforts are on-going to address the potential development of a commercial fishery for the *Ophichthus rex* eel. This species is commonly referred to as the rex eel, giant snake eel, and previously as the keoghfish.

Previous fisheries work with this eel is limited to one research note on occurrence (Bohlke and Caruso, 1980) and there has been no prior published work on utilization of this eel as a food item. Based on abundant incidental catches which indicated harvest potential, commercial interest requested initial investigation to determine the feasibility for this alternative fishery to compliment existing longline fisheries along the northern Gulf of Mexico. This interest lead to this project being funded by the annual MARFIN allocations.

METHODS

The initial work reviewed in the text is based on a preliminary study of the keoghfish by Burgess et al (1987) that was funded by the Florida Sea Grant Program. The work of this project is in conjunction with commercial vessels that have the gear and experience used to harvest shark and grouper/snapper, which is directly applicable to harvesting the rex eel.

The previous three eel expeditions have been aboard a contracted commercial shark fishing vessel out of Bayou La Batre, AL. The general area for harvesting these eel was located approximately 50 miles south of Bayou La Batre, AL in the Gulf. Specific fishing sites were based on previous experience of the captain and the senior investigator for harvesting rex eel on earlier trips.

Eels are caught alive using a longline set over the muddy bottom. The "soak" time for each longline set was approximately 3 hours. The longline sets were made in depths of 170-500 ft. during morning, afternoon and evening hours. Fresh eel was the bait of choice for both eel and shark. All eels were placed in an ice/salt slush after capture and held there until death. The eels were placed on ice and transported back to Gainesville for butchering and laboratory analyses. The meat is then destine for fish processing plants and a portion is used for food analysis and product development.

RESULTS AND DISCUSSION

This large eel, with lengths reaching 5 feet plus and maximum weights of 30 or more pounds, is shown respect by it captors due to its abundant razor sharp teeth. It is thought that the rex eel is located primarily on the muddy bottoms of the Gulf of Mexico.

At present, the rex eels are a by-catch harvested using longlines set over the muddy bottom. Special handling precautions are necessary when landing the eel. Due to their aggressive nature, the eels are immediately headed upon being landed. Once the longline is hauled in, the eels are gutted and placed on ice. When the eels are harvested for research, they are placed in an ice/salt slush after they are landed. Following death, they are packed on ice and transported whole to the laboratory for analyses.

A major problem for both the processor and the consumer is the numerous bones, the so called free-floating bones. It is difficult, if not impossible to process out a boneless fillet. The location of the cut determines the arrangement of the bones. For example, the bones in the front and middle

sections are scattered throughout the back and rib areas and become dense throughout the tail section. Therefore, a boneless fillet is not practical and makes the task of marketing more difficult.

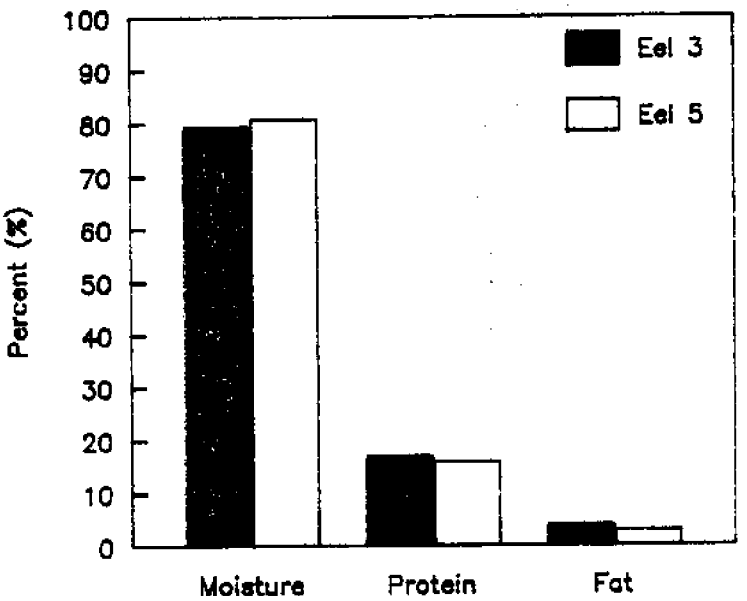
Post-harvest losses of headed, gutted and skinned eel has ranged from 28-32% (Table 1). With the tail section being dense with bones and with little obtainable meat, there is an additional loss of up to 20%. Another 20% is lost in the smoke house. After processing and cooking (i.e. smoking) the final yield of edible meat which still contains a number of small free-floating bones is approximately 30%. Due to the low final yield, options are being considered to recover some of the cost of processing, thereby keeping the cost of the final product affordable and marketable. The options include using deboning machines to recover lost eel meat and using the eel skins in the tannery industry.

Table 1. Postharvest Losses and Percent Yield Of The Rex Eel

Processing Stage	Loss (%)	Yield (%)
Whole Eel	——	100%
Gutted, Headed & Skinned	30%	70%
Tail Section	20%	50%
Smoked	20%	30%

The high protein/low fat content of the O. rex eel makes it nutritionally appealing to the health conscious consumer. The protein content is 15-20% and the fat content is 3-4% (Figure 1). No appreciable differences exist with sex, size, depth of habitat or anatomically for either protein or fat content. All analytical methods used followed AOAC (1980) procedures. The present date for protein and fat content is from a summer sampling of the species. Seasonal samples will be analyzed to determine any seasonal changes in protein and fat content.

Figure 1. Proximate Composition of Ophichthus rex Eel



The cooked and smoked eel have received very good consumer reviews. The meat color is white and has firm texture. The single negative quality expressed by domestic and foreign consumers is the abundant number of bones. The white meat with its firm texture is attracting the attention of at least one foreign market for possible use as a product in sushi. The shelf-life of vacuum packaged smoked eel has proven to be very good. After storing at -20°C for 4 months there were no negative organoleptic characteristics. Following a 42 day refrigerated (3°C) shelf-life study, there were slight negative changes in color, texture and flavor. Fresh eel, refrigerated at 3°C started showing negative quality values after 33 days.

Preliminary analyses were preformed on fresh and smoked rex eel for microbial counts, moisture content, water activity and salt content. The standard plate count gives a comparative measure of the overall degree of contamination. The standard plate count for days 0 and 20 for fresh and smoked rex eel were relatively low as compared to bacterial

counts of similar products (Table 2). The preliminary results from the microbial, compositional and shelf-life analyses suggest that fresh and smoked eel, when packaged and refrigerated properly, could have a shelf-life of at least 30 days.

Table 2. Microbiological And Compositional Data For The Rex Eel

Sample	Microbial Load (cfu/gram) Day 0	Day 20	Moisture (%)	Aw *	Salt (%)
Smoked Eel	1.5×10^{2}	7.1×10^{3}	75	.85	4.9
Fresh Eel	3.9×10^{3}	5.1×10^{5}	80	—	0.4

* Water Activity

Vacuum packaging the eel has been used successfully in presenting an eye-appealing package to the consumer. Due to the abundant bones in the product, special precautions must be taken when cutting the eel meat to prevent the bones from puncturing the vacuum bags. This problem has been remedied by using a band saw to cut frozen eel into the desired lengths before smoking. The result is bones cut flush with the meats edge with no jagged or extending bones to puncture the bag. Interest has recently been expressed in having the smoked eel packed in vegetable oil and thermally processed in cans. This has been done with success on a one time experimental bases in our lab. The meat stayed intact and pull away from the bone slightly. Also, there were no color or flavor changes in the smoked eel.

Marketing of the rex eel has proven to be one of the most challenging aspects of this project. The rex eel appears most suitable for unique domestic markets and traditional international markets (i.e., England and Japan). Since culture largely determines the extend to what products are acceptable, marketing strategies will be different in each country. Likewise, consumer's familiarity with a new product, the product form presented to the consumer, and how its packaged will determine whether or not the product is accepted by the consumer.

To develop a successful commercial fishery for the Ophichthus rex eel several potential areas of concerns must be addressed. Those areas needing to be addressed include:

(1) the abundant number of bones making it less palatable; (2) the low final yield of the marketable product may result in it being priced off the market; (3) determine the population size and reproductive cycles of this species so advise can be given on proper harvesting, so as not to endanger the species, and (4) continued consumer education in the domestic market to assist with consumer acceptability of this new product.

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DISTRIBUTION OF OVARY WEIGHTS FROM THE COMMERCIAL ROE MULLET FISHERY

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INTRODUCTION

The Alabama roe industry processed approximately 130,000 pounds and 325,000 pounds of Alabama caught mullet in 1986 and 1987 respectively (Hosking et. al. 1987, Perkins et. al. 1988). The directed fishing effort at the spawning stock of striped mullet (Mugil cephalus) raised concern over the long-term future of the fisheries. In 1987, the Alabama Department of Conservation and Natural Resources restricted the mesh size of gillnets to 3 3/4 inches or larger between October 15 and December if fishing for mullet. While a great deal of information is available on the effect such regulations would have on the size of mullet caught, little is known about the effect on the weight of roe harvested.

Roe mullet processing consists of removing the ovaries intact, packing in plastic bags and freezing in boxes. The larger ovaries (roe sacks) command a premium price over the smaller ovaries. For example, in 1987 the price of 2-4 ounce roe sacks was $3.50 to $4.00 a pound, while 6-8 ounce roe brought $6.00 a pound (Perkins et. al. 1988).

We gathered information on the weight of ovaries for various length striped mullet and related the length of fish caught by different gill net mesh sizes to weight of ovaries as part of a larger project conducted in cooperation with the Alabama Deparment of Conservation and Natural Resources, Division of Marine Resources. Funds for the overall project were provided by the National Marine Fisheries Service through the Marine Fisheries Initiative (MARFIN).

METHODS

Stiped mullet were sampled at random from processors in Alabama during the fall of 1986 and 1987. Total length (mm), weight of fish and weight of ovaries (g) were recorded for each fish sampled and regression analysis conducted to investigate the relationship between ovary weight and length using SAS. Frequency distributions of ovary weight were further analyzed in relation to fish length and gill net selectivity.

RESULTS AND DISCUSSION

Initial analysis revealed little difference between 1986 and 1987 samples and they were combined (n = 336) for regression analysis. The relationship between ovary weight and length was significant (Table 1) and is described by the equation, ovary weight = -250.4 + 0.906 x total length (Figure 1). However, analysis of the variance revealed a low R-square (0.549) indicating a large portion of the variation in ovary weight was unexplained.

TABLE 1. Analysis of variance for the relationship between total length of mullet (mm) and the weight of ovaries (g).

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	244457.31	244457.31	409.16	0.0001
Error	335	200145.47	597.44		
C Total	336	444602.79			

Root MSE	24.44	R-square	0.549
Dep Mean	112.93	Adj R-square	0.548

Because processors and managers work with ounces and inches, we converted our data to English units and examined the percentage distribution of ovary weights by length. Fish 14 inches or less produced 4 ounce ovaries (8% of total). Fifteen-inch fish had predominantly 4 ounce ovaries (30% of total) and some 6 ounce ovaries (6% of total). Sixteen-inch fish yielded mostly 6 ounce ovaries (19% of total) and some 4 ounce roe (13% of total). Seventeen-inch fish produced mostly 6 ounce roe (8% of total), while 18 and 19-inch fish produced 4 to 8 ounce ovaries but contributed only 10% of the total roe.

Gillnet selectivity curves, provided by the Alabama Department of Conservation and Natural Resources, Division of Marine Resources revealed that 3.5 inch, stretch mesh gill nets caught 13 to 16 inch fish, 3.75 inch mesh caught 15 to 16 inch fish and 4.0 inch mesh caught 16 to 17 inch fish. Based on these data, the Marine Resources Division recommended regulations requiring 3.75 inch mesh nets. This would allow almost all two-year-old fish (11 to 13 inches), which had probably never spawned, to escape. Likewise, a significant number of three-year-old fish (13 to 15 inches), which would be spawning for the first time, would also escape (Tatum, personal communication). Our

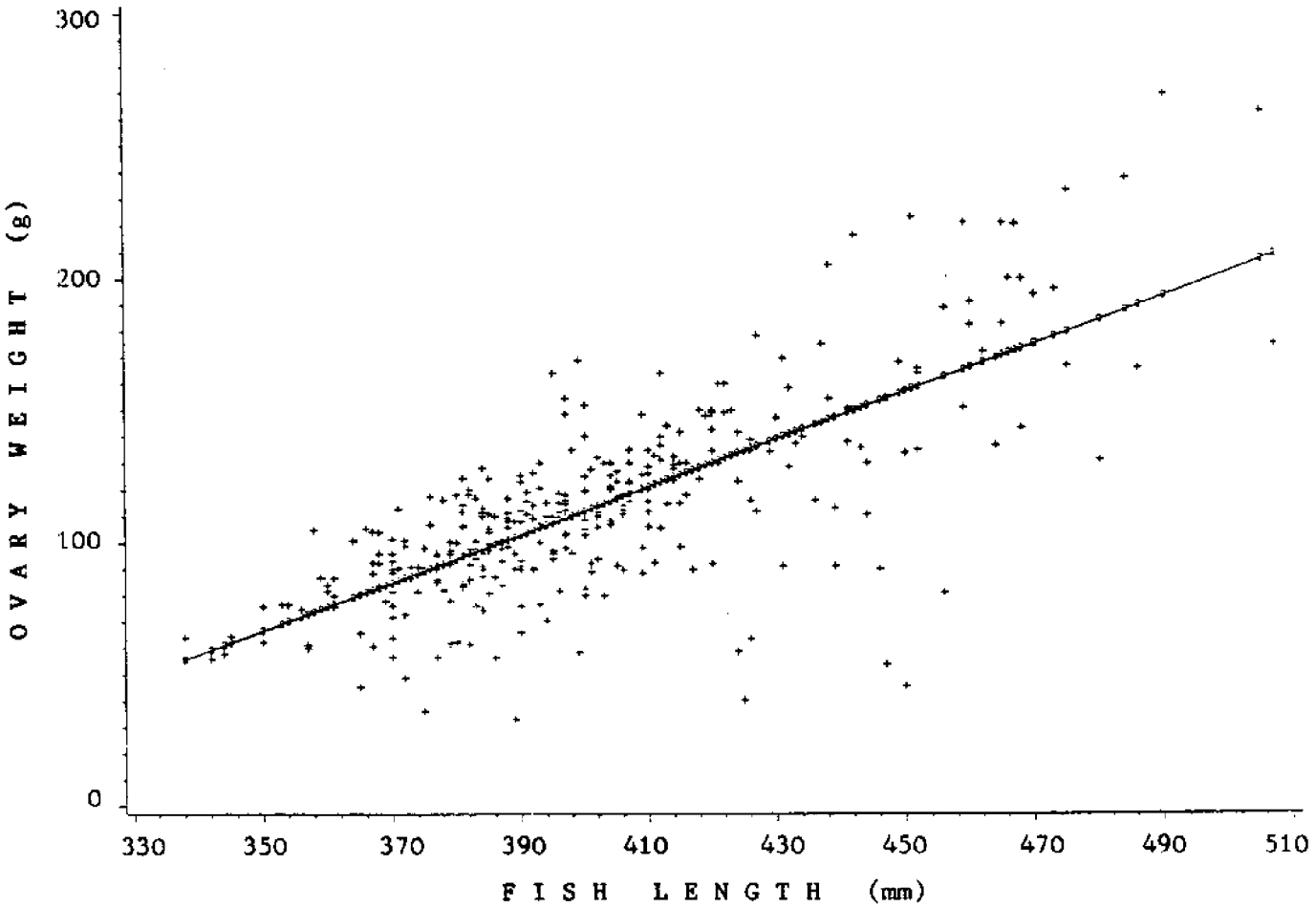

FIGURE 1. Ovary weight (g) versus total length (mm) of striped mullet. Circles and line represent predicted values.

analysis of ovary weight distribution by fish length indicated that the 3.75 inch mesh regulation will reduce the potential production of roe by only 8% and the reduction will only affect the 2 to 4 ounce ovaries which are least valuable. The 3.75 inch mesh or larger will catch predominantly 15 to 17 inch fish which produce proportionally more and larger ovaries.

We conclude that the mesh size regulations, based on the reproductive biology of the striped mullet, will not harm mullet roe production and should work to ensure a long-term future of the industry in Alabama.

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AN ECONOMIC ANALYSIS OF THE 1987 ALABAMA MULLET ROE INDUSTRY

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INTRODUCTION

The 1987 Alabama mullet roe season began with a few changes. Several smaller processors who had processed mullet roe during 1986 did not re-enter the industry during 1987. Those who remained modified their method of buying roe mullet. Instead of paying for the fish on a per-pound basis as they did in 1986, the processors paid the fishermen on the basis of roe yield. Another change, which processors attributed to the larger 3 3/4-inch mesh size, was the greatly reduced poundage of male mullet. During 1986, Alabama processors purchased and handled 402,000 pounds of male mullet (or 19.6% of the total) compared with only 128,000 pounds (or 9.6%) during 1987.

Alabama roe mullet processors generally waited until later in the 1987 season to begin buying roe mullet in an effort to maximize their purchases of the larger, more valuable sizes. As a result, Alabama processors purchased and processed fewer total mullet than they did during 1986.

The Alabama Sea Grant Extension Service gathered the following poundage and value data as part of a larger project conducted in cooperation with the Alabama Department of Conservation and Natural Resources, Marine Resources Division. Funds for the overall project were provided by the National Marine Fisheries Service through the Marine Fisheries Initiative.

METHODS

The data presented in this report were collected from three Alabama roe processors. In some instances, processors provided access to their purchase and sales receipts. In other cases, processors simply provided total product volume and value statistics for the various commodities purchased and sold.

RESULTS

Table 1 lists the poundages, mean prices, and total values of female roe mullet purchased from fishermen in four Gulf Coast states. Primary points of purchase included: Venice, Hopedale, and Ysclosky, Louisiana; Pascagoula, Mississippi; Mobile and Dauphin Island, Alabama; and, Pensacola, Carabelle, Pine Island, Tampa/St. Petersburg, and Marco Island, Florida. Prices paid were based on roe yield.

TABLE 1. 1987 Female Roe Mullet Purchases by Alabama Processors.

SOURCE	POUNDS	COST/POUND ($)	TOTAL COST
Florida	440,000	.92	$ 404,800
Alabama	325,000	.75	243,750
Mississippi	75,000	.70	52,500
Louisiana	375,000	.97	363,750
TOTALS	1,215,000	----	$1,064,800

Several changes in purchase patterns occurred during 1987. In 1986, Alabama processors purchased 44.1% of their roe mullet from Louisiana, but 1987 purchases dropped to 30.4%. The reduction in purchases from Louisiana was largely due to Louisiana prices escalating early in the season. Thus, Alabama processors redirected their attentions to Florida, which provided 36.3% of the female roe mullet to the Alabama processing industry during 1987, as compared to only 22.5% in 1986. Alabama's contribution varied little, down from 31.4% in 1986 to 27.1% in 1987. And, the amount of female roe mullet from Mississippi increased from 2% in 1986 to 6.2% in 1987.

Table 2 lists poundages, mean price, and total values of "white roe" male mullet purchased from fishermen in the same four Gulf Coast states. Mean prices paid for male mullet were equal from state to state, and steady throughout the year. The major change in the male mullet fishery was that males comprised only 9.6% during 1987, as previously noted. Several processors felt that the reduction in numbers of smaller sized male fish resulted from the use of larger 3 - 3/4-inch mesh gill nets during 1987.

TABLE 2. 1987 Male Roe Mullet Purchases by Alabama Processors.

SOURCE	POUNDS	COST/POUND ($)	TOTAL COST
Florida	47,500	.15	$ 7,125
Alabama	38,400	.15	5,760
Mississippi	8,300	.15	1,245
Louisiana	34,300	.15	5,145
TOTALS	128,500	----	$19,275

Table 3 shows that a total of 342 people in four Gulf Coast states were employed in jobs directly related to the 1987 Alabama mullet roe industry. The jobs held by the 115 processing employees were nearly equally divided among processing facilities located in three, small communities in lower Alabama. Some of the 208 Gulf Coast fishermen worked part-time, and some were full-time fishermen. Catch per unit effort was not determined in this portion of the study.

TABLE 3. Employment Related to 1987 Alabama Roe Mullet Processing.

STATE	FISHERMEN	PROCESSING EMPLOYEES	TRUCK DRIVERS
Florida	45	-0-	7
Alabama	57	115	5
Mississippi	6	-0-	1
Louisiana	100	-0-	6
TOTALS	208	115	19

As shown in Table 4, percent yield of female mullet roe varied according to geographic region. The mean percent yields listed in Table 4 show little range between the minimum and maximum values. However, roe yields as low as 16 percent were noted in fish landed in Mississippi and Alabama during the early part of the season. Roe yields as great as 21 percent were found in mullet purchased from South Florida during the latter portion of the season. The total number of pounds of female roe produced during 1987 was 23.7 percent less than the 280,000 pounds produced during 1986.

TABLE 4. 1987 Female Mullet Roe Yielded by Alabama Processors.

SOURCE	POUNDS OF FEMALE MULLET	PERCENT YIELD	POUNDS OF FEMALE ROE
Florida	440,000	17.8	78,320
Alabama	325,000	17.3	56,225
Mississippi	75,000	17.0	12,750
Louisiana	375,000	17.7	66,375
TOTALS	1,215,000	----	213,670

Table 5 provides a grade-by-grade analysis of the value of female mullet roe produced during the 1987 season. Although the percentage of 2-4 ounce and broken roe produced during 1987 was roughly equivalent to the percentage produced in 1986, the 1987 mean sale price of $3.83 per pound was nearly 60% greater than the 1986 mean sale price of $2.40 per pound. The very narrow ranges of sale prices for 4-6 ounce and 6-8 ounce roe, the two most economically important grades, indicate that there was very little room for discussion about price between buyer and seller. The mean price per pound for which 8-ounce-and-up roe were sold during 1987 was on par with the 1986 mean sale price. While 8-ounce-and-up roe accounted for less than 10 percent of total volume during 1986, they provided nearly 16.2% of 1987 roe volume.

TABLE 5. Value of Female Mullet Roe Produced by Alabama Processors During 1987.

SIZE OR GRADE	NUMBER OF POUNDS	SALE PRICE PER POUND ($)		TOTAL ($) VALUE
		Range	Mean	
2 - 4 oz and broken	47,485	3.50 - 4.00	3.83	$ 181,867
4 - 6 oz	74,035	5.20 - 5.25	5.24	387,943
6 - 8 oz	57,600	6.00 - 6.00	6.00	345,600
8 oz and up	34,550	6.70 - 7.25	6.75	233,212
TOTALS	213,670	- - - - - -		$1,148,622

The importance of the secondary products enumerated in Appendix Table 1 cannot be overemphasized. With the exception of 64,000 pounds of female carcasses which were rendered for feed, all of the secondary products added to the 1987 season's gross profit. And, the sale and utilization of secondary products kept waste to a minimum.

The 1987 mean mullet gizzard sale price of $2.07 per pound was up 29.3 percent over the 1986 price of $1.60 per pound. The frozen male mullet (exported to Egypt) and fresh male mullet (purchased by local smoking operations) sale price was $.25 per pound, or 19 percent higher than the 1986 sale price of $.21 per pound. Sale prices for whole male mullet and female carcasses were unchanged from 1986 to 1987.

Table 6 lists mullet roe production expenses incurred by Alabama processors in 1987. Female mullet and male mullet costs of $.876 and $.15, respectively, are arithmetic means of all mullet purchased in all states throughout the 1987 season. The 1987 production labor cost of $.085 per pound was 32.8% greater than the corresponding 1986 labor cost of $.064 per pound. Packaging costs increased slightly during 1987, while transportation costs remained the same. Substantial savings in frozen storage costs (56 percent less than in 1986) were realized during 1987 for two reasons: fewer total pounds of carcasses were stored for less time during 1987 than in 1986. Although over 64,000 pounds of carcasses were rendered into feed during 1987, that unit process added only $555 in additional processing expense to the 1987 total.

TABLE 6. 1987 Alabama Mullet Roe Industry Production Expenses.

EXPENSE TYPE	POUNDS	MEAN $/LB EXPENSES	EXPENSE
Cost of:			
Female Mullet	1,215,000	.876	$1,064,800
Male Mullet	128,500	.15	19,275
Labor	1,343,500	.085	114,197
Packaging	1,279,300	.0057	7,292
Frozen Storage	123,000	.05	6,150
Transportation	219,679	.015	3,295
Rendering	64,200	.0086	555
TOTAL	----------	-----	$1,215,564

Total income values from Table 5 and Appendix Table 1 were combined to yield the total income figure of $1,293,177 listed in Table 7. The total expense value from Table 6 was listed as total expense in Table 7. The three Alabama mullet roe processors yielded a combined total net profit of $77,613 during the 1987 season. Thus, each processor realized a mean net profit of $25,871 during the eight-week season.

TABLE 7. 1987 Alabama Mullet Roe Industry Net Income.

TOTAL INCOME	$1,293,177
TOTAL EXPENSE	1,215,564
NET INCOME	$ 77,613

In summary, the 1987 Alabama mullet roe industry was profitable because:

- Processors purchased fewer "low value" male mullet.
- Processors waited until later in the season to begin purchasing female mullet, thus increasing the number of larger sized, higher value roe.
- Processors made much better economic use of secondary products during 1987.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to the following individuals and organizations for their support and cooperation:

- The National Marine Fisheries Service, Marine Fisheries Initiative, for providing initial project funding.
- The Alabama Department of Conservation, Marine Resources Division, for providing project funds.
- The members of the Alabama roe mullet industry, for providing data and statistics.

APPENDIX TABLE 1. Disposition of Secondary Roe Mullet Products by Alabama Processors During 1987.

PRODUCT TYPE	NUMBER OF POUNDS	SALE PRICE PER POUND ($)		TOTAL ($) VALUE
		Range	Mean	
Mullet Gizzards	18,225	1.75 - 2.50	2.07	$ 37,726
Male Mullet - Exported to Egypt	81,021	.22 - .25	.25	20,255
Male Mullet - for Fresh Domestic Market	15,000	.25 - .25	.25	3,750
Male Mullet - Frozen for Bait	32,479	.08 - .15	.14	4,547
Female Carcasses - for Fresh Domestic Market	126,854	.15 - .25	.18	22,833
Female Carcasses - Frozen for Bait	792,051	.06 - .08	.07	55,444
Female Carcasses - Rendered for Feed	64,200	- 0 -	- 0 -	- 0 -
TOTALS	1,129,830	------	---	$144,555

A SURVEY TO DETERMINE THE AWARENESS OF HAZARDS RELATED TO RAW SEAFOOD INGESTION IN AT RISK PATIENT GROUPS

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INTRODUCTION

Vibrio vulnificus is a lactose fermenting, halophilic bacteria which has been isolated from waters in the Gulf of Mexico, the Atlantic and Pacific Oceans (1). This bacteria has been associated with the development of primary septicemia and severe wound infections (2). Infection with V. vulnificus can lead to severe illness, amputation and even death in persons belonging to selected "high risk" groups (3).

Epidemiologic and case-control studies (2,3,4) have defined those populations at risk for development of the severest form of infection. Those include persons with hepatic dysfunction, specifically cirrhosis, hepatitis and hemochromatosis. Also included are persons with various forms of cancer, especially those receiving chemotherapy. Other risk include any chronic illness, chronic alcohol consumption, use of immunosuppressive agents, chronic renal disease, hematopoietic disorders and gastric disease. These factors in conjunction with ingestion of infected raw oysters appear to lead to a greater risk of primary septicemia.

Blake et. al. (3) found that 75 percent (18/24) of those cases with primary septicemia had pre-existing liver disease. Specific diagnoses included hemochromatosis, chronic hepatitis, hepatic disease of unknown origin and various forms of cirrhosis, including alcoholic cirrhosis. Of the remaining persons with primary septicemia, three had histories of alcohol abuse, one had thalassemia major, one had diabetes mellitus and only one had no apparent underlying disease.

Similar results were obtained by Tacket et. al. (2). Thirteen out of eighteen patients with primary sepsis had a history of liver disease. Thirteen also had a history of raw oyster ingestion just prior to the onset of illness.

Risk factors (4) which were significantly associated with development of primary sepsis included use of immunosuppressive agents ($p<0.001$), consumption of more than one ounce of alcohol per day ($p<0.001$), and raw oyster consumption ($p<0.01$).

In several cases, the bacteria has been isolated from raw oysters ingested by the patient prior to becoming ill (5, 6). Raw oysters appear to be the primary vehicle for V. vulnificus. Oysters are filter feeders. They receive their oxygen and nutrients by pumping large amounts of water through a complex gill system. Oysters and other bivalve mollusks are efficient at concentrating microorganisms in their gut. Oysters can contain 3-20 times more bacteria than is contained in the surrounding water (7).

The Food and Drug Administration (FDA) has reported that there are no known sanitation or public health controls to ensure that shellfish are free from V. vulnificus. The FDA also stated that five to ten percent of the raw shellfish on the market may contain V. vulnificus or other pathogenic species of vibrio (1). Even oysters harvested from FDA approved waters can harbor this bacteria.

Although incidence of V. vulnificus infection is rare, mortality rate for infected persons is between 46-61 percent (1). Persons who fall into the risk categories should be aware of the potential hazards associated with raw oyster ingestion. However, there is presently no such information on any segment of the population which belongs to these various "risk" categories. The purpose of this study was to obtain descriptive data about a sub-group of mobile, independent persons who belonged to "risk" categories and were being followed in clinic. This study attempted to describe possible trends in patient knowledge about the potential hazards of raw seafood ingestion.

METHODS

A. SAMPLE:

Subjects for this survey (n = 57) were identified from three North Central Florida sources. Due to limited finances and time these subjects did not represent a random sample. There was also no control group sampled. Patients with various forms of hepatic disease were identified in an out patient gastrointestinal clinic at Shands Hospital. Patients who were undergoing chemotherapy treatment for various forms of cancer were identified in an outpatient oncology clinic at the Veteran's Administration Hospital in Gainesville. The mean age of 56 of the 57 respondents was 47.4 years, with a standard deviation of 16.1. Age was not available in the medical record for one respondent who reported the month and day of birth but not the year. Males represented 89.5% of the sample.

B. QUESTIONNAIRE DEVELOPMENT:

The instrument for data collection was designed specifically for this study. Reliability of the questionnaire was established by a review process involving faculty and lay persons. When the wording was found to be free of ambiguity the pilot test was conducted. Which

established how long it took to complete the form. It took anywhere from 5-10 minutes. In instances where respondents were unsure of medication which they were taking, they were told that the medication list could be obtained through the medical records.

Validity of a questionnaire is difficult to evaluate in large studies and even more difficult to evaluate in a small scale study. Often, tests of validity really test for reliability (11). Therefore, in order to increase validity of this survey, several guidelines were followed. All answers to the questions could be secured from either the patient or the medical record. The respondent was not asked to recall information over a six month period. The questions were tested for clarity and the survey did not require that the participants give out information which they may be unwilling to make public.

It was necessary to obtain the medications list, the actual diagnosis of the respondent and information about alcohol consumption through a review of each subject's medical record. Although the respondent was asked to recall some information from a six month period, raw oyster ingestion was considered to be a memorable experience. All participants in the pilot test stated that the questions were clear.

C. DATA COLLECTION PROCEDURES

This protocol was approved by the University of Florida Health Center Institutional Review Board. Persons that met the criteria for being at risk for V. vulnificus infection were signed by respondents and placed into their medical records. Questionnaires were self-completed in most cases. Age, diagnosis and medications were checked against the information contained in the medical records. Data was collected for 57 respondents during July 1988.

RESULTS AND DISCUSSION

Respondents were categorized according to illness and medications. Two out of the 57 respondents were dependent on substances other than alcohol and were deleted from the study. Only 15 out of the 55 respondents were taking medications that placed them at risk for V. vulnificus infection. All cancer patients (n = 11) studied were receiving chemotherapy. One respondent was taking immunosuppressants, one was taking antacids and one respondent was on steroid therapy. One patient with hematopoietic disorder was receiving chemotherapy. All other respondents were at risk for infection based on diagnosed illness alone. Table 1 summarizes the number of respondents in each risk category.

TABLE 1: FREQUENCY OF RESPONDENTS FOR EACH ILLNESS

ILLNESS	FREQUENCY	PERCENT
Liver disease	22	38.6
Cancer	11	19.3
Alcohol dependence	19	33.3
Hematopoietic disease	3	5.3
Other	2	3.5
TOTALS	57	100.0

Twenty-eight (50 percent) of the respondents indicated they consumed raw oysters. Nineteen respondents indicated that they has consumed oysters within the last six months. Fourteen of those nineteen stated that the oysters were raw. Twenty-one respondents indicated that they had received special diet instruction. However, only eight respondents remember being told to avoid raw oysters or to cook oysters before eating them. Table 2 summarizes the respondents that were aware of the potential hazards and the risk category to which they belong. Of those eight respondents that remember being told to avoid raw oysters or to cook oysters before consumption, three received this information from a doctor. Dietitian, friend and relative was the source of information for three respondents. One received the information from a book and another received the information from a science class.

TABLE 2: RESPONDENTS IN ILLNESS CATEGORIES THAT AVOID RAW OYSTERS OR COOK THEM BEFORE CONSUMPTION

ILLNESS	FREQUENCY	N	PERCENT
Liver disease	4	22	7.0
Cancer	1	11	1.8
Alcohol dependence	3	19	5.3
Hematopoietic disease	0	3	0.0
TOTAL	8	55	13.1

DISCUSSION

The sample studied in this survey was not intended to be representation of the entire population of persons who fall into the "high risk" categories for *V. vulnificus* infection. These persons were selected over a short period of time, within a limited geographic area.

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DISCUSSION

The sample studied in this survey was not intended to be representation of the entire population of persons who fall into the "high risk" categories for *V. vulnificus* infection. These persons were selected over a short period of time, within a limited geographic area. There was no control group with which to compare the results.

Therefore, differences between this sample and a sample that differs only with respect to criteria which places them at risk for V. vulnificus infection, cannot be elucidated. However, these results are illustrative of a sub-group of persons at "risk" for V. vulnificus infection including persons who are chronically ill and who are followed in clinic in the north central Florida area.

Because the persons in this sample have many avenues available to them to obtain this knowledge, they may be more aware than other populations which may not be receiving the medical attention that they require. However, even in this sample, the results indicate that only a small number of respondents were aware of the potential hazards of raw oyster ingestion.

Although Becker (8) has concluded that having knowledge and information does not ensure patient compliance, he also concluded that patients must have knowledge in order to comply. Therefore, measuring a patient's knowledge base is not the same as measuring compliance. However, the knowledge must be present in order for compliance to occur.

In summary, this preliminary study of chronically ill patients "at risk" for V. vulnificus infection showed lack of awareness of potential hazards of raw seafood ingestion. Further research is necessary to verify these results on a larger, more randomized sample. Also, attention needs to be directed into the best ways to make this information available to those persons who may not be receiving medical treatment.

Survey.MAN

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ABSTRACTS

VIBRIO VULNIFICUS IN RAW OYSTERS: EFFECTS OF PROCESSING AND ICE STORAGE

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Shellstock oysters shipped in interstate commerce and received at processing plants in Mississippi usually contain V. vulnificus. Since the consumption of processed oysters is rarely associated with V. vulnificus septicemia, experiments were undertaken to determine if processing and ice storage reduced the levels of naturally occurring V. vulnificus in raw oysters.

Oysters from each lot tested were sampled before and after processing (shucked and washed) and after three days and one week of ice storage in retail containers. Evaluation of three lots indicaterd that V. vulnificus was reduced minimally by the processing, but reduction greater than 99% was observed following one week of ice storage.

EVALUATION OF IMPROVED COOLING TECHNIQUES FOR BLUE CRAB PROCESSING

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By using forced air cooling, the temperature of crab cores resulting from debacking whole cooked crabs can be significantly reduced. Incorporating improved cooling techniques reduces blue crab processing time from 18 hours to less than 3 hours, increasing shelf-life and improving quality.

NUTRITIVE VALUE OF PASTA CONTAINING SURIMI

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Amino acid profiles and animal assay were examined to evaluate protein quality of pasta containing surimi. Pasta samples included control (100% durum semolina), 20%, 40% levels oof Alaska pollock surimi substitution. Lysine content was increased when surimi level was increased. Pasta with 40% Alaska Pollock surimi was utilized for body weight gain more efficiently than other protein sources.