

ABSTRACTS

REPORT ON FOOD PROTECTION ACTIVITIES OF THE PAN AMERICAN HEALTH ORGANIZATION

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525 23rd Street, NW
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The food protection activities of PAHO/WHO conducted within the framework of the Regional Plan of Action for Technical Cooperation in Food Protection mandated by the Pan American Sanitary Conference and the Ministers of Agriculture and Health of the region of the Americas. Technical cooperation activities in food protection are carried out by the Veterinary Public Health Program of the Division of Disease Prevention and Control.

The staff involved in the delivery of technical cooperation in food protection include a total of some 66 professionals distributed as follows: Program Coordination at the PAHO Headquarters in Washington, DC-4; the Pan American Institute of Food Protection and Zoonoses (INPPAZ) in Argentina-15; the Pan American Foot-and-Mouth Disease Center in Brazil-3 1; and Inter-Country and Country Veterinary Public Health Advisors- 16.

Delivery of technical cooperation is operationalized through: development of policies, plans and norms; resource mobilization; dissemination of information; training; applied research; and expert advisory services.

The program of technical cooperation in food protection encompasses 4 components: (1) Organization of comprehensive, integrated programs, (2) Development and strengthening of inspection and analytical services, (3) Epidemiological surveillance and control food borne disease, and (4) Sanitary control of street foods and consumer protection_

FDA'S REGIONAL PROGRAMS

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No abstract submitted

**REPORT ON THE TECHNICAL COOPERATION ACTIVITIES OF THE
PAN AMERICAN HEALTH ORGANIZATION ON CIGUATERA
AND OTHER MARINE TOXINS**

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Summary of PAHO/WHO activities on Marine Toxins and Ciguatera - 1995

As part of its efforts to implement Epidemiological Surveillance of Foodborne Diseases, PAHO has convened a workshop/conference with the University of Miami on Marine Seafood Intoxications "Pan American Implications of Natural Toxins in Seafoods", to strengthen the diagnostic and epidemiological skills of health officials of the Region for a better identification and treatment of such important food poisoning. The conference was attended by 81 participants from almost all countries in the Region.

Another workshop will be held in Santo Domingo, Dominican Republic November 8- 10, 1995 to discuss specifically the aspects of laboratory diagnostics of Ciguatera. This meeting will be attended by participants of the Caribbean.

PAHO also serves as "ex-officio" Secretary for two Subregional Networks for the Epidemiological Surveillance of Marine Toxins. One of them covers Central America, Mexico and the Northern part of South America (Colombia and Venezuela). The other one was just formed this year and comprehend the countries of Southern Cone (Argentina, Brazil, Chile and Uruguay).

YEASTS: MICROORGANISMS WITH THE COMPETITIVE EDGE IN PRESERVED SEAFOOD

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A relatively large number of yeasts have been isolated from fish or fish products. Unlike most bacteria, some yeasts can grow at extremely low a_w levels. Nearly all yeast species can grow within a wide range of pH values. They can also be found growing at temperatures as low as 0°C and as high as 45°C. Those that are encountered in foods are favored by +Eh conditions but have been isolated from vacuum-packed products. All of these characteristics describe the type of organism that could represent a problem in **preserved** foods.

Mechanisms used for food preservation, such as dehydration, acidification, low temperatures and modified atmosphere packaging, do not represent an obstacle to a considerable number of yeasts. With these preservation techniques, the vast majority of bacteria (competitors) are culminated, giving yeasts the competitive edge in the medium (food). We have studied the possible contribution of several yeasts to seafood spoilage, with special emphasis on the production of biogenic amines.

CHARACTERISTICS OF BREADED POPCORN SHRIMP PACKAGED IN MODIFIED ATMOSPHERE THERMOFORMED CONTAINERS DURING SEVEN MONTHS OF FROZEN STORAGE

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This study investigated the effects of modified atmosphere packaging on the quality and shelf life of frozen breaded popcorn shrimp held in an upright retail display freezer for seven months. Popcorn shrimp were packaged in the following containers: (1) the current retail pack, a 2-mil polyethylene bag placed in a ½ mil polyethylene-coated paper carton; (2) a medium barrier clear nylon-polyethylene thermoformed container under air, (3) a high barrier clear nylon-saran-polyethylene thermoformed container under air; (4) a medium barrier container under CO₂; and (5) a high barrier container under CO₂. The following analyses were completed: (1) percent O₂ and CO₂ (2) Hunter L, a, b; (3) aerobic plate counts; (4) TBA; (5) FFA, and (6) odor, taste and appearance sensory analyses. The CO₂ MAP high and medium barrier thermoformed packages reduced moisture loss when compared to control polyethylene-coated cartons and polyethylene bags. Shrimp packaged under a CO₂ atmosphere in both high and medium barrier thermoformed packages maintained significantly elevated CO₂ levels during five months of frozen storage. Oxygen levels remained low in CO₂ packaged shrimp. Fresh control moisture levels were not significantly different from percent moisture in CO₂ packaged shrimp at the end of five months. The FFA correlation coefficients for CO₂ packaged shrimp were lower than those found for shrimp held under air in high and medium barrier films, and the industry standard poly-bag/poly-box. FFA production maybe reduced by MAP packaging. All packaged shrimp except shrimp held under CO₂ in high barrier thermoformed packages produced a strong correlation relationship between time and increasing TBA values. The high barrier CO₂ package reduced the rate of rancidity development as defined by the TBA value. Thermoformed packages with CO₂ atmospheres appeared to reduce moisture changes in popcorn shrimp and slow rancidity development as measured by TBA levels.

An ammonia electrode was connected to a computer through an analog-to-digital converter. The electrode quantified ammonia in the headspace of intact shrimp kept in an air-tight box. No sample preparation was required except for NaOH to be sprayed in the shrimp. Less than two minutes were needed to obtain results. The measurement of ammonia in the headspace was comparable to that of wet chemical methods. Ammonia levels increased during storage time, and were correlated with a sensory panel, who determined that the level of ammonia at which the shrimp should be rejected is 230 ppm. This procedure could be used as a fast, simple and objective method in the evaluation of shrimp quality.

STORAGE CHARACTERISTICS OF CRYOGENICALLY AND BLAST FROZEN BLUE CRAB MEAT HELD IN IMPROVED PACKAGING FOR TEN MONTHS OF STORAGE - A PRELIMINARY REPORT -

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Cryogenic and blast freezing of special blue crab meat held in improved packaging materials were investigated. The experimental packages were (1) vacuum packaged Cryovac boil-in-bags, (2) Dynopack trays with an air atmosphere, and (3) Dynopack trays with a nitrogen atmosphere. Packaged meat was either frozen in a commercial carbon dioxide tunnel maintained at -54°C or in a commercial blast freezer held at -19°C. Crab meat packed into Crown, Cork, and Seal Co., Inc. 307 x 401 steel-tin cans and pasteurized at 85°C for 2 hours to an F_{165}^{16} 40.3 minutes served as the control for the study. Frozen crab meat was transferred from the commercial blast freezer to a walk-in freezer and held at -12°C for ten months. Pasteurized control meat was kept on ice in a walk-in cooler for the duration of the study. The following analyses were completed at 1, 2, 4, 6, 8 and 10 months of storage: headspace oxygen and CO₂ levels, ammonia concentrations, pH, aerobic plate counts, percent moisture, and Hunter L, a, b color appearances, odor, taste, and texture profiles for the meat. The profiles used a continuous scale from zero to ten with zero being none detected and ten representing the strongest possible response. Visual attributes evaluated by the panel included: color, general appearance, pearlescence, and wet/dry appearance. Meat odor attributes were: ammonia, cooked crab odor, cereal odor, freezer odor, musty odor, and off odors. The panel evaluated several flavors: crab flavor, astringent taste, sour taste, stale/freezer taste, old seafood taste, cereal taste, sweet taste, and off-flavors. Texture analyses included: moistness, chewiness, fibrousness, adhesiveness, and particle size.

BIOGENIC AMINES IN SEAFOOD

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The presence of certain biogenic amines in seafood have been used as indicators that decomposition of the product has occurred. These compounds, which include histamine, cadaverine and putrescence, can be retained in the product even after volatile organoleptically detectable compounds have been removed by processing or masked by processing aids. These compounds have also been used as markers of fish that may cause a clinical toxic syndrome which includes such signs and symptoms as erythema, facial flushing, headache, nausea, abdominal cramps, diarrhea, vomiting and dizziness. Such fish are also thought to be involved in episodes of toxicity and weight loss in poultry. As one result of the move to formalized HACCP plans, the development of rapid tests has accelerated, especially for histamine. The formation and levels of those compounds in seafood and their advantages and limitations as components in quality control programs will be discussed.

IMMUNOANALYSIS OF HISTAMINE

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High-affinity, specific monoclonal antibodies were obtained against derivatized histamine. We demonstrated that immuno-recognition of histamine is modified with derivatization, pH and temperature. In order to determine histamine in human plasma, the first histamine immunoassays were designed and optimized to reach maximum sensitivity (0.2 nM~2 pg/ml, for the Immunotech radio-and enzyme **immunoassay**). Such great sensitivity is probably not necessary for histamine determinations in food. When selecting the monoclonal antibody for the immunoassays, specificity was emphasized. Comparison with other monoclonal antibodies, described in the literature, shows that this was not always the case. Today, we offer very sensitive and specific histamine immunoassays. The histamine values obtained with these kits correlate well with fluorimetric and radioenzymatic histamine assays.

PER CAPITA FISH AND SHELLFISH CONSUMPTION IN FLORIDA

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A comprehensive survey of seafood consumption by Florida residents was recently completed at the University of Florida. The survey solicited information on away-from-home and at-home consumption by volume of seafood by species, product form (recreational and commercial), preparation method, and product source. **Annual** consumption by race, income level, age, and gender was estimated. The methodology employed was an aided recall technique designed to estimate the away-from-home consumption (total portion size) for the adult respondent and at-home consumption for all household members during the previous seven-day period. A telephone survey was utilized to sample 8,000 households statewide and 1,000 additional households within a five-county region of north Florida where contaminants from paper mills is prevalent within local streams and rivers. A total of 500 face-to-face interviews were conducted at Health and Rehabilitative Services offices within five counties in an attempt to collect data on low income consumption. Finfish represented the majority of consumption reported. Consumption of seafood was positively related to income levels. The majority of finfish and shellfish consumed was of saltwater origin. Asian and American Indians reported the highest levels of seafood consumption. Total annual per capita seafood consumption was estimated to be as high as 4 1.89 pounds. The study provides strong evidence that Floridians consume more seafood per capita than previously assumed on the basis of published estimates for the U.S. in general.

ANALYTICAL METHODS TO ENSURE SEAFOOD SAFETY AND SEAFOOD QUALITY

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Analytical methods for compounds associated with seafood toxicity or poor quality pose considerable challenges. The methods must perform well on a wide variety of species and products of interest without compromising speed, ruggedness, and specific forms of specificity. High sensitivity, although required for the detection of toxic compounds such as ciguatoxins, other potent marine toxins, and trace analytes, is not sufficient. In monitoring and regulatory applications the complexity and variability of toxin mixtures and decomposition profiles indicate that even detection methodology which is highly specific may actually fail. The specificity must be directed towards the property of interest, such as odor or health risk. High tolerance for sample matrix components is also crucial; time-consuming sample workup procedures bog down otherwise rapid analyses. Elements of successful testing methods for seafoods will be discussed from the standpoint of our current knowledge of seafood toxins and quality indicators.

APPLICATION OF MICROPLATE RECEPTOR ASSAYS TO THE DETECTION OF BIOTOXINS IN SEAFOOD

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The Center for Disease Control has estimated that more than 50% of all seafood-borne outbreaks in the U.S. are the result of naturally occurring biotoxins of algal origin. Most classes of biotoxin in seafood occur as suites of structurally related compounds which possess different toxic potencies and which are present in varying proportions in contaminated seafood. Thus, for regulatory purposes, the analysis of total toxin content is not so critical as it is the measurement of the combined toxic potency. Receptor assays are suitable for measuring combined toxic potency since, within a toxin class, all congeners bind to the same receptor and relative binding affinities correlate well with their relative toxic potencies. Because the binding affinities of seafood toxins for their receptors are in the nM range, the detection limits of receptor assays are the nanogram level. We have developed microplate receptor assays for toxins implicated in ciguatera fish poisoning (CFP), neurotoxic shellfish poisoning (NSP), paralytic shellfish poisoning (PSP), and amnesic shellfish poisoning (ASP), and report here on the performance of these assays relative to the mouse bioassay and to HPLC analysis. Modifications of the traditional receptor assays to the microplate format and the use of microplate scintillation technology provides for high sample throughput and rapid analysis time (three hours), with sufficient automation to be applied to routine sample analysis.

APPLICATION OF CELL-BASED ASSAYS FOR DETECTION OF CIGUATOXINS AND RELATED TOXINS

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Assuring quality and safety of seafood production requires the availability of test methods that are accurate predictors of potential risk to the consumer. The quality of these test methods are expressed by attributes including: correlation with *in vivo* toxicity, reproducibility, time to complete the test, amount of sample necessary, and the degree of sample preparation required. We have demonstrated that cell-based bioassays are effective biological screening tools for ciguatoxins and other toxins that interact at the level of the voltage-gated sodium channel. Furthermore, these *in vivo* methods exhibit many of the preferred embodiments of a toxicity test method and may prove to be suitable alternative to the mouse bioassay. The practical application of screening for ciguatoxicity by cell bioassay will be discussed.

METHODS FOR DETERMINATION OF CIGUATOXINS

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The performance of 4 methods was evaluated for the determination of ciguatoxins: **LC/MS**; *in vitro* **brevetoxin** competitive binding; *in vitro* cytotoxicity; and mouse implicated in ciguatera outbreaks. **LC/MS** identified Caribbean ciguatoxin (MH+ 1123.6), in 4 tissue samples also containing the highest levels of toxicity determined by binding competition and cytotoxicity assays. *In vitro* assays detected lower level toxicity in 2 additional samples not shown by **LC/MS** to contain ciguatoxin. Mouse bioassay was clearly positive for 2 of the samples found toxic by *in vitro* methods. Nonlethal responses were observed from 5 other samples, including those found toxic *in vitro* and by **LC/MS**. *In vitro* assays provided greater sensitivity and specificity than mouse bioassay but quantitative differences between laboratories ranged up to one order of magnitude reflecting systematic error. Mean ciguatoxin concentrations estimated by binding assays ranged from 1.7 to 21.3 ppb. Cytotoxicity estimates ranged from 0.6 to 5.4 ppb. **LC/MS** which identified only C-CTXI provided estimates ranging from 0.7 to 1.3 ppb. The study documents improved capabilities for screening and confirmation of ciguatoxins.

OXYGEN-DE RIVED FREE RADICALS AND LIPID PEROXIDATION AS POTENTIAL CELLULAR MECHANISMS INVOLVED IN CIGUATERA FISH POISONING

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Toxins produced by marine dinoflagellates are among the most potent no-proteinaceous poisons known. The toxic dinoflagellate *Ostreopsis lenticularis* grows in coral reefs which exhibit substantial daily variability in oxygen tension (pO_2). Changes in pO_2 is thought to be a factor influencing cellular levels of oxygen-derived free radicals which can in turn modify the structure of certain ciguatera toxins; polyether compounds which are highly oxygenated. The initial objective of these studies was to determine whether pO_2 regulated the toxicity of *O.lenticularis*. A 2.9-fold increase in toxicity (LD_{50}) was found in extracts obtained from cells cultured at 63% pO_2 relative to normoxic (21% pO_2) controls. Four oxygen-detoxifying enzymes (superoxide dismutase; SOD, catalase, ascorbate peroxidase and glutathlone peroxidase) were found to be present in the supernatant fractions of the normoxic and hyperoxic *O.lenticularis* cell-free extracts assayed. The specific activities of SOD and catalase increased 10.7-fold and 8.6-fold in cells cultured under hyperoxic conditions. These data indicated that the increased toxicity of was regulated by enhanced oxidative stress resulting from exposure to elevated ambient pO_2 . Subsequent studies, examined the role of lipid peroxidation in the increased toxicity of *Olenticularis*. This species after being cultured under hyperoxic conditions, showed a mean increase of 42% in lipid peroxidation [measured as malonaldehyde (MDA)-TBARS] relative to normoxic controls. Mice were injected (ip) with one sublethal dose of pure preparations of either ciguatoxin 1, maitotoxin 2, brevetoxin 2, or saxitoxin and sacrificed at 12, 24, 48 and 72 h after injection. A highly variability (0.003-24.10) in the MDA ratio (experimental/control) was found in the livers (cytosolic fraction) of these animals which depended on the type of toxin and the time in which samples were obtained. Cytosolic preparations of livers (n=3) of large (5.9-6.8 kg) highly toxic barracuda also showed a mean increase of 85% in MDA when compared with livers (n=2) from small (1 kg) and presumably non-toxic barracuda. Hyperoxia may cause an increase in peroxidation of polyether toxins present in *O. lenticularis* which may also result in an increased peroxidation of polyether toxins present in *O.lenticular* which may also result in an increased peroxidation of membrane phospholipids. Similarly, exposure of fish and humans to these toxins may cause elevated levels of liver lipid peroxidation.

TESTING FISH AND SHELLFISH: PRACTICALITY, TECHNICALITY, AND REALITY

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Toxins in seafood represent an ever increasing human health hazard and an industry liability. At the heart of the issue is a conflict between minimization of human intoxication risk, and concurrently a maximization of the fisheries resource utilization. Tests based on either chemistry or biology have been employed to detect a variety of contaminants in food products. Receptor based assays are exquisitely sensitive for detection, specific for toxin type, provide rapid results, are relatively insensitive to tissue (matrix) effects, and can readily be reduced to tests which offer color changes as the "reporter". Sodium channel tests for brevetoxins and ciguatoxins, protein phosphate tests for okadaic acid, and saxiphilin tests for saxitoxin will be discussed.

AQUATIC BIOTOXINS: PREVENTION, CONTROL AND SEAFOOD SAFETY MONITORING PROGRAMS

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Although the potential risk associated with aquatic biotoxins have been well documented, there is an increased need to obtain further information for the development and implementation of an international risk management plan. Aquatic biotoxins are distributed worldwide and have caused a great variety of acute and chronic syndromes. The challenge for international research institutes and regulatory agencies will be the development of seafood safety programs that will reduce the occurrence of acute toxicity syndromes and in the long-term, reduce chronic risk associated with consumption of foods from aquatic resources. The development of monitoring programs which would include analytical and decontamination procedures should be an international goal.

Shellfish and finfish have become important terms in the world's food supply. Several factors should be considered in the development of a seafood safety program in a given country: public health significance of the contaminant effects on the countries' economy, legal infrastructure, analytical resources, producer/consumer education, and the effectiveness of communication systems. An effective seafood safety program must include the ability to monitor fish/shellfish harvesting areas, the establishment of regulatory limits or a level of concern for the toxin(s), the ability to screen for suspect fishery products in the marketplace, and the management of unsafe fishing/harvesting areas and products. These programs are designed to identify toxic or high risk products and divert them into less-risk uses and allow acceptable product to proceed in commercial channels.

The components of the development of safety monitoring program are the following: determine commodity(s) and aquatic biotoxin(s) of concern for the program, evaluate analytical capabilities for each commodity(s)/aquatic biotoxins (s), determine appropriate monitoring program components, establish sampling plan, and establish regulatory policy for violative product.

Efforts should be made to develop and maintain international collaborative links and information exchanges. Early identification of the toxicologic syndrome is necessary to permit effective therapeutic intervention. Rapid and simple tests should be developed and used to screen potentially hazardous fish or shellfish at the point of harvest to reduce costs to the fishermen and to protect the consumer from toxins and dangerous contaminants. For most physotoxins, the establishment of adequate seafood safety program has been hampered due to the lack of adequate standards and analytical methods which could be used for monitoring the presence or absence of these toxins in fish or shellfish at various points in commercial channels. Once recognized, proper and prompt reporting will alert officials agencies to implement regulatory directives and prepare a well-defined plan for biotoxin standards isolated or synthesized. Educational programs for safe preparation and service of seafood in commercial and homesettings must also be developed and delivered as a part of a integrated seafood safety monitoring program.

WHAT YOU SEE IS WHAT YOU GET

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Ciguatera affects multiple organ systems having impressive gastrointestinal, neuromuscular, cardiovascular, dermatological, genitourinary and emotional components. Onset of symptoms is usually between 15 minutes to 30 hours of fish ingestion, ushered in typically by gastrointestinal upset including hypersalivation, nausea, abdominal cramps, vomiting and watery diarrhea. Neuromuscular manifestations usually follow, including dysesthesias of the extremities, itching, either generalized or of the palms and soles, or after alcohol, numbness, prickling and burning sensations, and temperature reversal, where cold feels like hot. Headaches, vertigo, dizziness, circumoral tingling, metallic tastes, dry mouth, pain or tingling or a "loose" sensation of the teeth are often complained of. Cranial nerve palsies, aphonia, ptosis, or miosis may occur. Pain in the muscles and joints, low back pain, muscle stiffness or spasm or fasciculations are not uncommon. Carpopedal spasm, trismus, menigismus, opisthotonus, weakness, ataxia, muscular incoordination and inability to stand have all been reported. Hyporeflexia, areflexia, stocking and glove hypesthesia or numbness, malaise or profound fatigue, stupor, peripheral flaccid paralysis, respiratory failure, generalized seizures and rarely, death may occur. Cardiovascular manifestations include any combination of hypotension, bradycardia, hypertension, tachycardia, arrhythmias, heart block, pulmonary edema and congestive heart failure. Dermatologically, the patient may develop rash, erythema, sweating or loss of hair and nails. Genitourinary signs include painful urination (dysuria), pain in the perineum, penis or vagina and pain in the penis during erection. This has been reported to be intensified during ejaculation and transmitted to the female partner causing painful intercourse (dyspareunia). The toxin can cross the placental barrier causing hypoactivity of the fetus by sonography and transient hypoactivity of the newborn. The toxin can also be secreted in the breast milk causing hypersensitivity of the nipples and interfere with breastfeeding. Emotionally, the patient may be depressed, hyperexcitable, anxious, nervous, giddy, apprehensive and restless, progressing to shouting. Hysteria, delirium, hallucinations and irrational behavior may be seen.

After the acute phase has passed, many bothersome symptoms may linger on for months. This "chronic ciguatera" includes itching after alcohol, persistent dysesthesias, sensitivity to cold, loss of energy, fatigue, malaise, and depression, among others. Chronic fatigue has also been linked to other toxic polyethers and ciguatera should be considered in the differential diagnosis. Two cases of biopsy proven polymyositis have been reported occurring several years after ciguatoxin exposure.

The disease is vastly under-diagnosed and diagnosis is made almost entirely by history, and usually in retrospect. There are few physical findings, none of which are diagnostic. Re-exposure may cause significant worsening of symptoms. There are no formal criteria for diagnosis. A high index of suspicion is needed and one must have heard of the disease to consider the diagnosis. Any unusual combination of gastrointestinal, neuromuscular, cardiovascular, dermatological, genitourinary or emotional symptoms should arouse the clinical suspicion to inquire if fish had been ingested. The initial gastrointestinal phase may be the only manifestation, or only neurological involvement may occur. Occasionally, only the inverted sensory phenomenon, or itching after alcohol are seen, with no other symptoms. The usual patient is quite uncomfortable.

LEGAL ASPECTS OF CIGUATERA FISH POISONING IN PUERTO RICO

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In Puerto Rico for many years any person who became intoxicated with ciguatera by eating fish in a restaurant was certain to obtain compensation for damages from the restaurant in which the plaintiff had eaten the fish. The Supreme Court of the Commonwealth of Puerto Rico had applied legal precedents in cases of food poisonings that did not require that a poisoned patron had to prove negligence on the part of the restaurant in order to obtain damages. Thus cases, claiming intoxication by ciguatera were generally settled out of court. As a consequence of this rather unjust or unfair situation with respect to the restaurant owners, two test cases were put before the courts regarding ciguatera fish poisoning. Subsequently, the Supreme Court supported the conclusion of the courts that fishermen, distributors and restaurant owners could not be held legally responsible for providing ciguatoxic fish given that there were no known practical methods to detect ciguatoxicity prior to human consumption.

***VIBRIO VULNIFICUS* RESEARCH AT GULF COAST SEAFOOD LABORATORY**

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Research during 1994-95 has focused on the hazards associated with *Vibrio vulnificus* in Gulf Coast oysters. The major objective has been to gather information on infectious dose and presence of specific strains that may increase the risk of *V. vulnificus* illness in humans consuming raw oysters. Weekly environmental sampling from areas traditionally implicated in human cases has produced a number of samples linked to cases in 1994-95. *V. vulnificus* has been enumerated and strains have been phage typed to determine any differences in these implicated samples.

Other significant research involves the evaluation of rapid DNA probe methods for enumeration of *V. vulnificus*, the effects of temperature abuse on post-harvest multiplication and studies of remediation involving relaying of oysters to high salinity areas and freezing of oysters.

DEVELOPMENT OF NEW METHODS FOR THE DETECTION OF PATHOGENS IN OYSTER TISSUES

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The detection of *Vibrio* spp. in the environment is difficult as the result of the lack of selectivity of the culture media used. The high concentration of the non-specific microbiota (especially **H₂S-producing** microorganisms) makes the easy and rapid isolation of the target microorganism almost impossible. Similarly, the protozoan parasites *Giardia* spp. and *Cryptosporidium* spp. which are emerging enteric pathogens have not previously been detected in oyster tissues, possibly as a result of the lack of methods for their recovery. This project presents data on the modifications to the currently used medium for the detection of *Vibrio* spp. in environmental waters as well as the development of a method for the detection of *Giardia* spp. in oyster tissues.

Thiosulfate-Citrate-Bile Salts (TCBS) agar was supplemented with 3%, 6% and 8% NaCl and the pH was buffered at 8.6 with Tris buffer (0.05 M final concentration). The same modifications were done to Alkaline Peptone Salt Broth to be used directly in the Most Probable Number technique. These modifications have allowed us to detect and isolate *Vibrio* spp. in estuarine water samples and oyster tissues. The pH conjointly with the high NaCl concentrations inhibited almost 100% of the background microbiota, thus significantly reducing the masking of the target organisms. This makes the modification easily adaptable for the screening of waters and oyster tissues for the presence of enterotoxigenic *Vibrio cholerae* strains as well as other important *Vibrio* spp. (such as *V. vulnificus*) using strain-specific gene probes.

The development of a method for the detection of *Giardia* and *Cryptosporidium* in oysters has enabled us to detect these pathogens in oysters collected from contaminated sites and at least in one case from market-brought oysters. Studies are under way to determine if oysters can in fact serve as vectors of these pathogens.

HACCP & 3M PETRIFILM PLATES IN THE SEAFOOD INDUSTRY

Ivonne Ruiz-Garcia

3M Corporation

Puerto Rico

The 3M Petrifilm plates are ready to use microbiological plates which have been approved by several countries regulatory agencies. Different plate types determine the amount of aerobics, coliforms, E.coli, Yeasts and molds on raw material and finished goods in the seafood processing plants.

Worldwide scientific papers demonstrated that the conventional method and the 3M Petrifilm plates are statistically equivalent.

**BRETTANOMYCES CLAUSSENII AS A CONTRIBUTOR IN
YELLOWFIN TUNA SPOILAGE**

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The role of yeasts in seafood spoilage is probably being underestimated. We have isolated several histidine decarboxylating yeasts from seafood, one of them identified as Brettanomyces claussenii. Common chemical indicators of spoilage were used in our study in order to establish the degree of decomposition in yellowfin tuna due to this yeast.

The ability of B. claussenii to produce ethanol, total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) at different temperatures (5° C, 25° C, 37 ° C) determined over a period of 14 days. Proteolytic activity was also determined.

The threshold value of spoilage for TVB-N (30mg/100 g) was reached between the second and fourth day of storage at 25 ° C and 37° C. Under the same conditions, TMA-N concentration was 10 mg/100 g. TMA and TVB-N concentration at 5 ° C, increased after seven days. Proteolysis was observed after 24h at 25 ° C and 37° C. This shows yeasts as contributors of spoilage at these temperatures.

**EFFECTS OF TEMPERATURE AND HUMIDITY ON SURVIVAL
OF *MACROBRACHIUM ROSENBERGII*, TRANSPORTED IN A
NON WATER ENVIRONMENT**

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A series of studies were undertaken to develop a method that could be used to maintain recently harvested, stressed freshwater shrimp (*Macrobrachium rosenbergii*) alive out of water for an extended period of time to facilitate transport under commercial conditions. Survival in the dry state was tested in foam containers at three temperatures (5, 17 or 27°C), using three packaging materials after the animals were submitted to a low temperature pretreatment to induce semi-hibernation at with 5 or -10°C. The best survival was obtained for shrimp that were pretreated before placing in foam containers were 10% for the first 12 hours of storage dropping to 17,12 and 12% after 18, 26 and 31 hours, respectively. Since 21% of the harvested shrimp routinely died prior to receiving the cold pretreatment, the above mentioned results could be improved by decreasing the stress the harvested shrimp received as they were transported (for 45 minutes) from the pond area to packaging site.

Lactic acid levels in the tails of adapted, via the cold pretreatment, and nontreated shrimp were measured as a function of survival time. Using analysis of variance and linear regression there difference ($p < 0.05$) in lactic acid levels with survival time in the pretreated shrimp, however, there were significant difference ($p < 0.05$) in lactic acid levels with survival time in the pretreated shrimp, however, there were **significant** differences ($p < 0.05$) encountered in the nontreated group. For the cold pretreated shrimp the r^2 for the relationship between survival time and lactic acid concentrated was an 0.063 when $n = 18$, while for **the** nontreated shrimp it increased to 0.519 when $n = 8$.

The results of the study to date indicate that it is possible to take shrimp directly from a production pond, induce a semi-hibernation state and package for live transport without water with no apparent changes in muscle lactic acid levels, and consequently, maintaining a high quality product.

USE OF ANTIOXIDANTS IN FRESH AND FROZEN SEAFOOD PRODUCTS

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Fresh and frozen fish consumption is increasing as consumer limit their intake of red meats and saturated fats. High in unsaturated omega-3 fatty acids, fish is highly susceptible to oxidative degradation, exhibiting off-odor, color and taste upon oxidizing. Adding antioxidants to fish products increases the oxidative stability of fish products.

An antioxidants effectiveness in delaying oxidation depends on its volatility and stability at different temperatures. Autoxidation of a fat in a fish is a very complex occurrence influenced by myriad of factor, such as moisture, pH, saturation of the fat and processing temperatures. Antioxidant efficacy varies in different applications, and may be enhanced by the addition of synergists. Synergism occurs when the effect of an antioxidant and another material is greater than the sum of the effect of either material is greater than the sum of the effect of either material alone. Synergists such as citric acid, ascorbic acid, tocoferols are incorporated with antioxidants to enhance stability.

CONTINUOUS IMPROVEMENT OF A HACCP PROGRAM AN UPDATE OF THE NMFS HACCP-BASED INSPECTION PROGRAM

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The National Marine Fisheries Service unveiled its HACCP-based Inspection Program in July of 1992. Since that time a number of policy and procedural changes have occurred. These program modifications were brought about for several reasons, political needs, enrolling the field inspector, the concepts of the industry and the consumer. Specific procedures for aquaculture, the vessel operator, the retailer, and for food service systems have helped in making the NMFS HACCP-based Inspection System truly a harvest to table program. New concepts and ideas have also been utilized in transforming the inspector to the system evaluator. Finally, the Inspection Services Division is looking to the future as to what other changes must be made to keep the program on the cutting edge.

NFI PERSPECTIVE ON HACCP

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HACCP FOR MULTINATIONAL CORPORATIONS

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Hazard Analysis for Critical Control Points (HACCP) program was adopted by many large corporations. World-Wide **Kraft, Inc.**, the largest food company in the USA, is actively implementing HACCP programs in all its operations world wide. Cross functional teams from the legal area, Scientific Relations and Quality Assurance were formed to refine, harmonize and establish the corporate policy for HACCP. A decision tree was derived to identify the **CCP's**. Factors such as acute vs. chronic, risk assessment vs. risk management are among the criteria used in constructing the decision tree. **WW Kraft, Inc.** also established the "Safety Pyramid" for food processing in which other "prerequisite" safety programs are mandatory for HACCP to achieve the expected food safety.

THE HACCP SYSTEM AT DARDEN RESTAURANTS, INC.

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In the 1960's, Pillsbury developed the first HACCP system for NASA to ensure that all critical food safety **checkpoints** were identified, monitored, verified, and documented during the production, packaging, transport, and use of all foods. The purpose was to prevent foodborne illness while the astronauts were in space.

This **successful** system is not only used in food production and manufacturing industries, but in the **foodservice** industry as well. In the restaurant industry, HACCP helps ensure food safety and prevent foodborne illnesses. It also results in less food waste, labor hour savings, higher food quality, and overall cost constraints.

At Darden Restaurants, Inc., hazards were identified by product type, food storage practices, production techniques, cooking methods, and cooling/reheating procedures. Depending on the degree of concern, controls were then implemented to eliminate, prevent or reduce the identified risk.

The key to the success or failure of this system is to ensure that the employees and management have the proper forms and tools (calibrated thermocouples) to do the job correctly. Equally important to a successful system is the documentation to monitor/verify at each of the identified critical control points. Examples of this are the temperature checks of different foods at different locations every two hours and corrective action taken when a problem is discovered.

INTERNATIONAL ASPECTS FOR HACCP

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HACCP is rapidly becoming recognized as the premiere food safety control system. Internationally, HACCP is well accepted. Nonetheless, HACCP from a regulatory perspective is still evolving and as such, organizations as the International Codex Alimentarius Food Standards Programme, the International Life Sciences Institute, the International Commission on Microbiological Specifications for Foods, and the European Union will play a major role in shaping the HACCP evolution of the future. This paper will describe the aforementioned international agencies' role in HACCP to date and forecast their future activities in sorting out the broader aspects of HACCP for international food trade dealing with a wide variety of issues such as the safety vs. quality concerns; mandatory U.S. voluntary considerations; the role of risk assessment in the Hazard Analysis equation; defining the role of regulatory agencies; determining HACCP performance requirements and benchmarking those requirements; training requirements and industry assistance scenarios; and the role of third party certification organizations in HACCP verifications.

LABORATORY ROLE IN A HACCP-BASED INSPECTION PROGRAM

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The National Seafood Inspection Laboratory (NSIL) conducts and coordinated chemical, physical, and microbiological sample analyses to provide measurement and indices of seafood product quality and safety to the U.S. Department of Commerce, National Marine Fisheries Service (USDA/NMFS) National Seafood Inspection Program(NSIP).

The Laboratory serves an important verification role ensuring that under the HACCP-based system, plants are producing safe, wholesome products. Important components of a successful laboratory testing program include: representative sample selection, aseptic sampling techniques, sample shipment, chain of custody procedures, sample security, reporting and interpretation of results. These components will be discussed during the presentation. In addition, **results** will also be shown from the Laboratory's Regional Analytical Surveillance Testing Program, the recent Retail Market Surveillance Program, and NSIP's HACCP-based analytical program.

SEAFOOD HACCP ALLIANCE TRAINING AND EDUCATION PROGRAM: AN UPDATE

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The **activities** of the Seafood HACCP Alliance have now advanced through the production of a core **training** manual **with** model HACCP plans to serve as classroom practicals. The core program is based on the 7 basic steps in developing HACCP programs for food safety. The Alliance approach is consistent with **the 1994** recommendations of the National Advisory Committee on Microbiological Criteria for Foods and the expected FDA mandates for HACCP programs for processing, handling and importing fishery products. In the core manual, individual quick frozen, cooked shrimp were selected to illustrate the progressive development of a HACCP plan. The practicals include HACCP plans for processing oysters, vacuum packed smoked mackerel, dried shrimp, pickled fish and pasteurized blue crab meat. These selections were based on concerns for various processing procedures rather than the extent of commercial activity.

In keeping with the objectives of this national Sea Grant funded project (1994-97) the training materials will be used to prepare a cadre of trainers with uniform programs of instruction aligned with the various Association of Food and Drug Official (AFDO) affiliates about the United States. The training programs are designed for a dual audience of inspectors and commercial interests. The first trial training program is scheduled for December 4-6, 1995 in Washington, DC.

PROBLEMS ON IMPLEMENTING HACCP IN THE FOOD INDUSTRY

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Hazard Analysis Critical Control Points (HACCP) is apparently an already familiar term in the seafood and food processing industries. The understanding of the HACCP concept by **the** food industry **appears** to be straight forward and the method is actually embraced due to its simplicity and potential benefits to control food safety.

A major problem that may exist at this time is the actual implementation by small and medium size industries. Very often the implementation of HACCP is **"Put** on the back burners' or placed down in the priority list, companies are waiting for final ruling by the USDA or the FDA, or simply, companies do not have the resources to be specifically assigned to implement HACCP.

Other problems is the misuse of basic HACCP terminology, including the term "Critical Control Points" (**CCP's**), resulting in some confusion about the importance of performing a very detailed and methodological analysis to establish such **CCP's**.

Some simple suggestions are presented to begin HACCP implementation for a company that has HACCP plans already developed.

