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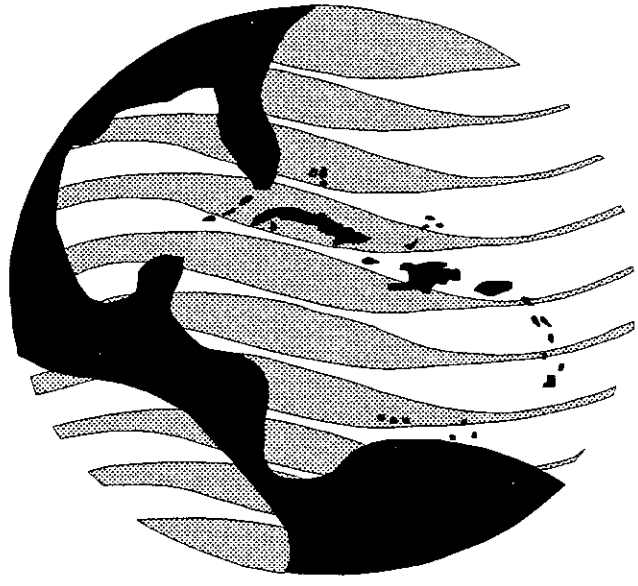
NINETEENTH and TWENTIETH ANNUAL CONFERENCES

TROPICAL AND SUBTROPICAL

SEAFOOD SCIENCE AND TECHNOLOGY SOCIETY

OF THE AMERICAS



Papers & Abstracts

19th Annual Conference
New Orleans, LA
September 11-13, 1994

20th Annual Conference
Humacao, Puerto Rico
November 5 - 8, 1995

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The PROCEEDINGS are a compilation of papers presented at an Annual Conference of the Tropical and Subtropical Seafood Science and Technology (SST) Society of the Americas. The previous (before 1994) name of the organization was the Tropical and Subtropical Fisheries Technological Society of the Americas. Joint proceedings have been held with the Atlantic Fisheries Technological Society (AFT) and the Gulf and Caribbean Fisheries Institute (GCFI). Some presentations are included only as abstracts based on the author's request. The intent in issuing this PROCEEDINGS is to disseminate results from ongoing research and advisory services, and commercial and regulatory issues. This intent will facilitate communications between regional investigators, industries, and government entities.

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NINETEENTH and TWENTIETH ANNUAL CONFERENCES

TROPICAL AND SUBTROPICAL SEAFOOD SCIENCE AND TECHNOLOGY SOCIETY OF THE AMERICAS

(previous Tropical and Subtropical Fisheries Technological Society of the Americas)

September 11-13, 1994
Monteleone Hotel
New Orleans, LA

November 5-8, 1995
Palmas del Mar Resort
Humacao, Puerto Rico

Proceedings compiled by:
W. Steven Otwell, Ph.D

The Tropical and Subtropical **Seafood Science and Technology Society of the Americas** (acronym "SST Society") is the new name for the previous Tropical and Subtropical Fisheries Technological Society of the Americas. This change was discussed and unanimously adopted by the SST Executive Committee during their 18th annual conference in Williamsburg, Virginia, September 1, 1993. This change was considered necessary to better reflect the intent and practice of the Society. Likewise, this new name better focuses and communicates the Society's future plans which are mindful of the increasing concerns for seafood product quality and safety. SST's concern for more technical exchange realizes the growing importance of the neighboring countries that share resources, common problems and opportunities. It is hoped that this name change will be recognized by mutual concerns and interests throughout the tropical and subtropical regions of South, Central and North America.

The Tropical and Subtropical Seafood Science and Technology Society of the Americas is a professional, educational association of aquatic food product technologists interested in the application food science to the unique problems of production, processing, packaging, distribution, storage and preparation of the tropical and subtropical species be they harvested, cultured or fabricated for domestic or international commerce. Their principle objective is to advance the use and understanding of applied and basic science as relates to product quality and safety. Their membership is open to commercial interest, government agencies, academic expertise and students. Their topics typically involve products and settings about the Gulf of Mexico and South Atlantic regions from Texas through Virginia and including the Caribbean Basin and countries about the Gulf of Mexico. They welcome and encourage participation from similar tropical and subtropical regions about the world.

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AN OVERVIEW OF THE GULF OF MEXICO PROGRAM INITIATIVES IN RELATION TO SEAFOOD

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Gulf of Mexico Propram Background

In the summer of 1984, the U. S. Environmental Protection Agency (EPA) held a public meeting in Brownsville, Texas on the subject of incineration of toxic waste at sea. The large turnout of concerned citizens made it clear that the public was interested in the well-being of the Gulf of Mexico. The response of these citizens, coupled with indications of environmental degradation in the Gulf, prompted the EPA, Region 4, to propose a Gulf-wide strategy termed "the Gulf Initiative." The intent of this initiative was to identify Gulf-wide problems and set goals and research needs while developing a strategy to respond to those problems. During an initial Gulf Initiative Workshop held in Gulf Shores, Alabama in August of 1986, 59 persons, representing a broad spectrum of organizations concerned with marine pollution, identified critical issues and activities causing problems in the Gulf, made recommendations for a program management structure, and provided a strong consensus that such a program was needed.

In August of 1988, the Gulf of Mexico Program was established as an intergovernmental program at Stennis Space Center, Mississippi under the sponsorship of the EPA.

On December 10, 1992, eleven federal agencies, the governors of all five Gulf Coastal states and the Citizens Advisory Committee (CAC) chairman, signed an agreement for cooperation to build the support and obtain the resources necessary to meet nine environmental challenges within five years and to attain the long-term goal of protecting the Gulf of Mexico. Those federal agencies included the EPA; Soil Conservation Service (SCS); National Oceanic and Atmospheric Administration (NOAA); Fish and Wildlife Service (FWS); National Park Service (NPS); Food and Drug Administration (FDA); National Aeronautical and Space Administration (NASA); U.S. Army; Department of the Navy; U.S. Air

Force. and the U.S. Coast Guard. This symposium signatory document was termed "A Partnership for Action."

The goal of the Gulf of Mexico Program is to protect, restore, and enhance the coastal and marine waters of the Gulf of Mexico and its coastal natural habitats; to sustain living resources; to protect human health and the food supply; and to ensure the recreational use of Gulf shores, beaches and waters in ways consistent with the well-being of the region.

Program Purpose

The main purpose of the Program is to develop and implement an agenda for protecting, restoring, and maintaining the health and productivity of the Gulf and ensure that the waters and products of the Gulf are safe for use. Such a strategy should achieve a balance between the impacts and demands of human-related activities and the preservation and enhancement of the marine resources of the Gulf.

Program Framework and Implementation

By building on, enhancing, and coordinating existing programs and missions the Gulf of Mexico Program serves as a catalyst for change. As stated in the first Five-Year Stategy the framework for action provides

* A mechanism for addressing complex problems that cross federal, state, and international jurisdictional lines;

* Better coordination among federal, state, and local programs, thus increasing the effectiveness and efficiency of the long-term effort to manage and protect Gulf resources;

- A regional perspective to address research needs, resulting in improved transfer of information and methods for supporting effective management decisions; and

* A forum for affected groups using the Gulf, for public and private educational institutions, and for the general public to participate in the solution process.

Implementation is directed through a Policy Review Board (PRB), a Citizen's Advisory Committee (CAC), and a Management Committee with guidance from a Technical Advisory Committee (TAC).

The PRB consists of 22 senior level representatives of state and federal agencies. The PRB is now assisted by a smaller 12 person MC in guiding and reviewing activities of the Program. The MC assists the PRB in carrying out its mission. The PRB is still

the final approval body for the Program.

The CAC is made up of five citizens, one from each Gulf coast state, appointed by the state governor. They represent environment, agriculture, business/industry, development/tourism, and fisheries. The CAC provides public input and assistance in disseminating information relevant to the goals and results of the program. The chairman of the CAC also sits on the PRB and MC. Also sitting on the MC is the CAC co-chair.

With approximately 65 members, the TAC is made up of representatives of state and federal agencies, academia, and private and public sectors, appointed by governors or by the PRB. The TAC provides technical support to the PRB and directs and receives input from the ten issue committees.

The ten issue committees listed below have representation similar to that of the TAC, but do not require governor or PRB appointment. The issue committees are those entities that develop the action items and projects that address the goals and challenges assigned to the program. Eight of those committees address specific Gulf issues. The other two (*) are not issue-specific but are of importance to all other committees as well as the Program in general.

1. Habitat Degradation
2. Public Health
3. Nutrient Enrichment
4. Coastal and Shoreline Erosion
5. Marine Debris
6. Toxic Substances and Pesticides
7. Freshwater Inflow
8. Living Aquatic Resources

* Public Education and Outreach

* Data and Information Transfer

Projects are implemented through the Program office, the committees, or the agencies and organizations involved with the program. The primary tools for identification of environmental problems and implementation of projects to remediate the associated adverse impacts are the committee action agendas.

Symposium Signatory Document

Endorsed by the five Gulf states as well as by key federal agencies, this document increases the level of awareness and commitment from these groups to the issues that threaten the Gulf of Mexico. As the document itself states, it helps to harmonize the diverse interests focused on the Gulf. Entitled "A Partnership For Action," this agreement lays out nine five-year environmental challenges, which will be a major driving force for program activities for the next five years. It is a landmark document

for the program, showing that state and federal agencies can work together as a team and agree upon a common set of challenges aimed at improving the environmental status of the Gulf It also underscores the commitment of these agencies to continue to work as a team to meet these challenges. The nine challenges are as follows:

1. Significantly reduce the rate of loss of coastal wetlands.

2. Achieve an increase in Gulf Coast seagrass beds.

3. Enhance the sustainability of Gulf commercial and recreational fisheries.

4. Protect human health and food supply by reducing the input of nutrients, toxic substances, and pathogens to the Gulf.

5. Increase Gulf shellfish beds available for safe harvesting by 10 percent.

6. Ensure that all Gulf beaches are safe for swimming and other recreational uses.

7. Reduce by at least ten percent the amount of trash on beaches.

8. Improve and expand coastal habitats that support migratory birds, fish and other living resources.

9. Expand public education/outreach tailored for each Gulf Coast county or parish.

A tenth challenge was added following the signing which was: Reduce critical Coastal and shoreline erosion.

The first five-year strategy set out two goals. The first is to establish an effective infrastructure for resolving complex environmental problems associated with human use of the Gulf of Mexico, and the second is to establish a framework-for-action for implementing management options for pollution controls, remedial and restoration measures for environmental losses, and for research direction and research direction and environmental monitoring protocol. The second five year strategy set out the environmental goals resulting in the development of what is termed second generation action agenda,

Action Agendas

Each subcommittee has produced an action agenda delineating action items that must taken to address the challenges and goals. There are three primary purposes for each of the issue specific action agendas: (1) reflect the public input with regard to addressing the issue; (2) communicate what activities are planned or that need to be

planned for corrective action, and (3) provide baseline information from which success can be measured and refined. Each issue specific action agenda sets forth a structured set of strategies, objectives, and action items to achieve these purposes. Their current status is provided as follows:

1. Marine Debris; completed, one year status report now available
2. Public Health; finalized in 1993 and currently in print
3. Habitat degradation; final working draft
4. Coastal and Shoreline Erosion; final working draft
5. Nutrient Enrichment; under committee review
6. Toxic Substances and Pesticides; under committee review
7. Freshwater Inflow; first draft submitted to committee
8. Living Aquatic Resources; under committee review

International Activities

The Program participates in the cooperative effort between EPA and Mexican Federal agencies in the development of the U.S./Mexico border operating plan and provides technical assistance for several United Nations Caribbean Environmental Program (CEPPOL) activities as they address the complex problems of land based sources of pollution and water quality in the Caribbean region. Ongoing efforts to complete Gulf of Mexico Special Area Designation will prevent the dumping of garbage from ships under an international treaty for the "Prevention of Pollution From Ships" (MARPOL Annex V). Collaboration and information exchange has been established with EPOMEX (the Gulf of Mexico Ecology, Fisheries, and Oceanography Program), a Mexican program that supports and coordinates regional research of the marine environment. EPOMEX is geared towards the optimum exploitation and preservation of the marine environment.

Program Inertia

Gulf Coast problems are often very complex and thus solutions are not straightforward. With over 1600 miles of linear coastline and 17,000 miles of tidal shoreline, the Gulf Coast is an immense geographical region. The variability of both the natural and anthropogenic influences on the system presents a large number of issues that need to be addressed. Given the size of the area, priorities must be set so that resources can be used where they are most needed. Setting priorities can be difficult when so many parties with very diverse and often unique interests are involved. Public education and outreach activities for such a large and diverse geographical region is an immense task. The five states that border the Gulf agree on many issues, but disagree on others because of major differences in the driving forces of the economies of each state. There are also numerous state organizations and agencies to coordinate with in addition to the federal agencies that are involved, and conflicting interests, such as development versus habitat preservation, recreational versus commercial fishing interests, and freshwater consumption versus ecological use

such as diversion. The latter issue is important because many marine organisms depend on specific salinity ranges for reproduction or survival.

Interagency Cooperation

In addition to my position as FDA liaison, there are currently liasons for NOAA/National Marine Fisheries Service, the U.S. Fish and Wildlife Service, and the Soil Conservation Service (SCS). The Corps of Engineers has a representative dedicated to Program activities who spends some time in the Program Office. These representatives provide guidance and support from their agencies on the ten issue areas (mentioned under Action Agendas), and their assistance has been invaluable in the success of this Program. SCS has had particularly strong involvement and provided a great deal of support to the Nutrient Enrichment committee; NOAA has similarly assisted with the Living Aquatic Resources committee, and the Corps has taken the lead with the Coastal and Shoreline Erosion committee. I have provided similar support as the federal co-chair of the Public Health committee.

Two state representatives are located within the office, one from the Mississippi Cooperative extension Service and the other, who represents all Gulf States Soil and Water Conservation agencies, comes from the Mississippi Soil and Water Conservation Commission.

Seafood and Public Health

Of the nine short term environmental challenges, six deal predominantly with sealife or 'the water itself and three deal with seafood and public health. Although all directly or indirectly effect seafood quantity or quality, these three interrelate:

> PROTECT THE HUMAN HEALTH AND FOOD SUPPLY BY REDUCING INPUT OF NUTRIENTS, TOXIC SUBSTANCES, AND PATHOGENS TO THE GULF
>
> INCREASE GULF SHELLFISH BEDS AVAILABLE FOR SAFE HARVESTING BY 10 PERCENT
>
> EXPAND PUBLIC EDUCATION/OUTREACH TAILORED FOR EACH GULF COAST COUNTY OR PARISH

The four goals of the public health subcommittee are

1. PREVENT ADVERSE HEALTH EFFECTS RESULTING FROM CONSUMPTION OF RAW SHELLFISH HARVESTED FROM THE GULF OF MEXICO.

2. PREVENT ILLNESS RESULTING FROM EXPOSURE TO MARINE BIOTOXINS IN COASTAL WATERS OF THE GULF OF MEXICO.

3. REDUCE LONG-TERM HEALTH RISKS FROM EXPOSURE TO TOXIC SUBSTANCES IN GULF OF MEXICO SEAFOOD WHILE MAINTAINING THE BENEFICIAL EFFECTS OF SEAFOOD CONSUMPTION.

4. PREVENT EXPOSURE TO PATHOGENS IN COASTAL WATERS OF THE GULF OF MEXICO.

The prioritized categories of concern are: (1) exposure to pathogens in Gulf waters via consumption of raw molluscan shellfish; (2) marine biotoxins and their effect on human health; (3) toxic substances in the food chain, the potential for biomagnification and human health effects caused by consumption; and (4) exposure to pathogens in Gulf waters from recreational or occupational contact.

Four strategies have been designed to meet the challenges of the program: (1) research and demonstration; (2) monitoring/assessment; (3) standards/enforcement; and (4) public outreach. Objectives are grouped according to these strategies and are the specific short-term targets for attaining the goals. Each objective is followed by action items that identify those actions to be taken to meet the goals and objectives of public health,

1. RESEARCH AND DEMONSTRATION (R&D)
(action items l-6)

OBJECTIVES

a. Conduct scientific studies designed to contribute new empirical information pertinent to the prevention of illness in those exposed to Gulf waters or from consumption of products harvested from Gulf waters.

b. Identify and characterize marine biotoxins and progenitors that have not been investigated and continue to develop and improve methods for the identification and management of marine biotoxins.

2. MONITORING/ASSESSMENT (M&A)
(action items 7-18)

OBJECTIVES

a Determine the extent of human fecal pollution of shellfish growing areas in the Gulf of Mexico.

b. Establish a formal database on the occurrence of seafood-borne disease.

c. Determine Gulf Coast residents at risk from consumption of potentially contaminated seafood.

d. Survey Gulf Coast bathing beaches for the presence of human fecal wastes and other pathogens (i.e., Vibrio sp.)

e. Assess the public health significance of marine biotoxins in the marine environment.

f. Implement remote-sensing technologies for the identification of phytoplankton blooms/red tides in the early stages of development.

3. STANDARDS AND ENFORCEMENT
(action items 19-36)

OBJECTIVES

a. Reduce the level of human wastes in the coastal waters of the Gulf of Mexico.

b. Establish a Gulf Coast Fish Contaminants Group.

c. Encourage states to adopt compatible policies for management of public health hazards.

d. Standardize methods for marine biotoxin identification and analysis from phytoplankton producers and seafood vectors.

e. Organize a Gulf-wide marine biotoxins management and consulting group.

4. PUBLIC OUTREACH (PO)
(action items 37-44)

OBJECTIVES

a. Undertake an educational campaign to

(1) help the public understand and control or minimize potential risks associated with the consumption of raw molluscan shellfish;

(2) help health care professionals understand the potential risks associated with the consumption of raw molluscan shellfish, especially high risk patients, and to transmit information to these patients and encourage the reporting;

(3) help legislative and judicial members understand the seriousness of violations of shellfishing regulations related to public health,

(4) inform enforcement officials about public health aspects of shellfishing regulations.

b. Help the public understand the nutritional/health benefits and the potential risks from consumption of seafood, other than raw

molluscan shellfish, as well as how to maximize those benefits while avoiding, controlling, or minimizing risks.

c. Help the public, especially high risk populations, understand the risks associated with recreational and occupational exposure to naturally occurring pathogens (i.e., *Vibrio sp.*) in marine waters.

d. Help the public understand the risks associated with consumption of seafood contaminated with marine biotoxins and the risks associated with direct exposure to marine biotoxins.

In Fy 1994, projects were accepted through interagency agreements among federal agencies. Some of the projects were further meted out to academia through grant programs by those designated federal agencies. In FY 95 preproposals are being sent out and may be awarded directly to academia, states, other federal agencies, or to non-profit organizations depending on the projects developed and the degree of partnering cited.

There have been developed 44 specific action items aligned under the four strategies. The top priority action items have been highlighted for project development.

(R&D)

1. Methods for Identification of Indicator Organisms Originating From Human Fecal Sources (1995); goal 1; ISSC, NOAA, FDA, EPA, academia, industry)

2. Identification of sources of chemical contaminants and pathogens (1994; goals #l, 3 &4; EPA, states, Toxic Substances and Pesticides Subcommittee, GoMPPHC)

3. State ambient water quality assessment and monitoring needs (1993; goal **4;** EPA, states)

4. Atmospheric deposition research (1994; goal 3; EPA)

5. Discussion meeting- Coordination of Marine Biotoxin Research (1993; goal 2; GoMPPHC)

6. Coordination of Marine Biotoxins Research (1995; goal 2; FDA, NMFS, EPA, NIH)

(M&A)

7. Survey and classification of shellfish growing areas (1993; goal 1; states, FDA)

8. Standard reporting for seafood-borne disease (1993; goals 1, 2, 3; CDC)

9. Regular reporting of seafood-borne diseases (1993; goals 1, 2, 3; states)

10. Seafood consumption patterns study (1993; goals 1,3; NMFS, EPA)

11. Seafood contamination data collection (1993; goal 3; states)

12. Evaluation of seafood contamination and consumption information (1995; goal 3; GoMPPHC)

13. Comprehensive seafood contamination data monitoring program (1993; goal 3; FDA, states)

14. Survey of current practices for monitoring bathing beaches (1993; goal 4; GoMPPHC)

15. State bathing beach monitoring plans (1995; goal 4; states)

16. Toxigenic phytoplankton monitoring program for inshore waters (1994; goal 2; FDA, GoMPPHC, NMFS)

17. Monitoring program for marine biotoxin residues in shellfish and finfish (1994; goal 2; FDA, GoMPPHC, NMFS)

18. Remote sensing technologies for the identification of phytoplankton blooms/red tides (1993; goal 2; NMFS, GoMPPHC)

(S&E)

19. Reopening of Gulf coast areas currently closed due to poor water quality (1996; goal 1,4; states)

20. No discharge areas in state waters (1995; goal 1,4; states)

21. Gulf Coast Fish Contaminants Group (GCFCG) (1993; goal 1,3; GoMPPHC)

22. Consistent interagency policies and procedures relative to seafood contamination and public health (1993; goal 1,3; GCFCG)

23. GCFCG Workshops (1994; goal 1,3; GCFCG)

24. Review of state applications of developed guidelines (1995; goal 1, 3; GCFCG)

25. State review, evaluation & adoption of new bacteriological criteria for recreational waters (1994; goal 4; states)

26. State ballast exchange guidelines (1995; goal 1,4; states, EPA, USCG)

27. **Minimum criteria for septic systems in critical areas** (1993; goal 1,4; **states)**

28. **Adoption and implementation of state standards for septic systems (1994; goal 1,4; states)**

29. Alternatives to septic tank systems (1994; goal 1,4; states)

30. Microbiological and chemical standards for POTW permits in shellfish areas (1995; goal 1; EPA or states)

31. Limits on microorganisms in NPDES permits for POTWs near bathing beaches (1994; goal 4; EPA or states)

32. State adoption of new indicators of fecal pollution (1996; goal 1; FDA, states, ISSC)

33. Marine biotoxin identification and analysis manual (1993; goal 2; FDA)

34. Distribution of Marine Biotoxin Manual (1993; goal 2; FDA, GoMPPHC, NMFS)

35. Update/adoption of Marine Biotoxin Action Levels (1993, goal 2; FDA, NMFS)

36. Investigations for Biotoxin outbreaks (1993, goal 2; FDA, CDC)

(PO)

37. Educational materials on risks associated with consumption of raw molluscan shellfish (1993; goal 1; GoMPPHC, PEOC, CAC)

38. Education effort/materials on benefits & risks from consumption of seafoods (1993; goal 3; GoMPPHC, PEOC, CAC)

39. Recreational and subsistence fishing guide (1994; goal 1, 3; NOAA, Sea Grant, GCFCG)

40. Assessment of signs & warnings in limiting consumption of contaminated sports fish species (1993; goal 3; GoMPPHC)

41. Educational program on impacts of boat discharges on seafood (1993; goal 1,3; USCG, GoMPMDC)

42. Marine water contact advisories related to recreational and occupational exposure (1993; goal 4; GoMPPHC, PEOC)

43. Educational materials for physicians (1993; goal 4; GoMPPHC)

44. Public information releases on the effect of Marine Biotoxins on Public Health (1993; goal 2; GoMPPHC, PEOC, CAC)

ACTION ITEMS were prioritized by using the following criteria:

- Action contributed to one or more of the environmental challenges
- Likelihood of success is high and the impact on the issue is substantial
- Completion of the action is critical to the initiation of other important actions
- Action can be sufficiently initiated/completed in time to support reaching the environmental challenge in five years
- Success of the action can be defined or measured

This prioritization resulted in a list of top ten action items:

19. Reopening of Gulf coast areas currently closed because of poor water quality APPROVED BY POLICY REVIEW BOARD 2/94

2. Identification of sources of chemical contaminants and pathogens APPROVED BY POLICY REVIEW BOARD 2/94

29. Alternatives to septic tank systems

28. Adoption and implementation of state standards for septic systems

21. Gulf Coast Fish Contaminants Group (GCFCG)
APPROVED BY POLICY REVIEW BOARD 2/94

5. Discussion meeting- Coordination of Marine Biotoxin Research

27. Minimum criteria for septic systems in critical areas

37. Educational materials on risks associated with consumption of raw molluscan shellfish

1. Methods for identification of indicator organisms originating from human fecal sources

10&13. Seafood consumption patterns study; Comprehensive seafood contamination data monitoring program

GULF OF MEXICO PROGRAM 1995 PRIORITY PROJECTS

The second generation action agenda resulted in development of priority projects for action items addressing the ten challenges. There are five objectives in the call for preproposals:

- To seek the most technically sound and cost-effective proposals available to address the Gulf of Mexico Program's 1995 priority projects.

- To solicit participation from federal, state, and local governments throughout the Gulf region, as well as research, academic, nongovernmental, business, and industry participants in the Gulf of Mexico Program.

- To specifically address the Program's 1995 priority projects.

- To accomplish the objectives specified in the projects within one year.

- To encourage, to the maximum extent possible, the development of organizational partnerships between federal, state, and local governments, and research, academic, non-governmental, business, and industry stakeholders in the Gulf of Mexico's future ecological and economic viability.

ELIGIBILITY:

Federal, state, and local government agencies, academic institutions, and/or nonprofit groups are eligible to respond directly. Due to the restrictions on federal funding,

regional business and industry may not respond directly, but are encouraged to develop partnerships with eligible academic and nonprofit organizations.

Preproposals will be evaluated and ranked by a process administered by the Gulf of Mexico Program. Those submitting preproposals which are selected for award will be contacted by the Gulf of Mexico Program Office in February 1995, and will be asked to work with Program staff to develop more complete proposals of the work to be accomplished.

Examples of these preproposals with projects for FY 95 are:

PROJECT CODE: 95/PH/A5/P1

PROJECT DESCRIPTION: Develop reference and instructional materials for the training of technical/research and management personnel in handling toxic algal bloom phenomena. This is an effort to develop a well trained and consistent infrastructure to deal with toxic algal bloom phenomena in the Gulf states.

Type: Independent
Geography: Gulf State region
Statement of Product: A manual and accompanying video covering the elements of how to identify toxic algal blooms, how to identify the causative species, strategies for field sampling and monitoring, determination of toxins in water and seafood, and management strategies for dealing with exposure to toxins (closure of shellfish areas, monitoring for fish and other seafood products, etc.).
Cost: $75K
Start Date: Fall 1995
Completion Date: 12- 18 months after initiation of project

TASK 1: **Develop the** full text for the manual and accompanying video.

Start **Date:** Fall 1995
Completion Date: 12 months after project initiation
Statement of Product: A manual and accompanying video

TASK 2 Evaluate materials through a review process, improve where necessary and prepare for publication and distribution.

Start **Date:** 1 month after completion of draft materials
Completion Date: 3 months after project initiation

TASK 3: Implement use of materials in training sessions. **(Note:** outside **the** scope of current proposal.)

PROJECT CODE: 95/PH/A10,13/P1

PROJECT DESCRIPTION: Conduct a workshop with seafood consumption model developers and selected Gulf of Mexico Program participants to ensure that state public health needs are incorporated into the model, and begin familiarizing Gulf of Mexico users with the model.

Type: Dependent
Geography: Gulf States
Statement of Product: Workshop
Cost: 75K
Start **Date:** June 1995
Completion Date: October 1995

TASK 1: Complete a Plan of Operations (Planops) which details specifically how the project is to be planned, conducted, and completed. The Planops will include the designation of project leader, independent QA reviewer, all extramural cooperators, as well as a description of the background of the issue, methods to be used in conducting the project, schedules, and specific criteria for measuring performance.

TASK 2: Conduct workshop and evaluate performance.

TASK 3: Modify model if necessary to meet Gulf of Mexico needs.

PROJECT CODE: 95/PH/A10,13/P2

PROJECT DESCRIPTION: Train scientists to recognize that, regardless of the precision of scientific estimates, public issues of equity including the dimension of "outrage" need to be thoroughly understood and incorporated into risk management solutions. This project follows an FY94 project which familiarizes state managers with a new paradigm, Interactive Risk Communication, so that the techniques can be pilot tested and employed in the Gulf of Mexico. This process seeks to proactively involve an affected public in describing risks and seeking solutions for risk management strategies.

Type: Dependent on Action Item 21, Project 1 (FY94)
Geography: Gulfwide
Statement of Product: Workshop and report
cost: $75K
Start Date: 1995
Completion Date: 1996

TASK 1: Complete a Plan of Operations (Planops) which details specifically how the project is to be planned, conducted, and completed. The Planops will include the designation of project leader, independent QA reviewer, all extramural cooperators, as well as a description of the background of the issue, methods to be employed in conducting the project, schedules, and specific criteria for measuring performance.

TASK 2: Conduct and evaluate workshop.

TASK 3: Submit a report which evaluates the concept and recommends where the technique can be applied in the Gulf of Mexico.

PROJECT CODE: 95/PH/A10,13/P3

PROJECT DESCRIPTION: Compile and evaluate existing data and information on the occurrence and factors associated with *VIBRIO VULNIFICUS* in the Gulf of Mexico, and make recommendations to support appropriate control strategies and management plans.

Type: Independent
Geography: Gulfwide
Statement of Product: Report and data base
Cost $200K
Start **Date:** 1995
Completion **Date: 1996**

TASK 1: Compile existing data on occurrence and virulence of *VIBRIO VULNIFICUS* in the Gulf of Mexico, and identify any data gaps.

TASK 2: Identify and evaluate factors associated with occurrence and virulence (i.e., populations and loading information, temperature, time of year, water quality, nutrient loading, salinity, etc.).

TASK 3: Analyze data and information and make recommendations to fill data gaps and develop public health management strategies.

PROJECT CODE: 95/PH/A28/P1

PROJECT DESCRIPTION: Develop educational materials related to the problems, effects, and solutions of on-site sewage disposal system usage in coastal areas. Develop minimum criteria for such systems. Develop fact sheets to inform citizens of the need for improved regulation of on-site sewage systems in coastal areas and seek changes in state laws in the five Gulf states to incorporate these criteria.

Type: Independent
Geography: Coastal states
Statement of Product: Model code with criteria for septic system installation in coastal areas, fact sheets describing problems and benefits of septic system installation, display for citizens describing benefits and problems of different septic systems.
Cost: $40K ($4k for booth; plus resources for development of model legislation, fact sheets, and distribution of information within the five Gulf States by non-governmental organizations)
Start Date: Spring 1995
Completion Date: October 1995

TASK **1:** Develop model legislation containing minimum criteria for new and existing on-site sewage disposal systems in coastal areas. This could be developed through an existing organization of regulators in the Southeastern states.

TASK 2: Produce fact sheets targeted to the public and legislators that describe problems, effects, and solutions for inadequately regulated on-site sewage disposal systems in coastal areas.

TASK 3: Procure and make available to citizen groups a display booth showing information on the problems, effects, and solutions for inadequately regulated on-site sewage disposal systems in coastal areas.

PROJECT CODE: 95/PH/A29/291

PROJECT DESCRIPTION: Evaluate composting toilets, incineration toilets, and aerobic systems under intermittent use conditions. The objective of this project is to evaluate the treatment efficiency of representative types of cornposting toilets and aerobic sewage treatment devices under intermittent use conditions that would be applicable to the Gulf Coast climatic conditions. The tests should be conducted in order to meet the specifications of the National Sanitation Foundation (NSF Standard 40) if possible, since this is a requirement for approval of alternative on-site sewage treatment devices in some Gulf Coast states. If standard conditions for these evaluations do not exist, the conditions used should be determined in conjunction with the Public Health Issue Committee and included in the final report

Type: Independent
Geography: Louisiana
Statement of Product: A report that can be used by state health departments in rule making and permitting of on-site sewage treatment systems in situations where conventional septic tanks and leach fields will not provide adequate treatment and disposal.
Cost: $l00K
Start Date: Spring 1995
Completion Date: Spring 1996

PROJECT CODE: 95/PH/A29/P2

PROJECT DESCRIPTION: Enhance public awareness through demonstration projects.

Type: Dependent
Geography: Gulf States
Statement of Product: Project report on benefits of alternative systems
Cost: $500K
Start Date: Spring 1996
Completion Date: Fall 1996

TASKS: One demonstration project on an alternative system should be conducted in each state.

PROJECT CODE: 95/PH/A29/P3

PROJECT DESCRIPTION: Conduct public outreach activities.

Type: Independent
Geography: Gulf States
Statement of Product: Pamphlets and outreach implementation.
Start Date: 1996
Completion Date: Ongoing

PROJECT CODE: 95/PH/A29/P4

PROJECT DESCRIPTION: Evaluate the potential for a revolving fund with low interest loans (three percent) to upgrade on-site sewage treatment systems. Conduct a demonstration project in one community or a pilot project in one state to test criteria for qualifying homeowners for funding.

PROJECT CODE: 95/PH/A37/P1

PROJECT DESCRIPTION: Develop and implement a judicial and legislative educational program concerning the public health risks associated with the consumption of illegally harvested molluscan shellfish and how to avoid, control, or minimize those risks.

Type: Independent
Geography: Gulfwide
Statement of Product: Written material with possible video and oral presentations
Cost $65K
Start Date: 1995
Completion Date: 1996

TASK 1: **ISSC** Education Committee develops material for presentation.

Start Date: 1995
Completion Date: 1995
Statement of Product: Educational material may consist of video and/or printed material that outlines recent cases of illnesses that resulted from consumption of shellfish illegally harvested from closed shellfish growing waters. Material will also outline all possible illnesses that may be caused by consumption of illegally harvested shellfish and relate the history of the shellfish program and how shellfish water are classified. The Gulf of Mexico Program project officer will be informed of the type of material to be presented and a copy will be sent to the Gulf Program office.

TASK 2: State Shellfish Sanitation Program personnel will identify a proper time and date for delivery of material to the judiciary and legislators, and coordinate with Sea Grant and FDA Shellfish Specialists to arrange meetings to deliver materials.

Start Date: 1995
Completion Date: 1995
Statement of Product: A coordinated calendar that specifies who, what, when, and where material will be delivered to appropriate Gulf State members of the judiciary and legislators. The events should be timed for maximum participation by all parties. Copies of each calendar will be sent to the GMPO project officer.

TASK 3: Education materials will be delivered by respective State Shellfish Sanitation Program personnel, Sea Grant, and FDA Shellfish Specialists as indicated in the calendar.

Start **Date: 1995**
Completion Date: 1996
Statement of Product: Delivery of educational material to appropriate

PROJECT CODE: 95/PH/A37/P2

PROJECT DESCRIPTION: Develop an innovative public outreach program which explains the public health risks associated with the consumption of raw oysters and ***VIBRIO VULNIFICUS*** and how to control or minimize those risks. Certain activities should be targeted to potential high risk individuals.

Type: Independent
Geography: Gulfwide
Statement of Product: An outreach strategy and associated products, which could include a video, written materials, etc.
Cost: $50K
Start **Date: 1995**
Completion Date: 1996

The Gulf of Mexico Program is a "must" program. The federal and state agencies, industry and concerned citizens must work together to reach the lofty goal of protecting, restoring, and enhancing the coastal and marine waters of the Gulf of Mexico and its coastal natural habitats, to sustain living resources, to protect human health and the food supply, and to ensure the safe recreational usage of its waters and beaches. We must have total confidence in its seafood and we must accomplish this with efficiency and enthusiasm.

ISSC UPDATE

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Good morning, it is a pleasure to be here in New Orleans. I am David Heil, Chairman-Elect of the Interstate Shellfish Sanitation Conference (ISSC). I want to thank Dr. Steve Otwell for inviting me to speak to you today.

I was asked to present an update of current ISSC activities. Many of you are probably not familiar with the ISSC, and before updating you on ISSC activities, I would like to explain what the ISSC is, who is involved, why the organization was formed, and how it functions.

The ISSC is a molluscan shellfish regulatory development organization comprised of participants from state shellfish control agencies, the shellfish industry, members of the academic community, and federal regulatory agencies (most notably the U.S. Food and Drug Administration and National Marine Fisheries Service Branch of the National Oceanic and Atmospheric Administration–NOAA. Simply stated, that is the "what" and "who" of the ISSC.

The "why" is more complicated. Regulatory control governing the sanitary quality of shellfish dates back to 1658 when the Dutch Council of New Amsterdam (later renamed New York) passed an ordinance regulating the harvesting of oysters from the East River. Similar measures have occurred in the United States and eventually the U.S. Food and Drug Administration (FDA) established the National Shellfish Sanitation Program (NSSP) in 1924 as a voluntary state and industry program. A ***Manual of Operations*** was published at that time outlining the procedures that would govern the program.

The NSSP functioned well for approximately 50 years, but changes in state and federal governmental organizations and challenges to the federal portion of the program made participation by state regulatory officials and the shellfish industry ineffective. State programs began to diverge from the NSSP, and the federal program appeared unable to maintain the uniformity necessary to ensure the safety of shellfish as a food source. Faced with this deteriorating situation, representatives of fifteen shellfish producing states met in October, 1979, in Ocean City, Maryland, to

investigate the problem. The ISSC was established with the purpose of providing a. formal structure whereby state regulatory authorities, industry, and federal agencies could establish and periodically update guidelines and procedures which could be applied uniformly and would ensure sanitary control of the shellfish industry.

On March 4, 1984, the ISSC and FDA signed a memorandum of understanding which recognized the ISSC as the forum for establishing guidelines and FDA as the evaluator of state programs. During the first four years, the conference reviewed and updated part I and part II of the NSSP *Manual of Operations.*

One of the keys to the success of the ISSC is "how" the conference functions. Anyone who has an interest in molluscan shellfish may submit issues for consideration by the conference. These issues outline suggested changes and include rationale for the changes. The issues are reviewed by appropriate task forces at the annual meeting:

Task force I - growing areas
Task force II - processing and distribution
Task force III - administration

These task forces are comprised of state regulatory, federal regulatory, and industry representatives.

Upon review of the issues, the task forces make recommendations to a voting general assembly made up of state regulatory representatives with each shellfish producing state having one vote in all task forces and each non-producing state having one-half vote in task force I and a full vote in task forces II and III. Complex issues are referred to special committees for detailed review and recommendations. These committees pool expertise from regulatory agencies, industry, and the academic community.

The ISSC has a 22-member executive board which manages the affairs of the conference. ISSC executive board representatives for the Gulf Coast and South Atlantic regions are:

Region 4 (NC, SC, GA, FL)
Regulatory - David Heil (FL)
Industry - Oscar Reksten (FL)
Region 5 (MS, AL, LA, TX)
Regulatory - Joe Gill, Jr. (MS)
Industry - Al Sunseri (LA)

That is a summary of the "what", "who", "Why", and "how" of the ISSC. Now I will update you concerning current ISSC activities.

As some of you may be aware, we held our twelfth annual meeting August 6-12, 1994, in Tacoma, Washington. Attendance was the largest ever. The meeting had representation from 28 states, 3 federal agencies, and 6 foreign countries: Canada, Mexico, United Kingdom, Australia, New Zealand, and Republic of South Korea.

Noteworthy Accomplishments Of the Meeting

(1) ***Vibrio vulnificus*** has been a topic of concern and discussion by the ISSC for many years. The FDA submitted an issue at this year's annual meeting which, if adopted, would require that all oysters from states known to be affected by ***Vibrio vulnificus*** be tagged at time of harvest for shucking by a certified dealer. Oysters so identified would be required to be shucked and packed into containers that were clearly labeled "this product should be fully cooked and not consumed raw." This difficult issue received unprecedented attention and debate which extended into State Legislative Assemblies, Governors' offices, and the United States Congress. The conference voted not to impose the recommended requirement but did direct:

(A) The appointment of a committee to work jointly with FDA to develop manual language necessary for 1995 conference consideration of fFDAs proposed requirement. In addition, the committee was charged with development of proposed additional manual revisions which identify other harvesting and handling practices which may be considered an acceptable alternative to the harvest limitations provided for in fda's proposed requirement. The proposed alternative practices must provide comparable levels of protection to public health. Development of alternative harvesting practices should be based on a review of all available information and may include but not be limited to such considerations and at-harvest temperature controls, restrictions on shelf-life, tagging, and labeling procedures.

(B) In the interim, FDA and ISSC, utilizing funds made available from the National Marine Fisheries Service, will develop a more aggressive and effective education plan.

(C) The ISSC will work jointly with FDA and the conference for food protection to develop uniform language for consumer education advisories.

This issue presents a difficult challenge for the ISSC, and a meeting is scheduled for Thursday of this week in Charleston, South Carolina, to develop a

strategic plan for conference action. We have begun appointing the committee and notifications will be mailed immediately following the Charleston meeting.

(2) At this year's annual meeting, the voting delegates endorsed FDA's seafood HACCP initiative, and an ISSC committee will soon begin a series of activities which include:

(A) Preparation of issues for the 1995 annual meeting which would ensure consistency between the NSSP manual of operations language and final language of the seafood haccp regulation and the "fish and fishery products hazards and controls guide".

(B) Development, in consultation with FDA, of model shellfish haccp plans.

(C) Development of haccp training courses targeted for the shellfish industry and state regulatory agencies.

(D) Development of a schedule for ISSC actions regarding the proposed regulations.

(3) The conference established a committee to review "critical", "key", and "other" designations associated with the standardized processing plant inspection form.

(4) The conference endorsed the FDA retail food code requirement for providing consumer information messages on all potentially hazardous foods.

(5) The conference established a non-voting position of the executive board for the Indian treaty tribes of Western Washington.

(6) The executive board supported the development of a petition to FDA for an irradiation process for molluscan shellfish.

The 1994 summary of actions which includes all recommendations adopted at this year's annual meeting will be submitted for FDA review in early October. There will not be a 1994 printing of the ***Manual of Operations*** . All 1994 manual changes will become effective with the printing of the 1995 manual unless otherwise specified.

The conference referred several issues to committees for recommendations. The 1995 committees will include:

Biotoxin
Certification
Depuration
Education
Epidemiological Investigation Criteria
Foreign Relations
Haccp Review
Identity of Origin

Irradiation
Issue Review
Laboratory Checklist
Patrol
Plant Standardization Advisory
Procedures
Research Guidance
Resolutions
Shellfish Container
Shellfish Restoration
shipping
Standardization of Growing Areas
Strategic Planning
Survey
Tagging
Thermal Processing
Time/Temperature
Vibrio Vulnificus
Wet Storage

The ISSC has several ongoing efforts that I want to update.

(1) On October 1, 1993, the FDA implemented a revised state evaluation program referred to as "Focus '94". FDA invited ISSC comments prior to finalizing Focus '94, and ISSC recommendations were incorporated into the program. For many years the ISSC executive board has been encouraging a more effective compliance effort with ISSC involvement in state non-compliance issues. This compliance initiative provides for both. The safety of molluscan shellfish should not be judged by the least effective state shellfish control program, and I am very encouraged by this effort. I believe that consistent, uniform evaluations and sanctions are critical to ensuring the safety of molluscan shellfish and instilling consumer confidence. FDA invited ISSC executive Director, Ken Moore, to attend their Focus '94 workshop which was held in October, 1993. The meeting involved all shellfish regional specialists and focused on implementation of the initiative. Ken indicated he was impressed with the cooperative attitude of everyone present. The ISSC believes this effort will resolve many of the long-standing problems which have been associated with state evaluations.

(2) In 1994, the conference adopted a formal procedure for FDA to issue NSSP manual interpretations. FDA and ISSC jointly agreed to discontinue the issuance of formal manual interpretations approximately six years ago; however, the shellfish processing plant standardization and compliance initiatives require a mechanism for obtaining guidance and clarification regarding NSSP guidelines. The procedure, which is available to anyone involved in molluscan shellfish, became effective April 26 and has been provided to all members and participants of ISSC. If the responses received on the first three drafts are any indication, this is going to be a very helpful and productive process.

(3) The standardization of shellfish processing plant inspection became effective January 1, 1994 Standardization is a major step, and it has not come without problems. The ISSC executive board has established a plant standardization

advisory committee, and all concerns regarding plant standardization will be forwarded to the committee which will made recommendations to the executive board. This process should be helpful in fine tuning processing plant standardization.

(4) The ISSC education committee has developed a directory of shellfish education information which can be used by states in advising medically compromised individuals of the risk of consuming raw shellfish. In 1988 the conference recommended that states begin educational efforts of this type. To date, with a few isolated exceptions, very little has been accomplished. The education committee is being asked to assess the objectivity of available information and develop additional information for an educational package which ISSC will make available to states and to other groups to facilitate effective public health education.

(5) In an effort to establish better communication with state regulatory agencies and industry, the ISSC has developed a periodical newsletter. Those interested in receiving the newsletter should contact the ISSC executive office.

(6) The ISSC is continuing to assist National Marine Fisheries Service and the northeastern states in the development of a marine biotoxin dockside testing protocol to allow harvesting of shellfish from Georges Bank. The conference, with financial assistance from NMFS and FDA, held a workshop on June 28-29 in Boston. Conference staff was successful in mediating differences which have previously prevented implementation of this program.

(7) The ISSC is managing the development of the shellfish management information system (SMIS) which is an Outgrowth of the ***Shellfish Register of Classified Estuarine Waters.*** This project was formerly managed by National Marine Fisheries Service. It is the goal of the conference to develop an on-line data base which will be periodically updated to provide current and accurate harvest area information. It is our intent to make the information available to states, federal agencies, and industry. The project is expected to eventually include other seafoods and provide critical support to the FDA HACCP initiative. The ISSC has established a steering committee to provide project guidance. The committee includes FDA, NMFS, National Ocean Service (NOS), EPA, State Regulatory, and Industry Representatives.

(8) ISSC will be assisting FDA in providing training and technical assistance to states. This is an area which is going to require considerable attention with the present budget situations of state and federal agencies.

(9) ISSC will be assisting FDA in resolving issues of state non-compliance with the NSSP. ISSC has recently been involved with FDA in addressing several state non-compliance issues, and our joint efforts have been quite effective.

FDA's proposed seafood haccp regulations have received considerable attention. The ISSC provided comments on the proposed regulations. Those comments are available and can be obtained upon request.

In closing, I want to talk about the future of the national shellfish sanitation program. Twenty years from today, those of us who are still involved in molluscan shellfish will look back and be amazed at the duplication of food safety effort that occurred at the federal, state, county, and municipal levels in the "big government era" of the sixties, seventies, and eighties. In saying this I am suggesting that in twenty years there will be a noticeable reduction of duplicated effort This financially driven reduction will be the result of 1990's financial consciousness which is occurring throughout government. The FDA-proposed HACCP regulation is a by-product of that financial consciousness. Effective integration of HACCP in molluscan shellfish, seafood in general, and all foods must include every level of government involved in food safety. The potential for mandatory introduction of HACCP into seafood has created a unique opportunity for those of us involved in molluscan shellfish The size, structure, and process of the National Shellfish Sanitation Program gives the ISSC the opportunity to shape the future of food safety.

The sharing of public health responsibilities, which is the cornerstone of the NSSP, will soon be the rule rather than an exception called a cooperative program. The Food and Drug Administration is requesting that ISSC provide leadership in establishing this new way of doing business. As discussions occur regarding HACCP, reinventing government, and how best to regulate food, I ask that each of you give consideration to the unique opportunity before us, and I encourage your participation in the ISSC. We have a choice. We can shape the future, or the future will shape us.

The thirteenth annual ISSC meeting will be held August 19-25,1995, at the Sheraton Hotel in Orlando, Florida. I urge each of you to attend and support our efforts.

IRRADIATION TO ENSURE HYGIENIC QUALITY OF FRESH AND FROZEN SEAFOOD

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INTRODUCTION

There is clearly a significant increase in awareness of the risks from food-borne diseases among consumers in western countries in the past decade. The concern about the quality and safety of food appears to be driven by increased affluence, new scientific discoveries, by more sophisticated measurements and analyses, by new information about linkages between diet and health, by new food technologies and by mass communication (Kinsey, 1993). No doubt, growing demand for higher quality and safer food leads, also, to higher demand for effective government regulations of food quality and safety.

The outbreaks of E. coli 0157:H7 during the past year which claimed several children's lives and hospitalized hundreds of adults and children alike in the USA is a good case in point. The initial outbreak in the west coast of the USA in early 1993 attracted wide media attention as it not only caused fatalities in children but involved consumption of undercooked hamburgers served at a well known restaurant chains. It taught consumers that there are health risks associated with food of animal origin which, if not properly prepared, could lead to hazards or even death. It also provides a confirmation that raw foods of animal origin often harbour pathogenic bacteria which are dangerous for consumption if the food is not properly processed or prepared. Because of the serious outbreaks of this bacteria, policy makers and legislators are calling for a tighter control of national food protection programmes by introducing public statements and legislative bills (Anonymous, 1994; Lee, 1994). In the USA, warning labels of the risks of pathogenic bacteria in or on food have been mandated on some foods of animal origin.

Fresh and frozen seafood, although not widely implicated in incidences of food-borne diseases, are not without problems. Vibrio parahaemolyticus, a natural microflora of seawater and a common contaminant of fresh fish and shellfish, was responsible for 27 outbreaks in the USA from 1973 to 1991, 22 of which were attributed to contaminated shellfish (Potter, 1994). A survey conducted in shellfish growing areas in 9 States in the USA in mid-1980's showed that mean density of V. parahaemolyticus was 100 times greater in oysters than in water (DePaola et.al., 1990). In the past few years, oysters and clams, often consumed raw, from US States neighbouring the Gulf of Mexico were responsible for deaths of dozens of consumers, most of whom were immuno-compromised. The culprit of the fatality was Vibrio vulnificus which is part of the natural microflora of seawater in the ocean, especially during summer months. This species of bacteria cannot be cleaned up by routine depuration. The negative impact of media publicity of the incidences attributable to consumption of raw oysters and clams has devastated trade in these delicious and nutritious seafoods in the USA, especially in the Gulf States. Large retail buyers entirely stopped purchasing oysters in 1988; demand and resultant production of oysters dropped 41% nationwide; and per head consumption of domestic eastern oysters dropped 60% (Kilgen, 1993). The estimated added cost of US$0.05-0.08/lb for large volume commercial irradiation was considered a cost-beneficial value-added processing step to the oyster industry in the USA (Roberts, 1992).

Pre-cooked frozen shrimp can be mishandled and resulted in infection to consumers (Beckers et.al., 1981). This product, often consumed without further heating, represents a high risk if contaminated by pathogens during processing. In the Netherlands, shrimp cocktails served to elderly people in a pension home during Christmas of 1983, claimed 14 lives because of shigellosis. The pre-cooked frozen shrimp used for the preparation of shrimp cocktail, purported to originate from an Asian country, were found to be contaminated with a drug resistant strain of Shigella flexneri (Mossel and Stegeman, 1985). The Dutch authorities ordered a ban on importation of all Asian shrimp for 3 months following the incident. It was later discovered that the shrimp were caught in the North Sea, cooked on-board, frozen, exported to an Asian country for hand peeling, refrozen and imported back to the Netherlands. Shigella contamination was believed to occur during the hand-peeling process.

Economic losses because of food-borne diseases can be enormous. The U.S. Center for Disease Control and Prevention estimates that 6.5 to 33 million cases of food-borne diseases occur each year in the USA (Kvenberg and Archer, 1987). Salmonellosis alone accounts for about 2 million of these cases and is estimated to cost the US economy $2,540 million annually (Roberts, 1988). The incidences of contamination of V. vulnificus in oysters mentioned above have resulted in the loss of 50% of wholesale business and two-thirds of retail sales to the Louisiana oyster industry (Kilgen, 1993). The recent outbreaks of E. coli 0157:H7 will undoubtedly prove extremely costly to the responsible retailers and wholesaler of the implicated

food. Often, food manufacturers have to absorb the cost of recalling if their products were found to be contaminated by pathogenic microorganisms. For example, a potato chip product glazed with paprika had to be recalled by a large food manufacturers in Germany in 1993 because the paprika was found to be contaminated by Salmonella. Although no illness was involved, such a recall costs the company 30-40 million Marks (Anonymous, 1993).

IRRADIATION AS A COLD PASTEURIZATION PROCESS

While thermal pasteurization of liquid foods such as milk and fruit juices is a well established and satisfactory means of terminal decontamination/ disinfection of such commodities, this process is not suitable for solid foods such as meat, poultry, seafood and dry ingredients such as spices. Irradiation is instead a more efficient method, both from technological and economic points of view, to "pasteurize" these solid food to avoid changes in the physico-chemical and sensory quality of the product. Irradiation acts through changes induced in the DNA structure of the microbial cells, which results in prevention of replication or function. The energy level used for food irradiation to achieve any technological purpose is normally extremely small. At the maximum energy level or dose of irradiation recommended by the Codex Alimentarius Commission (which sets worldwide standards for food trade) for treating food, i.e. 10 kGy, this energy level is equivalent to the heat energy needed to increase the temperature of water by only 2.4° C (calculated on the basis that 10 kGy of ionizing energy is equivalent to a heat energy of 10 J/kg and the heat capacity of water is 4.2 J/kg; 10 J/kg 4.2 J/kg = 2.4° C). The dose of irradiation to ensure hygienic quality of fresh or frozen seafood is in the range of l-5 kGy depending on the product and its state. Thus, the increase in heat energy in such products is in the range of 0.24 - 1.2° C. Irradiated food, therefore, remains at essentially the original natural state after the treatment.

Being a cold process with penetrating power, irradiation is unique in its ability to "pasteurize" fresh and frozen food products including seafood without changing product quality. Irradiation therefore provides a new "critical control point" for eliminating pathogenic bacteria in these food.

In 1986, FAO, IAEA and WHO through their International Consultative Group on Food Irradiation (ICGFI) convened a Task Force on Use of Irradiation to Ensure Hygienic Quality of Food to evaluate the role of irradiation for this purpose. The Task Force concluded that at that time and for the foreseeable future, no technology was available to produce raw food of animal origin in which the absence of certain pathogenic microorganisms such as Salmonella, Campylobacter, etc., can be guaranteed. These foods, therefore, pose a significant threat to public health. Thus, the Task Force believed that where such food are important in the epidemiology of food-borne diseases, radiation disinfection must be seriously considered (WHO, 1987).

EFFECTIVENESS OF IRRADIATION AS A METHOD TO ENSURE HYGIENIC QUALITY OF FRESH AND FROZEN SEAFOOD

Irradiation is an effective method to ensure hygienic quality of fresh and frozen food of animal origin including seafood. Its use, however, should not be a substitute for good manufacturing practices (GMPs) required for such products.

A. Irradiation of Fresh Seafood

The primary objective of irradiation of fresh seafood is for shelf-life extension under refrigeration or melting ice and to inactivate any non-spore forming pathogenic bacteria which may be present. The optimum radiation dose levels for various seafoods is generally in the range of 1.0 to 2.5 kGy according to Table 1.

Irradiation of fresh seafood at these optimum dose levels does not significantly affect quality but reduces significantly the microbial content of the product, thereby increasing-the shelf-tie. The quality and shelf-life of radiation pasteurized seafood is a function of many variables such as:

initial quality (post-mortem age or microbial counts)
qualitative/quantitative composition of the microbial flora
packaging atmosphere
absorbed dose
presence or absence of microbial inhibitors such as sorbate, etc.
biochemical composition of the seafood product
post-irradiation storage temperature.

Fresh fish and shellfish can be contaminated with certain non-spore forming bacteria such as enteropathogenic E. coli Salmonella, Shigella, Vibrio parahaemolyticus, V. vulnificus, V. cholerae, faecal streptococci, Staphylococcus aureus and viruses if caught from polluted waters or through post-harvest handling. Viruses are generally resistant to radiation at the dose levels required for pasteurization or shelf-life extension. Other means of disinfection of viruses such as depuration would have to be considered in addition to irradiation. Non-spore forming pathogenic bacteria are, however, relatively radiation sensitive. Vibrio spp., in particular, is sensitive to radiation with D-value in various seafood ranged from 0.03 to 0.16 kGy depending on salt concentration and serotype Loaharanu, 1972; Grodner and Andrews, 1991; Kilgen, 1993). Quinn et.al (1967) estimated radiation resistance of various pathogens or non-pathogenic members of genera containing pathogenic species in different seafood as follows:

BACTERIA	D-VALUE (kGy)
E. coli	0.15 - 0.35
Proteus vulgaris	0.10 - 0.20
Shigella	0.25 - 0.40
Salmonella (7 serotypes)	0.50 - 1.0
Streptococcus faecalis	0.75 - 1.0
Staph. aureus	0.80 - 1.90.

The storage temperature of seafood following irradiation is of critical importance not only from the shelf-l&e point of view but also because of the risk from botulinum toxin. Clostridium botulinum, strain A-G, are spore-forming bacteria which are resistant to low dose irradiation. Types E and F are of particular concern as they occur in marine environments and could grow and produce toxin at refrigeration temperature. Similar to food sub-sterilized by other means, e.g. thermal pasteurization, modified atmosphere packaging, prevention of botulinum toxin in irradiated fresh seafood can be achieved by a storage temperature of 3 °C or below.

Of more recent interest is the possible use of irradiation to disinfect Vibrio spp. in live oysters and clams which are often consumed raw. This species of bacteria cannot be depurated from these live mollusks and could pose health problems to consumers, especially those immuno-compromised. Vibrio spp. are natural inhabitat of seawater especially in warm areas and can contaminate live fish and shellfish. Live mollusks are, however, relatively resistant to low dose irradiation. A dose of 2 kGy which is more than sufficient to inactivate Vibrio spp in the live mollusks, appears to have little affect on mortality of the shellfish during the first week of storage (Mallet et.al., 1987; Kilgen, 1993). Irradiation therefore appears to be the only known method for pasteurizing live mollusks of Vibrio spp. without significantly affecting their mortality.

B. Irradiation of Frozen Seafood

The primary reason for irradiating frozen seafood is to ensure its hygienic quality from pathogenic microorganisms such as Vibrio spp. which naturally contaminate the product, and Enterobacteriaceae which may contaminate product during handling and processing. With regard to the former species of bacteria, good manufacturing practices including HACCP employed pre-, during and post- freezing can only reduce but not eliminate the contamination. GMPs and HACCP are important tools to avoid contamination of the latter group of bacteria, however. Human handling of products such as peeled and de-veined shrimp prior to freezing can increase the risk of contamination by Salmonella and related group of bacteria as well as Staphylococcus spp.

Frog-legs are often processed and traded by the seafood industry. Live frogs are naturally contaminated by Salmonella and related bacteria. GMPs employed

during butchering and processing including chlorination do not eliminate this group of pathogenic bacteria from frog-legs. Frozen frog-legs are, therefore, often contaminated with Salmonella and cannot gain access to markets in countries which have strict hygienic standards

Irradiation is unique in inactivating non-spore forming pathogenic bacteria in frozen food without changing physico-chemical and sensory characteristics of the product. The dose levels of radiation required to "pasteurize" frozen food including seafood are slightly higher than that for comparable fresh products because of low water activity of the former. A minimum dose of 3kGy is normally required for pasteurizing frozen seafood and frog-legs to ensure hygienic quality from Salmonella and related bacteria.

C. Irradiation of Ready-To-Eat Seafood

In addition to contamination by Salmonella and related microorganisms because of human handling as mentioned above, a number of ready-to-eat foods including seafood have been found to be contaminated by Listeria monocytogenes. The U.S. Food and Drug Administration and the USDA introduced zero tolerance for L monocytogenes in ready-to-eat foods in 1989 (Tompkin et.al., 1992). Ready-to-eat foods such as cheeses, sausages, pate', cooked meat and Cole slaw have been implicated in human listeriosis (Tompkin et.al., 1992, McLaughlin, 1991). L monocytogenes is ubiquitous in nature and can resist heat, nitrite, salt, and acidity better than many other pathogenic microorganisms. It can multiply slowly at temperature as low as 1° C. Some strains of L. monocytogenes have developed resistance to antibiotics such as tetracycline, erthyromycin, co-trimoxazole, and clindamycin (Barbuti et.al, 1992).

Although data are limited, surveys suggested that cooked fish and other seafood may also be contaminated with L. monocytogenes. About 4-8% of cooked crabmeat and 3-4% of shrimp samples may yield the organism on analysis. One study on frozen, butterfly shrimp using a genetic probe suggested that 200 organisms per gramme may be present (WHO, 1988).

L. monocytogenes has a relatively high sensitivity to irradiation. Its D-values have been reported to range from 0.27 to 1.0 kGy depending on strains, growing media, and irradiation conditions (Patterson, 1989; Huhtanen et.al., 1989). In most cases, however, the D-values are similar to those reported for Salmonella spp. irradiated under similar conditions. Thus, L. monocytogenes in ready-to-eat seafood may be controlled by the same dose required for Salmonella and related pathogenic bacteria.

ECONOMICS OF IRRADIATION OF FISH AND SEAFOOD

Economics of food irradiation facilities is best characterized as capital intensive but low operating cost. It requires an economy of scale to justify investment in an irradiation facility. i.e. large quantities of food must be treated to achieve reasonable average unit costs. Factors which are important to determine the cost of operation of irradiation facility and consequent treatment cost include type of radiation, efficiency of radiation source, minimum throughput capacity, dose absorbed, number of hours the facility is operated over a given time period, types of product and their packaging configuration, etc. (Urbain, 1993).

A number of cost estimates on irradiation of fish and seafood have been made using a number of assumptions. These estimates together with actual cost charged by a commercial irradiation facility in France are listed below:

Product	Absorbed Dose (kGy)	Treatment Cost/kg (US$)
Frozen shrimp	2	0.08 - 0.10[1]
Frozen froglegs	4	0.10 - 0.12[1]
Fish fillet	1.75	0.06 - 0. 15[2]
Live oysters	1	0.10 - 0.15[3]

Source:
1. Y. Henon. Gammaster-Provence, France (Personal Communication)
2. Ref.: R. Morrison. 1985.
3. Ref.: K. Roberts. 1992.

CONSUMER ACCEPTANCE OF IRRADIATED FOOD

A. Consumer Attitude Surveys

As food irradiation is perceived to be associated with nuclear technology, any introduction of irradiated food can be erroneously connected with radioactive materials. Thus, it comes as no surprise that there appears to be a widely held opinion among national authorities and the food industry that consumers would be apprehensive about food treated by irradiation because of the perceived association with radioactivity.

Because of the controversy and public debate on food irradiation during the 1980's, a number of consumer attitude surveys were conducted in several advanced countries during this decade to ascertain whether consumers would be willing or prepared to accept irradiated food (Anonymous, 1984; Anonymous, 1986; Titlebaum

et.al., 1983; Weise, 1984, Bruhn et.al., 1986a). Most of these surveys were conducted through telephone interviews, home interviews or mailed questionnaires in countries such as Canada, U.K. and USA at the time when irradiated food were not available for retail sale. The outcomes of these surveys were far from conclusive and led to further debate as to whether or not consumers will accept irradiated food. An interesting outcome of these surveys showed that professional organizations gained a better understanding of the technology and often made positive statements about it. From these surveys, Bord and O'Connor (1989) concluded that the extent to which the public will accept or reject irradiated food depends on the presence or absence of information about the topic. Interviews with those who knew something about irradiation and responded correctly to information about the technology were significantly more willing to accept irradiated food.

Following the outbreaks of E. coli 0157:H7 in the west coast of the USA in early 1993 and the increasing interest in the use of irradiation to ensure hygienic quality of food of animal origin, the American Meat Institute funded a nation wide three-part-study conducted by the Gallup Organization, Abt Associates and the Center for Food Safety and Quality Enhancement, University of Georgia, to measure consumer attitude on irradiation in relation to food safety (AMI Foundation, 1993). The Gallup survey found that while most consumers were aware of food irradiation, few were knowledgeable of the process. After the benefits of irradiation were explained and endorsements by health organizations such as the American Medical Association, Food and Drug Administration and World Health Organization were mentioned, 54% of those interviewed said they were more willing to purchase irradiated meat rather than non-irradiated meat. Sixty percent of the survey participants said they would be willing to pay a 5% premium for hamburger with the bacteria count greatly reduced by irradiation. It was interesting to note from this survey that consumers viewed irradiation as more necessary for food of animal origin such as meat, poultry and seafood than for fruits and vegetables.

In a simulated supermarket selling study conducted by the University of Georgia, 50% of consumers tested chose irradiated ground beef over non-irradiated. After the consumers tested learned more about the process and how it affects raw meat, those choosing irradiated beef increased to 70% of the sample size.

The conclusion from various consumer attitude surveys, conducted mainly in advanced countries, showed that consumers at-large are still not knowledgeable about food irradiation. They need accurate information about the safety, benefits and limitation of the process to be able to make an informed decision whether they will accept irradiated food or not. Similar conclusions could be made about consumers in developing countries. A seminar jointly organized by the International Consultative Group on Food Irradiation (ICGFI), established under the aegis of FAO, IAEA and WHO since 1984, and the International Organization of Consumer Unions (IOCU), in the Netherlands in September 1993 showed that credible consumer organizations do not oppose the use of food irradiation for specific applications (ICGFI, 1993).

B. Market Testing of Irradiated Food

The opinion of consumers about irradiated food is quite different when they are given the opportunity to select and purchase the food. During the past decade a number of market trials on several irradiated food items, with clear labelhng indicating the treatment, were carried out in both advanced and developing countries. A variety of irradiated food, e.g. onions, garlic, potatoes, mangoes, papaya, strawberries, dried fish fermented pork sausages, etc. were put on sale, often alongside the non-irradiated. In none of these trials, carried out in market places where consumers could make their own choice whether to buy irradiated food or not, was there any evidence to indicate that informed consumers will not accept irradiated food (Loaharanu, 1993).

COMMERCIAL APPLICATION OF FOOD IRRADIATION

The number of countries which use irradiation for processing food for commercial purpose has increased steadily from 19 in 1987 to 28 at present. The majority of the increase in recent years is in developing countries which either need irradiated food for their domestic market or see an opportunity to develop market overseas. Spices and vegetable seasonings are the most common products which have been irradiated in some 20 countries. After the European Union issued a Directive which prohibits the use of ethylene oxide in food and food ingredients in January 1991, the use of irradiation to ensure hygienic quality of spices and vegetable seasonings has increased significantly, to over 30,000 tonnes in 1993.

The most significant event which created an awareness of food irradiation among the governments, food industry, and the media was the opening of the first commercial food irradiator in the USA, at Mulberry, near Tampa, Florida in early 1992. The successful sale of several fruits and vegetables both in Florida and other States was widely publicized by the media (Marcotte, 1992; Pszczola, 1992). In the Chicago area, a small grocery called “Carrot Top” reported that irradiated strawberries outsold non-irradiated ones by a margin of 10 to 1 to 20 to 1 depending on the time of sale. Apparently, the consumers were attracted by the premium quality of “natural field ripe" irradiated berries as compared to half- to-three-quarter ripe non-irradiated ones normally available. This retail store also reported significant saving by reducing spoilage losses from about 10% for non-irradiated strawberries to about 2% for irradiated ones. This reduction not only provided them with additional profit but enabled them to compete with larger retailers by offering better quality products at the same price charged by other store for lower quality products (Corrigan, 1993). The successful sale of irradiated produce has led to the sale of irradiated chicken at the retail level in the USA in late 1993 which was also successful.

A number of countries have approved the use of irradiation for extending shelf-life/ensuring hygienic quality of fresh and frozen seafood as well as frog-legs

(Table 2). Irradiation of fresh and frozen seafood in the USA is still not permitted although two companies submitted petitions to the US Food and Drug Administration to authorize commercial irradiation of these products in 1992. Currently, no country is using this technology for shelf-life extension or ensuring hygienic quality of fresh seafood. There is little potential in using irradiation on fresh seafood as long as regulatory authorities do not recognize health risks from consumption of this product which is naturally contaminated by Vibrio spp. which can cause illness and even death.

Irradiation is used commercially, however, to ensure hygienic quality of frozen seafood and frog-legs in Belgium, France and the Netherlands. Because of strict microbiological standards in France, frozen frog-legs and cooked, peeled and deveined shrimp are routinely irradiated for this purpose. The quantities of frozen seafood and frog-legs irradiated commercially in these three countries are relatively small, i.e. a few thousand metric tonnes per annum.

IMPLICATION FOR INTERNATIONAL TRADE IN IRRADIATED SEAFOOD

According to FAO (1992), the world trade in fresh/chilled/frozen fish and shellfish has increased steadily to over 10 million tonnes at the value of almost US$ 30 billion in 1992. Fiih and shellfish are important export commodities from developing countries. In 1992, developing countries exported about 3.5 million tonnes of these commodities at the value of US$ 13.6 billion. The demand for seafood consumption in western countries has increased recently for health and nutrition reasons. To satisfy increasing demand for seafood especially shrimp in western countries, developing countries including China, Ecuador, Indonesia, and Thailand have increased the shrimp production for export through shrimp farming with increasing outputs. Such farming practices could increase the risk from contamination of Salmonella and related pathogenic bacteria because of the close proximity to human and animals in the vicinity. The contamination by these bacteria could be detrimental to the export market of this high value commodity. Shrimp, regardless of whether caught from the open sea or shrimp farms are natnrally contaminated by Vibrio spp., however.

To ensure hygienic quality of seafood from non-spore forming pathogenic bacteria, terminal treatments such as irradiation which do not significantly affect the characteristics of the product must be considered. The treatment is critically important for seafood to be consumed raw or minimally cooked, to protect consumer health. There is already a worldwide standard, i.e. Codex General Standard for Irradiated Foods recommended by the Codex Alimentarius Commission of the FAO/WHO Food Standards Programme, since 1983. Such a standard recognizes the safety and effectiveness of irradiation as a method of food processing/preservation of any food commodities treated with a maximum overall average dose of 10 kGy.

The Agreement on the Application of Sanitary and Phytosanitary Measures, adopted during the Uruguay Round of GATT Multilateral Trade Negotiations in 1993 and to enter into force in 1995, should facilitate trade in seafood which have been processed according to a recognized international standard. Under this Agreement, governments which are signatories to GATT or a member of the World Trade Organization (about to be established) will have to justify the reason for denying entry of food (including irradiated food) which have been processed according to standards and recommendations of the following international organizations:

A. Codex Alimentarius Commission (food safety and human health)
B. International Office of Epizootics (animal health)
c. International Plant Protection Convention (plant health).

An exception is made if the importing country can provide scientific proof that the importation of such food could endanger health of their citizens, animals or plants.

Since there is a Codex General Standard for Irradiated Foods recommended by the Codex Alimentarius Commission in 1983, then represented by over 130 governments, non-tariff trade barriers by importing countries solely because of irradiation treatment can no longer be accepted under the GATT procedures. International trade in irradiated food, including seafood, is likely to increase based on their technical merits in the near future.

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Table 1. Optimum radiation dose levels and shelf-life at 0.6 C (33 F) for fish and shellfish aerobically packed in hermetically sealed cans (87).

Seafood	Optimum dose (kGY)	Shelf-life (weeks)
Oysters (shucked)	2.0	3-4
shrimp	1.5	4
Smoked chubs	1.0	6
Yellow perch fillets	3.0	4
Petrale sole fillets	2.0	2-3
Pacific halibut steaks	2.0	2
Ring crab meat (cooked)	2.0	4-6
Dungeness crab meat (cooked)	2.0	3-6
English sole filets	2.0-3.0	4-5
Soft-shell clam meats	4.5	4
Haddock fillets	1.5-2.5	3-4
Pollock fillets	1.5	4
Cod fillets	1.5	4-5
Ocean perch fillets	1.5-2.5	4
Mackerel fillets	2.5	4-5
Lobster meat (cooked)	1.5	

Source: Ref. Quinn et.al, 1967.

Table II **CLEARANCE OF IRRADIATED FISH AND SHELLFISH** February 2,1995

ITEM NAME	COUNTRY	TYPE OF CLEARANCE	DATE	DOSEMAX
DRIED FISH	BANGLADESH	UNCONDITIONAL	28/12/83	1
	BRAZIL	UNCONDITIONAL	08/03/85	2 . 2
	CHILE	UNCONDITIONAL	29/12/82	2 . 2
	SYRIA	UNCONDITIONAL	02/08/86	2 . 2
	THAILAND	UNCONDITIONAL	04/12/86	1
	UNITED KINGDOM	UNCONDITIONAL	01/01/91	3
	VIETNAM	CONDITIONAL	03/11/89	1
FISH	BANGLADESH	UNCONDITIONAL	28/12/83	2 . 2
	BRAZIL	UNCONDITIONAL	07/03/85	2 . 2
	CHILE	UNCONDITIONAL	29/12/82	2 . 2
	SOUTH AFRICA	CONDITIONAL	09/03/87	2
	SYRIA	UNCONDITIONAL	01/01/91	2 . 2
	THAILAND	UNCONDITIONAL	04/12/86	3 . 2
	UNTIED KINGDOM	UNCONDITIONAL	01/01/91	3
FISH PRODUCTS	BRAZIL	UNCONDITIONAL	07/03/85	2 . 2
	CHILE	UNCONDITIONAL	29/12/82	2 . 2
	THAILAND	UNCONDITIONAL	04/12/86	3 . 2
	BANGLADESH	UNCONDITIONAL	28/12/83	2 . 2
FROZEN SEAFOOD	INDIA	UNCONDITIONAL	02/03/91	6
	UNITED KINGDOM	UNCONDITIONAL	01/01/91	3
SHELLFISH	UNITED KINGDOM	UNCONDITIONAL	01/01/91	3
SHRIMPS	BANGLADESH	CONDITIONAL	28/12/83	5
	BELGIUM	UNCONDITIONAL	30/11/88	5
	FRANCE	UNCONDITIONAL	02/10/90	5
	INDIA	UNCONDITIONAL	02/03/91	6
	NETHERLANDS	UNCONDITIONAL	01/08/92	4 . 5
	THAILAND	UNCONDITIONAL	04/12/86	5
	UNITED KINGDOM	UNCONDITIONAL	01/01/91	3

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EFFECTS OF LOW-DOSE GAMMA IRRADIATION ON THE BACTERIAL MICROFLORA OF FRESHLY PICKED CRABMEAT

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ABSTRACT

Low-dose gamma irradiation is used to reduce or eliminate spoilage and pathogenic microorganisms that may contaminate seafoods during processing. Pathogenic bacteria that have been targeted for reduction or elimination in freshly picked blue crabmeat include ***V. parahaemolyticus, Listeria monocytogenes, Staphylococcus aureus, E. coli*** and other aerobic spoilage organisms.

Microbiological analyses were performed to enumerate the surviving organisms in freshly picked crabmeat following treatment with 0, 0.125, 0.25, 0.5, 0.75, 1.0, 1.5 and 2 kGy of gamma irradiation. Both non-selective and selective media were used for enumeration of spoilage and various pathogens on day 0, 7 and 14 of ice storage.

The results indicate that low-dose gamma irradiation significantly reduced all microorganisms in crabmeat. The D-values, calculated from linear regression analysis for specific bacterial genera varied accordingly to each individual ***L. monocytogenes*** had the highest D-value of 0.59 kGy and ***Vibrio parahaemolyticus*** had the lowest of level of radiosensitivity among the microorganisms analyzed. ***S. aureus*** had the D-value of 0.16 kGy. Gamma irradiation doses equal

or greater than 1 kGy was found to significantly reduce the growth and multiplication of the bacteria. Therefore, it was concluded that 1 kGy gamma irradiation was sufficient to reduce the risk of food borne pathogen in freshly picked crabmeat, and at the same time, extend shelf life.

Introduction

Seafood products, including crabmeat, are highly perishable and may lose much of their value prior to complete spoilage and production of an off flavor. The high value of crabmeat and its susceptibility to spoilage have promoted investigations into the microbial flora and organisms responsible for spoilage (Conkey, and Chai, 1991). The environment has a great influence on the bacterial flora of freshly caught crabs. This study is focused on the natural microbial flora of the Gulf Coast blue crab meat.

The meat of freshly caught blue crabs should contain very few bacteria. However, during processing including peeling and picking, bacteria that are on the body surface or in the intestine can be introduced into the crabmeat itself and cause contamination.

The objective of this study was to isolate and enumerate the presence of four species, *E. coli*, *L. monocytogenes*, *S. aureus* and *V. parahaemolyticus* if present in the natural microbial flora of fresh raw crabmeat and to determine the effect of low dose gamma irradiation on those specific microorganisms.

Materials and Methods

Preparation of samples

Raw, unblanched crabs were purchased from local seafood markets every month from March to June, 1994. Those fresh crabs were then hand picked and sealed in 24 sterile plastic containers. The crabmeat samples which were mixed with hemolymph and intestine were placed in an ice chest filled with ice.

Irradiation

The crabmeat samples were transferred on ice to the Nuclear Science Center, LSU, and 21 samples were placed into a water tight

diving bell and lowered into the water for irradiation from a ^{60}Co source which emitted 0.017 kGy per minute. The rest of the samples were labeled "control" and kept in the cooler. For a comparable length of time for the irradiation, one hour of exposure would equal 1 kGy. The diving bell was pulled up when desired exposure time and dose were reached. Four samples were removed from the diving bell, labeled and then placed in the cooler with ice. The diving bell was again lowered into the source for further exposure until all the samples were removed. The irradiated crabmeat samples were then transferred back to the Department of Food Science where the microbiological analyses were performed immediately. The unused samples were kept on ice and stored in the walk-in refrigerator in the Department of Food Science. The temperature of the ice stored crabmeat was 2°C. Every microbiological analysis procedure was repeated three times in order to calculate the average.

Preparation for Microbiological Evaluation

Aseptically weigh 10 g of crabmeat into a sterile plastic bag. Add 90 ml of sterile 0.1 % peptone dilution fluid with 8.5 % of NaCI, blend for 90 seconds with a stomacher (Lab-Blender 400, Tekmar Co., Cincinnati, OH). This represents the 10^{-1} dilution. Make further sequential dilutions using peptone dilution fluid.

Standard Plate Count

Procedures

1. Pipet 0.1 mL from the sequence of dilutions onto pregelled Plate Count Agar (Difco Laboratories, Detroit, MI) using the spread plate method.

2. When the surface of the plate count agar becomes dry, invert the plates and incubate at 35° C for 48 hours.

3. Count all plates which contained 25 to 250 colonies and calculate the total plate count per gram crabmeat.

4. Repeat the same procedures after 7 and 14 days, respectively.

Results and Discussions

The results of different irradiation doses and storage times effects on the standard plate count of both irradiated and unirradiated crabmeat are listed in Table 1 The numbers listed in the table are Log_{10} Colony Forming Unit aerobic or Total Plate Count per gram (CFU/g) of crabmeat.

Table 1. The Log_{10} numbers of the surviving microorganisms on Plate Count Agar

Day	Control	0.125 kGy	0.25 kGy	0.5 kGy	0.75 kGy	1.0 kGy	1.5 kGy	2 kGy
0	7.1	6.9	6.2	5.9	5.3	4.3	3.5	2.7
7	9.2	7.2	6.4	6.1	5.4	4.3	3.4	2.4
14	10.4	9.6	7.8	7.4	6.8	5.8	4.1	3.9

By using the linear regression analysis, the D value, which is -l/slope, for the standard plate count is 0.42 kGy. This D value means that 1 kGy of gamma irradiation is capable of reducing 2.4 log number of the microorganisms in the crabmeat. The log of the numbers of CFU of irradiated samples remained stable or decreased in the first week and went up after one week of storage on ice. The log number of CFU control sample increased rapidly and slowed down after one week. The survival curves for standard plate counts are shown in Figures 1 and Figure 2.

Vibrio parahaemolyticus

Procedures

1. Pipet 0.1 mL from the sequence of dilutions onto pregelled thiosulfate citrate bile salt sucrose (TCBS) agar (Difco Laboratories, Detroit, MI) using the spread plate method.

2. When the surface of the TCBS agar becomes dry, invert the plates and incubate at 35°C for 48 hours.

3. Count all plates which contain 25 to 250 typical ***V. parahaemolyticus*** colonies. Typical ***V. parahaemolyticus*** colonies appear round, opaque green or bluish on TCBS agar.

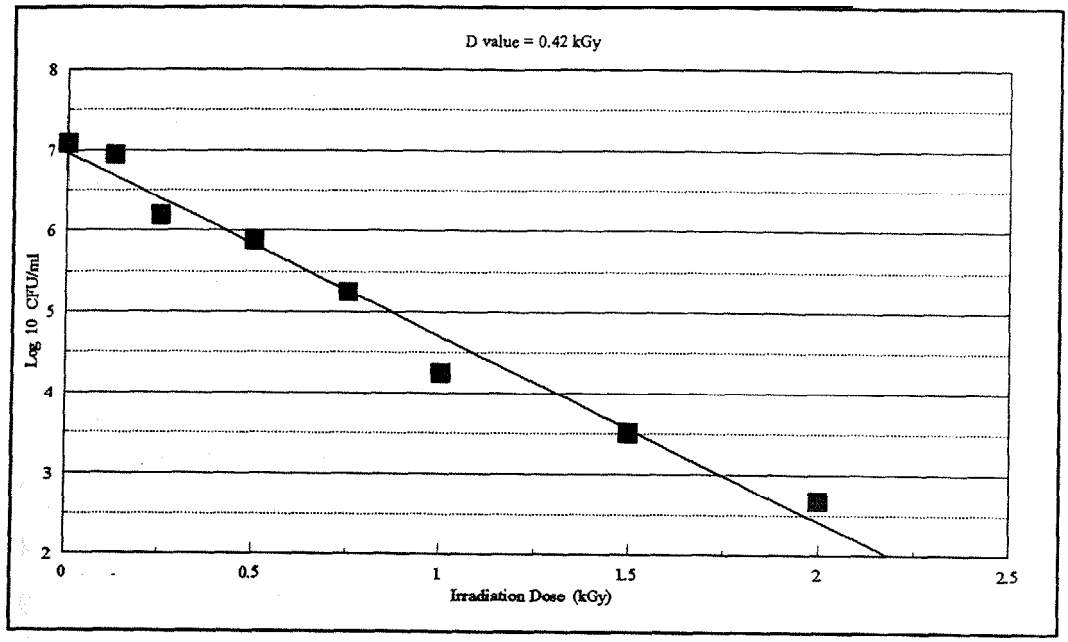

Fig. 1 Survival Curve for Standard Plate Count

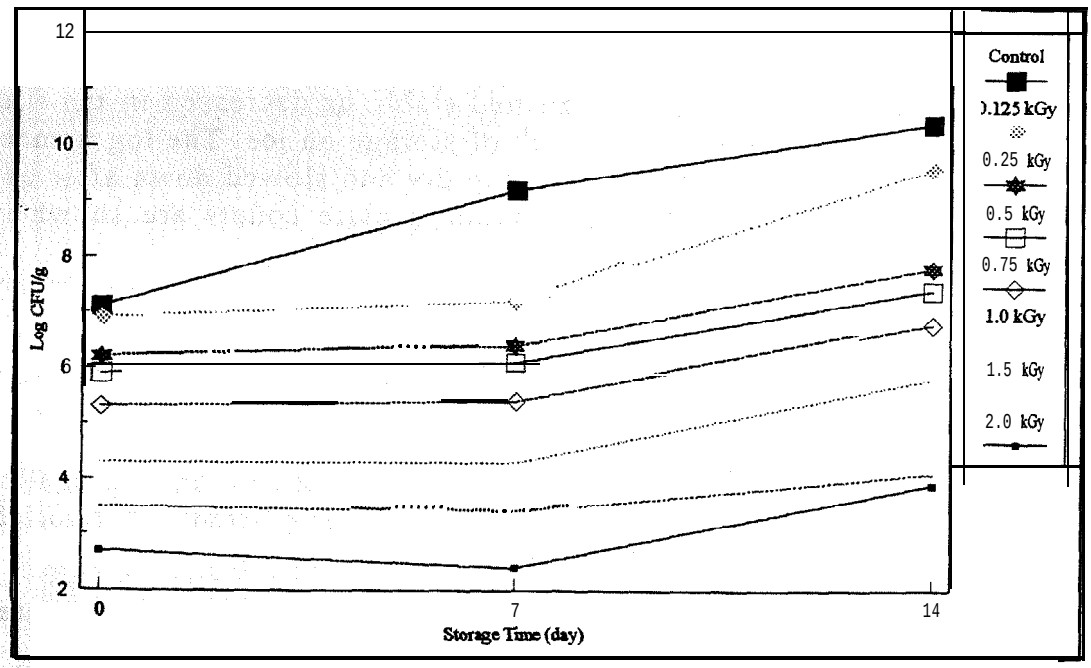

Fig.2 Survival Curves of Standard Plate Count of Control and Irradiated Crabmeat during Ice Storage

4. Differentiate *V. parahaemolyticus* and *V. vulnificus* by halophilism and ONPG tests.

5. Repeat the same procedures after 7 and 14 days, respectively.

Results and Discussions

V. parahaemolyticus is very sensitive to gamma irradiation. The D value is only 0.05 kGy. They were totally eliminated by 0.5 kGy of gamma irradiation. The results of gamma irradiation on *V. parahaemolyticus* counts are shown in Table 2

Table 2. The effect of gamma irradiation and 2°C ice storage on *V. parahaemolyticus*

Day	Control	0.125 kGy	0.25 kGy	0.5 kGy	0.75 kGy	1.0 kGy	1.5 kGy	2 kGy
0	4.88	3.8	NC	0	0	0	0	0
7	4.32	3.4	NC	NC	0	0	0	0
14	4.18	NC	NC	NC	0	0	0	0

The numbers are expressed in Log_{10} CFU/g *; NC:* Not Countable, the number of CFU from the maximum dilution < 25 in one plate.

The D value and effect of ice storage on survival numbers of *V. parahaemolyticus* are shown in Figure 3. and Figure 4.

The numbers of *V. parahaemolyticus* kept decreasing during ice storage, indicating that low temperature (2°C) is inhibitory to this organisms.

Listeria monocytogenes

McBride Listeria medium was employed to detect and enumerate *Listeria monocytogenes.*

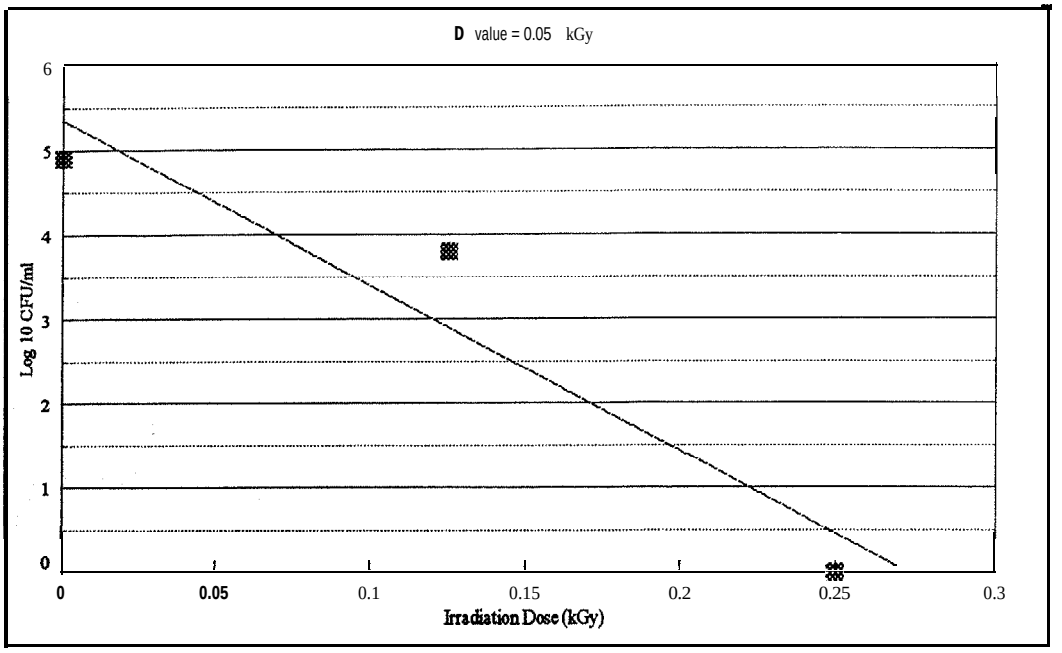

Fig. 3 Survival Curve of *Vibrio parahaemolyticus*

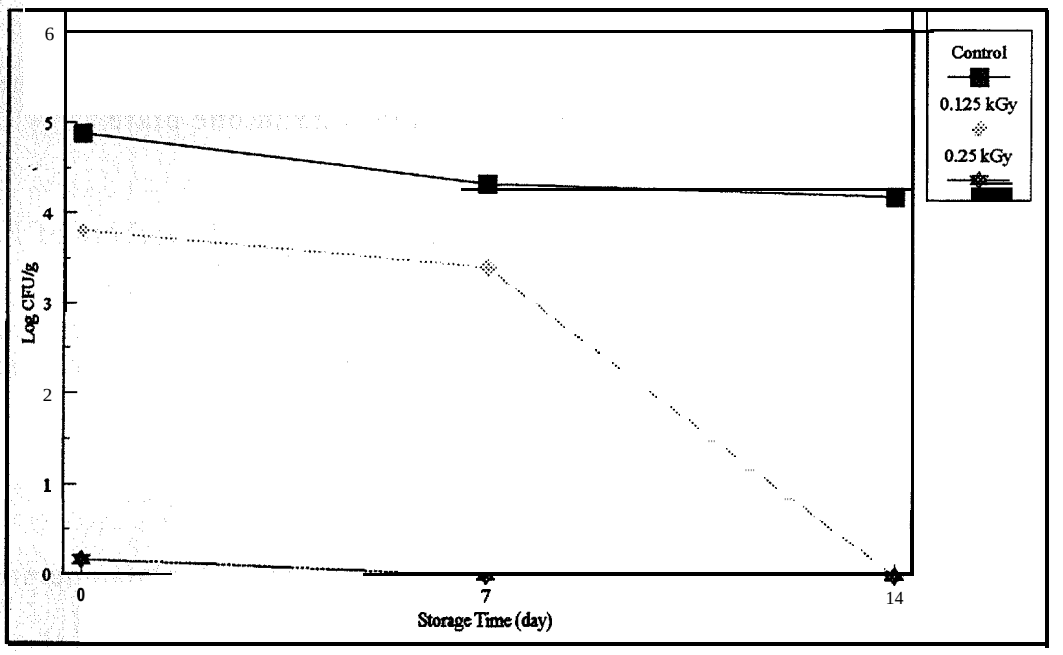

Fig. 4 Survival Curves of *V. parahaemolyticus* in Control and Irradiated Crabmeat During Ice Storage

Procedures

0.1 mL of each dilution prepared in aerobic plate count was spread evenly onto three plates of McBride Listeria medium. The plates were then inverted and incubated at 35° C for 48 hours.

Typical *L. monocytogenes* colonies are round translucent, slightly raised bluish-gray colonies with fine textured surface that varies from 0.3 to 1.5 mm in diameter and have a narrow zone of beta-hemolysis. API Listeria, a rapid method for the identification of Listeria was employed to identify the suspected colonies.

Results and Discussions

The results showed that *L. monocytogenes* was very resistant to gamma irradiation. With a D value of 0.59 kGy, a large portion of the original microbial population survived the irradiation process. *L. monocytogenes* can survive and grow during the ice storage. Obviously, these organisms are capable of self-repair to damage by ionizing radiation. The results of the effect of gamma irradiation and ice storage on *L. monocytogenes* are shown in Table 3.

Figure 5 shows the reduction of *L. monocytogenes* by gamma irradiation.and Figure 6 shows the relationship between ice storage time and log number of survival colony forming units

Table 3. The effect of gamma irradiation and ice storage on *L. monocytogenes.*

Day	Control	0.125 kGy	0.25 kGy	0.5 kGy	0.75 kGy	1.0 kGy	1.5 kGy	2 kGy
0	4.7	4.6	4.2	3.8	3.5	NC	NC	NC
7	5.2	5.0	4.4	3.7	3.7	NC	NC	NC
14	6.7	6.9	6.3	5.9	6.4	5.8	5.4	NC

Staphylococcus aureus (Coagulase +)

Procedures

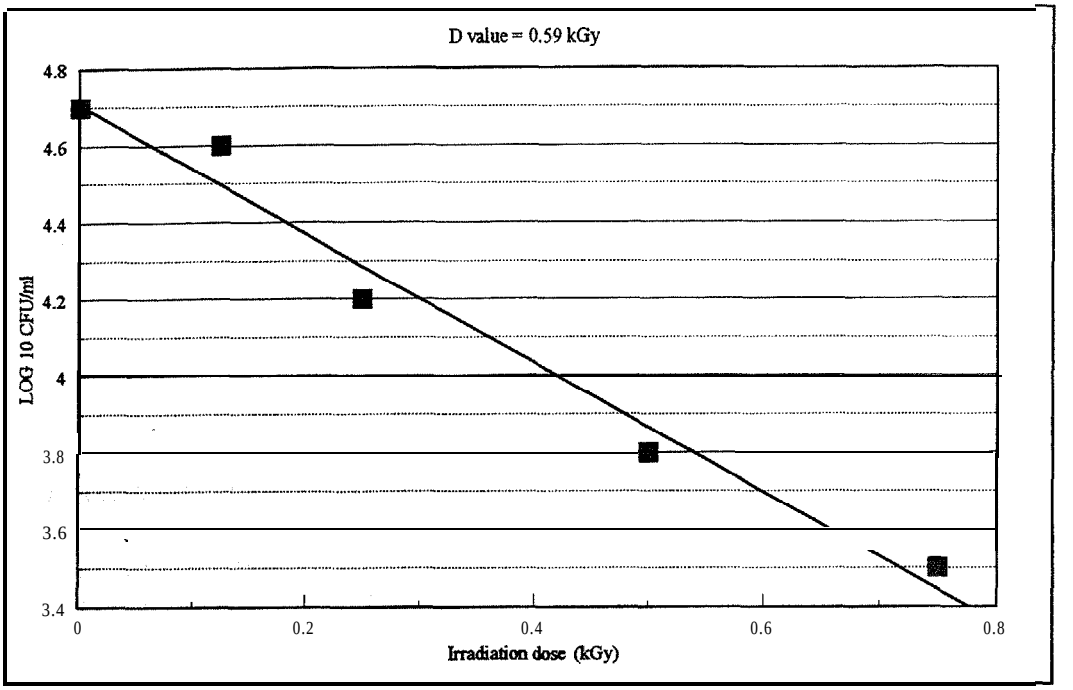

Fig. 5 Survival Curve of *Listeria monocytogenes*

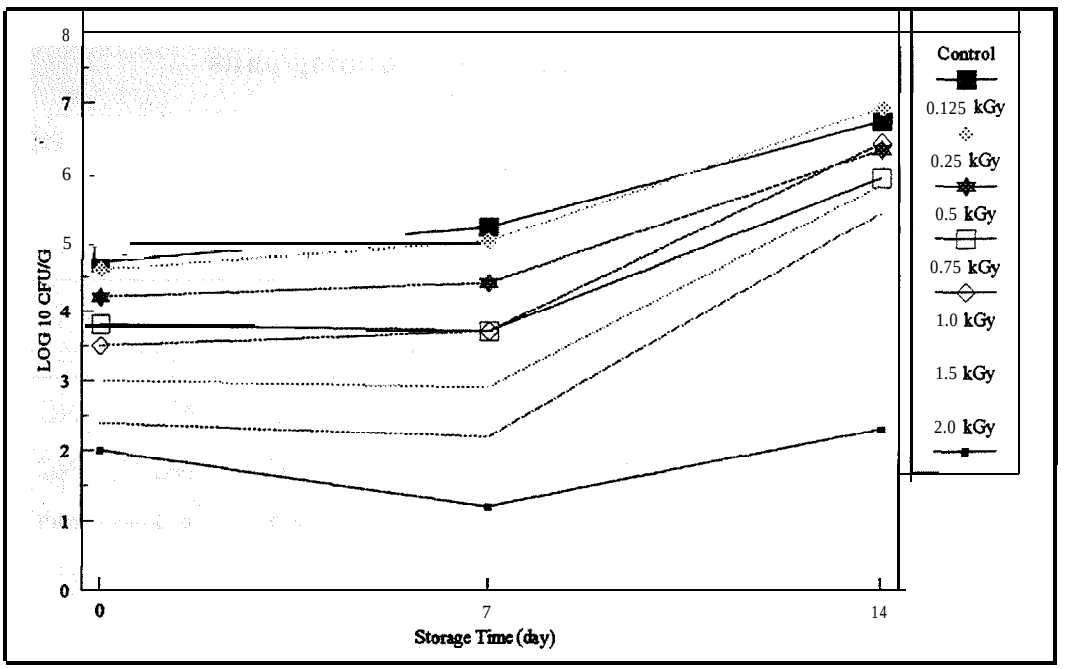

Fig. 6 Effect of Ice Storage and Irradiation on *L. monocytogenes*

Sequential dilutions were made by the same procedures as in the standard plate count. For each dilution to be plated, a 0.1 mL of sample suspension was aseptically distributed onto three plates of Baird-Parker agar surface and was spread evenly by using sterile hockey sticks. After the inoculum was absorbed by the medium, the plates were inverted and incubated at 35° C for 48 hours.

The plates containing 25 - 250 colonies with the typical appearance of *S. aureus* were counted. Colonies of *S. aureus* were circular, smooth, convex, moist, 2-3 mm in diameter on uncrowded plates, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by an opaque zone and frequently with an outer clear zone (Bennet, 1984).

Colonies with a negative coagulase test reaction were subtracted from the initial colony count.

Results and Discussions

S. aureus was quite sensitive to gamma irradiation. The D value of this organism was found to be 0.16 kGy. The large initial population of this organisms indicated that the raw crabmeat was contaminated probably by improper handling and hand picking. However, due to its irradiation sensitivity, the total population was eliminated by 0.75 kGy of gamma irradiation. During ice storage, the log number of CFU/g of untreated sample increased and the log number of CFU/g of irradiated sample decreased, indicating that low dose gamma irradiation is very effective in destroying or injuring *S. aureus.* The results of the effect of gamma irradiation and storage time on the survival of *S. aureus* are given in Table 4, Figure 7 and 8.

Table 4. The effect of gamma irradiation and ice storage on S. aureus

Day	Control	0.125 kGy	0.25 kGy	0.5 kGy	0.75 kGy	1.0 kGy	1.5 kGy	2 kGy
0	5.7	4.3	3.2	NC	0	0	0	0
7	6.1	4.2	3.1	0	0	0	0	0
14	6.7	4.7	4.5	0	0	0	0	0

NC: Not countable, the number of CFU from the maximum dilution< 25 in one plate.

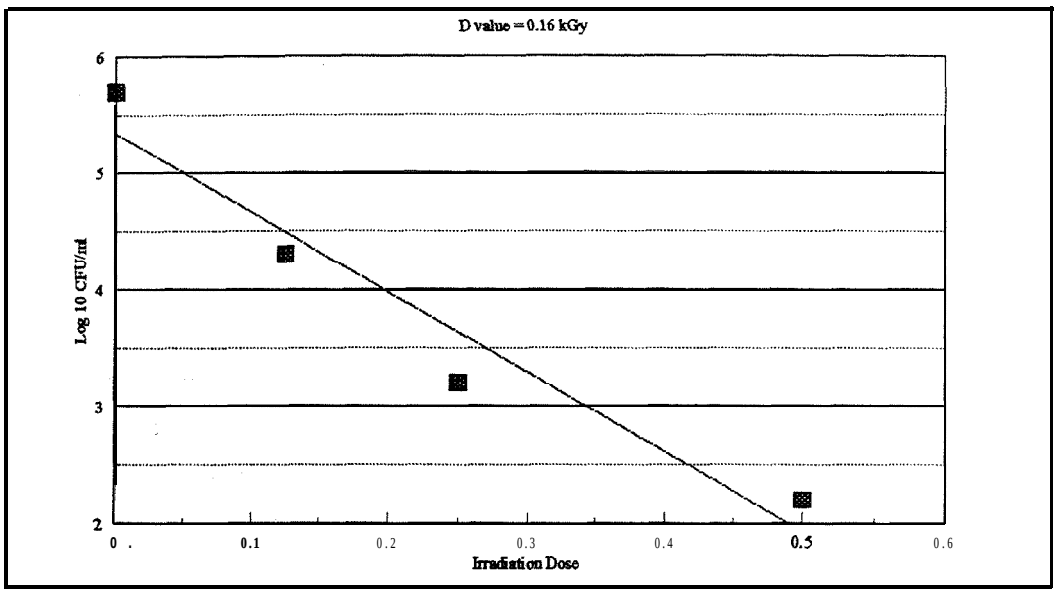

Fig. 3.7 Survival Curve of ***Staphylococcus aureus***

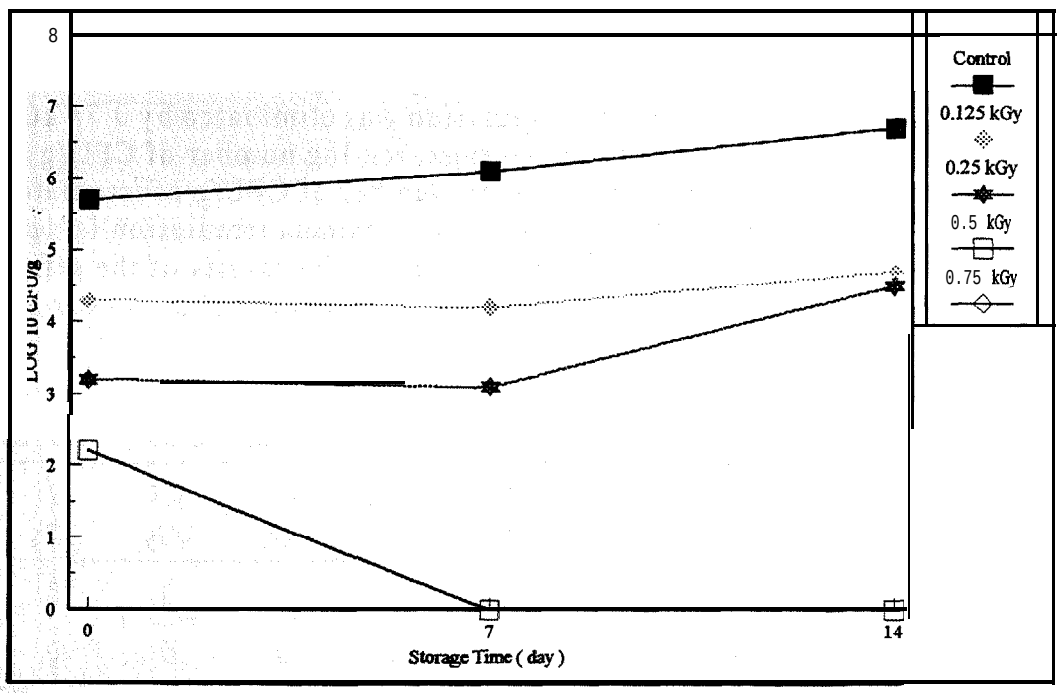

Fig. 8 The Effect of Ice storage and Irradiation on the Survival of *S. aureus*

E. coli

Procedures

5-tubes MPN technique was used to estimate the number of *E. coli* as well as the coliform number. 10 grams of fresh crabmeat was mixed with 90 mL of lauryl sulfate tryptose (LST) broth. Two lower sequential dilutions were also prepared. The tubes were then incubated at 35°C for 48 hours. The tubes with gas production were observed and recorded. The positive LST tubes showing gas were subcultured into EC (E Coli) broth and incubated in a 45.5°C hot water bath for 48 hours. The EC broth tubes showing gas production were subcultured onto the surface of McConkey agar and incubated at 35°C for 24 hours. The typical *E. coli* colony appeared bright red on the McConkey agar. The typical *E. coli* colonies were subcultured on to the non-selective plate count agar and the organisms from the colonies on the plate count agar were confirmed by API 20E rapid identification technique. The 5-tubes MPN table was used to estimate the MPN/g of both coliform group and *E. coli* in the crabmeat.

Results and Discussions

The results indicated that the numbers of *E. coli* as well as other coliform group member were very low in the crabmeat. For the control sample, the number of the coliform group and E. *coli* are 4 MPN/g and 2 MPN/g respectively. The coliform and *E. coli* were both eliminated after irradiation and did not appear after the ice storage. The results of the MPN analysis of coliforms and *E. coli* listed in Table 5

Table 5. The effect of gamma irradiation and ice storage on *E. coli*

Day	Control	0.I25 kGy	0.25 kGy	0.5 kGy	0.75 kGy	1.0 kGy	1.5 kGy	2 kGy
0	2	<2	<2	<2	<2	<2	<2	<2
7	<2	<2	<2	<2	<2	<2	<2	<2
14		<2	<2	<2	<2	<2	<2	<2

Numbers in MPN/g

Conclusion

In this study, low dose gamma irradiation was found to be very effective on the reduction of targeted microorganisms except for *L.monocytogenes,* which required higher dose to achieve radurization. For the irradiation sensitive species like *S. aureus* and *V. parahaemolyticus,* 0.5 kGy of gamma irradiation was sufficient to eliminate them from the crabmeat. However, for *L. monocytogenes,* which can survive the low dose irradiation and multiply rapidly later during ice storage, the irradiation dose had to be higher in order to control this organism for a longer time. The numbers of *E. coli* and coliform are very low in crabmeat.

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EFFECT OF SPLIT DOSE APPLICATION ON THE RADIOSENSITIVITY OF *LISTERIA MONOCYTOGENES*

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INTRODUCTION

The ability of *Listeria monocytogenes* to survive and proliferate at refrigeration temperatures in a variety of food products, and its potential to survive minimal thermal processing (Doyle, 1988), have caused food safety concerns among food scientists and technologists. Due to the ubiquity of this genus, *Listeria spp.* have been shown to contaminate a variety of food products including red meats, seafood and dairy products either from harvest or during processing (Donnelly, 1994). The United States Food and Drug Administration (USFDA) currently mandates a zero tolerance level for *Listeria* in food products (USFDA 1994) due to the potential severity of infection in immunocompromised individuals due to liver disease, immune disorders, age, and/ or pregnancy.

One method suggested as a possible solution to reducing the risk of this bacteria has been irradiation processing. In minimally processed foods such as fresh seafood, dairy, and sous vide products, low dose irradiation might be used to reduce this risk while preserving the fresh quality of these products. Although *L. monocytogenes* has proven to be among the more radiation resistant vegetative bacteria, if present in small numbers, below 10^4 CFU/g(ml), it has been shown to be sensitive to doses of 2 kGY (Andrews and Grodner, 1992; Huhtanen et al., 1989). In contrast, higher concentrations of the bacterium have been shown to resist 2 kGy of gamma radiation and recover with logarithmic growth (Andrews and Grodner, 1992; Juneau, 1989;Patterson et al., 1993).

In general, the radiosensitivity of bacteria varies depending on many different factors including medium in which the irradiation occurs (Urbain, 1986), temperature at time of irradiation (Josephson and Peterson, 1982), initial cell concentration (Kelner, 1955), and many other environmental factors such as oxygen level, pH, and water activity (Urbain, 1986; Josephson and Peterson, 1982). Traditionally, irradiation processing of food products has focused on single dose application to a

processed and packaged product. A method of radiation application used extensively to treat cancerous tumor cells has been to administer radiation doses in fractions over a period of time. This method was developed as a method to reduce the "good" tissue damage while effectively denaturing the activity of deoxyribonucleic acid of the tumor cells. It is not known whether use of fractionated doses will alter the overall sensitivity of bacterial cells. The purpose of this research was to determine if differences in the radiation sensitivity of a common food pathogen, *Listeria monocytogenes,* could occur following a split versus single dose gamma irradiation application.

MATERIALS AND METHODS

Test Organism

Listeria monocytogenes strain Scott A was obtained from the Louisiana State University Food Microbiology Culture Collection (August 1991). The bacterium was maintained on tryptic soy agar (TSA) at 4°C with quarterly subculturing. Following each subculture, the bacterium was tested (API Listeria 10-300, System for identification of *Listeria*) for typical physiologic and biochemical attributes to ensure consistency in growth and identification (Bille et al., 1992).

Preparation of Inoculum

Listeria monocytogenes strain Scott A was grown in tryptic soy broth (TSB-9 ml in a 20 x 125 mm pyrex screw cap tube) at 35°C for 18 h to achieve stationary phase cells at a concentration of 10^9 CFU/ml broth. For a lesser cell concentrations of 10^6 CFU/ml, dilutions were performed using 9 ml TSB to achieve the desired concentration. Cell dilutions were held at room temperature (20°C) and immediately irradiated.

Irradiation

Listeria monocytogenes samples stored in 20 x 125 mm screw cap tubes were maintained at constant temperature (20°C) until exposure to a 1.25 MeV cobalt-60 radiation source in the Nuclear Science Center at Louisiana State University. For the control samples, single dose, the radiation absorbed doses ranged from 0-5 kGy For split dose irradiation application, cultures were irradiated with equally split doses of gamma radiation (0.0-0.0 kGy; 0.25-0.25 kGy; 0.5-0.5 kGy; 1 .0- 1 .0 kGy 1.5- 1.5 kGy 2.0-2.0 kGy, and 2.5-2.5 kGy) to achieve the same total dose as the controls (0-5 kGy). The "Time Between Fractions" (TBF) varied from 0 in the controls to 0.25, 0.50, 1.0, and 2.0 hours(h) in the test cultures. Test cultures were maintained at initial temperatures during intervals between dose application. Surviving cells were enumerated by serial dilution and spread plated onto TSA as described according to standard plating methods recommended by the USFDA (BAM, 1987). Initially negative cultures were incubated at 35° C for one week and examined for growth by visual observance of turbidity followed by plating on TSA. This procedure was to allow for the possibility of repair and recovery of injured cells exposed to sublethal radiation doses and for enumeration of very low numbers of survivors. The procedure schematic is presented in Table 1.

Statistical Analysis

Irradiation D-Values (the irradiation required to eliminate 90% of the cell population[D_{10}]) in kGy were calculated [D_{10} = -l/slope] from the linear portion of irradiation survival curves using standard linear regression analysis (LRA) using Statistical Analysis System (SAS,1987). Mean differences were compared using Tukey's Pairwise Comparison (SAS, 1987).

The second method used to calculate irradiation D-Values was based on the reduction of the initial cell population to zero. This method, to be termed total dose, TD, used the beginning population in logs and the approximate amount of irradiation required to have no culturable survivors:

Irradiation D-Value = dose in kGy/log population

This method was used to account-for survival curves that exhibited a shoulder, tail, or two linear portions (Huhtanen et al., 1989) that might skew regression analysis. All experiments were performed three separate times (three replicates) with double plating of duplicate samples for each replicate.

RESULTS AND DISCUSSION

Irradiation of food products for the purpose of reducing spoilage bacteria or reducing the risk of pathogenic microorganisms has traditionally been applied in a single dose to achieve a targeted irradiation exposure. However, some foodbome pathogenic bacteria, like *Listeria monocytogenes,* are resistant to federally approved low doses (<3 kGy), when present in numbers exceeding 10^4 CFU/g. When irradiation is applied in doses above 2 kGy, changes in the sensory quality may occur in some products including seafood products (Andrews and Grodner, 1992).

Alteration of the radiosensitivity of *L. monocytogenes* was attempted by using split dose application of gamma radiation. This method of application is feasible in a commercial cobalt-60 facility, by simply shielding the product from the source for a desired time. When comparing the single versus split dose irradiation application to *L. monocytogenes* in a nutrient broth, two levels of cellconcentration were selected, 10^6 and 10^9 CFU/ml. Cell cultures of *L. monocytogenes* at or less than 10^3 CFU/ml have proved to be sensitive to irradiation doses of 2 kGy with D-Value below 0.50 kGy (Andrews and Grodner, 1992). As many as 1 million Listeria/gram of food product may be present with no apparent spoilage detected by the usual sensory means (Marshall, 1993). The higher cell concentrations used in these experiments provided sufficient data points to construct irradiation survival curves in an attempt to predict the behavior of this bacterium

Table l.Procedures schematic for effect of split dose irradiation on radiosensitivity

Listeria monocytogenes, Strain Scott A (LSU)

Tryptic Soy Broth - 18 h
(Dilution to log 6, or 9 CFU/ml)

↓

Storage (20°C)

↓

Irradiated - 0-5 kGy
(1.25 MeV gamma - $Cobalt^{60}$)

Split Dose
(0-2 h)

↓

Enumerate Survivors
(Neg. cultures held 7 days & S/C for recovery)

↓

D-Value Calculations
Linear Regression Analysis (LRA)
(SAS, 1987)
Total Dose Method (TD)

Temperature is an important factor to be considered in relationship to the food product of concern. In preparing, packaging and shipping food products, temperatures may fluctuate during handling. In this discussion, a single temperature of irradiation (20°C) will be considered.

The use of 20°C, as an irradiation processing temperature, is commercially applicable to dry spices, fruits and vegetables. The generation time for ***L.*** *monocytogenes* in TSB at 20°C was approximately 1.39 h (Andrews, 1994). Generation time is an important factor to consider in determining the ability of *L.monocytogenes* to recover from sublethal irradiation. This was especially a concern during these experiments since the available irradiation source was low with an emission rate of 18 Gy/min, compared with commercial irradiation facilities emitting 100's Gy/min.

Results of split dose irradiation of *L.monocytogenes* at 20°C with an initial cell concentration 10^9 CFU/ml TSB are presented in Figure la. When comparing the control versus the composite of split dose, the survival curves appear to be quite similar. However, in calculating the D-Values using linear regression analysis, there were statistical differences ($p < 0.05$) in the slopes of the 1 and 2 h time between fractional doses (TBF) when compared with the control and the two shorter TBF By regrouping the survival plots, according to regression calculated D-Values, differences in the slopes become clearer. Figure 1 b groups the survival curves for the control (single dose) and samples with the 0.25 and 0.50 h TBF. The slopes of all three curves were similar with no statistical differences among regression or total dose calculated D-Values (Table 2). Figure lc compares the survival plots of control sample with the 1 and 2 h TBF. In these plots, it is clear that the split applications were successful in reducing the total dose necessary to reduce the bacterial population to zero recovered. A 1 kGy reduction in total dose increased the slope of regression with a significant drop in D-Values from 0.50 kGy for the control to 0.42 and 0.41 kGy in the 1 and 2 TBF samples, respectively. Similar results were obtained using total dose calculation, Table 2. When the numbers of surviving bacteria were presented in a cumulative fashion for the control and each of the split dose conditions (Figure 2), it was apparent that the total numbers of bacteria recovered were less with the 1 and 2 h TBF than with the control or 2 shorter TBF (0.25 and 0.50 h). Noticeable differences occurred beginning with the 2 kGy total dose. At 2 kGy *Listeria,* when present at 10^3 CFWml, has been shown to be highly sensitive and unable to recover (Andrews and Grodner, 1992). However, Patterson et al. (1993) reported that 10^4 CFU/g *L. monocytogenes* was able to recover from a dose of 2.5 kGy with exponential growth after 2.5 days storage at 15°C. It appeared on cumulative plots, that at the 2 kGy level the radiosensitivity of *Listeria,* may have been most affected by using split dose application. One possibility for an increase in sensitivity with the 1 and 2 h TBF was that this time frame allowed for the bacterium to become actively involved in the repair and recovery of radiation damage. Subsequently, there may have occurred a lessening or depletion of enzymatic activity which might otherwise block the ionization (hydroxyl radical) or toxic products

(hydrogen peroxide) produced from the indirect effects of irradiation. Another explanation was that the bacterium was attempting to regenerate and thus more likely susceptible to irradiation damage. Bacteria have been shown to be most susceptible to irradiation damage during growth phase (Urbain, 1986; Josephson and Peterson, 1982; Kelner et al.,1955). In fact, all cells exhibit an inverse relationship between resistance and level of metabolic activity (Johns and Cunningham, 1983).

Results of the lower initial cell concentration (10^6 CFU/ml) at the same temperature (20°C) were similar to those just described in the previous paragraph. Figure 3a shows the plot of all split doses at this temperature and cell concentration. As indicated by the plot and subsequent regression analysis, D-Values for the control, 0.25, and 0.50 h TBF were the same 0.58 kGy (Figure 3b). D-Values for the 1 and 2 h TBF plots, 0.42 and 0.41 kGy, respectively, (Figure 3c), under the same conditions, were significantly ($p < 0.05$) lower than that of the control (0.58 kGy). Total dose D-Value calculations gave similar results, Table 2. The similarity of the results implied that survival plots were linear throughout with no significant shoulders or tails. Cumulative plots of survivors, Figure 4, indicated that, at 10^6 CFU/ml, the radiosensitivity of this bacterium was most susceptible to changes in dose application at the 1 and 2 kGy level. Again, this may have been related to the bacteriums level of metabolic activity.

Table 2. Comparison of D-Values for *Listeria monocytogenes* at 20°C

TBF_a/hr	LRA_b D_{10}	TD_c D_{10}
10^9 CFU/ml		
None	0.50_d	0.51_d
0.25	0.51_d	0.53_d
0.50	0.50_d	0.46_{de}
1.00	0.42_e	0.43_e
2.00	0.41_e	0.43_e
10^6 CFU/ml		
None	0.58_f	0.55_h
0.25	0.58_f	0.50_h
0.50	0.58_f	0.50_h
1.00	0.42_g	0.45_j
2.00	0.41_g	0.41_j

[a] TBF = time between fraction of equally split doses.
[b] LRA = linear regression analysis $r^2 > .95$
[c] TD = total dose/bacterial counts
Statistical differences ($p < 0.05$) occurred between
$_d$and$_e$;$_f$and$_g$;$_h$and$_j$

CONCLUSION

Statistical differences occurred in the radioresistance of *L. monocytogenes* with a split dose of 1 and 2 h TBF. From an irradiation processors point of view, it is important to know that in the event of a brief shut down (up to 2 hours) of an ongoing process, little adjustment (if any) in exposure time would be necessary to complete the process as prescribed. There are situations in an irradiation facility when the radiation source is shielded and process temporarily halted. As long as the product remains in the plant, it is legal to resume and complete the process as prescribed (Everett, 1994). It is not legal, however, to apply a dose, ship a product out of the plant, and return the product to the plant for further processing.

Better understanding of the mechanisms involved in bacterial resistance to radiation exposure need to be explored. Biochemical activities, enzyme and growth metabolites, that may have occurred in these or similar experiments should be tested. This type of testing may be possible when using a higher output from the radiation source and under a more controlled environment such as self contained machine sources available today and in the future.

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List of Figures

Fig. la Survival plots for 10^9 CFU/ml L. monocytogenesall times between fractions (TBF).

Fig. lb Survival plots for 10^9 CFU/ml L. monocytogenes0.00, 0.25, and 0.50h TBF.

Fig. lc Survival plots for 10^9 CFU/ml L. monocytogenes0.00, 1.0, and 2.0h TBF.

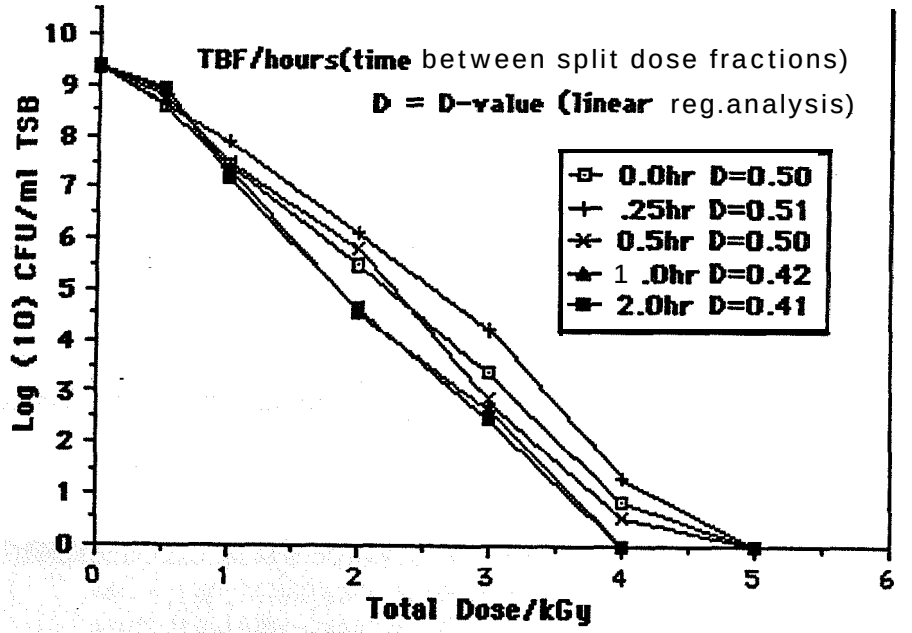
Fig. 2 Cumulative survival plots 10^9 CFU/ml L.monocytogenes all time between fractions

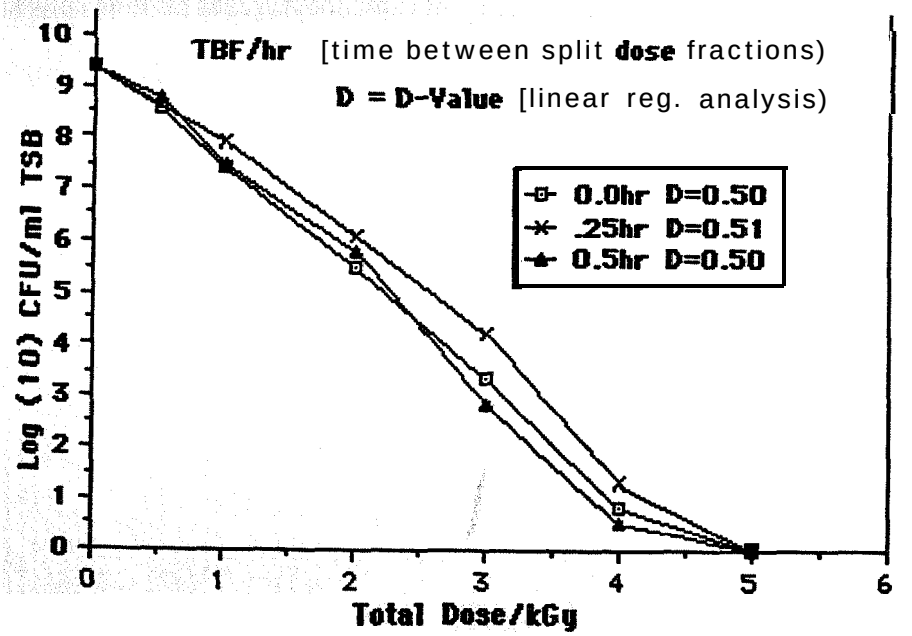
Fig. 3a Survival plots for 10^6 CFU/ml L. monocytogenes all times between fractions (TBF).

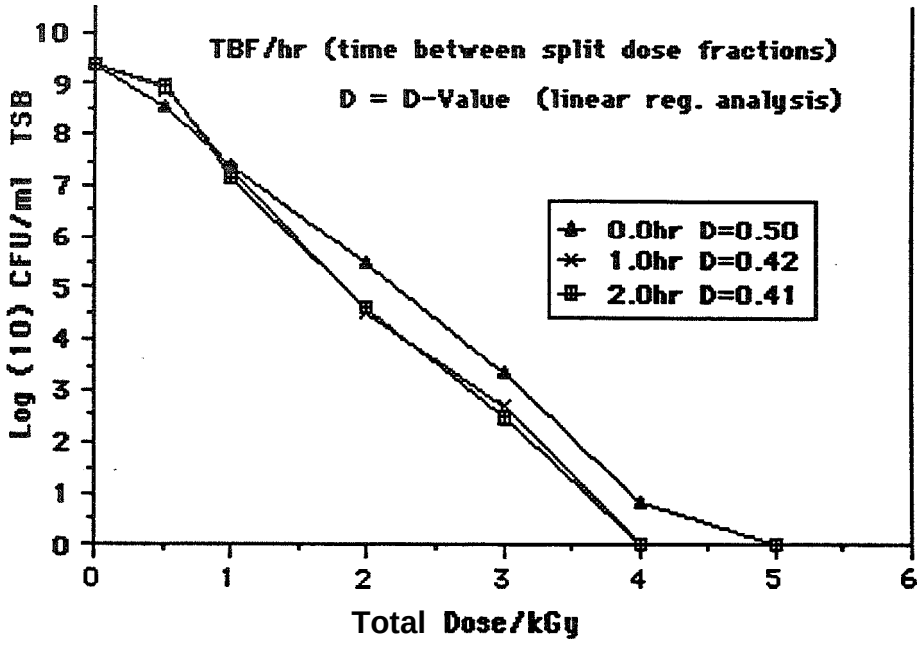
Fig. 3b Survival plots for 10^6 CFU/ml L. monocytogenes 0.00, 0.25, and 0.50h TBF.

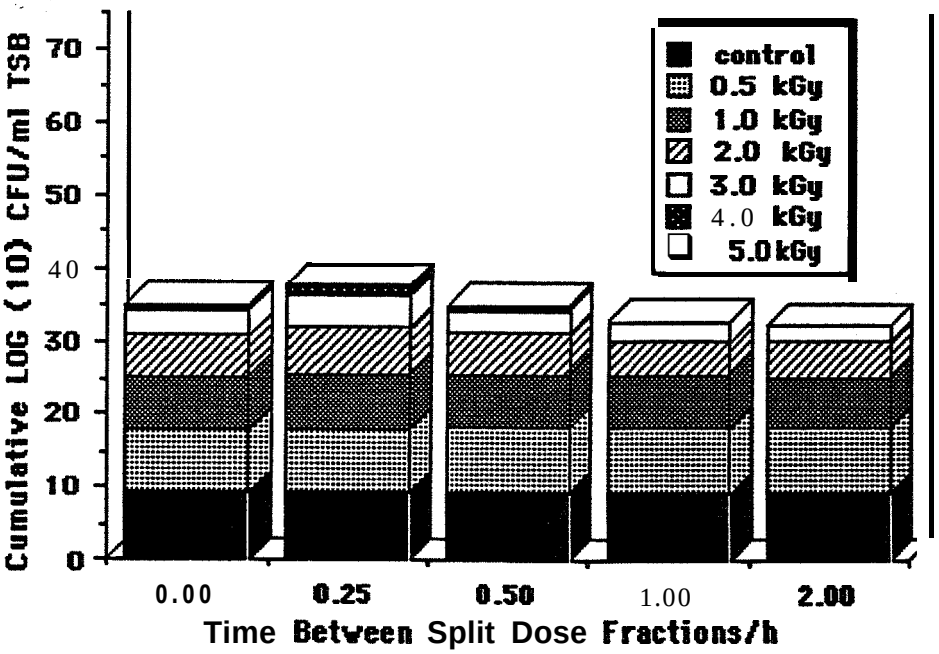
Fig. 3c Survival plots for 10^6 CFU/ml L. monocytogenes 0.00, 1.0, and 2.0h TBF.

Fig. 4 Cumulative survival plots 10^6 CFU/ml L.monocytogenes all time between fractions.


TBF/hours(time between split dose fractions)
D = D-value (linear reg.analysis)
0.0hr D=0.50
.25hr D=0.51
0.5hr D=0.50
1 .0hr D=0.42
2.0hr D=0.41
Log (10) CFU/ml TSB
Total Dose/kGy


TBF/hr [time between split dose fractions)
D = D-Value [linear reg. analysis)
0.0hr D=0.50
.25hr D=0.51
0.5hr D=0.50
Log (10) CFU/ml TSB
Total Dose/kGy


TBF/hr (time between split dose fractions)
D = D-Value (linear reg. analysis)
0.0hr D=0.50
1.0hr D=0.42
2.0hr D=0.41
Log (10) CFU/ml TSB
Total Dose/kGy


control
0.5 kGy
1.0 kGy
2.0 kGy
3.0 kGy
4.0 kGy
5.0kGy
Cumulative LOG (10) CFU/ml TSB
0.00
0.25
0.50
1.00
2.00
Time Between Split Dose Fractions/h

AUTOMATED OHMIC THAWING OF SHRIMP BLOCKS

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INTRODUCTION

The totalU.S. shrimp catch and imports in 1993 were 133 million kg, and 273 million kg, respectively (Current Fishery Statistics, 1994). Shrimp constituted 37% of total edible imports in 1993. Seventy percent of shrimp processed in Florida is imported, mostly in the form of rectangular frozen blocks (Miyajima, 1993). These need to be thawed before fiuther processing and handling. The conventional process to thaw shrimp blocks is to place them into large warm water vats. Water is a good heat transfer medium (Singh and Heldman, 1993), and immersion in water reduces thaw drip loss in shrimp (James and Bailey, 1984). Henderson (1993) reported 1 - 2% increase in the moisture content of Shrimp thawed in water. However, there are problems with this method. Warm water carries heat to the surface of the block by convection, and then heat is transferred by conduction from the surface to the center. This is a slow process. Since thermal conductivity of food is greater in the frozen than in the non-frozen state, and the heat capacity of food is greater in the non-frozen than in the frozen state (Karel and Fennema, 1975), the thawed layer of food on the surface of the block acts as a thermal insulator; thus, the thaw times are long. For 2.3 kg shrimp blocks (30 cm x 22 cm x 5 cm) the thawing time is on the order of 1.5 to 2 hours. The possibility of microbial growth is high for the outer layers exposed to the warm water for long periods of time. Another disadvantage is the loss of some soluble proteins during thawing. This reduces the nutrient quality of the shrimp (Peplow, 1975), and increases the load in the wastewater generated. The most important disadvantage is environmental. Gulf Coast schrimp processors use an estimated 3.4 bilion L water annually (Bough and Perkins, 1977). In locations where availability of fresh water is limited, this is an impediment to the operations and growth of the plants. Wastewater generated by the Gulf Coast shrimp industries is estimated to contain 4.5 million kg/year dissolved and suspended solids (Bough and Perkins, 1977). Large shrimp processing plants pay up to$75,000/month on sewer costs (Miyajima, 1993). Therefore, they must find an alternative method to thaw frozen shrimp blocks.

When an electrical current passes through food, its resistance converts electrical energy to heat This is called ohmic heating, and it is being seriously considered as a method to heat foods (Sastry, 1992). This method can also be used to thaw frozen foods by placing them between two

electrodes and applying an alternating current. The advantages of this process are (1) there is no water used in thawing and no wastewater generated, (2) thawing can be relatively uniform due to volume heating, (3) the process can be easy to control. Previous research conducted in our lab (Henderson, 1993) pointed to a potential problem with ohmic thawing. As the frozen product thaws, current passes through the thawed portion of the block more readily, since frozen shrimp has 100 times lower electrical conductivity than thawed shrimp (Luzuriaga et al., 1994). With the current flowing through the thawed portion of the block, shrimp in that portion may cook, while the rest of the block is still frozen This is called runaway heating, or the formation of hot spots. If this problem is solved, a new method to thaw foods would be available. In this study, an automated prototype ohmic thawing unit to eliminate the runaway heating was designed, built and tested. The specific objectives were: (1) To design a prototype portable ohmic thawing unit, with surface temperature sensing and computer automated capabilities, that can thaw two shrimp blocks at the same time. (2) To conduct extensive testing of the operation of the unit to assure the elimination of runaway heating, and to develop guidelines and suggestions for scale-up. (3) To compare the quality attributes (moisture content, microbial loads, and taste) of ohmically and conventionally thawed shrimp in a processing plant environment.

MATERIALS AND METHODS

Previous work showed that hot spots occur at both sides of the blocks simultaneously. Our objective was to automatically shut the current off when a point on the surface starts to heat. We decided to have four electrically isolated quadrants per electrode, so that when power to a quadrant is shut off the others will still continue to heat the rest of the block. The electrodes (24.13 x 30.48 cm x 1 cm) consisted of 304 stainless steel. Each quadrant has 32 thermistors covering the surface uniformly ; the maximum distance from any point to a sensor was less than 1.91 cm (3/4") The thermistors resided in holes drilled into the plates. The thermistor leads from each electrode quadrant were fed to an electronic circuit board designed by MicroTherm, Inc., Gainesville, FL. Temperatures were compared to an adjustable set point (above the melting point of shrimp and well below cooking temperatures). If the temperature of any of the 32 thermistors is greater than the set point, a microprocessor automatically turns off the power relay to that quadrant. A time delay is allowed for heat to dissipate. Then power to the quadrant is turned on by the computer. This is a positive method to control overheating regardless of size, moisture content, salinity etc. of diferent shrimp blocks.

At the beginning of the operation relatively high voltages are needed since the resistance of the block is high (Henderson, 1993). As thawing continues, the temperature of the block increases and resistance decreases. Therefore, reduction of voltage is necessary to keep the current at a given level and prevent overheating. The control of current was automated by a stepper motor and circuit board (designed by Dr. S.Yeralan, Ind. Eng. Dept.) connected to a transformer (602OCT-25, Staco Energy, Dayton, OH). The system monitored the current and voltage of each quadrant continuously. Smce the control "intelligence" is not concentrated at the computer but distributed to several boards, the flexibility reliability, and speed of the operation is increased. A mobile cart was built to transport the prototype device to Singleton Seafood in Tampa, FL for in-plant testing.

A computer program was developed in Turbo Basic to supervise the operation. The logical algorithm of the program is as follows:

1. Turn on the heating relays to all four quadrants of an electrode.
2. Setup a fast loop where the amps, currents, temperature alarm status, and relay on/off status is read, the watt-hrs calculated and accumulated for each quadrant, and for the total shrimp block.
3. At predetermined intervals, these values are shown on the computer screen, and printed to an output file.
4. The time interval in which a quadrant is turned off due to a temperature alarm is monitored. When the alarm for that quadrant is off, additional time is allowed for the temperature to equilibrate before the relay is turned on again.
5. The maximum amp flowing through the system is kept constant by the stepper motor. Amperage levels are set by the operator.
6. The end point of the process is reached when the total accumulated watt-hrs reaches the heat necessary to thaw that particular block.

Experiments

Lab Tests

Frozen rectangular shrimp blocks supplied by Singleton Seafood, Tampa, FL were transported to the University of Florida in Gainesville on dry ice, and placed into a freezer at -20°C. Four different kinds af shrimp were thawed using the ohmic system: (1) Yolita Brand, white shrimp, butterflied, tail-on, peeled and deveined, 41- 50 count to the lb; (2) Moon Star Brand, pink shrimp, round, peeled and undeveined, 91 - 120 count; (3) Marbella Brand, pink shrimp, round, peeled and undeveined, 130 - 150 count; and (4) white shrimp, butterfly, tail-on, peeled and deveined, higher count than Yolita Brand.

Seven thawing experiments with two blocks thawing simultaneously were conducted in the lab. Before placing between the electrodes, each block was measured and weighed. The weight information was entered into the computer program. During the thawing process, the set amperage was recorded whenever it was changed. After thawing was complete, the shrimp and thaw drip were collected separately and weighed., Thawing times at diferent conditions were compared, and the proper functioning of the unit was tested.

In - Plant Testing

The mobile unit was transported to Singleton Seafood for two one-week periods. Conventional thawing experiments were conducted in the steam injected thawing tanks with 2,888 L capacity. Only similar types of shrimp were thawed at the same time. Each block was weighed and measured. The time necessary for the blocks to thaw was recorded. Then, samples for microbiology and moisture analysis Were taken. Sanitary bags (Fischer Scientific, Pittsburgh, PA) were used to collect shrimp and water samples.

Energy Input Calculations

The actual energy input Q_{Actual} was calculated in the program based on:

$$Q_{actual} = P \Delta t = V I \Delta t \quad (1)$$

where V = voltage (V), I= current (A), P = power (Watts), t = time (hrs), and E = energy (KJ). The theoretical energy, Q_{thcor} needed to thaw the shrimp block was calculated as:

$$Q_{theor} = \left[m\ Cp_{below}(T_{fp}-T_{initial}) + m\ \lambda + m\ Cp_{above}(T_{final}-T_{fp}) \right]_{ice} + \left[m\ Cp_{below}(T_{fp}-T_{initial}) + m\ \lambda + m\ Cp_{above}(T_{final}-T_{fp}) \right]_{shrimp} \quad (2)$$

where: m = mass of the shrimp, kg
Cp_{below} = heat capacity of the product below freezing, KJ /kg · °C
Cp_{above} = heat capacity of the product above freezing, KJ /kg · °C
T_{fp} = freezing point of the product, °C
$T_{initial}$ = initial temperature of the block, °C
λ = latent heat of freezing, KJ /kg
T_{final} = final temperature after thawing the block, °C

The theoretical energy calculated was compared to the actual amount of electricity used to thaw the blocks by defining the coefficient of performance (COP) which is the percentage of total energy that came from the electricity to thaw the shrimp blocks.:

$$\frac{Q_{actual}}{Q_{theor}}(100) = C.O.P \quad (3)$$

Quality Attribute Comparison

Microbiology

Culture tubes were filled with 3.6 ml of Butterfield's buffer (Speck, 1976). Twenty five g shrimp and 225 ml Butterfield's buffer were homogenized. Shrimp samples were serially diluted to 10^{-6} and water samples to 10^{-8} One-tenth ml of each dilution was plated on four aerobic plate-count agar (DIFCO Labs, Detroit, MI) plates. The incubation time was 48 hours at 25°C. The plates were evaluated by counting visible colonies, and averages taken.

Moisture Content Determination

Several shrimp were peeled, and diced in a food processor. A 10 g sample was weighed in the moisture balance (MB200, Ohaus Corporation, Florham Park, NJ). After 25-30 minutes the reading stabilized, and moisture values were recorded. A t-test was used to determine whether the moisture contents between immersion thawed shrimp and ohmic thawed shrimp were significantly diferent at the 5% level.

Sensory Comparison

Thawed shrimp from water immersion and ohmic methods were steamed in Singleton's Stem Counterflow Oven at 104°C. The smaller shrimp (71- 90 count) were cooked for 120 secs, and the larger shrimp(31 - 40 count) for 165 secs. They were then iced and transported to Gainesville, FL. A triangle test was given to untrained panelists who were asked to select the odd sample by considering sensory attributes of appearance, smell, texture and taste. They were given 2 consecutive tests. Statistical significance of difference was evaluated at α = 5%, (Meilgaard et al., 1991).

RESULTS AND DISCUSSION

The computer program saved voltage, current, and cumulative watt-hrs vs time data. From these, plots were generated to determine a given shrimp block's thawing characteristics. With two ohmic units runnmg simultaneously, eight quadrants were analyzed during a single experiment. One of the eight quadrants determined the voltage level applied to the entire ohmic system. This quadrant was the "limiting quadrant" and indicated the area in the shrimp block with the greatest conductivity. This limiting quadrant was determined by observing from the graphics which quadrant first allowed current at the given voltage level. During the initial period of thawing, the block has a high resistance, anda high level of voltage is necessary to pass the set current. During the later stages the temperature of the block is close to the freezing point, portions of it are partially thawed, and the resistance is lower. The level of voltage necessary to pass the set current is low. In this period, the operation is controlled by the surface temperature, indicated by frequent LED and relay off /on sequences. In all experiments, the temperature of the shrimp was below the set point temperature (12.7° C) at all times. This was also confirmed at the end of each-experiment by touching the thawed shrimp. In all cases, the ohmically thawed shrimp was colder than the water immersion thawed shrimp.

Lab Test Analysis

Table 1 summarizes the results of the lab experiments. The thickness, net weight, time required to thaw, electrical energy used, and the C.O.P. for each experiment is shown.

Table: 1. Operational Results of Lab Tests.

Test	Thick. cm	Net. Wt. g	Shrimp / water	Thaw time min	Calc. Watt-Hr	Theor . Watt-Hr	COP
Set 1:1	5.4	2027	2.48	124	57.5	305.85	18.8
2	6.03	1918	2.07	124	55.8	307.42	18.2
Set 2 :1	3.81	1950	3.76	190	34.1	261.1	13.3
2	3:81	1482	1.79	100	87.3	250.9	35.5
Set 3:1	4.45	1814	2.12	176	60.9	288	21.6
2	4.45	2054	3.02	161	34.9	291.5	12.2
Set 4 :1	4.76	2223	3.84	137	87.89	295.98	30.4
2	4.45	1953	2.27	87	32.1	302.9	7.4
Set 5 :1	4.45	1759	2.59	97	69.1	261.5	26.4
2	4.45	1556	2.47	97	36	234.6	15.3
Set 6:1	5.72	1878	2.16	155	42.2	296.1	14.3
2	5.08	1606	2.54	119	32	239.8	13.3

In plant Comparison Tests

The results of the lab tests showed that the ohmic system worked as designed, with the capability of thawing two blocks simultaneously. Since the approximate thawing time of similar blocks by immersion thawing was reported in previous experiments, the lab test results proved that the ohmic units could thaw shrimp blocks in a time period competitive with water immersion, without the incidence of hot spots. Thus, the major obstacle of ohmic thawing has been solved, and the ohmic

unit could be used with confidence to thaw shrimp blocks and be competitive, if not better, than the conventional method. The next step was to use the unit in a plant environment, and to compare quality attributes of shrimp thawed with both methods. Table 2 summarizes the results of the in-plant experiments. The thickness, net weight, time required to thaw for the ohmic and control experiments, the electrical energy used, and the C.O.P. for the ohmic experiments are shown.

Table 2. Operational Results from In-Plant Tests.

Test	Thick. cm	Net. Wt. g	Shrimp /water	Thaw time min	Calc. Watt-Hr	Theor. Watt-Hr	COP
Single 1: 1	6.35 - 6.67	1956	2.5	45	13.2	293.5	4.5
2	6.35 - 6.67	1930	2.17	45	81.2	304.4	26.7
Control :1	5.72	1784	1.47	50	---	---	---
2	6.99	1867	1.49	75	---	---	---
Single 2	5.4	1914	5.01	85	20.2	240.9	8.4
Control : 1	5.4	1860	4.04	75		---	---
2	5.72	1890	3.03	75	---	---	---
Single 3 : 1	6.99	2066	3.93	121	13	273.0	4.8
2	7.62	2150	4.37	121	12.6	278.1	4.5
Control : 1	7.62 - 8.89	1993	2.86	40		---	---
2	7.62 - 8.89	2089	3.01	40	---	---	---
Single 4	6 - 7.5	1807	2.23	77	32.3	287.4	11.2
Control : 1	5.8 - 7.1	1714	1.52	71	---	---	---
2	5.8 - 6.0	1896	2.24	71		---	---
Single 5	6.7	1855	1.92	170	81.6	305.9	26.7
Control : 1	6.7	1880	1.99	130	---	---	---
2	5.7	1851	2.08	130	---	---	---
Single 6	5.72 - 6.99	1486	1.18	104	101.9	303.3	33.6
Control :1	5.4 - 6.35	1812	1.81	75	---	---	---
2	6.03	1794	1.89	75		---	---

Note : The control samples were the shrimp thawed in the immersion tanks.

Table 2 shows the shrimp blocks used in the ohmic system and in the immersion were similar in thickness, net weight, and shrimp/water ratio within each experiment. Generally, the immersion thaw method was faster than the ohmic thaw method. However, optimization of the ohmic process should reduce the time. One noticeable difference between the two methods is the temperature of the shrimp after thawing. The temperature of the shrimp from the immersion method was much warmer than the shrimp from the ohmic thawed shrimp. The temperature of the water in the immersion tank was 28°C so the shrimp from this method was near 28°C. The shrimp from the ohmic unit never reached above 12.7°C. Typical voltage, current, and cumulative watt-hrs of quadrants vs. time are shown in Figure 1.

Microbiology Comparison

The shrimp and water from both methods of thawing were compared for aerobic microbial plate counts. Table 3 gives the results of this study. The microbial count of the ohmic thawed shrimp and water were on the same order of magnitude as that of the immersion thawed shrimp and water.

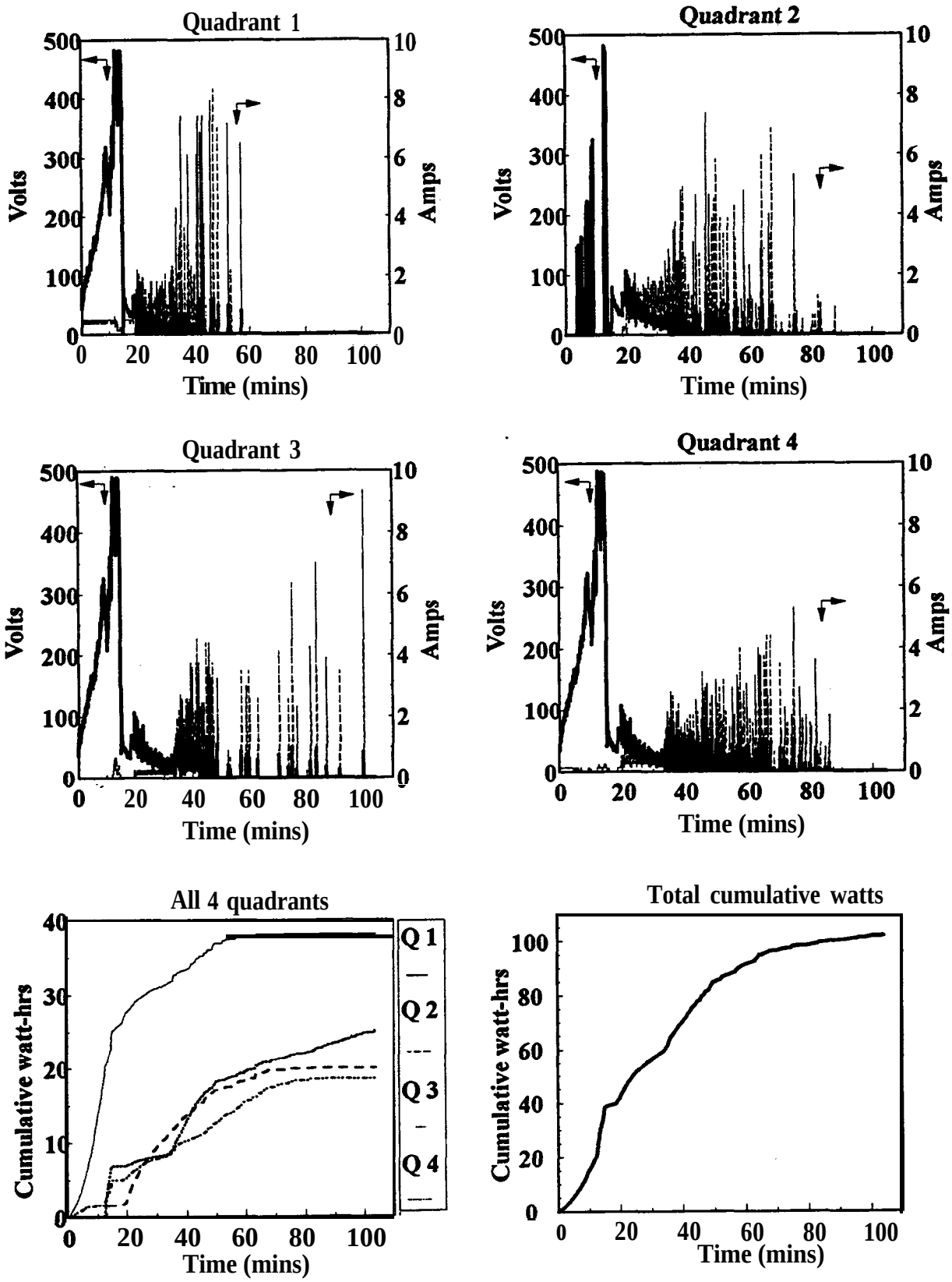

Figure 1. Typical volt, amps, and cumulative watt-hrs vs time profiles.

Table 3. Microbial Comparison Results.

Thaw Method	Shrimp (per gram)	Water (per ml)
Initial (n= 3)	1.35 $x10^5$	2.13 $x10^5$
Ohmic (n = 4)	2.75 $x10^5$	5.45 $x10^4$
Immersion (n = 5)	3.34 x 10^5	6.63 x 10^4

Moisture Content Comparison

The moisture content of large shrimp showed no significant difference (5% level) between the two methods (Table 4). Small shrimp, however, showed significance differences. This can be explained by the larger surface-to-volume ratio of small shrimp, and is consistent with the findings of Henderson (1993). However, any difference in moisture content of the two thaw methods would disappear during storage in ice water prior to processing, as confirmed by Henderson (1993).

Table 4. Moisture Content Comparison Results.

Sample	Immersion	Std. Dev.	ohmic	Std. Dev.	Diff.(@ 5%)
Pink peeled, l00-130 count	81.6% (n=4)	0.79	80.4 % (n=4)	0.63	No
Yellow, peeled, tail-on 9l-00 count	83.2 % (n = 4)	0.4	82 % (n=2)	0.57	No
Brown, tail-on 150-200 count	83.1 % (n = 4)	0.48	80.9 % (n=4)	0.76	Yes
Pink, peeled 150-200 count	83.4 % (n = 10)	1.28	80.1 % (n= 6)	1.61	Yes

Sensory Test Comparison

Table 5 shows the results of sensory comparison tests. For 54 panelists taking the test, at least 25 panel members need to answer correctly to report a significant difference at the 5% level (Meilgard et al., 1991).There was no significant difference (5%) between the two shrimp samples in either test. Many of the panelists who answered correctly said that they guessed. Trained panelists at Singlenton thought the ohmic thawed shrimp had better texture than the immersion thawed shrimp.

Table 5. Taste Test Results.

Sample	Correct Responses	Incorrect Responses	Significance (at P <0.05)
71-90 count (n=55)	**21**	**34**	**No**
31-40 count (n=53)	**15**	**38**	**No**

FUTURE WORK

The ohmic system performed to its design specifications. These results should encourage further work for improvement of the ohmic thawing unit towards scale-up and commercial operation. These are:

1. The thermistor-based surface temperature monitoring method should be replaced by a fiber optics-based method. When a liquid crystal that changes its color at the desired set point temperature is coated to the tip of a plastic fiber, it can detect temperatures much safer, since there is complete isolation of the circuit boards from the high voltage and current of the electrodes. This method is also much less expensive than using thermistors.

2. The current system operates in batch mode. Industrial applications require continuous operation. A system of rollers can be designed that would act both as the electrodes, and as the transport mechanism for the blocks on a conveyor system.

3. The inherent disadvantage of the current system is that it supplies the same voltage to all 8 quadrants, bound by the "limiting quadrant". The high conductivity of the limiting quadrant causes that all other quadrants have lower amps passing through them. Since heat generated is proportional to the square of the current, they thaw slower. A system that can supply diferent levels of voltage to diferent quadrants would solve this problem. Ideally, each quadrant would have its own voltage, set current and control system.

4. A positive method of monitoring the electricity used, and therefore predicting the end-point of thawing would be to attach a watt-meter to the unit. This is quite inexpensive, and should be the first modification to the current system.

5. Optimization of the set current profile during thawing depending on shrimp species, size, shell on/off, shrimp to water ratio in the block, etc. This will also build the database of knowledge necessary to apply ohmic thawing effectively to commercial operations.

6. There are reports in the literature that coating of the electrodes may minimize or prevent electrolysis at the electrode in the high voltage and high current density operations. This would extend the life of the electrode, and minimize the deleterious effects of the interaction of gases evoking at the electrode (oxygen, chlorine, etc.) with the food.

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Using Edible Film to Improve Smoked Fish Quality

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INTRODUCTION

Edible film is defined as thin layers of material which can be eaten by the consumer and provide a barrier to moisture, oxygen, oil and solute movement for the food. It can improve mechanical-handling properties, impart added structural integrity to foods, and retain volatile flavor compounds. It is sometime used to carry food additives. Edible film has been used for many years. The earliest recorded use was in 12th and 13th centuries in China. later in 16th century, "Larding" was used in England. Preservation of meats and other foodstuffs by coating with gelatin films was proposed in late 19th century. In 1930's, hot-melt paraffin waxes became commercially available for citrus fruits. In 1950's, carnauba wax oil-in-water emulsions were developed for coating fresh fruits and vegetables.

Smoked fish is a ready-to-eat product; however, this value added seafood product has a short shelf life. Rainbow trout is a high valued commodity in seafood markets in the US. In recent years, many small rainbow trout farmers have begun to produce smoke product. Due to several commercial smoked salmon products have been recently recalled for the contamination of Listeria monocytoeenes, eliminating this pathogen becomes a timely task. In order to insure a high quality and safe smoked fish, this study was designed to evaluate edible film incorporated with an antimicrobial agent to reduce psychrotrophic bacteria population as well as eliminate the contamination of L. monocytogenes on rainbow trout.

MATERIALS AND METHODS

Smoked Rainbow Trout

Smoked rainbow trout fillets were purchased from a commercial trout farm in north Georgia. Each fillet was cut into 5X5 cm pieces.

Edible Films

Five percent mixture of 3 : 1 of hydroxy propyl methyl cellulose (I-IPMC) and methyl cellulose (MC) was suspended in 30 ml 95% ethanol. Seventy ml distilled water was added while stirring. Potassium sorbate was added at 0.3% level. After 20 min mixing, the solution was allowed to rest for 30 min to remove entrapped air. Ten ml of solution was then poured on a 20X20 cm glass plate and dried at 45-47°C for 7 hrs.

Inoculation and Packaging

For inoculated samples, fillets were inoculated with 1 ml of 10^4 CFU/ml of mixed strain of Listeria monocytogenes (Scott A, LCDC, V-7, and Brie) (Huang et al., 1993). Each fillet section will be wrapped either with or without film before packaging. The packaging method included overwrapped and vacuum skin packaging. All samples stored at 4 and 10°C for 24 and 15 days, respectively and sampled at 3-day intervals.

Microbiological Analysis

Samples were massaged for 1 min with 25 ml sterile Butterfield's phosphate buffer. Appropriate serial dilutions were plated onto the following agars by spread plate methods. Listeria Selective Medium (Oxid, Basingstoke, UK) agar was used to enumerate the L. monocytogenes population. Plate were incubated at 35°C for 48 hrs. The psychrotrophic population was determined on plate count agar (Difco, Detroit, MI) incubated at 20°C for 72 hrs. The aerobic and anaerobic population were incubated at 32°C for 48 hrs (Speck, 1984).

Data Analysis

Analysis of variance was performed on the data by means of PC SAS (SAS, 1987). Ducan's multiple range test was used to determine any significant differences among F-values at different packaging treatment and microbial populations on smoked fillets with and without edible film wrapping.

RESULTS

Effect of Edible Films on Microbial Populations of Smoked Trout

Aerobic, anaerobic and psychrotrophic populations of vacuum skin packaged smoked rainbow trout stored at 4°C were shown in Fig. 1.1, 1.2 and 1.3. Statistically significant difference ($p<4.05$) between fillets wrapped with and without edible films was observed. Edible films reduced microbial populations on fillets by over 1 log. No significant difference on pH values was also found (Fig. 1.4).

FIG-1.1 AEROBIC COUNT OF SMOKED TROUT (4°C)

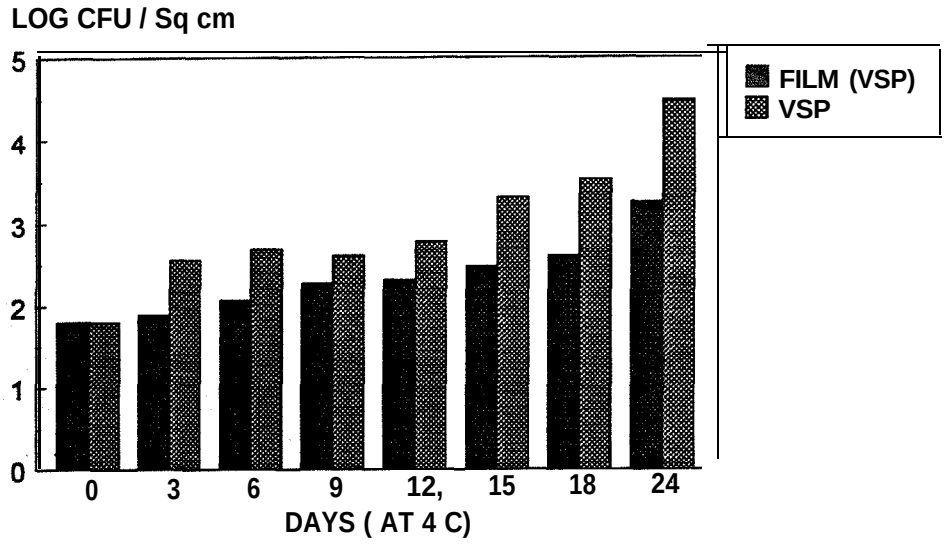


FIG-l.2 ANAEROBIC COUNT OF SMOKED TROUT (4° C)

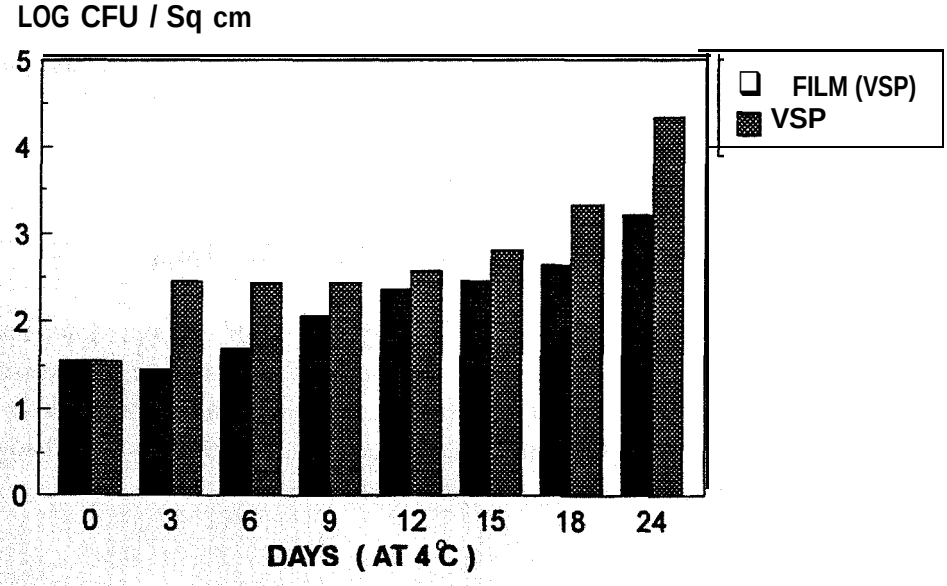


FIG-1.3 PSYCHROTROPHIC COUNT OF SMOKED TROUT (4° C)

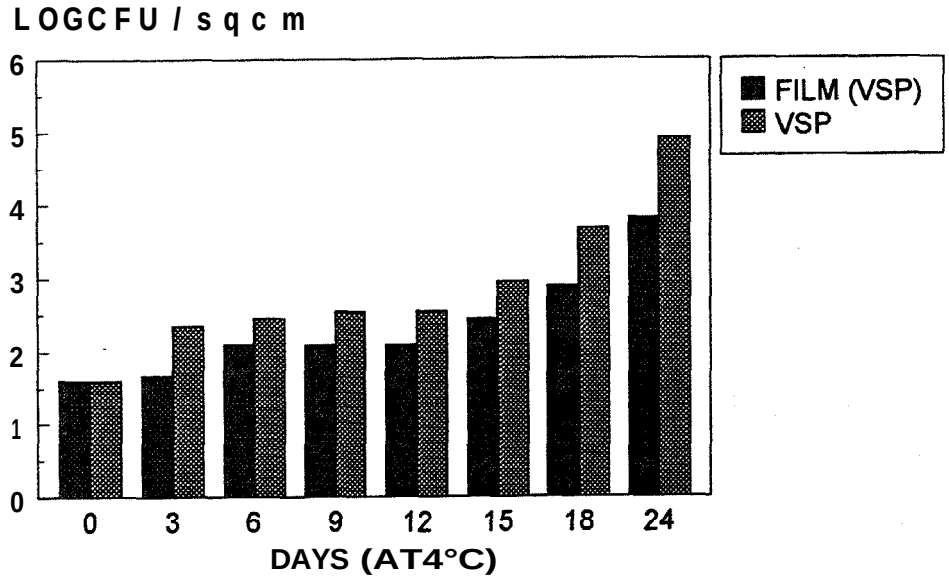
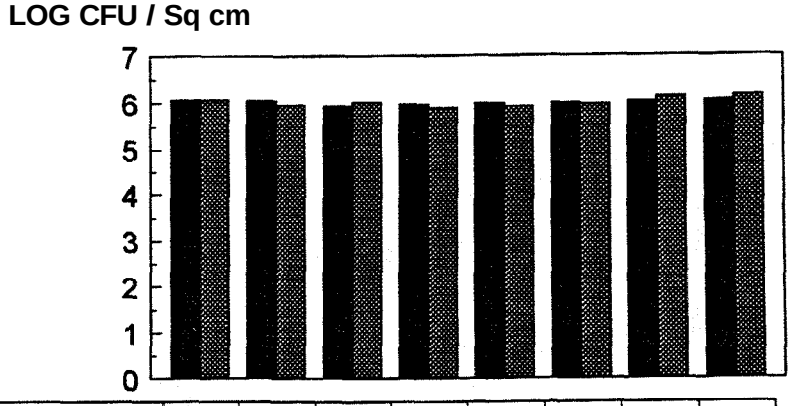


FIG-l.4 pH PROFILE OF SMOKED TROUT (4°C)



DAYS (AT 4℃)	0	3	6	9	12	15	18	24
FILM (VSP)	6.06	6.04	5.92	5.96	5.98	5.97	6.00	6.03
VSP	6.06	5.95	6.01	5.87	5.90	5.95	6.10	6.13

FIG-l.5 AEROBIC COUNT OF SMOKED TROUT (10°C)

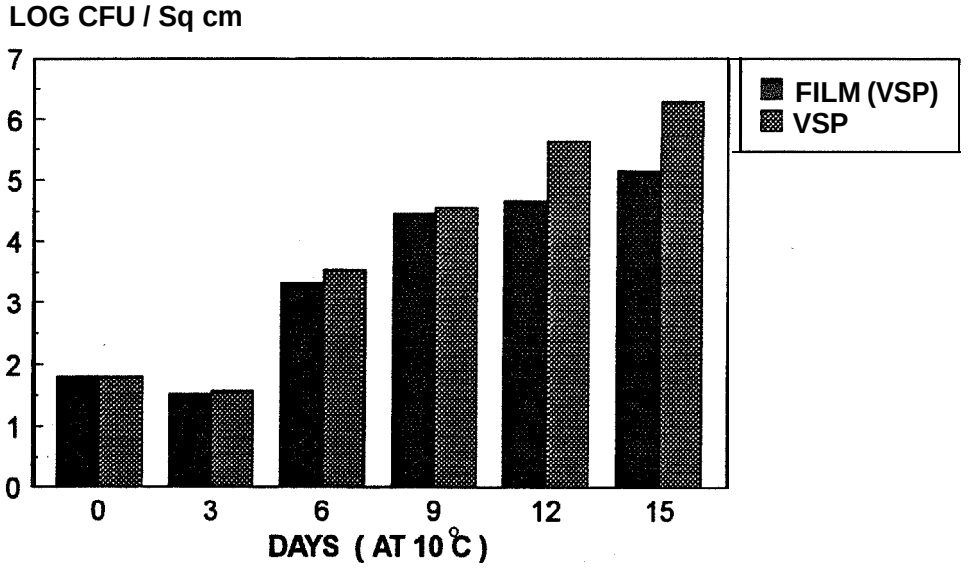


FIG-l.6 ANAEROBIC COUNT OF SMOKED TROUT (10° C)

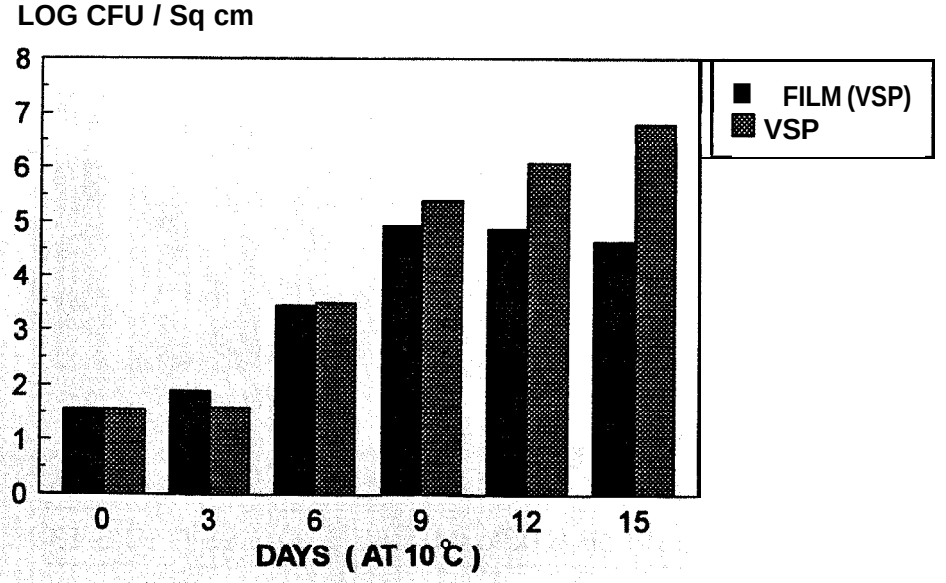


FIG-1.7 PSYCHROTROPHIC COUNT OF SMOKED TROUT (10°C)

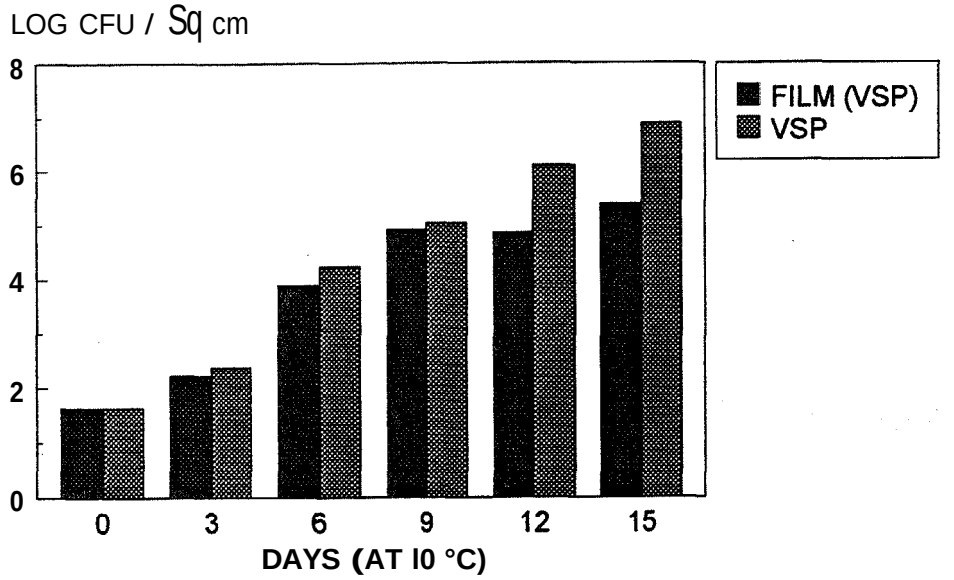
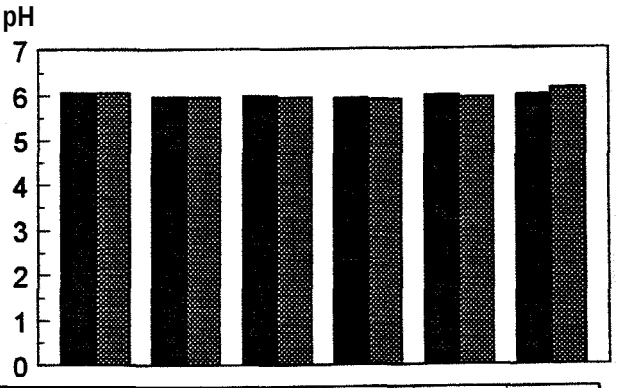


FIG-l.8 pH PROFILE OF SMOKED TROUT (l0° C)



DAYS (AT 10°C)	0	3	6	9	12	15
FILM (VSP)	6.06	5.97	5.99	5.93	5.98	5.98
VSP	6.06	5.95	5.94	5.90	5.93	6.13

FIG-2.1 PSYCHRQTROPHIC COUNT OF SMOKED TROUT (4°C)

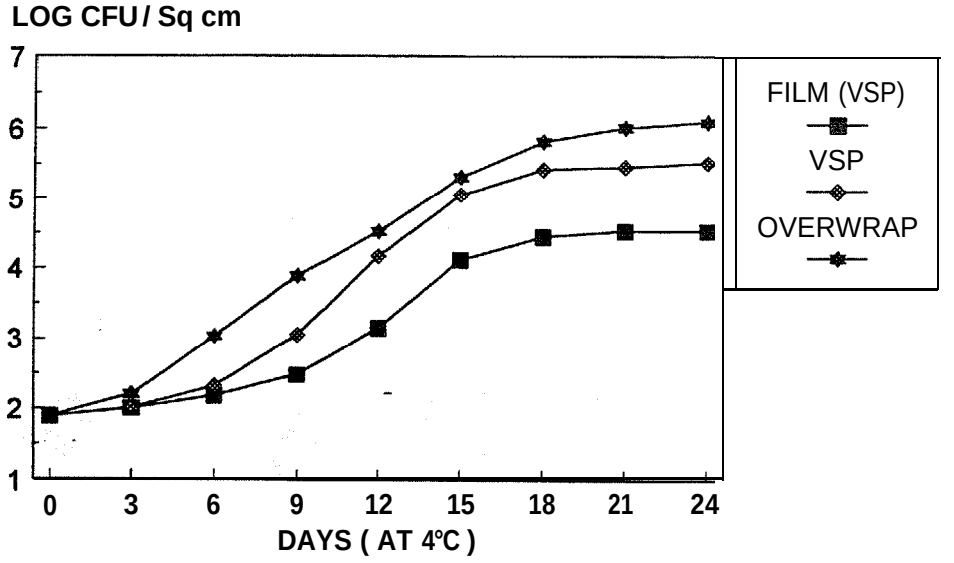


FIG-2.2 *L. monocytogenes* COUNT OF SMOKED TROUT (4°C)

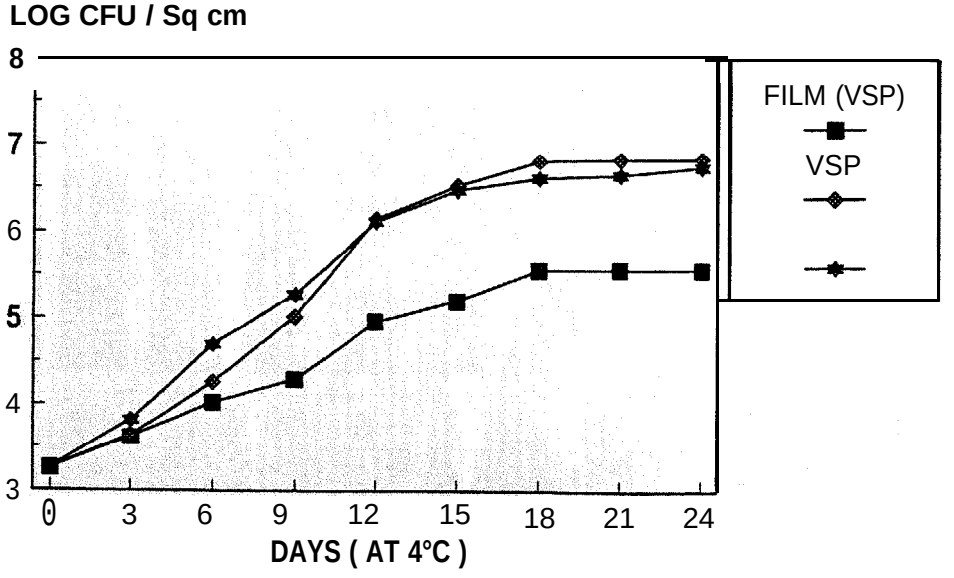


FIG-2.3 PSYCHROTROPHIC COUNT OF SMOKED TROUT (10°C)

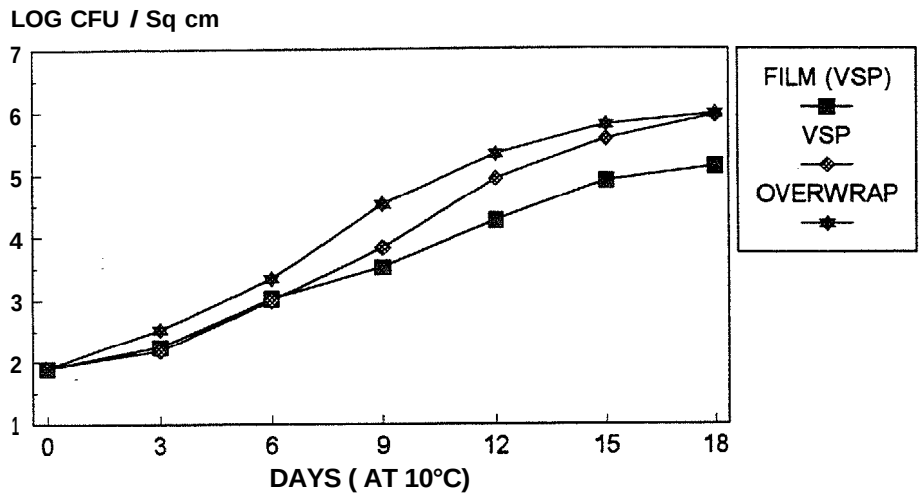
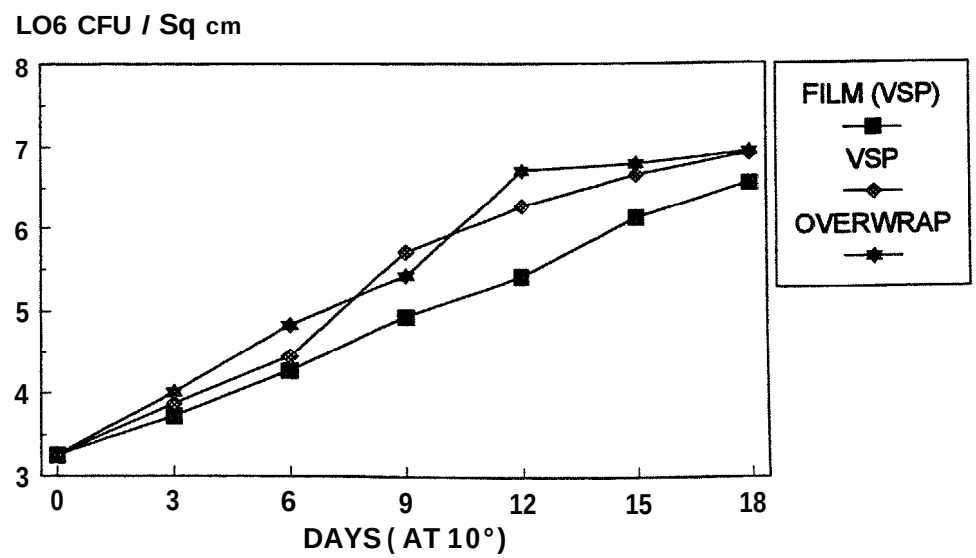


FIG-2.4 *L.monocytogenes* COUNT OF SMOKED TROUT (10°C)



For samples stored at 10°C, no significant difference on microbial population was observed (Fig. 1.5, 1.6 and 1.7). No significant difference on pH values was found (Fig. 1.8).

Effect of Edible Film on Growth of L. monocytogenes on Smoked Trout

Smoked trout wrapped with edible before vacuum skin packaging showed a significant lower Listeria count throughout the entire storage period at 4°C (Fig. 2.2). However, no significant difference was found when samples stored at 10°C (Fig. 2.4). Similar results for psychrotrophic population at both temperatures were observed (Fig. 2.1 and 2.3).

In summary, edible film incorporated with potassium sorbate had potential antimicrobial effect on growth of psychrotrophic bacteria and L. monocytogenes. However, the concentration of antimicrobial agent need to be study to further eliminate the Listeria contamination.

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MICROBIOLOGICAL AND SENSORY CHARACTERISTICS OF FRESH BLUE CRAB MEAT HELD IN TAMPER-EVIDENT CONTAINERS

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INTRODUCTION

On April 1, 1993, North Carolina regulators at the Department of Environment, Health, and Natural Resources announced that all blue crab meat sold or processed within the state must be packaged in tamper-evident containers. The regulators were addressing concerns about consumers opening crab meat containers to examine the contents before purchasing the product. State authorities approved several general types of tamper-evident packaging. Similar requirements have been adopted in other states, are pending, or are being considered for action.

This report describes our efforts to learn the effects of new tamper-evident packaging on the quality and safety of fresh blue crab meat. Little or no information was available to the blue crab industry, regulatory officials, or packaging manufacturers detailing the effects of the tamper-evident containers on head-space gases, microbial growth, chemical decomposition, sensory quality, or shelf life. The probability of toxin production by CJostridium botulinum at refrigeration temperatures also needed to be evaluated. Crab processors and regulators needed this basic information to determine the safety and effectiveness of the new containers.

METHODS

Chemical, microbiological, physical, and sensory changes in fresh special blue crab meat were monitored during 18 days of iced storage at O° C and 13 days of refrigerated storage at 4°C. The meat *was* packaged in four types of retail containers:

(1) 12 oz copolymer polyethylene *cups* that are currently used by most crab processors. The containers were supplied by the Virginia Design Packaging Corporation of Suffolk, VA

(2) 12 oz *copolymer polyethylene cups with* heat-shrink *tamper-evident low density polypropylene seals* from Virginia Design Packaging

(3) *10 oz copolymer polyethylene cans with aluminum easy-open ends from the* King Plastic Corporation, Orange, CA, and

(4) 12 oz *copolymer polypropylene cups with an integral tamper-evident pull-tab* from Triplas Incorporated of Monroe, NC.

Fresh special blue crab meat was packed into 18 containers each of the four packaging types. Three packages of each container type were randomly selected for sampling on the following iced storage days: 3, 6, 9, 12, 15, and 18. An initial composite of fresh meat used to pack the four cup styles served as the zero day sample for all container types.

Oxygen and CO_2 levels were measured from the headspace of each container using an Illinois Instruments 3600 headspace analyzer. Crab meat quality was estimated through chemical, microbiological, sensory, and physical analyses of a three-container composite sample for each of the package types. Ammonia levels (Ward et al., 1978) and pH (Helrich, 1990) were measured with specific ion electrodes. Total volatile base values were analyzed by $MgSO_4$ extraction with a micro-Rjeldahl steam distillation unit (Woyewoda et al., 1986). Aerobic, anaerobic, and psychrotrophic plate counts were enumerated by standard dilution and culture techniques (Speck, 1984). Anaerobic plate count samples were incubated in anaerobic jars at 32°C for 48 hours. A Minolta Chroma Meter CR-200 (Minolta Corporation, Ramsey, NJ) was used to detect Hunter L, a, b color values of the meat (Hunter and Harold, 1987). A seven member trained panel developed sensory odor profiles on a continuous scale from zero to six with zero being none detected and six the strongest possible response for ammonia, sour, putrid, and crab odors (Cardello, 1981; Civille and Liska, 1975; Civille and Szczesniak, 1973; Gates et al., 1984a, Gates et al., 1984b, Jellinek, 1985). The panel's subjective like or dislike of meat color and appearance was rated on a continuous scale from zero to six.

Statistical analyses were performed on physical, chemical, sensory, and microbiological data by means of PC SAS (SAS, 1987). SAS GLM and Duncan's multiple range test at the 0.05 level were used to detect significant differences among sample containers and days of storage. Correlation analyses detected significant relationships among measured parameters and storage time for iced and refrigerated crab meat samples.

RESULTS AND DISCUSSION

Iced Storage

There were no significant differences in pH levels among the four sample containers (Figure I). Ammonia levels gradually increased between six and twelve days of storage. The North Carolina pull tab containers had significantly greater mean ammonia levels than the cups with the heat-shrink seals on day 12 and higher levels than the control and the heat-shrink sealed samples on day 15. Mean ammonia levels increased rapidly for all container types between 12 and 18 days of storage (Figure 2). Total volatile base nitrogen increased gradually after nine days of storage. Levels in the control sample were significantly less than all other samples on day 18 (Figure 3).

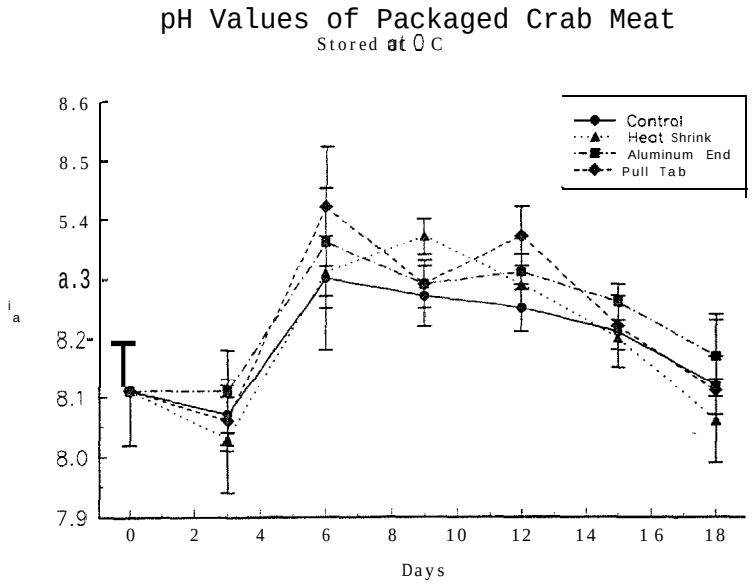

Figure 1. Mean pH levels of packaged crab meat stored at 0°C in the control and three tamper-evident containers

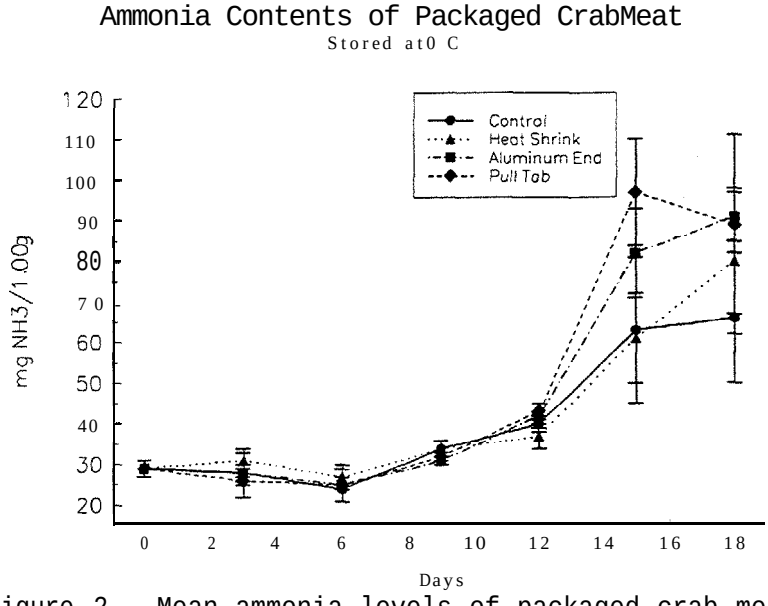

Figure 2. Mean ammonia levels of packaged crab meat stored at 0°C in the control and three tamper-evident containers

Oxygen levels decreased with time and varied considerably among the four iced containers. Levels in the control samples ranged between approximately 20% and 3% 0_2. Oxygen levels in the other containers dropped to less than 1% by the end of the study. On day three the control samples had significantly greater 0, levels than the other cups. The North Carolina tamper-evident tab containers contained significantly less oxygen than any other containers on day three. Oxygen levels in the tamper-evident-tab containers were lower than those found in the control samples on day six. No significant differences were measured for days 9, 12, and

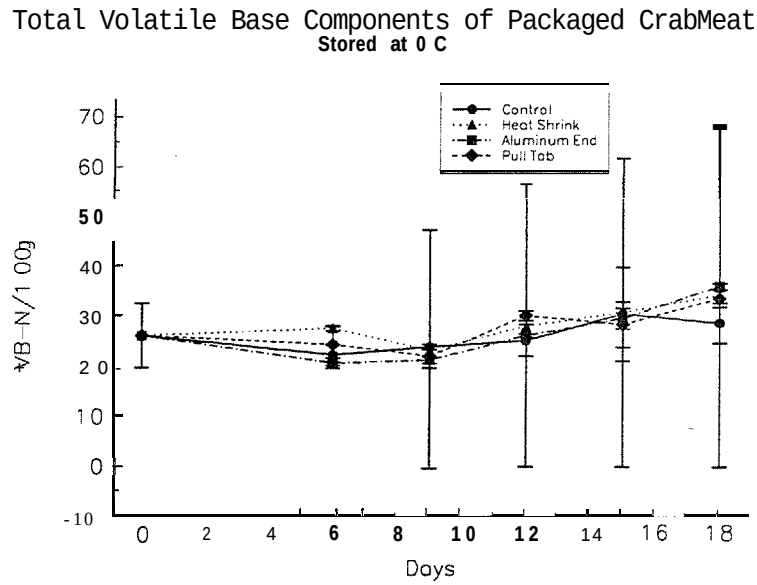

Figure 3. Mean total volatile base nitrogen levels of packaged crab meat stored at **0°C in** the control and three tamper-evident containers

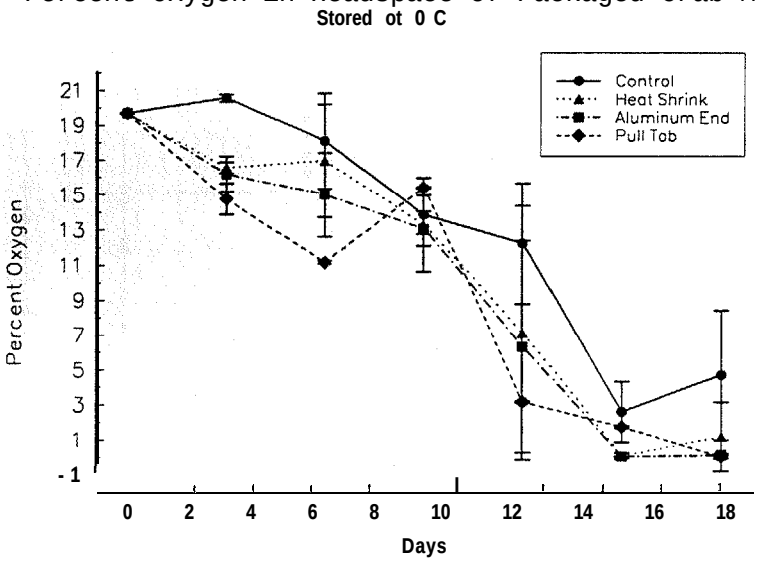

Figure 4. Mean percent oxygen levels of packaged crab meat stored at 0°C in the control and three tamper-evident containers

15. On day 18, the control samples contained more oxygen than the aluminum end and North Carolina tamper-evident tab cups (Figure 4). Carbon dioxide levels increased with time. Levels in the aluminum-end cans were significantly greater than those found in the control cups on days 3 and 18. The shrink-wrap sealed containers had greater CO_2 levels than the control and the North Carolina 'tamper-evident tab containers on day nine (Figure 5).

Aerobic plate counts were not consistently different among the four containers over 18 days of iced storage. Plate counts increased rapidly after nine days of storage. All meats exceeded

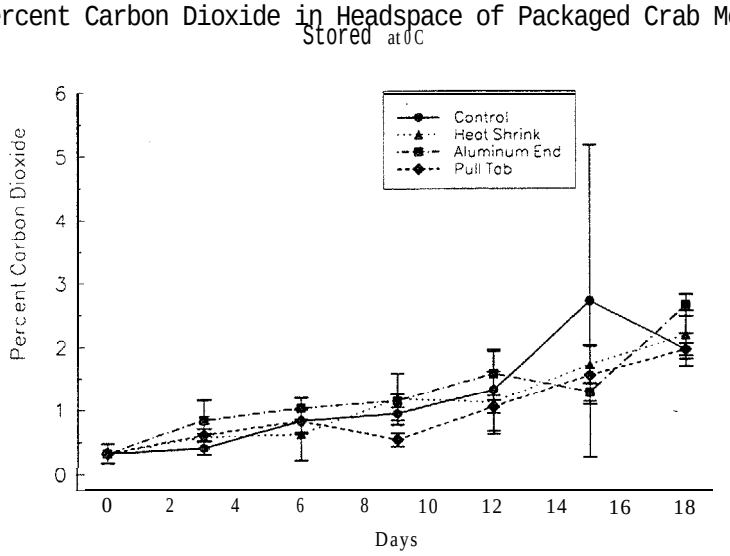

Figure 5. Mean percent carbon dioxide levels of packaged crab meat stored at 0°C in the control and three tamper-evident containers

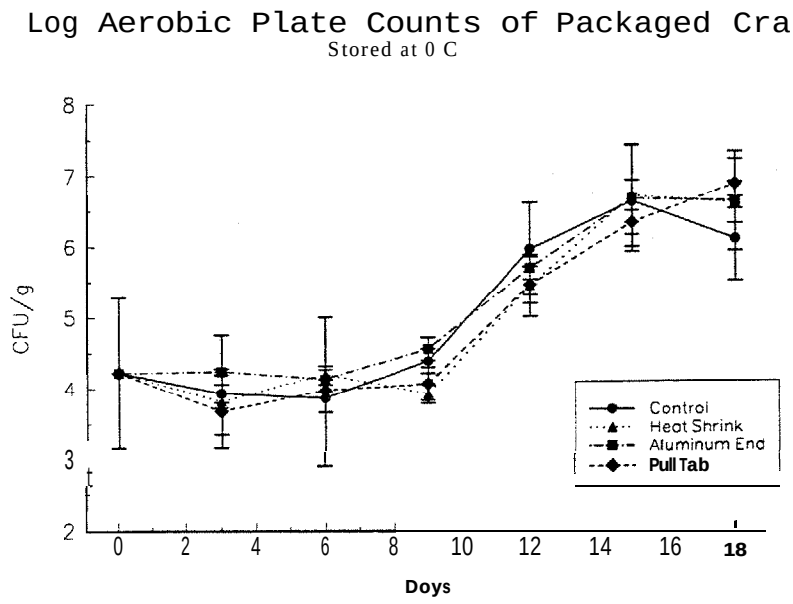

Figure 6. Mean log aerobic plate count populations determined for packaged crab meat stored at 0°C in the control and three tamper-evident containers

10^6 CFU/g by day 15, reaching the end of their microbiological shelf life (Figure 6) (Gates et al, 1993). Anaerobic plate counts increased with time, most rapidly between 6 and 18 days of Storage. No consistent differences among the four storage cups were observed (Figure 7). Psychrotrophic plate counts showed no consistent differences among the containers during iced storage (Figure 8).

Although Hunter L or whiteness values were lower for meat collected from the aluminum-end cups through 15 days of storage, the differences were not statistically significant (Figure 9). Hunter a or relative redness and Hunter b or relative blueness levels

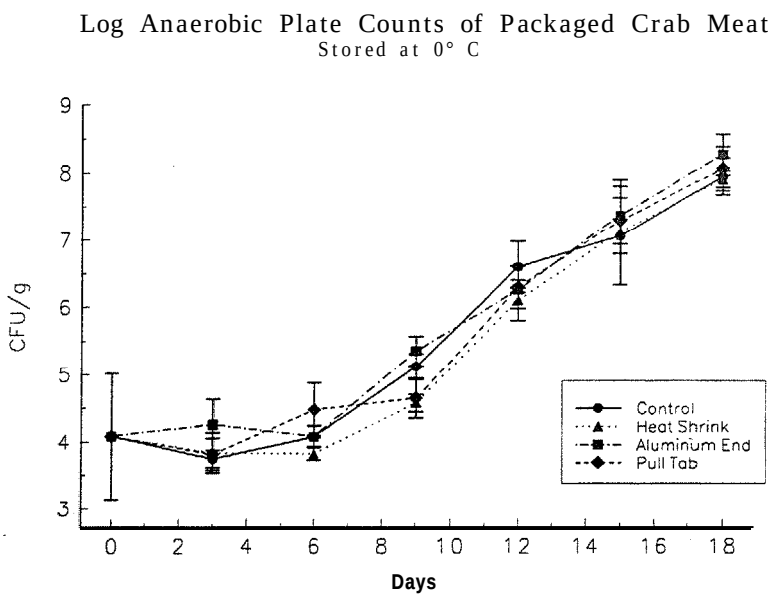

Figure 7. Mean log anaerobic plate count populations determined for packaged crab meat stored at 0°C in the control and three tamper-evident containers

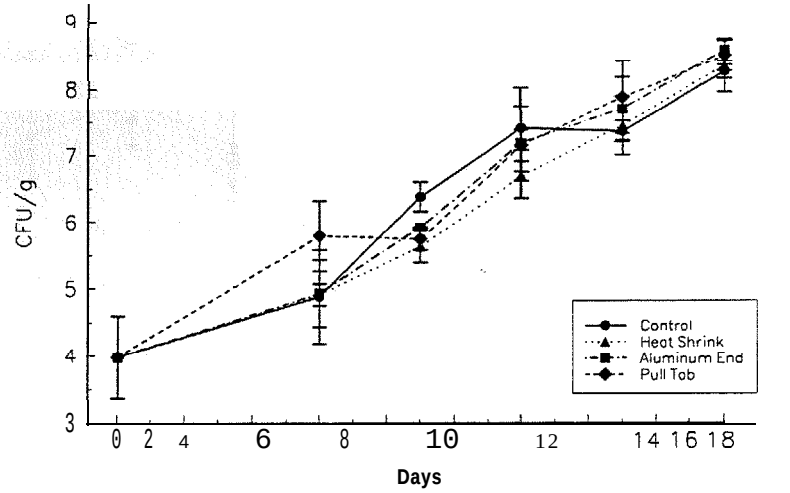

Figure 8. Mean log psychrotrophic plate count populations determined for packaged crab meat stored at 0°C in the control and three tamper-evident containers

showed no consistent differences. The sensory panel found no subjective differences in meat color or appearance among the four sample containers.

Panel analyses found no consistent significant differences among the four container types for ammonia, sour, putrid, or crab odors.

Several monitored parameters had significant correlation coefficients with storage time. The results were statistically

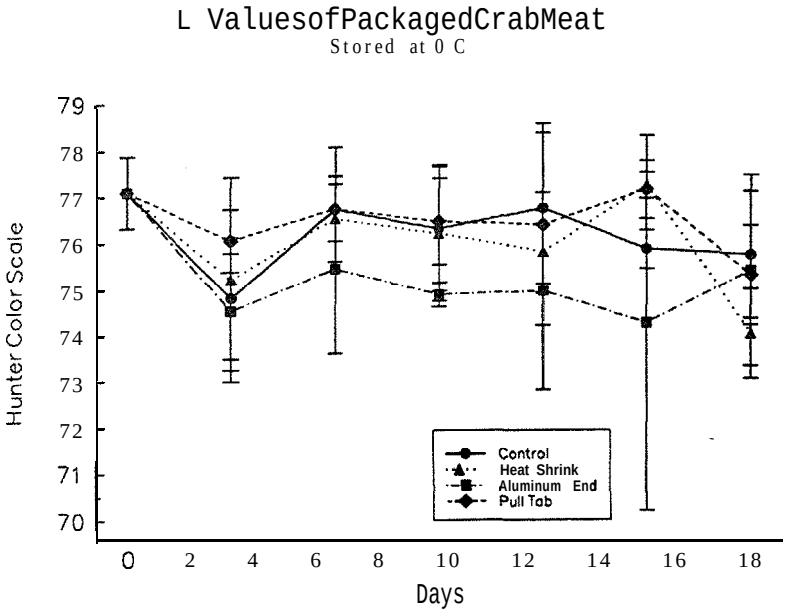

Figure 9. Mean Hunter L color values determined for packaged crab meat stored at 0°C in the control and three tamper-evident containers

significant at the 0.05 level and had correlation coefficients greater than 0.7. Combined percent carbon dioxide (0.7381, aerobic plate counts (0.8121, and ammonia concentrations (0.8451, correlated well with storage day for all containers. Psychrotrophic plate counts (0.956), aerobic plate counts (0.9191, and oxygen levels (-0.868) had the highest correlation coefficients. The putrid odors recorded from control packages also correlated well with storage time (0.702).

Refrigerated Storage

As shown in the iced storage part of the study there were no consistent pH differences among the containers. However, pH levels

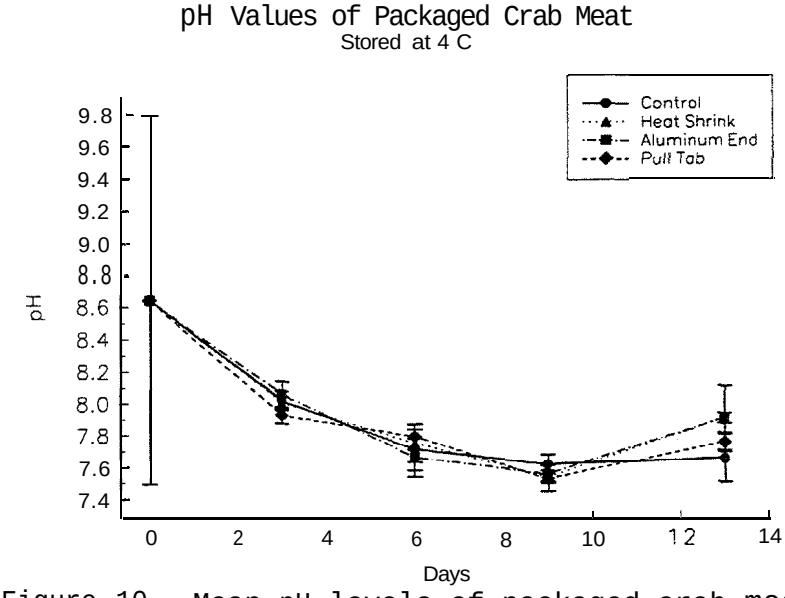

Figure 10. Mean pH levels of packaged crab meat stored at 4°C in the control and three tamper-evident containers

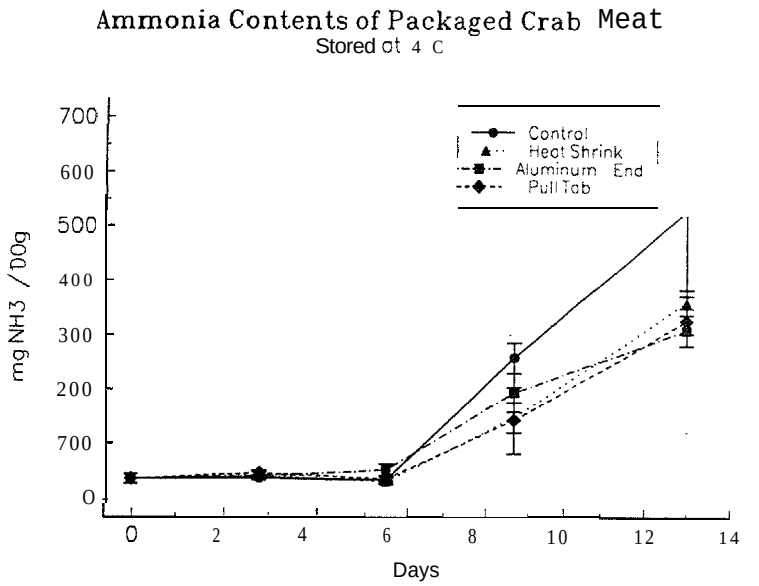

Figure 11. Mean ammonia levels of packaged crab meat stored at 4°C in the control and three tamper-evident containers

decreased between zero and nine days of storage (Figure 10). Ammonia levels increased between 6 and 13 days of storage. On day six the aluminum-end cups had greater ammonia levels than the other containers. The control containers had significantly higher ammonia concentrations than the North Carolina tamper-evident tab containers on day nine and greater concentrations than all other containers by day 13 (Figure 11). Total volatile base nitrogen increased rapidly between 6 and 13 days of storage. The aluminum-end containers measured greater TVB-N than all other packages on day six. TVB-N levels in the aluminum-end containers and the control samples were greater those found in the other containers on day nine (Figure 12).

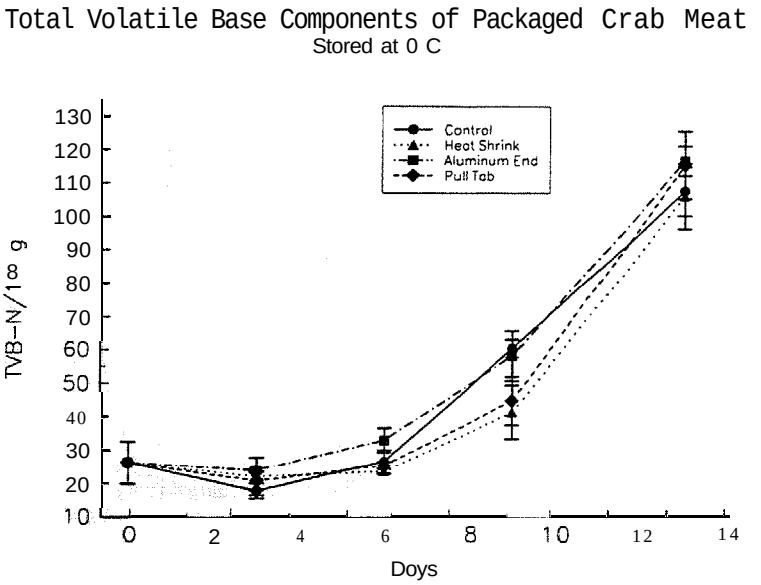

Figure 12. Mean total volatile base nitrogen levels of packaged crab meat stored at 4°C in the control and three tamper-evident containers

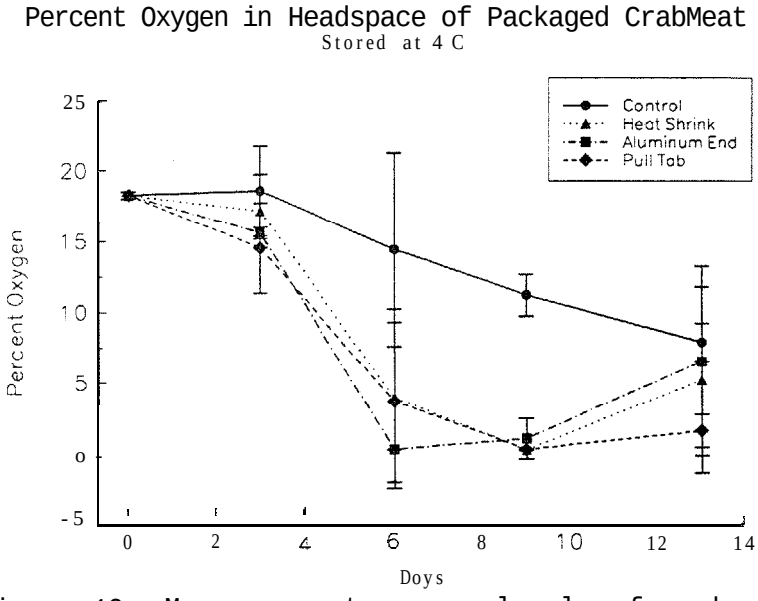

Figure 13. Mean percent oxygen levels of packaged crab meat stored at 4°C. in the control and three tamper-evident containers

Oxygen levels decreased with time and again varied considerably among the four cups. Levels in the control containers ranged between approximately 18% and 7.5% 0_2. Oxygen levels in the other containers approached zero during storage. Refrigerated control samples had higher oxygen levels than the other containers on all sample days and were significantly greater on day six (Figure 13). Carbon dioxide levels increased with time. Levels in the control containers were significantly greater than those found in the other cups on days 9 and 13 (Figure 14).

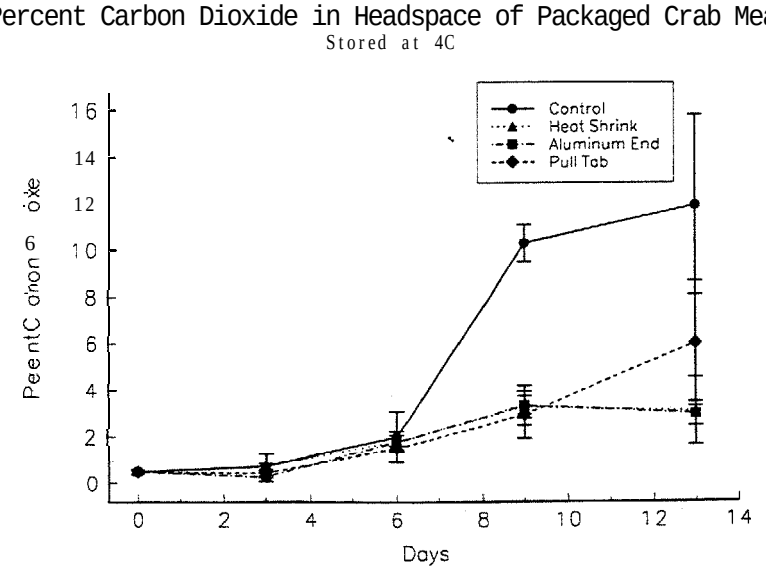

Figure 14. Mean percent carbon dioxide levels Of packaged crab meat stored at 4°C in the control and three tamper-evident containers

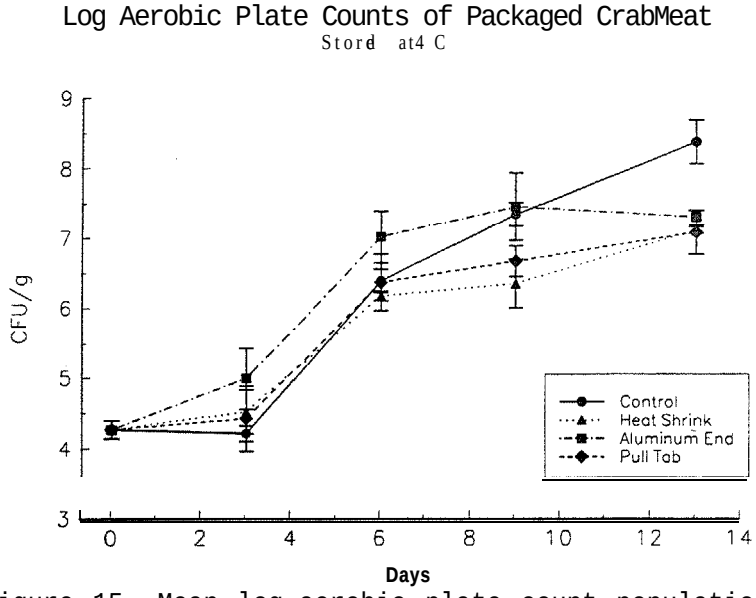

Figure 15. Mean log-aerobic plate count populations determined for packaged crab meat stored at 4°C in the control and three tamper-evident containers

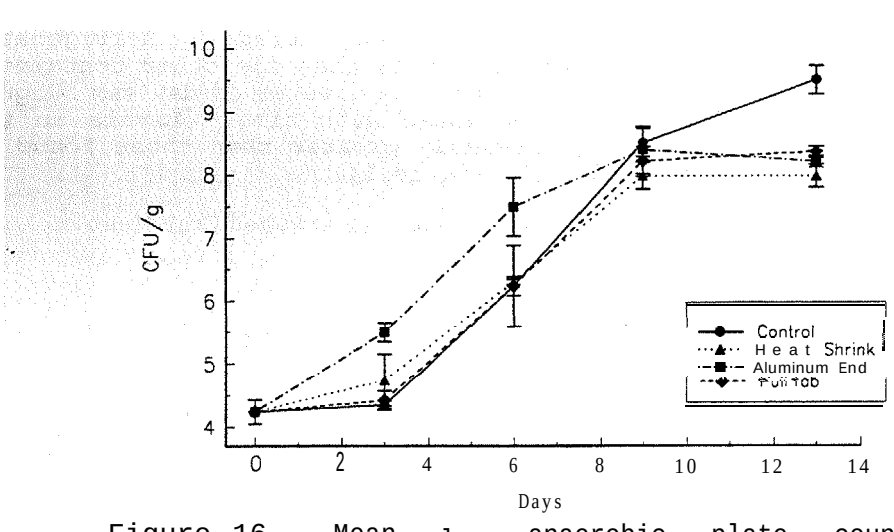

Figure 16. Mean log anaerobic plate count populations determined for packaged crab meat stored at 4°C in the control and three tamper-evident containers

The aluminum end plastic cans had the highest aerobic plate counts on days three and six. Plate counts from the control samples were greater than other samples on days 9 and 13. All samples exceeded 10^6 CFU/g by day six (Figure 15). The results from the anaerobic plate counts were similar. The aluminum end plastic cans had the highest counts on days three and six. Counts from the control cups were greater than other samples on day 13 (Figure 16). Psychrotrophic plate counts showed no consistent differences among the four package types (Figure 17).

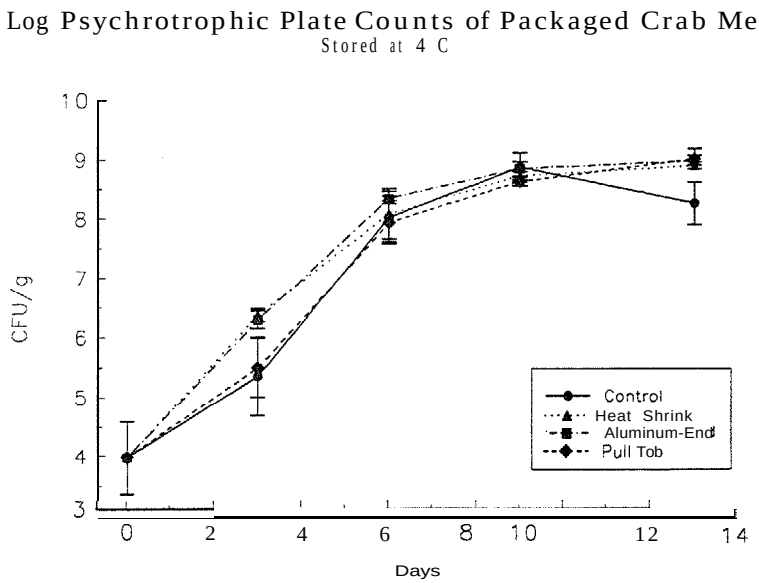

Figure 17. Mean log psychrotrophic plate count populations determined for packaged crab meat stored at 4°C in the control and three tamper-evident containers

Aluminum-end containers had the lowest Hunter L values or whiteness levels on days 3, 6, and 9. Aluminum-end L values were significantly less than control cup and North Carolina tamper-evident tab containers on days six and nine. Control samples were significantly whiter than the North Carolina cups on day nine. The controls were whiter than the heat-shrink sealed products on day 13 (Figure 18). Hunter a and Hunter b measurements showed no consistent differences among the containers. As with the iced samples, the sensory panel revealed no significant differences among the sample containers.

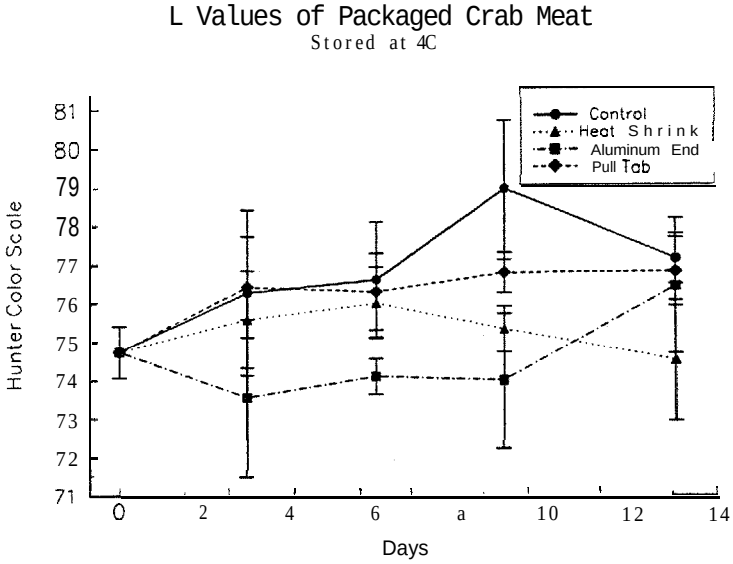

Figure 18. Mean Hunter L color values determined for packaged crab meat stored at 4°C in the control and three tamper-evident containers

The variables that correlated well with storage day for all combined containers were the same parameters found during 0°C storage except for the deletion of percent CO_2 and the addition of total volatile base. The microbiological determinations had the highest correlation values with storage time at 4°C: anaerobic plate counts (0.928) psychrotrophic plate counts (0.913), and aerobic plate counts (0.894). Total volatile base (0.857), ammonia (0.845), and oxygen (-0.703) levels also correlated significantly with storage time. Putrid odor did not correlate well with storage time for combined samples. Control (0.769), shrink-wrap (0.713), and aluminum-end (0.703) containers developed relatively high individual putrid odor correlation coefficients. Meat packed in control containers also had high correlation values for ammonia (0.768) and crab odors (0.751).

A collateral study (Harrison et al., 1994) looked for toxin production by *Clostridium botulinum* during inoculated pack studies at 4°C and at the abusive temperature of 10°C. No toxin was detected following 18 days of storage at 4°C or following 15 days of storage at 10°C. The crab meat was obviously spoiled at 18 and 15 days, respectively.

CONCLUSIONS

Control samples in industry standard plastic fresh meat cups generally maintained higher oxygen levels than meat packaged in tamper-evident containers. No consistent differences in quality or shelf life were detected among the four package types. Market shelf life was limited to six days for meat held at 4°C and 15 days for meat held at 0°C in all sample containers. The collateral work by Harrison et al. (1994) shows that toxin production by *Clostridium botulinurn* neither occurred following 18 days of storage at 4°C nor after 15 days of storage at an abusive temperature, 10°C. Spoilage occurred before any toxin production.

The study suggests that blue crab processors can safely use new tamper-evident packaging. New blue crab meat packaging options have little or no effect on product quality or shelf life. Processors may choose appropriate packaging options using price, packaging quality, market appearance, and ease of production as the deciding criteria.

ACKNOWLEDGEMENTS

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EVALUATION OF COOLING RATES FOR ATMOSPHERIC STEAMED BLUE CRAB MEAT

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The application of moist heat (atmospheric) to fresh (cooked) blue crab meat offers several commercial advantages and disadvantages for processors. Some of the advantages are improved microbiological (Rippen et al., 1988) and sensory (Gates, 1977) qualities for hand-picked meats. In addition, research indicates that the heat process is effective for assuring the control of the potential pathogen, Listeria monocytogenes (Rippen et al, 1993). Use of atmospheric steam when applied to crab meat was determined to provide a high margin of safety, at least a 20 D-process for Listeria monocytogenes (Scott A), when heated internally to 75°C (Hackney et al., 1991). Disadvantages in the process are reduced lump meat integrity, the need for strict controls to prevent product recontamination and overcoming regulatory concerns for its proper industry use (Fulcher, 1994).

This presentation summarizes some of the experiences gained in a commercial setting with atmospheric steam for in-line quality assurance. Specific objectives of the study were to evaluate the heat process for adequacy to control Listeria monocytogenes and to evaluate the cooling process for the purpose of reducing the opportunity for post-process contamination.

MATERIALS AND METHODS

An atmospheric steam tunnel of approximately six feet in length was constructed of stainless steel and operated by Craven Crab Company of New Bern, N.C. The tunnel was equipped with a temperature (steam) controller, time-temperature recording thermometer and indicating thermometer. Fresh crab meat hand-picked from blue crabs cooked under pressure at 15 psig for 10 minutes was

used to evaluate both heating and cooling curves by four product types; lump, backfin, special and claw.

Part I

Preliminary evaluation of steam distribution within the tunnel was performed using a portable potentiometer (Model No. 3087, Yokowgawa Corp. of America, Newman, GA) equipped with copper-constantan (type-T) thermocouple wire and two inch stainless steel (s.s.) needle thermocouples (Ecklund-Harrison Technologies, Inc., Fort Myers, FL). Air temperatures were monitored within the tunnel at 12 inch intervals (6 locations). Heat penetration studies were performed on the four product types to establish standard operating procedures (SOPs) and to determine a process schedule (i.e., time and temperature).

After process standardization was achieved, a total of nine heat penetration profiles were obtained for each of the four product types studied. These data were obtained by inserting a thermocouple in the largest portion of crab meat centered on a perforated s.s. tray. Each tray held two pounds of meat that was leveled to less than one inch in thickness. Heat penetration profiles were monitored with a hand-held thermometer (Model HH-21, Omega Instruments, Inc., Stamford, CT) equipped with type-T thermocouple wire and a two inch s.s. needle thermocouple (Ecklund-Harrison Technologies, Inc., Fort Myers, FL).

Part II

Exit temperatures for the atmospheric steamed crab meat were measured and air cooling monitored with the hand held thermometer during transfer from the perforated S.S. trays to sanitized solid s.s. trays. Samples were allowed to air cool approximately 10-15 minutes in the solid s.s. tray before packing into standard 16 oz. copolymer polyethylene cups (Venture Plastics, Inc., Monroeville, Ohio). The containers were weighed, capped and sealed with tamper evident low density polyethylene seals and packed on ice (50 pounds per waxed corrugated cardboard box). Product cooling rates on ice were monitored with use of molded plastic thermocouples (Ecklund-Harrison Technologies, Inc., Fort Myers, FL) inserted through the top of the snap-on lids for the 16 oz. polyethylene containers. Temperature profiles for packed crab meats during cooling were monitored until the core meat temperature approached 10 °C.

Air-cool versus hot-fill packing

A comparison of cooling rates for crab meat allowed to air cool prior to packing on ice versus direct hot-filling and then packed on ice was performed in order to evaluate the effects of initial meat temperature on cooling rates. The hypothesis was that the rate of cooling would be faster for "hot-fill" packing compared with "air-cool" packing since the driving force (e.g., heat) would be greater. The hot-fill

technique would reduce the potential for recontamination of product after steaming by decreasing the time that meat is exposed to the plant environment (i.e., air).

Special crab meat was chosen for the purpose of this comparative study. Cooling rate profiles of crab meat were obtained for air-cool and hot-fill process techniques, respectively, according to procedures described in Part II. Due to the difficulty in handling crab meat at high temperatures, no samples were packed by hand with temperatures exceeding 65.5°C.

Model simulations

In order to simulate temperatures greater than 65.5°C, a mathematical spreadsheet (Quattro Pro for Windows, Version 5.0, Borland International) and model generator (MathCAD 2.0, Addison-Wesley) were used to curve fit cooling data and extrapolate initial meat temperatures outside the experimental range obtained at the processing plant. By generating models for these cooling rates a better understanding of how to improve the atmospheric steam process could be obtained. Models were developed using the following exponential equation:

$$T_t = T_{sur} + T_0 * e^{-(K*t)} \qquad (1)$$

T_t is the meat temperature at any time, t. T_{sur} is the surrounding temperature. T_0 is the initial temperature minus the surrounding temperature. K is the rate constant and t is time. This model clearly shows that the temperature at any time t is dependent not only on the surrounding temperature but also on the initial meat temperature.

The constant K which determines exactly how fast the meat will cool can be calculated with the following equation:

$$\frac{\ln\left(\frac{T_t - T_{sur}}{T_0}\right)}{t} = K \qquad (2)$$

It can be seen from equation (2), that if time remains constant and the surrounding temperature is constant then crab meat with the higher initial temperature will have a faster cooling rate. This translates simply into the fact that the hotter a body is the faster that it cools. Knowing this, the question now becomes at what initial meat temperature does this make a significant difference in the cooling time? Since only a small difference in cooling time was actually observed between air-cool and hot-fill packing of meat, it was further hypothesized that there must be an optimum temperature in which to pack crab meat to obtain the maximum cooling rate and

minimize the chances for product recontamination.

Laboratory analyses

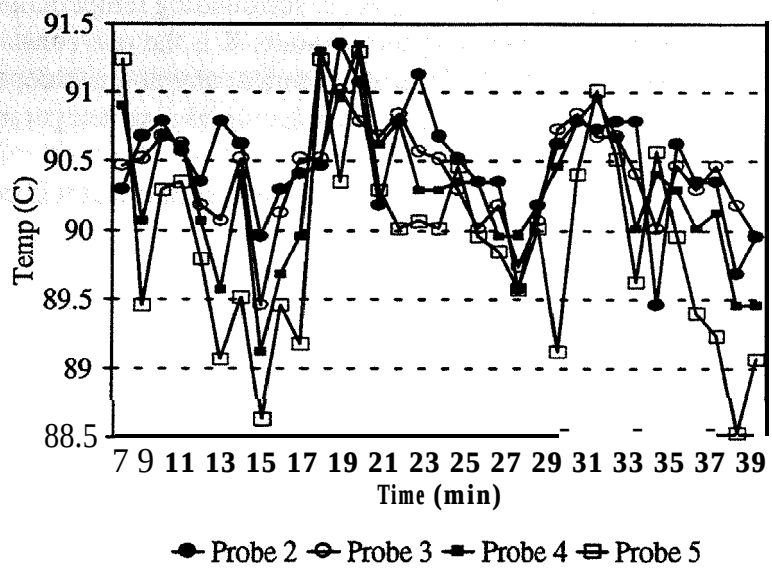
Duplicate 16 oz. samples of lump, backfii, special and claw crab meat before (control) and after atmospheric steaming (air cool only) were collected. Samples were evaluated at the NCSU Seafood Laboratory for pH, moisture, color, aerobic plate count (35°C) and sensory qualities according to methods described in Henry (1991). Meat stored on ice for 21 days was evaluated for aerobic plate counts by standard methods (APHA, 1984) in order to determine shelf life of steamed product and non-treated controls.

RESULTS AND DISCUSSION

Part I Evaluation of the atmospheric steam process.

A representative steam distribution pattern for the atmospheric tunnel under standard operating procedures is shown in Figure 1. Positions 1 and 6 are not given due to lower temperatures near inlet and exit-positions of the tunnel.

Figure 1. Steam Distribution Pattern



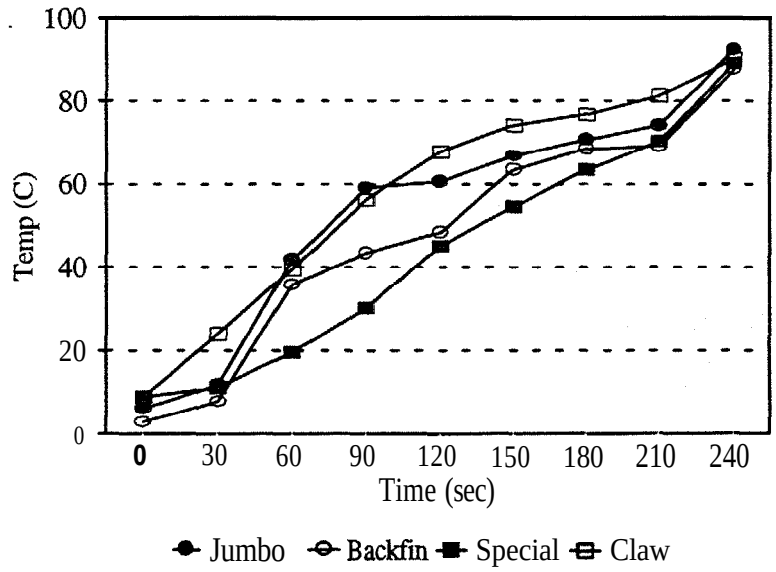
Two significant observations were made. First, temperatures inside the tunnel fluctuated as a result of the steam controller. And secondly, temperatures recorded were higher (88.6 to 91.4°C) than the indicating thermometer (87.8°C). Reasons for the fluctuations and higher temperatures observed may be explained by the relative heat sensitivity of the potentiometer compared to the less sensitive steam controller

and indicating thermometer.

In addition to steam distribution patterns, a series of heat penetration profiles were obtained for the various product types in order to establish standard operating procedures and a process schedule. It was determined that a process schedule of 4 minutes and 15 seconds at 87.8°C would deliver sufficient heat to all four product types.

Figure 2 shows the slowest heating curves from nine observations for each of the four product types studied. Internal meat temperatures reached 79.4°C in 3 minutes and 45 seconds. Final temperatures exceeded 85°C for all product types at 4 minutes.

Figure 2. Heat Penetration Profiles



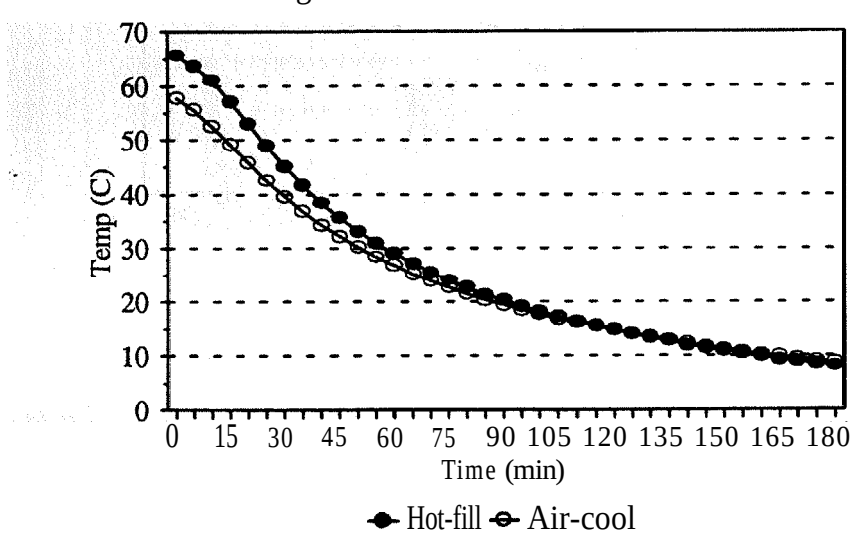
Harrison and Huang (1990) first determined decimal point reduction times (D-values) for inactivation of Listeria monocytogenes (Scott A) in blue crab meat. D-values of 40.43, 12.00 and 2.61 minutes at 50, 55 and 60°C were found. Hackney et al. (1991) found similar D-values of 12.4 and 2.4 at 55 and 60°C for Listeria in blue crab meat. Based on an inoculated pack study with Listeria monocytogenes, a process achieving an internal temperature of 75°C was adequate to achieve at least a 7D process (Hackney et al., 1991). The heat penetration profiles shown in this study (85°C) would be sufficient to provide a D-value for L. monocytogenes of several hundred fold.

Part II Evaluation of the cooling process.

Air cooling of atmospheric steamed crab meat was accomplished by transferring meat from perforated s.s. trays on exit from the tunnel to solid s.s. trays. This transfer resulted in a temperature drop of 15°C from exit temperatures. Allowing the meat to cool in the tray for an additional l0-15 minutes prior to packing further lowered meat temperature depending upon the relative thickness of the sample. Hot-filled crab meat was immediately packed into plastic cups after transfer (not allowed to air cool) to the solid s.s. trays.

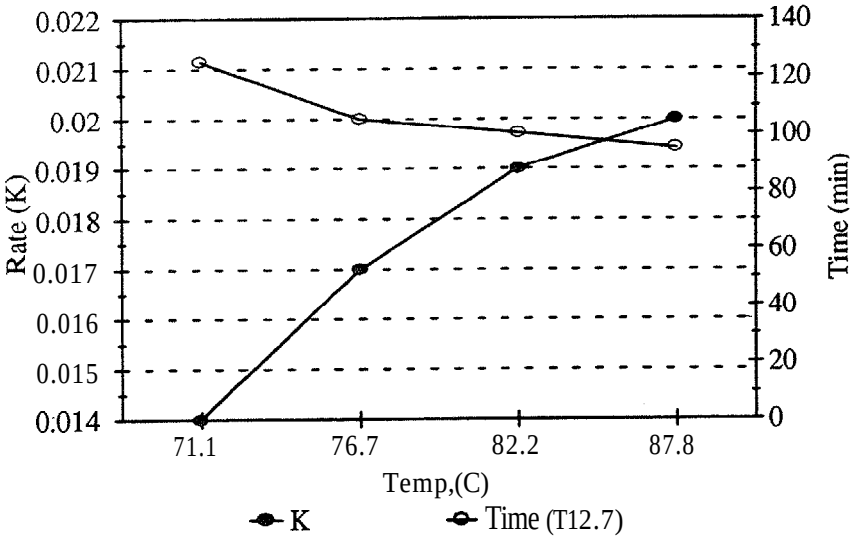
Figure 3 shows cooling rate differences between air-cool and hot-fill packing of crab meat after placing on ice. Both samples rapidly cooled to below 37.7°C in less than one hour and were virtually identical (14.5°C) after two hours on ice. The primary difference observed was the initial cooling rate. Even though the two packing techniques yielded only a small difference in cooling time, the results are significant. Further studies were conducted to simulate conditions where crab meat would be directly filled into containers from the end of the tunnel without handling. This packing technique would minimize exposure of crab meat to environmental conditions in the packing room.

Figure 3. Air-cool vs. Hot-fill Pack



By using a mathematical spreadsheet and model generator, cooling times for initial temperatures outside the experimental conditions could be approximated. This task was accomplished by noting trends in cooling rate constants observed forinitial meat temperatures. These trends were used as a base line and extrapolated in order to achieve a good estimate on rate constants at higher initial temperatures. Figure 4 shows that as the initial meat temperature increases, a rise in the rate constant occurs resulting in a drop in time to reach a specified temperature.

Figure 4. Estimated Rate vs. Time



The accuracy of the model was tested by comparing predicted cooling times with a set of actual data obtained in the field. The $T_{12.7}$ model estimates were found to be within +/- 10 minutes of the actual experimental values. From the results found in this study, the benefits of hot packing can be seen. Direct filling of crab meat at an exit temperature of 82.2°C would reduce the amount of time to achieve a final product temperature of 12.7°C by 25% (i.e., 135 min at 65.5°C compared with 100 min at 82.2°C) and minimum the opportunity for the occurrence of post-process contamination of meat.

Crab meat quality

Product quality is critical when using hot-filling techniques especially where plastic containers are subjected to static cooling conditions (e.g., packed on ice). The plastic insulates the meat, trapping heat inside, and reduces heat transfer rates. The importance of this is significant because of the relative heat sensitivity of blue crab meat to discoloration (Boon, 1975; SFA, 1988). For the most part, blue crab meat is pasteurized at temperatures less than 87.8°C. Due to this concern, crab meat samples were examined for moisture, pH, color and sensory qualities.

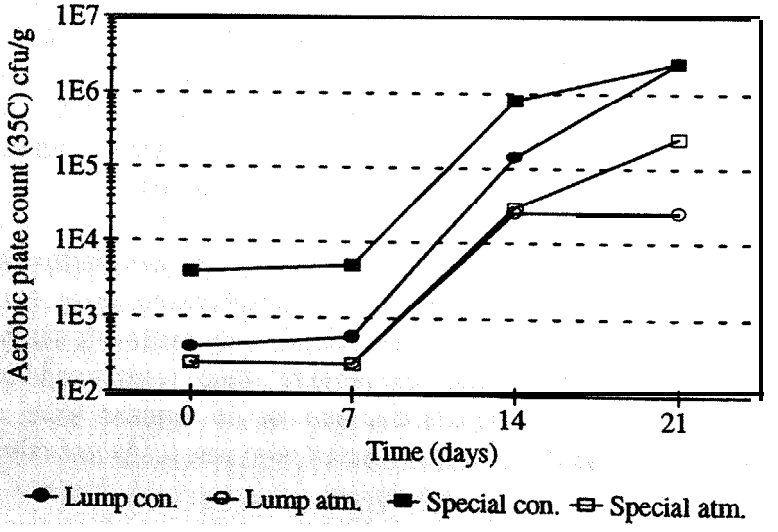
Table 1 contains the results from laboratory analyses performed on lump, backfin, special and claw meats before and after atmospheric steaming. No differences were found in pH, moisture, or color as a result of steam treatment. Higher moisture content and color differences (darker and more red-blue) were found between body and claw meat samples. An insufficient number of samples were analyzed to statistically compared these differences and no attempt was made to evaluate changes over time of storage.

Table 1. Laboratory analyses on control and treated crab meat samples.

Type/ Treatment	pH	Moisture, %	Color L	a	b
Lump					
Control	7.47	74.96 + 0.05	64.43 + 0.43	-0.49 + 0.23	11.20 + 0.70
Steamed	7.47	74.93 + 0.07	64.3 1 + 0.47	-0.44 + 0.18	11.21 + 0.52
Backfin					
Control	7.46	75.01 + 0.01	65.13 + 0.41	-0.47 + 0.11	10.82 + 0.18
Steamed	7.46	75.38 + 0.33	64.87 + 0.74	-0.45 + 0.12	10.87 + 0.11
Special					
Control	7.46	74.88 + 0.10	64.02 + 0.15	-0.20 + 0.05	10.72 + 0.08
Steamed	7.46	74.93 + 0.15	63.30 + 0.11	-0.38 + 0.06	10.66 + 0.07
Claw					
Control	7.46	79.98 + 0.01	51.80 + 0.11	4.00 + 0.07	6.49 + 0.11
Steamed	7.46	79.99+ 0.02	49.65 + 0.20	4.22 + 0.09	5.87 + 0.10

Figure 5 shows the relative shelf life of lump and special crab meat before and after steaming. Aerobic plate counts were performed over 21 days storage of meat on ice. Steamed samples showed an extended shelf life of 7 days over controls with lump meat exhibiting the lowest APC of all samples tested, less than 25,000 APC/g after 21 days at 0°C. It can be observed in Figure 5 that initial meat qualities (APC, 35C) were well below the state health standard of 100,000 cfu/g. Enhanced GPMs and employee training were implemented by Craven Crab for Listeria control prior to use of atmospheric steam for in-line quality control..

Figure 5. Bacteriological Qualities



Sensory evaluations of lump, special and claw meats before and after steaming were performed by trained staff at the NCSU Seafood Lab. No (lump, claw) or little (special) free liquid was observed in one pound containers of meat stored for 48 hours on ice. Steamed lump meat was judged to be less yellow and glossy and slightly gray, drier and more fibrous that controls. Steamed claw meat was slightly darker, drier and noticeably sweeter that controls. Steamed special was slightly darker with a noticeable dark concentric ring at the meat surface.

CONCLUSIONS

The present method of air cooling atmospheric steamed crab meat prior to packing requires additional handling by employees and has poential for air-borne contamination of the product. Hot filling of crab meat reduces this risk with a slight increase in the cooling rate obtained. The potential for developing direct filling techniques with atmospheric steamed crab meat is evident. This study demonstrated the adequacy of the atmospheric steam process for control of Listeria monoctyopenes. It offers industry several opportunities for reducing cooling rates of atmospheric steamed crab meat thereby reducing the opportunity for post-process contamination. Develop of rapid cooling techniques and the proper application of the atmospheric steam process by the U.S. blue crab industry would provide consumers with crab meat of higher bacteriological quality and a greater margin of safety.

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AN ECONOMIC ANALYSIS OF THE DOMESTIC AND FOREIGN MARKETS OF VARIOUS COASTAL HERRING AND ASSOCIATED SPECIES

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INTRODUCTION

This paper describes the potential domestic and foreign markets of selected coastal herring and associated fish species, namely: chub mackerel (Scomber japonicus), Gulf butterfish (Peprilus burti), round herring (Etrumeus teres), and Spanish sardine (Sardinella aurita The coastal herrings and associated species have great potential as human food. Potential domestic and foreign markets exist for Gulf fishes as food products. It would be beneficial if the factors limiting the consumption of-these underutilized species are identified

Limited economic information are available on the domestic and foreign markets of the coastal herring species. Lea and Roy (1976) conducted an economic feasibility study on processing of groundfish from the Gulf of Mexico. Perkins (1977) prepared an economic evaluation on canned fish products for export to Nigeria. Perkins (1978, 198l) also studied the economic potential for Spanish sardines, and the possibility for a sardine fishery in the Northern Gulf of Mexico. Raizin and Regier (1986) evaluated the impact of domestic wholesale demand for canned sardines on market accessibility of potential Gulf of Mexico Products. Thrash (1986) evaluated the commercial feasibility of harvesting, processing, and marketing Gulf butterfish and concluded that a strong market potential existed for the fish species. Dufrene (1988) reported that the lack of a good, consistent market for the fish was the most significant factor affecting a major fishery developing for Gulf butterfish. A one-month commercial exploratory fishing operations conducted in the Gulf of Mexico showed that quantities and sizes of Gulf butterfish that were commercially valuable can be caught and that the fish can be successfully marketed in Japan. Additional

market and economic studies focusing on other Gulf species are needed in order to more fully exploit their potential.

MATERIAL AND METHODS

Several methods were used to identify product forms of selected fish species landed, produced and consumed in existing and potential domestic and foreign markets. First, mailed interviews were conducted with seafood marketing firms handling mackerel products. Second, letters requesting for information regarding prices and product forms were sent to Commercial or Fisheries Officers of U.S. embassies in countries where landings of the selected fish species were reported. Third, relevant market data were collected from various statistical publications covering domestic and foreign markets. The statistical data collected include potential yields of selected domestic fisheries, landings and values, ex-vessel and wholesale prices, product forms, processing production and trade.

Potential yields Three levels of potential yields of the selected domestic fisheries are reported, namely long-term potential, current potential and recent average yields. The long-term potential yield (LTPY) is the maximum long-term average yield or catch that can be achieved through conscientious stewardship, by controlling the fishing mortality rate to maintain the population at a size that would produce a high average yield or harvest (USDC 1991). Current potential yield (CPY) is the yield or catch that will maintain the current population level or biomass or stimulate a trend toward a population that will produce the long-term potential yield (USDC 1991). The recent average yield (RAY) is the reported fishery landings averaged for the three-year period (USDC 1991). These yield levels are used to determine the status of utilization of the fishery resource.

Annual world and domestic landings. Annual landings by producing countries, by domestic and by state commercial fisheries are reported for a period of at least ten years starting from 1980. National and state landings were obtained from the Fisheries Statistics Division and Regional Fishery Statistics offices of the National Marine Fisheries Service (NMFS). The world and by country catches were obtained from the Yearbook of Fishery Statistics of the Food and Agriculture Organization of the United Nations (FAO, 1980-90a).

Ex-vessel and wholesale prices The domestic ex-vessel price was obtained from the ex-vessel value and the quantity of commercial landings. The ex-vessel prices in foreign commercial fisheries were solicited through mailed questionnaires to Commercial Sections of U.S. embassies abroad, and NMFS and other publications. The data collected on ex-vessel prices in foreign commercial fisheries, however, are very limited. The weekly ex-vessel price of the North Carolina butterfish landings reported by the NMFS office in New Orleans is published in the Fisheries Market News Report by Urner Barry Publications (NMFS, 1991-93).

The wholesale prices of selected fish species at six wholesale markets in Japan are monitored by the NMFS office in Long Beach. These prices are reported on a monthly basis at the Fisheries Market News Report published by Urner Barry Publications (NMFS, 1991-93). The wholesale price of fresh butterfish at the New York Fulton Fish Market collected by NMFS in New York is reported, at most, three times per week by the Fisheries Market News Report published by Umer Barry Publications (NMFS, 1991-93).

Marketing margins were computed to show the relationship between prices of butterfish products in different market levels. These margins are expressed in absolute ($/lb) and relative (%) terms. The marketing margin measures the difference between the average monthly wholesale price of fresh whole butterfish at the New York Fulton Fish market and the average monthly ex-vessel price of North Carolina butterfish landings.

Forms of fish and fishery products. The forms of fish products landed, produced, and consumed were identified by using NMFS (1988-89, 1987-91, 1991-93), FAO (1980-90b) and GLOBEFISH (1991) publications, interviews with commercial officers of U.S. embassies, and a survey of fish marketing companies handling mackerel products. Mailed interviews were conducted with Commercial or Fisheries Officers in U.S. embassies abroad based on the list of major producing countries published by FAO (1980-90a). Limited amount of information on fish production, prices and product forms, however, were provided by Commercial or Fisheries Officers who responded to the questionnaire.

A survey of fish marketing companies handling mackerel was conducted to collect marketing data on Pacific mackerel. A total of 39 questionnaires were mailed to- companies based in California About 49 percent or 19 seafood marketing companies responded to the mailed interviews, and 13 completed interview schedules were used in the analysis of the survey results.

Production and trade. The data on the domestic production of processed fish products were collected from the Annual Summaries of Frozen and Processed Fishery Products (NMFS, 1988-89) and the Fisheries of the United States (NMFS, 1980-92). The prices of domestic fishery products were derived from the value and quantity of domestic fishery production. The foreign production data were obtained from the Yearbook of Fishery Statistics (FAO, 1980-90b) which did not include data on the value of foreign production.

Domestic wholesale trade data of butterfish products at the New York fish markets were collected from the NMFS-Economics Data Office in New York and NMFS (1991-93). NMFS (1991-93) reports these data, at most, three times a week, while the New York office of NMFS provided a monthly summary of these data.

The foreign demand for U.S. exports of fish and fishery products could be affected by several factors ranging from its own price, prices of related products, foreigners' disposable income, exchange rates, and foreigners' tastes and preferences. In addition, restrictions to foreign trade imposed by importing countries such as tariffs and non-tariff barriers could limit the flow of goods into their economies. The current trend toward trade liberalization under the new General Agreement on Tariffs and Trade (GATT) may bring about changes in international trade in fishery products. The specific tariffs and non-tariff barriers (NTB's) imposed by Asian, EEC, West African and North American countries on small pelagic fish species are discussed in Salapare (1991), INFOFECHE (1991), MacPherson (1990) and Barnett (1994). The economic implications of the above-mentioned trade barriers on the export demand of the selected underutilized fish species are not discussed in this report.

Harvesting and handling costs. The use of on board storage facilities would enhance the quality of fish upon delivery. The costs of harvesting and handling can be estimated by using existing data regarding the use of selected methods in the Gulf of Mexico by commercial or experimental fishing boats. Ernst and Brown (1982) prepared an overview of the engineering and economics of refrigerated (RSW) and chilled (CSW) sea water systems. Uebelhoer (1984) reported that an on board RSW system was much less expensive to operate than conventional icing and produced consistently superior fish at the dock.

Vecchione (1987) stated that the ability to process the butterfish catch adequately is of utmost importance in maintaining the quality necessary to sell the fish in Japan, the primary market. Vessels outfitted with onboard freezers could be improved further by equipping them with refrigerated-seawater holding tanks. This would prevent extremely large catches from spoiling on deck before they can be processed completely. The added costs of shipping the butterfish products from the landings points to the wholesale or export markets should not exceed the marketing margins between these market levels.

III. RESULTS AND DISCUSSION

Chub Mackerel

Potential yields. The long-term potential yield of the Pacific mackerel fishery is about 28,000 mt (USDC, 1993). A higher current potential yield amounting to 36,000 mt (USDC, 1993) has been reported. With a recent average yield of 23,000 mt (USDC, 1993), the Pacific mackerel fishery appears to be fully utilized.

World landings. The reported annual landings of chub mackerel by all producing countries are 2.0M mt in 1980-85 and 1.7M mt in 1986-90. The major producing countries of chub mackerel in 1980-90 are Japan, the former U.S.S.R., Ecuador, China, and South Korea The share of Japanese landings to world landings decreased from 44.0% in 1980-85 to 36.5% in 1986-90. The former U.S.S.R.

reported a fairly stable share of about 17.8% of world landings during the entire period. Ecuador, China, and South Korea landed about l0.0%, 7.8%, and 6.1% of world chub mackerel landings in 1980-90, respectively.

Domestic landings. Since there are no consistent official reports of commercial landings in the Gulf of Mexico states, domestic landings and ex-vessel prices reported in this paper refer to the California and Oregon fisheries. The average annual landings of the U.S. chub mackerel fishery increased from 34,285 mt in 1980-85 to 38,953 mt in 1986-91. This fishery contributed an increasing share to world chub mackerel landings from 1.7% in 1980-85 to 2.4% in 1986-90. Current data released by NMFS office in Silver Spring showed lesser Pacific mackerel landings in 1992 and 1993.

Domestic ex-vessel prices These nominal ex-vessel price of chub mackerel fell from $0.09/lb in 1980-85 to $0.07/lb in 1986-91. In deflated terms, ex-vessel prices deteriorated from $0.09/lb to $0.06/lb during the two time periods under consideration.

Japanese ex-vessel prices . The annual landings of fresh Pacific mackerel at 51 landings ports in Japan averaged 326,798 mt in 1987-92. Landings value was $238.8M per year. Average ex-vessel price was $73l/mt or $0.33/lb.

Japanese wholesale prices. In 1987-92, the six wholesale markets in Japan moved about 29,573 mt of fresh Pacific mackerel per year valued at $92.8M. Wholesale price of fresh fish in these markets averaged $3,139/mt or $1.42/lb.

About 13,444 mt of frozen Pacific mackerel worth $36.7M arrived each year at-the six wholesale Japanese markets in 1987-92. Typically, these frozen fish products were sold at $2,732/mt or $1.24/lb.

Product forms. Fish processors in the U.S. Pacific coast are canning Pacific mackerel for human consumption and pet food. The fish species is sold in the domestic market in several product forms namely: canned, fresh, frozen, dried, and smoked or kippered. This fish is also used by fishermen as bait either in live, fresh or frozen form. Frozen Pacific mackerel products are also exported. A staff member of NMFS office in Long Beach stated that U.S. Pacific mackerel products were exported to Japan in 1991 when Japanese landings were low.

The results of the FCA (1988) marketing project indicated that the preferred form of Pacific mackerel exported to Fiji Islands was one-pound size, whole or headed and gutted fish in frozen blocks. The project also experimented on seasoned, crumbed, precooked, and frozen nugget-shaped Pacific mackerel product, packed raw mackerel fillets, and headed, gutted, tail off, individually quick frozen mackerel product.

The survey of California-based seafood marketing companies handling mackerel products revealed different forms and sizes of Pacific mackerel bought and sold. The annual sales of these seafood companies ranged from $l.0M to $l00.0M and had been in the seafood business between 10 to 44 years. The most preferred forms bought and sold by seafood companies are frozen, fresh, chilled, canned, salted, smoked and dried. These companies like the fish whole, filleted, headed, gutted, skinned and tail off. The industrial uses of this fish are pet food or animal feed, baitfish, food additive and protein concentrate. The preferred size for whole fish ranged from 0.5 lb to 4.0 lb, with most of the companies specifying the 1.0 - 2.0 lb size. For those companies which handled filleted fish, the preferred size was 4.0 - 10.0 oz. Generally, seafood companies canning Pacific mackerel are using 15-oz containers.

The Japanese supply of Pacific mackerel consists primarily of fresh landings and frozen imports. Japanese fish processors produce several Pacific mackerel products including boiled and canned in water, canned in oil, and canned in other packs. Most of these processed fish products are exported by Japan to several countries (NMFS, 1991-93). Typically, Japanese exporters sold their frozen chub mackerel products mostly to North Korea, Taiwan, Singapore, Philippines, Thailand and Canary Islands. Saudi Arabia, Kuwait, Yemen and Angola are the major destinations of the canned in oil chub mackerel products exported by Japan. The major importers of Japanese chub mackerel canned in water are Italy, Greece, Ghana, Papua New Guinea, and Micronesia.

Processing. The average world production of frozen chub mackerel products are 396,000 mt in 1980-85 and 438,342 mt in 1986-89. Based on FAO (1980-90b) fisheries commodities statistics, the principal producing countries of frozen chub mackerel products in 1989 are Japan (94.9 %), Peru (3.7%), Ecuador (l.l%), Italy (0.2%), and Chile (0.1%).

The annual world output of canned chub mackerel fell from 76,582 mt in 1980-85 to 49,373 mt in 1986-90. Morocco and Japan produced 31.7% and 23.1% of world canned production of chub mackerel, respectively. The rest of the primary producing countries of canned chub mackerel products are Argentina (12.0 %), Brazil (12.0 %), South Korea (11.6%), Chile (6.7%), Ecuador (1.6%), and Peru (1.1%).

Foreign trade Japanese exports of canned in water chub mackerel products decreased from 23,888 mt in 1987 to 10,735 mt in 1992. The export price of canned in water chub mackerel products averaged $1,712/mt or $0.78/lb. The value of Japanese exports of canned in water chub mackerel products fell from $31.5M in 1987 to $25.4M in 1992.

Annual exports of canned in oil chub mackerel products from Japan decreased from 3,661 mt in 1987 to 227 mt in 1992. The typical export price of canned in oil chub mackerel products was $2,386/mt or $l.08/lb. The value of Japanese exports

of canned in oil chub mackerel products declined from $7.1M in 1987 to $1.0 million in 1992.

A declining trend in both the volume and value of Japanese exports of chub mackerel products in other packages was observed. Export volume fell from 4,249 mt in 1987 to 1,169 mt in 1992. The value of exports also fell from $6.1M in 1987 to $3.0M in 1992. However, export price rose from $1,440/mt or $0.65/lb to $2,543/mt or $1.15lb in 1992.

Japanese exporters generally sold their frozen chub mackerel products at average prices ranging from $663/mt or $0.30/lb in 1986 to $l,232/mt or $0.56/lb in 1992. The volume of frozen chub mackerel products exported by-Japanese traders consistently decline during the period.

The price of Japanese imports of frozen chub mackerel (and sardine or Atlantic mackerel) products averaged $942/mt or $0.43/lb in 1987. The average import price rose slightly to $l,003/mt or $0.46/lb in 1992.

Gulf Butterfish

Potential yields A comparison of the long-term or current potential yield with the recent average yield show that both the domestic Atlantic (Peprilus triacanthus) and Gulf butterfish (Peprilus burti) fisheries were underutilized fishery resources in 1988-90. The current or long-term potential yield of the Atlantic butterfish fishery was approximately 16,000 mt (USDC, 1991). During the years 1988-90, however, the recent average yield of the fishery was about 2,500 mt (USDC, 1991). This average yield represents around 15.6% of the potential yield of the fishery during the 1988-90 fishing seasons. An average 19,700 mt (SEFC, 1992) were harvested annually from the Gulf butterfish fishery during the 1986-90 fishing seasons. This yield accounts for about 74.3% of the estimated 26,500 mt (SEFC, 1992) current or long-term potential yield during the 1986-90 period.

World landings The combined landings of Atlantic butterfish, Gulf butterfish (and harvestfish) by producing countries generally declined during the period 1980-90. World landings averaged 5,441 mt annually during the 1980-90 period. Total world landings declined from 6,500 mt in 1980 to 4,836 mt in 1985, and 3,149 mt in 1990. Total world landings, however, rose to 8,520 mt in 1982 and peaked at 12,030 mt in 1984.

The Atlantic butterfish fisheries landed an average 5,328 mt/yr. The major producing countries of Atlantic butterfish are the U.S. and Japan. About 95.5% of total landings were harvested from the U.S. butterfish fisheries. Total landings persistently fell from 6,159 mt in 1980 to 4,724 mt in 1985 and 2,965 mt in 1990.

The Gulf butterfish (and harvestfish) landings were mostly reported by Venezuela at an average of 89 mt annually. The Venezuelan butterfish (and harvestfish) landings generally rose from 83 mt in 1980, 112 mt in 1985, and 184 mt in 1990 (FAO 1980-90a).

Japanese landings. The Japanese landings of Atlantic butterfish continuously fell from 661 mt in 1980, 32 mt in 1985, and negligible quantities since 1987 (FAO, 1980-90a). The average Japanese butterfish landings for the years 1980-86 was 294 mt.

The butterfish caught by the Japanese trawl fleet in the East China Sea as compiled by NMFS in Pascagoula, Mississippi averaged 2,237 mt for the Period 1980-88. Japanese butterfish catch generally rose from 827 mt in 1980 to 1,358 mt in 1985 and 3,160 mt in 1988. The 1988 butterfish landings, however, were less than half the 6,703 mt landed by the Japanese trawl fleet in 1987.

The decline in Japanese butterfish landings generated a shortage of the fish species in the Japanese markets. This situation created market opportunities for similar species from both domestic and foreign fisheries to fill up the shortfall in Japanese butterfish landings.

Domestic landings. The annual butterfish landings in the U.S. showed a downward trend over time. The landings in 1980-85 averaged 6,352.9 mt/yr. However, annual landings declined to about 3,434.2 mt in 1986-9 1. Recent data from NMFS-Silver Spring office indicated higher butterfish landings in 1992 and 1993. The total deflated value of butterfish landings averaged $4.5M and $3.8M in 1980-85 and 1986-9 1, respectively.

The three major producing states of butterfish are Rhode Island, New York and New Jersey. The state of Rhode Island landed an average 71.7% of total domestic butterfish landings in 1980-91. Each of the New York and New Jersey fishery landed less than 9% of total landings during the entire period.

Regionwise, the New England and Middle Atlantic regions reported most of the butterfish landings during the period. The New England butterfish fisheries landed 78.7% of total domestic landings. An average 16.2% of total landings was registered by the Middle Atlantic butterfish fisheries.

Gulf of Mexico landings Due to the confidential nature of butterfish landings data, no consistent time-series data can be presented for the Gulf of Mexico states. The published commercial landings data from NMFS office in New Orleans seem to suggest that limited quantities of butterfish were caught in the Gulf of Mexico states. However, it was reported that an average 19,700 mt (SEFC, 1992) were harvested annually from the Gulf butterfish fishery from 1986 to 1990. It has been cited,

however, that off-shore Gulf of Mexico shrimp fleet incidentally caught 80-97% of the total annual butterfish catch since 1986 (SEFC, 1992).

Ex-vessel prices. The domestic ex-vessel price of butterfish averaged $0.32/lb in 1980-85 and $0.59/lb in 1986-91. In New England, the ex-vessel price of butterfish averaged $0.29/lb and $0.64/lb in 1980-85 and 1986-91, respectively. Similar trends are observed in the Middle Atlantic ($0.42/lb and $0.54/lb), the Chesapeake ($0.30/lb and $0.39/lb), and the Gulf Coast ($0.22/lb and $0.46/lb). The average ex-vessel price of the Pacific butterfish (Peprilus simillimus) fell from $0.98/lb in 1980-85 to $0.73/lb in 1986-90.

The butterfish ex-vessel prices in the Gulf of Mexico states also showed the same trend over the entire period. In Alabama, the ex-vessel price rose from $0.17/lb in 1980-85 to $0.30/lb in 1986-91. An increase from $0.20/lb in 1980-85 to $0.40/lb in 1986-91 was noted in the Louisiana butterfish landings. Similarly, the Mississippi butterfish landings were valued from $0.27/lb in 1980-85 to $0.42/lb in 1986-91.

Forms of fish and fishery products. In the U.S., butterfish has been generally processed into fresh or frozen fillets or dressed form (NMFS, 1988-89). In New England, NMFS (1988-89) reported that butterfish was also processed into fresh or frozen fillets form. Fresh whole butterfish are delivered to the NY Fulton fish market from several states by truck and imported from Ecuador by air (NMFS, 1991-93 and NMFS office in New York). A senior staff member of NMFS office in Long Beach indicated that butterfish landed in California are probably sold fresh in the local markets. The results of a survey conducted in New York (NYSDS, 1989) showed that several seafood establishments were selling fresh or frozen whole, dressed or fillets, and smoked butterfish products.

Exports of fresh and frozen butterfish products were reported in various countries (MAFMC, 19991). A Commercial Specialist of the U.S. embassy in Hamburg wrote that butterfish products were imported into the former Federal Republic of Germany exclusively for human consumption only in frozen and smoked forms. This information was gathered by the specialist from German fish importers, wholesalers, frozen fish processors, Federal Fish and Seafood Institute, and Fish Industry and Wholesalers' Association. Responses to letters of inquiry sent to Commercial Officers in other selected U.S. embassies suggested that there were no specific market information available at that time regarding butter-fish.

Domestic wholesale trade. Based on the data provided by the NMFS office in New York, the deliveries of fresh whole butterfish at the New York Fulton fish market from all states of origin fell from 338.5 mt in 1990 to 187.1 mt in 1991, 197.9 mt in 1991 and 298.5 mt in 1993. The major suppliers of butterfish to the New York Fulton fish market are New York, Rhode Island, New Jersey, Connecticut, and North Carolina. The New York butterfish fishery supplied between 30.6% to 50.9% of total deliveries from 1990 to 1993. Rhode Island delivered between 7.7% and 28.7 percent

during the same period. The butterfish fisheries of New Jersey, Connecticut, and North Carolina sent about 10% each to the New York Fulton fish market.

The total deliveries from the Gulf of Mexico states to the New York Fulton fish market were about 50.9 mt in 1990. After that year, no significant deliveries of Gulf butter-fish to the New York Fulton fish market were reported.

Domestic wholesale prices. The monthly wholesale prices of fresh whole butterfish at the New York Fulton fish market were reported by size of fish. The grades of butterfish based weight are as follows: small, 80-100 g; medium, l00-150 g; and large, >150 g (Vecchione 1987). The average wholesale price of small butterfish were $0.48/b in 1991, $0.58/lb in 1992 and 0.59/lb in 1993. The medium and mixed-sized butterfish were sold at $0.78/lb in 1991 and 1992, and $0.60lb in 1993. The large butterfish were sold on the average at $0.98/lb in 1991, $0.9l/lb in 1992, and $l.03/lb in 1993. For all sizes of butterfish, the average wholesale prices were $0.81, $0.71, and $0.77/lb in 1990, 1992, 1993, respectively.

The marketing margins between the average monthly ex-vessel price in North Carolina and the average monthly wholesale price in New York were measured in both absolute and percentage terms. In absolute terms, the marketing margins fell from $0,55/lb in 1991, to $0.30/lb in 1992, and to $0.39/lb in 1993. In relative terms, the marketing margins decreased from 234.9% in 1991 to 93.1% in 1992, and to 125.2% in 1993.

Japanese wholesale prices Differences in auction prices for Atlantic and Gulf butterfish were observed at the Tokyo Central Wholesale Market from April 1988 to March 1990. The butterfish from the Gulf coast states were sold at relatively lower prices (64.1%) than those coming from the New England states. The average wholesale price for Gulf butterfish was $1.36/lb as compared to $2.13/lb for the Atlantic butterfish Gulf butterfish were sold at wholesale prices ranging from 29.7% to 72.8% of the wholesale prices of Atlantic butterfish auctioned in Japan during the period. About 71.4 mt of Gulf butterfish and 74.8 mt of Atlantic butterfish were auctioned each month at the Tokyo wholesale market. Due to the differences in the average auction prices, the average total value of Gulf butterfish was about 58.4% of that of the Atlantic butterfish.

Foreign trade The annual volume of butterfish exported by the U.S. to foreign markets averaged 4,050 mt in 1981-85 valued at $6.6M or $0.75/lb. The average butterfish export price rose to $l.05/lb in 1986-90 with 2,422 mt valued at $6.3M. As th e average annual butterfish exports fell further to 1,420 mt in 1991-93, total value decreased to $3.9M while average price rose to $1.22/lb On the average, about one-quarter of U.S. butterfish exports were sold in December. Between 10.4% to 18.1% were exported in November, January, February and March.

The major importing country of U.S. butterfish products is Japan. Japan imported an average 4,038 mt, and 2,304 mt of butterfish in 1981-85 and 1986-90, respectively. The concentration of U.S. butterfish exports to Japan fell from 99.6% in 1981-85 to 92.3% in 1986-90. The Japanese market paid a relatively higher price (103.4 %) than the average price received by U.S. butterfish exporters during the later period. The list of importing countries includes Canada, UK, France, former West Germany, Italy, Taiwan, Belgium, Luxemburg, and Spain. Varying quantities of butterfish were exported by the U.S. to these countries at different prices.

Harvesting and handling costs. A one-month commercial exploratory butterfishing operations was conducted by a New England firm in the Gulf of Mexico in May-June 1986 (Vecchione, 1987). The total amount of butterfish landed by two fishing vessels was 212.9 mt. All the butterfish shipped were sold at prices ranging from $0.58 to $0.7 l/lb or at an average price of $0.63/lb The total cost of unloading and storage amounted to $18541.76 or an average of $0.04/lb It was further cited that the cost of unloading and storage differed between Pascagoula, Mississippi ($0.02-0.03/lb) and Lake Charles, Louisiana ($0.04/lb). Different shipping routes and destinations resulted to different shipping costs ranging from $0.02/lb to $0.07/lb, or an average of $0.03/lb. The estimated net profit, excluding the special marketing costs in Japan, was $137,081, $2,285/vessel/day or $0.29/lb. The total cost of landing the fish can be imputed from the bid price made by the commercial fishing firm amounting to $2,100/vessel/day, or $0.27/lb.

Dufrene (1988) reported that the cost of retrofitting an 88-ft steel hull shrimp trawler in the northcentral Gulf of Mexico was about $60,000. Good catches of butterfish were made from June 14 to October 28, 1988 at both west and east of the Mississippi Delta. A total of 77.1 mt of various sizes of butterfish were reported landed in 11 landing dates or an average of 7.0 lb per landing date.

Round Herring

Potential yields. According to Houde (1977), there are eight discrete populations of round herring worldwide, namely: western Atlantic, eastern, central and western North Pacific, Indo-Pacific, western Indian Ocean, Red Sea and Eastern Pacific. In the U.S., recorded populations of round herring occur from Cape Cod to the Gulf of Mexico. The best estimate of potential annual yield of the eastern Gulf of Mexico fishery ranges from 50,000 to 250,000 mt. Round herring are fished commercially off Japan and South Africa.

World landings The world landings of red-eye round herring (Etrumeus teres) rose from 38,696 mt/yr in 1980-85 to 51,042 mt/yr in 1986-90. The major producers of red-eye round herring are Japan and Romania. Japan landed about 94.4% of all Etrumeus teres landed in 1980-90. The world landings of whitehead's round herring (Etrumeus whiteheadi) increased from 32,911 mt/yr in 1980-85 to

48,693 mt/yr in 198690. The primary producing nations are South Africa (99.99%) and Romania (0.01%).

Japanese landings. According to the Commercial Section, U.S. Embassy in Tokyo, Japanese round herring are mostly landed in Tottori, Kumamoto, Yamaguchi and Nagasaki Prefectures in western Japan. Fishing season is primarily in September to October. Fresh package for auction at landing ports is 30 kg random weight. Average annual landings of round herring (E. teres) or *urume iwashi* in 1980-85 were 35,666 mt, valued at 129 yen/kg or $0.25/lb. In 1986-90, annual landings rose to 49,240 mt and sold at 101 yen/kg or $0.32/lb.

Domestic landings There were no records of commercial landings of round herring in the Gulf of Mexico before 1993. The NMFS office in Miami reported that about 0.01 mt of round herring were landed in Florida (West Coast) and valued at $19 or $0.09/lb. However, there are other domestic fisheries in the U.S. which can provide insights into the trends in landings, values and prices of herring species. The commercial Atlantic herring (Clupea harengus) fisheries landed about 43,182 mt annually in 1980-91 valued at $5.2M or $0.05lb. The Pacific herring (Clupea pallasi) fishery landed an average 55,021 mt/yr in 1980-90 worth $ 36.4M or $0.30/lb. The Florida (West Coast) thread herrings (Opisthonema oglinum) were harvested at an annual rate of 158.4 mt in 1980-85 and 1,315.4 mt in 1986-90. The annual value of the fishery was $0.244M in 1986-90 or 0.05/lb. Thread herrings in Florida are mostly landed between the months of May and October. Based on the landings data provided by the Beaufort Laboratory of NMFS, thread herrings are also caught in North Carolina waters. Annually, about 1,224.7 mt were landed in 1980-85, 1,179.3 mt in 1986-90, and 2,177.2 mt in 1990-93. The commercial value of the fishery was limited to about $0.239M in 1993 or $0,04/lb.

Product forms. *The* Commercial Section of the U.S. Embassy in Tokyo provided a good description of the various forms of round herring products in Japan. Japanese herrings are sold fresh at retail outlets but the most common product is *maruboshi* or fish dried in the round. Whole fish is first soaked in about 3% salinity brine water, then dried naturally or mechanically to a moisture level below 30%.Salt content of the finished *maruboshi* is about 5%. This process is said to enhance fish flavor and shelf life at retail outlets. Two kilograms of finished *maruboshi* are laid on flat plastic or card board trays, six of which are packed in master cartons. They are stored frozen and consumed lightly grilled at home. The meat of fresh *urume iwashi* harvested in winter is said to be firm and good for grilling. A limited quantity is said to be frozen for bait in the longline tuna fleet. Landed quantity is said to be too small to allowfish meal production The use for pet food is unknown. The Embassy is not aware of any Japanese imports of fresh or frozen round herring from overseas. It may be imported in cans but statistics are difficult to identify since this import category includes other species.

Japanese wholesale trade. An average of 19.8 mt were handled annually in Japanese fresh wholesale market for round herring in 198690. The typical wholesale price of fresh round herring was about 337 yen/kg or $l.06/lb. The wholesale quantity of dried in the round Japanese round herring or *maruboshi in* 1986-90 remained relatively stable at about 499 mt. The average wholesale price of dried round herring was about 1,350 yen/kg or $4.30/lb. The U.S. Embassy in Tokyo reported that retail prices of *maruboshi are* about 300-300 yen per 100 gram package.

Spanish Sardine

Potential yields. According to Johnson and Vaught (1986), Spanish sardine (Sardinella aurita) occurs throughout the Gulf of Mexico, northward to Massachusetts and southward to Rio de Janeiro, Brazil. Fisher (1978) as cited in Johnson and Vaught (1986) also stated that the species is also common in the eastern Atlantic, Mediterranean Sea and the Western Pacific Ocean. There are no current USDC (1991; 1993) estimates of the potential yields for the Gulf of Mexico Spanish sardine fishery. Johnson and Vaught (1986) cited Reintjes (1979) estimate of the potential yield of the Florida Spanish sardine fishery as between 60,000 to 120,000 mt. The recent average yield based on the 1989-91 landings of the Florida (West Coast) Spanish sardine fishery was about 928.2 mt.

In the U.S. Pacific Coast, the Pacific sardine (Sardinpos sagax) fishery has been managed by the state of California (Leet et al. 1992). Since the early 1980's, sardines were taken as incidental catch to southern California fishery for Pacific and jack mackerel. A growing directed Pacific sardine fishery has been allowed under an increasing annual quota since 1986. USDC (1993) estimates for current and long term potential yields are 22,000 mt and 250,000 mt, respectively. Based on the 1990-92 annual landings of 10,000 mt (excluding Mexican landings) of the Pacific sardine fishery, USDC (1993) categorized the fishery as fully utilized.

World landings. Annual world landings of Spanish sardines rose from 137,061 mt in 1980-85 to 320,797 in 1986-90. Based on the reported landings in 1980-90, the major producing countries of Spanish sardine are the former U.S.S.R. (50.17%), Venezuela (28.94%), Ghana (10.91%), Romania (6.72%) and Mexico (1.94%). The reported Spanish sardine landings in the U.S., Togo, Cuba, Gambia, Benin and Grenada accounted for less than 1% of the average annual world landings during the period. A report made by INFOFECHE (199 1) indicated that the Spanish sardine fishery in Ghana landed about 33,417 mt/yr in 1980-85 The average landings in Ghana during the same period based on the previously reported landings by FAO (1980-90a) was about 8,800 mt. In addition, the same INFOFECHE (199 1) report also indicated that the Spanish sardine artisanal and industrial fisheries of Cote D'Ivorie landed about 19,940 mt in 1980-85 and 26,529 mt in 1986-89.

Domestic landings. The Florida (West Coast) Spanish sardine fishery is currently the most important if not the only commercial Spanish sardine fishery in the

U.S. Landings consistently increased since 1980, reached a peak in 1987 and started faking since 1988. A Florida Sea Grant Extension Agent estimated that in the 1990's, the number of boats fishing for Spanish sardine fell to about one-fourth of its size in 1988. The average landings of the Florida Spanish sardine fishery in 1980-85 was about 1,45 1.5 mt valued at $0.29M. A higher average annual landings was reported in 1986-91 amounting to 1,678.3 mt and valued at $0.41M. The peak of the monthly Spanish sardine landings in 1977-86 occurred in June with most of the landings concentrated from April to August.

Ex-vessel prices The deflated ex-vessel price of the Florida Spanish sardine fishery averaged $0.10/lb during the entire period. The rapid increase in annual landings forced the deflated ex-vessel price down from $0.l5/lb in 1980-85 to $0.06/lb in 1986-90.

Forms of fish and fishery products INFOFECHE (1991) describes the West African market for small pelagic fish species including Spanish sardine. Benin imported the species in frozen form packed in 27-kg cartons. The fish species was imported frozen whole by Cote D'Ivorie. The consumers in this country preferred the fish species over 20 cm in size, 7-8% in fat content, and boxed in 20-kg cartons. Ghana, Nigeria and Togo liked round sardinella which are either sun dried, smoked dried or hot-smoked. Johnson and Vaught (1986) reported that in the U.S., Spanish sardine are generally used as bait or frozen animal food. They also suggested possible use of the species in the production of surimi or minced fish products.

Harvesting and handling costs. The findings of the deepwater purse seine-refrigerated sea water system exploratory fishing project conducted by Raffield Fisheries (Uebelhoer, 1984) showed that the profitability of a vessel can be improved by harvesting large quantities of surplus species and using an on-board refrigeration system to quickly chill them. This project used a new 62-ft fiberglass fishing vessel (Fisherman's Bride) equipped with an independently powered onboard marine refrigeration and brine spray freezing system.

The refrigerated seawater (RSW) chilling system consisted of two parts, namely: the refrigeration section and a brine spray freezing unit. The fishing vessel was also equipped with fish locating electronics and communication equipment. A spotter plane was used to search for and locate fish over a much larger area during the project. The initial investment cost on the fishing vessel, electronics and communication equipment, RSW system and purse seine was about $0.41M.

During the 60-day purse seining operations, the Fisherman's Pride landed about 676.2 mt of assorted fish. The major fish species caught were ladyfish (31.0%), Spanish sardine (15.9%), jack crevalle (14.5%), bonito (13.9%) and menhaden (10.2%) The total landing value of all fish species caught was $113,066.36 or $0.0825/lb. Using one-half of the actual harvest rate during the project, it was estimated that the annual total cost of fishing and handling can be fully covered by the

ex-vessel value of landings. The estimated average cost of fishing and handling was $0.0839/lb, consisting of average fixed cost ($0.0188/lb) and average variable cost ($0.065l/lb) With the opportunity costs of operator's labor, management and capital included, the return on investment for the fishing project was $56,682/yr or 13.7%.

CONCLUSION

Chub mackerel, Gulf butterfish, round herring and Spanish sardine products were traded in both domestic and foreign markets. In the domestic markets, the consumption of these fishery products were generally limited to fresh and frozen whole and fillets, dried, smoked and canned product forms. Excluding butterfish, these species were also widely used as fishing bait and pet food. Another possible use of Spanish sardine is in the production of surimi or minced products.

Fresh and frozen forms of mackerel, sardine and herring were traded domestically and internationally in many Asian countries. Canning in water, in oil and in other packs were also widely practiced in these countries to provide both for their domestic markets and foreign countries. Production of fish products from these species also included salting and smoking. The decline in landings and development of processing industries (mainly canning) in these countries enhanced the demand for imports of these species. The Japanese demand for fresh and frozen imported butterfish was constrained by the size of the Japanese landings of the species.

Several West African countries imported mackerel, herring and sardine products generally in frozen and canned form. Consumers in these countries preferred these species because they were cheap and had high fat content. The high fat content of the species allowed fish smoking, a preferred means of preserving fish due to the lack of freezing or refrigeration facilities at home. The traditional suppliers of these fish products were the fishing fleets of the former Eastern European countries.

Western European nations imports of herring, mackerel and sardines consisted of fresh and frozen whole and unprocessed forms, prepared mainly canned products, and dried, salted and smoked fish products. Several European countries imported butterfish in fresh and frozen form. Smoked butterfish were also imported by Germany for human consumption.

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EFFECT OF WASHING WATER pH ON COLOR AND LIPID OXIDATION OF MINCE AND SURIMI MADE FROM GULF MENHADEN

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INTRODUCTION

The period 1975-1987 witnessed a market growth in the consumption and/Or acceptance. Of surimi, minced and water-washed fish muscle tissues, in the form of manufactured items such as shellfish analogs. The estimated U.S. supply of surimi products increased from 2.7 to over 45.4 million kg during this same period (Vondruska, 1987).

Diverse species of fish have been used as raw material for surimi. Alaska pollock (*Theragra chalcogrumma)* has been the primary fish used for crableg analogs but recently access to U.S. fishermen has been limited by the U.S. declaration of a 200-mile fisheries conservation zone. In addition, due to fishing pressure, alternative species such as Atlantic and Gulf menhaden ***(Brevoortia patronus*** and ***B. tyrannus,*** respectively) and Pacific whiting ***(Merluccius productus)*** have been investigated for surimi production. A growing interest in the use of dark-fleshed fish species for mince and surimi has developed during the so-called "Age of Engineered Seafoods". Dark-fleshed, oily fish have been traditionally used for feed, oil and fertilizer production. With the increasing realization of the potential health benefits of engineered seafoods, red-fleshed, oily fish species are now regarded as possible sources of omega-3 unsaturated fatty acids in the diet (Lanier et al., 1988).

U.S. commercial fisheries landings for Gulf menhaden were 539,200 mt in 1993 (NMFS, 1993). Gulf menhaden are regarded as an industrial fish species. Before these fish can be utilized for human consumption, various problems need to be addressed For instance, the improper on-board handling and storage of these fish, the lack of processing equipment suitable for heading and eviscerating small-sized fish, and the fact that these fish are fatty and dark-fleshed fish make them unacceptable for direct human consumption (Babbitt, 1986). However, due to its good gelling properties (Anonymous, 1989), menhaden has a potential as a protein

matrix in which other seafoods could be combined to formulate various seafood products if flavor and color of the mince could be improved.

This work was to evaluate the effects of various pH buffered water-washing treatments on the Hunter color, hematin content, carotenoid content, TBA and, carbonyl values of menhaden mince and on moisture content of the gel.

MATERIALS AND METHODS

Preparation of Fish Mince

Gulf menhaden were caught off the Florida coast, chilled and held in slush ice (1 to 3°C) on the boat until processed (< 48h). The fish were transported to the Mississippi State University (MSU)/National Marine Fisheries (NMFS) Experimental Seafood Processing Laboratory at the Coastal Research and Extension Center, Pascagoula, MS. About 113 kg of fish were processed (Fig. 1) in June 9, July 23, and July 28, 1992 using commercial equipment: a Model M-072 Lapine Fish Heading Machine and a Model M-017 Lapine Fish Gutting Machine (Pisces Industries, Ltd., Wells, MI).

The dressed fish exited the cutting area to enter a Model NDX13 deboner with 5-mm holes (Bidun Machine Construction Co., Ltd., Japan) to separate fish flesh from bones, skin and scales. The deboning process is also called a mincing process. A portion of the nonrefmed menhaden mince recovered from the dressed fish (about 14kg for each replication) was placed in wax coated storage boxes. The boxes (1.6 to 1.65kg) were stored in the walk-in freezer (-30°C) until they were shipped overnight on dry ice to the MSU Food Processing Laboratory, Miss. State, MS. The boxes were then stored in a walk-in freezer (-17°C) upon arrival on campus.

pH Washing Treatments

The mechanically deboned fish muscle (MDFM) was thawed at 4°C for 48 h before use.

Buffer Preparation

Phosphate buffers of pH 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0, ± 0.2 and same ionic strength were prepared with analytical grade $NaH_2PO_4.H_2O$ and Na_2HPO_4, (Fisher Scientific, Fair Lawn, NJ) by the procedures of Gomori (1955). Chilled distilled water was used to make the different buffer solutions which were then stored at 4°C until used.

Buffer and Water Washings

There were seven pH treatments, including a control (potable water, pH = 6.8). The washing process called for a batch wash system as follows:

- First cycle (4 parts of water to 1 part of mince): One kilogram of MDFM was

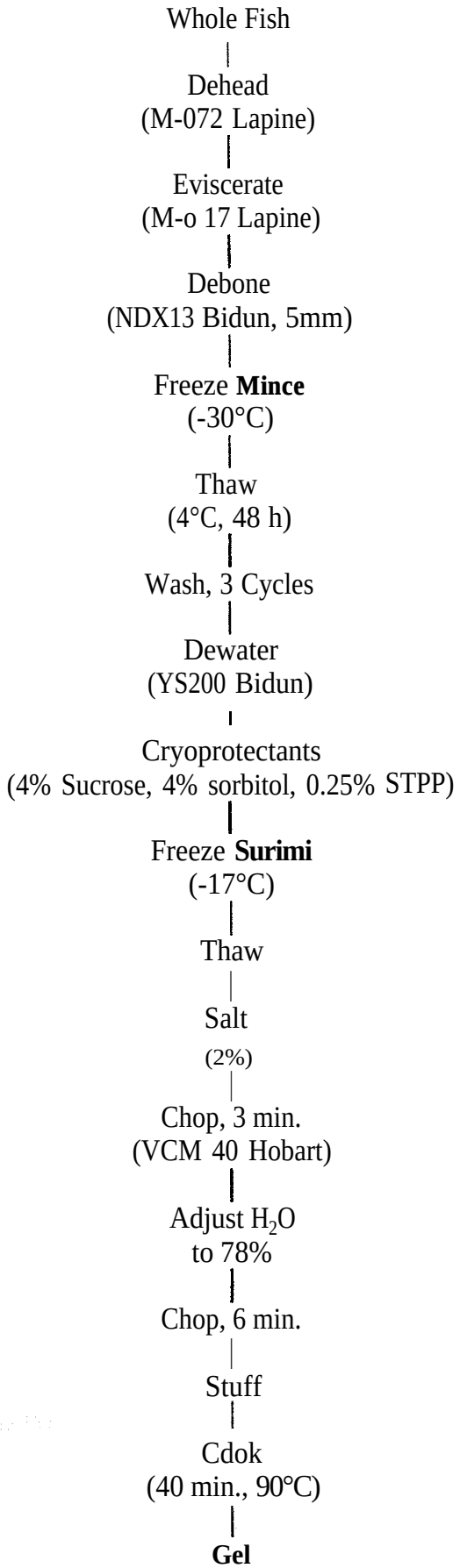

Figure 1, Menhaden Mince and Surimi Processing Flowchart.

washed with four liters of phosphate buffer at different pHs at 4°C. The mixture was manually stirred for 3 min, allowed to settle for 15 min and filtered through a double layer of fine cheese cloth. The water was pressed out by hand and floating particles of fat were removed.

- Second and third cycles (5 parts of water to 1 part of mince): The fish paste from the first cycle was washed with five liters of potable water at 4°C. In the 3rd cycle, 0.15% (w/w) of NaCl was added. After each cycle, the mixture was hand stirred for 3 min, allowed to settle for 5 mm and then filtered through a double layer of fine cheese cloth. The fish paste was further pressed using a Model YS200 Screw Dehydrator (Bidun Machine Construction Co., Ltd., Japan) to reduce the moisture content. Washed meat was weighed to separate samples used for different chemical analyses from the one used for the preparation of the gels.

Preparation of Surimi Gels

The fish paste was mixed with 4% sucrose (commercial granulated refmed sugar), 4% sorbitol (Neosorb 20/60, Roqueete Corporation, Gurnee, IL) and 0.25% sodium tripolyphosphate (BK-Landenburg, Corp., Cresskill, NJ) on a w/w basis in a household mixer (Kitchenaid, Hobart Manufacturing Company, Troy, OH) (Fig. 1). The total mixing time was 3 min at speed 4; subsequently, the surimi was stored in a 0.076-mm polyethylene bag at -20°C until used.

The surimi was thawed overnight in a refrigerator then chopped with 2% (w/w) NaCl in a silent cutter (Model VCM40, Hobart Manufacutring Company, Troy, OH) for 3 min. An additional 6 min of chopping was performed with a sufficient amount of ice-chilled water (2 ± 1 "C) to adjust the moisture content to 78%. The chopped paste was stuffed into 3cm diameter cellulose casings, and cooked at 90°C for 40 min in a water bath. The heat induced surimi gels were cooled in running tap water for 20 min The prepared gels were allowed to equilibrate to room temperature overnight and then cut into 3 cm dia. and 2 cm long cylindrical shapes for color measurements_

Chemical Analysis

The fish paste saved for chemical studies was stored for 24 h at 4°C prior to analysis.

Total carotenoid pigments were determined calorimetrically (OD,) after extraction on 25g of fish paste, as described by Saito and Regier (1970). The OD at 460 nm was multiplied by a factor of 2 to express the results as µg carotenoids/g sample. The concentration of the carotenoids were determined at pH 5.0, 6.0,7-0, 8.0, 9-0, 10.0 and control.

The hematin content of the menhaden fish was determined on 5g of fish paste by the alkaline hematin method as described by Karlsson and Lundstrom (1991).

Hematin concentration, expressed as ppm hematin/g sample, was calculated using the following equation: $ppm=(5/50)[97.261(OD_{700}) - 0.0751$

Oxidative deterioration of the lipid components (rancidity) was evaluated on log sample using the 2-thiobarbituric acid test of Tarladgis et al. (1960). The optical density was read at 538 nm and readings multiplied by a factor of 7.8 to express the TBA reactive substances (TBARs) results as mg of malonaldehyde/kg sample (Sinnhuber and Yu, 1958).

The carbonyl content (moles/g) was determined by the method for trace quantities of carbonyl compounds as described by Siggia (1963). Results were converted to µmole/g by multiplying by 10^6.

Moisture content of unwashed menhaden mince, pH treated menhaden paste and the control were determined by method 950.46, drying under vacuum at 95 to 100°C (AOAC, 1990). Values were reported as percent moisture on a wet weight basis.

Color Measurement

Hunter "L" (lightness), "a" ("+" indicating redness) and "b" ("+" indicating yellowness) values were measured by a Hunter Labscan 6000 0/45° Spectrocolorimeter (Hunter Associates Laboratory Inc., Reston, VA). The instrument was calibrated using a white Hunterlab color standard tile no. LS- 13601 (L_0=90, a_0= 0.16 and b_0=0.48). The fish samples (at room temperature) were placed on to a 60x15mm (dia x ht) clear Pyrex© culture petri dish cover and over a 2 in. port. Hunter "I", "a", and "b" values were recorded for all pH treatments of both menhaden paste and heat-induced surimi gel.

The hue angle, $\tan^{-1}(b/a)$ and total color difference $AE=[L-L_0)^2+(a-a_0)^2+(-b_0)^2]^{1/2}$ were calculated for menhaden mince.

Statistical Analysis

All data were analyzed using a randomized complete block design with three replications (blocks). Blocks consisted of three batches of menhaden fish processed June 9, July 23 and July 28 1992, respectively. The treatments consisted of unwashed mince, paste/gel washed with tap water (control), and paste/gel washed with phosphate buffers at pH 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. Analyses were performed using the GLM procedures of the Statistical Analysis System (SAS, 1985). Means were separated where significant differences were found using least significant differences (LSD) test (SAS, 1985) at the 5% level of significance.

RESULTS AND DISCUSSION

Chemical Analysis

There were no differences ($P>0.05$) in carotenoids content, on a dry basis, due to wash treatments (Table 1). Due to their aliphatic and aliphatic-cyclic molecular structure composed of carbon isoprene groups, carotenoids are fat-soluble pigments. Consequently, aqueous washing treatments do not extract these pigments.

Total heme pigment extraction was affected ($P>0.05$) by washing medium pH (Table 1). Washing with phosphate buffers of pH 8.0 or lower resulted in lower ($P>0.05$) hematin than unwashed products. The pigment content decreased by 74%, from 1.07 ppm in the unwashed fish mince to 0.27 ppm in the tap water washed sample. Dawson et al. (1988) reported that heme pigments are more soluble in low salt concentrations (Froning and Niemann, 1988), possibly facilitating their removal by the washing solution.

There were no differences ($P>0.05$) in TBARs due to wash treatments. The TBARs varied between 8.40 for unwashed mince and 4.53 mg of malonaldehyde/kg for mince washed with pH 5.0 water (Table 1). These values are considered to be high for frozen seafoods (Sinnhuber and Yu, 1958) and may result in unacceptable products by consumers. Disruption of muscle membrane system caused by deboning was postulated to increase the rate of lipid oxidation by exposing labile lipid constituents to oxygen (Sato and Hegarty, 1971). Hall (1987) stated that a rupture of the organized cellular structure brought together lipids, catalysts and enzymes involved in lipid oxidation.

Nakayarna and Yamamoto (1977) reported that rancidity was detected at **TBA** values of 1.50 and 3.00 in different fish species. However, malonaldehyde is not the only end product of lipid peroxidation (Buege and Aust, 1978). Furthermore, Slabyj and True (1978) reported that high TBA values did not necessarily reflect rancidity in some samples.

There were differences ($P<0.05$) in carbonyl values due to wash treatments (Table 1). The application of water washing to raw fish mince decreased carbonyl values in mince. Carbonyl values decreased by 50% from 0.463 in the unwashed raw fish mince to 0.233 μmole/g in the tap water-washed sample but were not different ($P>0.05$) among washed minces.

Carbonyl values reported in the fish mince washed with a pH 5.0 phosphate buffer were usually higher than in those washed with alkaline pHs. This may be attributed to lipid oxidation by nonheme proteins (proteins associated with free inorganic forms of iron, copper, or cobalt), which have been reported to be responsible for about two-thirds of oxidation (Decker and Schanus, 1986) and found to be pH sensitive; the catalytic effect of the iron being higher at acidic pH values.

Table 1. Mean carotenoids, hematin, 2-thiobarbituric acid reactive substances (TBARs) and, carbonyl values of menhaden mince as affected by different washing treatments.

Treatments	Carotenoids (dry basis) (ppm)	Hematin (dry basis) (ppm)	Carbonyls (µmole/g)	TBARs (mgmalon-aldehyde/kg)
pH 5.0	1.07[NS]	0.27 bc	0.320 ab	4.53[NS]
pH 6.0	0.70	0.17 c	0.270 bc	6.73
pH 7.0	0.87	0.23 bc	0.170 bc	5.90
pH 8.0	0.90	0.17 c	0.157 c	5.00
pH 9.0	0.90	0.67 ab	0.203 bc	4.77
pH 10.0	1.03	0.63 ab	0.150 c	6.37
Tap water	0.57	0.27 bc	0.233 bc	6.67
Unwashed	0.63	1.07 a	0.463 a	8.40
Overall Mean	0.83	0.43	0.246	6.05
CV (%)	45.24	64.17	36.01	22.59
SEM	0.14	0.08	0.078	1.87
LSD (0.05)	0.66	0.49	0.155	2.39

abc- Means within columns not followed by a common letter differ ($P<0.05$).
NS- No significant differences
CV - Coefficient of Variability
SEM - Standard error of the mean
LSD - Least signifcant differences at 5% level of probability

Labuza (1971) suggested that the protein part of hemoprotein molecules normally hinders the catalytic function of the iron, and in the denaturation of the protein part of hemoprotein molecules could expose iron to lipids.

Mean moisture values of menhaden mince and menhaden gel as affected by different washing treatments are shown in Table 2. Menhaden mince from meat washed with water pH 8.0, 9.0 and 10.0 had higher ($P<0.05$) moisture than those washed with low pH buffer or tap water. Moisture content for any washed mince sample was higher ($P<0.05$) than for the unwashed mince sample. No differences ($P>0.05$) in gel moisture were found, since this was made to a target 78% moisture (Fig. 1).

It was noticed that water removal from the washed menhaden mince during the dewatering step was particularly difficult as the water pH increased (data not shown). Ball et al. (1984) reported a similar direct relationship between increased washing solution pH and increased moisture content in washed poultry meat. The increase of water binding properties of muscle proteins was attributed to an adjustment of pH away from the protein isoelectric point, in mechanically deboned poultry meat (Dawson et al., 1988).

Color

Hunter "L", "a", hue value, and, total color difference (AE) of menhaden mince of washed treatments was different ($P<0.05$) from the unwashed. The most dramatic effect of water washings was a 84% reduction in redness ("a" value), from 4.49 to 0.72 (Table 3). The fish mince Hunter 'L' value was reduced by 10 units after washing, but wash pH did not have any effect.

Hue angle values (Table 3) increased from 68.74 in the unwashed fish mince to 86.97 in the tap water washed sample. There was no additional increase in the hue values when different phosphate buffers were used. As the red color was removed, the fish mince decreased in darkness, and hues other than red tended to predominate. Total color difference (AE) values between untreated and treated samples decreased ($P<0.05$) with washing.

The changes in color of the washed mechanically deboned fish mince during the cooking step could be attributed to the denaturation of any remaining myoglobin to denatured metmyoglobin (Francis and Clydesdale, 1975). Fogg and Harrisson (1975) reported that myoglobin in pure solution denatured when heated to 85°C. Francis and Clydesdale (1975) stated that upon cooking, salmon flesh was lighter in color and hue shifted from red to orange-red. Contamination of menhaden mince with skin, bone and scale indicated too high deboner belt pressure and an improper feeding of the deboner. Fish fed skin side down were used, forcing more bone and scale through the drum perforations along with the fish mince. This outcome could have been avoided by adjusting the belt pressure of the deboner and orienting the split fish so that the deboner perforated drum was in direct contact with the white muscle (bone

Table 2. Mean moisture values of menhaden gel as affected by different washing treatments.

Treatments	Mince Moisture (%)	Gel Moisture (%)
pH 5.0	81.30 b	77.90 ab
pH 6.0	81.63 b	78.30 ab
pH 7.0	84.77 ab	79.20 a
pH 8.0	85.47 a	79.23 a
pH 9.0	86.60 a	79.77 a
pH 10.0	86.30 a	78.67 a
Tap water	78.63 b	77.13 b
Unwashed	71.87 c	N/A
Overall Mean	82.07	78.54
CV (%)	2.24	1.14
SEM	3.37	0.80
LSD (0.05)	3.22	1.57

abc - Means within columns not followed by a common letter differ ($P<0.05$).
N/A - Data not available
CV - Coefficient of Variability
SEM -Standard error of the mean
LSD - Least significant differences at 5% level of probability.

Table 3. Mean Hunter "L", "a", hue, and AE color values of menhadedn mince as affected by different washing treatments.

Treatments	L	a	Hue[1]	ΔE^2
pH 5.0	51.92 a	0.11 bc	89.70 a	39.35 b
pH 6.0	51.70 a	0.33 bc	88.61 a	39.59 b
pH 7.0	51.44 a	0.16 bc	89.53 a	39.73 b
pH 8.0	52.98 a	0.28 bc	88.42 a	38.15 b
pH 9.0	53.27 a	-0.06 c	90.68 a	37.97 b
pH 10.0	51.95 a	0.13 bc	89.86 a	39.22 b
Tap Water	51.28 a	0.72 b	86.97 a	40.04 b
Unwashed	41.41 b	4.49 a	68.74 b	49.97 a
Overall Mean	50.74	0.77	86.56	40.50
cv (%)	3.18	54.27	2.55	3.87
SEM	2.60	0.19	4.86	2.46
LSD (0.05)	2.82	0.76	3.86	2.75

'Hue = Tan^{-1} (b/a)

[2]ΔE = Total color difference = $[(L-L_0)^2 + (a-a_0)^2 + (b-b_0)^2]^{½}$

abc - Means within columns not followed by a common letter differ($P < 0.05$).

CV - Coefficient of Variability

SEM - Standard error of the mean

LSD - Least significant differences at 5% level of probability.

side). These adjustments are essential to control the quantity of dark muscle incorporated as well as skin, bone and scale.

CONCLUSIONS

Washing with tap water or pH-buffered water (acid or alkaline) resulted in a lighter ($P<05$) color mince than unwashed product. However, there were no differences ($P>0.05$) in washing treatment on color of mince and gel made from menhaden. There was no effect ($P>0.05$) of washing treatment on carotenoids or TBARs, but there were ($P<0.05$) on hematin and carbonyl values of the mince.

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EXTENDED SHELF-LIFE OF CATFISH FILLETS TREATED WITH SODIUM ACETATE, MONOPOTASSIUM PHOSPHATE, AND BIFIDOBACTERIA

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The shelf **life** and safety of refrigerated fish and fish products are dictated by the presence of food spoilage and pathogenic microorganisms (Vilemure et al., 1986; Ingham, 1989; Harrison et al., 1991; Kim and Heamsberger, 1994). Temperature abuse during handling, transportation, and storage of fish without additional preservation methods allows for proliferation of these microorganisms (Barnett et al., 1982; Fey and Regenstein, 1982; Josephson et al., 1985; Scott et al., 1986; Baker et al., 1990). Growth of undesirable spoilage bacteria in refrigerated fish causes deterioration of keeping quality and reduces consumer acceptance.

Efforts to enhance the quality and safety of refrigerated foods have been primarily directed to preventing the growth of or to destroy aerobic spoilage bacteria and foodbome pathogens during storage and handling by using combinations of food additives and/or biopreservatives (Gilliland and Ewell, 1983; Lindgren and Dobrogosz, 1989). Several researchers have suggested that biopreservatives such as lactic acid bacteria or their metabolites in refrigerated foods have the ability to suppress aerobic bacteria that cause food spoilage (Raccach and Baker, 1978; Schaack and Marth, 1988; Laroia and Martin, 1990; Modler et al., 1990; Hughes and Hoover, 1991; Ray, 1992).

Phosphates have broad spectrum antimicrobial activity and are used for improving the microbiological quality of muscle foods and to improve shelf life (Molins, 1991). Molins (1991) reported that changes in pH induced by phosphate addition may play an important role in the ability of these compounds to chelate metal ions essential in bacterial metabolism andgrowth. He suggested that, in general, gram-positive bacteria are more susceptible to inhibition by various pyro- and polyphosphates than are gram-negative bacteria Although there has been limited

work on combining acetates and phosphates in meat products (Mendonca et al., 1989) as antimicrobial surface treatments, this has not been studied with fish.

Lindgren and Dobrogosz (1989) observed that a combination of *Lactobacillus leuteri* and 250 mM glycerol inhibited aerobic spoilage bacteria in herring fillets stored in N_2 at 5°C for 6 d. Raccach et al. (1979) and Schaack and Marth (1988) showed similar effects with lactic acid bacteria on food spoilage bacteria and pathogens in poultry meat and skim milk, respectively. Use of *Pediococcus cerevisiae* (Accel^R) and *Lactobacillus plantarum* (Lactacel^R) in refrigerated deboned poultry meat decreased the microbial population, reduced off-odor, and extended shelf-life by 2 d (Raccach et al., 1979). Kim and Heamsberger (1994) reported that combination of *Lactococcus lactis* ssp. *cremoris* ATCC 19257 with sodium acetate and potassium sorbate effectively inhibited growth of gram-negative bacteria on refrigerated catfish fillets. Bifidobacteria may control food spoilage bacteria and foodbome pathogens through production of lactic and acetic acids as well as other antibiotic substances (Laroia and Martin, 1990; Modler et al., 1990; Hughes and Hoover, 1991; Ray, 1992). Previous work in our laboratories has shown that sodium acetate is effective in suppressing the growth of aerobic spoilage bacteria on refrigerated catfish fillets (Kim and Hearnsberger, 1994). Because there are no studies on the combined effect of sodium acetate with phosphates or bifidobacteria as an antimicrobial treatment of fish or fish products, the present study was designed to evaluate shelf-life of refrigerated (4°C) channel catfish fillets surface-treated with sodium acetate, monopotassium phosphate and/or selected bifidobacteria.

MATERIALS & METHODS

Preparation of bifidobacteria cultures

Bifidobacterium infantis ATCC 15697 and *B. longum* ATCC 15707 were obtained from the American Type Culture Collection (Rockville, MD). B. *adolescentis* 9H Martin was obtained from the culture collection of Dr. J. H. Martin, Mississippi State University. Stock cultures were maintained in sterile skim milk medium (SM; 10% skim milk, 0.5% yeast extract, and 0.5% glucose) at 37°C for 24 h in an anaerobic jar (Gas-Pak; BBL). For fish inoculation, the cultures were prepared by three successive 1: 10 transfers at 24-h intervals into fresh SM incubated as above. Bifidobacteria cultures in SM (6.0×10^7 to 1.0×10^8 CFU/ml) were added at a given percentage (V/W) to fresh catfish fillets as described below. Final SM pH was 4.4-4.8.

Catfish preparation and treatment

Fresh channel catfish (*Ictalurus punctatus)* fillets were obtained from a commercial source, transported to MSU on ice, and used within 3 h. For each treatment, 2 kg of fillets (average weight of 110 g per fillet) were placed in a precleaned (Jettson General Purpose Cleaner, Diversy Group, Cincinnati, OH) and sanitized (hot water at 88°C) tumbler (Polymaid Model SS 350, Lax-go, FL) at room temperature. Food preservatives were added to the tumbler and fillets were tumbled

for 15 min at 18 r-pm. Untreated controls were tumbled without preservatives. The tumbler was cleaned and sanitized between treatments. Duplicate experimental trials consisted of the following treatments: (a) 0.0, 0.25, 0.5, 0.75, or 1 .0% (W/W) sodium acetate (SA; Fisher Scientific Co., Norcross, GA), (b) 0.0, 0.25, 0.5, 0.75, or 1.0% (W/W) monopotassium phosphate (MKP; FMC Corp. Philadelphia, PA), (c) 0 or 2.5% (V/W) *B. adolescentis, B. infantis,* or *B. longum* (V/W), (d) 0.5% SA and 0.1, 0.2, 0.3, or 0.4% MKP, (e) 0.4% MKP and 0.3, 0.5, or 0.7% SA, (f) 0 or 2.5% of *B. adolescentis, B. infantis,* or *B. longum* combined with 0 or 0.5% SA, (g) 0 or 0.5% SA combined with 1.5%, 2.5%, 3.5% or 4.5% *B. infantis,* and (h) 0 or 0.5% SA combined with 2.5% *B. infantis* incubated for 24, 48, or 72 h before addition. Treated catfish fillets were removed from the tumbler and stored individually in "Ziploc^{R}" (DowBrands L.P., Indianapolis, IN) bags at 4°C. Storage bag gas transmission rate is 1.65 cc/cm^2 per mil in 24 h at 1 atm. Microbiological analysis was performed for each treatment over a 12 d storage period. Selected treatments were scheduled for pH measurements and sensory evaluations during storage.

Microbiological analyses

Each fillet was weighed and 0.1% sterile peptone water was added to make a 1:1 dilution (W/V). A Stomacher Lab Blender 400 (Tekmar, Cincinnati, OH) was used to homogenize the specimen for 2 min and then appropriate serial dilutions were made for spiral plating. Total aerobic plate counts (APC) were determined by duplicate spiral-platings (Spiral System, Inc., Bethesda, MD) on standard plate count agar (Difco Detroit, MI) incubated at 30°C for 2 d before counting (Swanson et al., 1992). APC values were expressed as mean log$_{10}$ CFU/g for 2 fillets per treatment per sampling day. Generation times were calculated as described elsewhere (Marshall and Schmidt, 1988).

Bifidobacteria were enumerated by pour plating appropriate serial dilutions in 0.1% peptone water with neomycin-paromomycin- nalidixic acid-lithium chloride agar Teraguchi et al, 1978; Laroia and Martin, 1991) followed by incubation at 37°C for 48 h in an anaerobic jar (Gas-Pak; BBL) prior to counting colonies.

Measurement of pH

Fillet pH was measured with a standardized pH meter (Accumet^{R}, Model 50, Fisher) by placing a surface electrode directly onto the fillet surface. Mean pH values were reported as the average of quadruplicate readings for each duplicate fillet.

Sensory evaluation

Sensory evaluation of samples was performed by an eight member untrained panel. Uncooked odor and appearance of treated fillets were evaluated every 3d during storage at 4°C Samples were warmed to room temperature prior to analyses. Treated samples were judged against a fresh control (fresh fillets were used each analysis day) which was assigned a score of 5. Samples liked less than the control were scored 1 to 4, where 1 = most disliked. Samples liked more than the control were scored 6 to 9, where 9 = most liked. Untreated fillets also were compared with

untreated fresh fillets and treated fillets. Two fillets per treatment per sampling day were analyzed.

Statistical analyses

APC, pH, and sensory data were analyzed using ANOVA, and means were separated by the least significant difference test (SAS, 1992).

RESULTS & DISCUSSION

Combined effect of sodium acetate and monopotassium phosphate

APC of refrigerated (4°C) catfish fillets were affected by increasing levels of SA (Fig. 1). Use of 0.75% and 1.0% SA significantly lowered ($P<0.05$) initial APC by 0.6-0.7 log units compared to the control. Generation times (GT) of aerobic bacteria on fillets treated with SA were significantly higher ($P<0.05$) than the control (Fig. 2). Spoilage of most muscle foods is thought to occur when APC reach 10^7 CFU/g or greater (Ayers, 1960). Therefore, SA treatments, at 0.75% or greater, of fillets could increase shelf-life (as measured by APC) by 6 d under present experimental conditions. These results confirm previous work that demonstrated the antimicrobial potential of SA on catfish fillets, where 0.5-1.0% SA suppressed growth of gram-negative bacteria at 4°C for at least 6 d (Rim and Hearnsberger, 1994).

MKP in refrigerated catfish fillets had no effect on APC values (results not shown) or generation times (Fig. 3).
Treatment with MKP lowered fillet pH values by 0.5-0.9 units (results not shown). Kim and Heamsberger (1994) reported that the combined antimicrobial effects of SA, potassium sorbate, and lactic acid bacteria in refrigerated catfish fillets were not pH related. Results of the present catfish study are consistent with previous work by Molins (1991) who reported that other phosphates (sodium acid pyrophosphate or sodium tripolyphosphate) did not improve the microbiological quality of cooked, vacuum-packaged bratwurst held at 5°C for 7 d.

Combining MKP and SA effectively inhibited growth of aerobic microorganisms (Figs. 3 and 4). All samples treated with the combination had sigficantly ($P<0.05$) lower APC after 3 d of storage than the control. Combining MKP with 0.5% SA reduced numbers during latter stages of storage more than did SA alone (results not shown), which was reflected by increased generation times with increasing MKP concentration (Fig. 3). Conversely, when MKP concentration remained at 0.4%, no differences ($P>0.05$) in inhibition were observed with increasing Concentrations of sodium acetate (0.3-0.7%) (Fig. 4). Results indicate that the interaction between MKP and SA is likely additive.

Although MKP alone had no inhibitory effect, it may cause a decline of water activity values in treated fish fillets (Synder and Maxcy, 1979) and have indirect antimicrobial effects due to chelation of metal ions essential for bacterial metabolism and cell integrity (Scott et al., 1986). Microbial spoilage of fish is caused by gram-

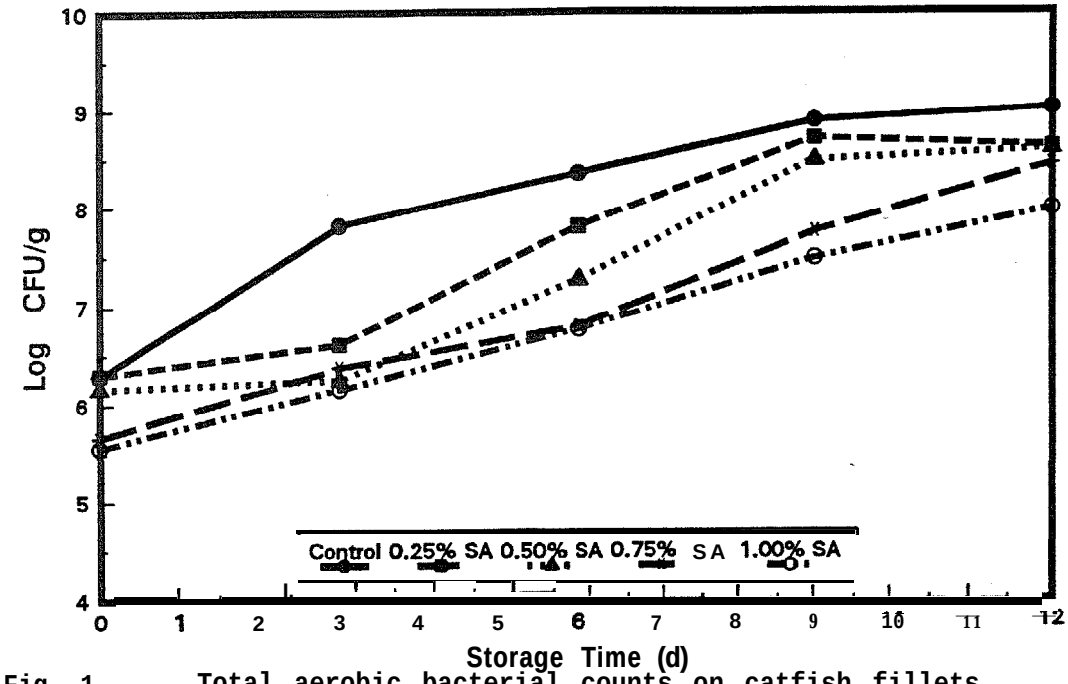


Fig. 1. Total aerobic bacterial counts on catfish fillets treated with sodium acetate (SA) during storage at 4°C.

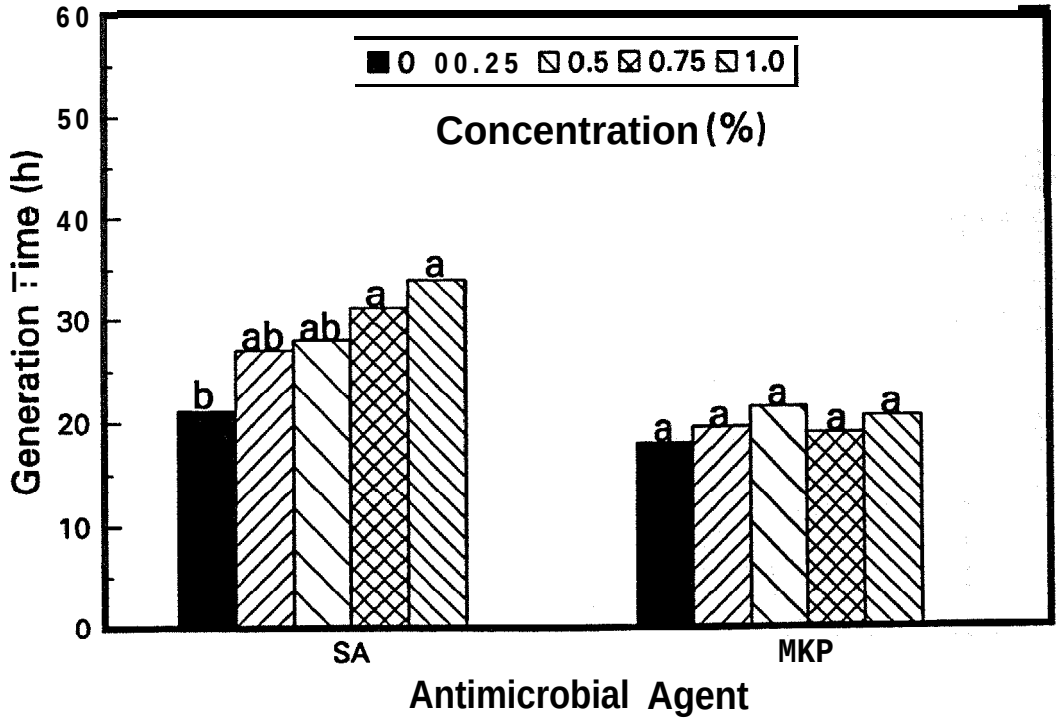


Fig. 2. Generation times of aerobic bacteria on catfish fillets treated with sodium acetate (SA) or monopotassium phosphate (MKP) and stored at 4°C. Means within the same cluster having the same letter are not significantly different (P>0.05).

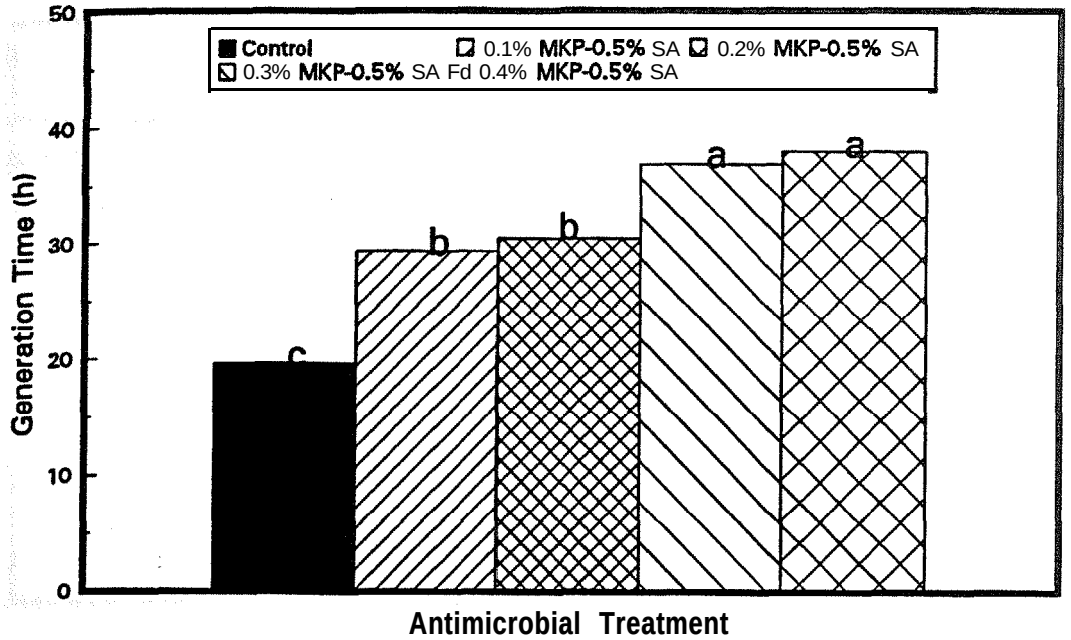
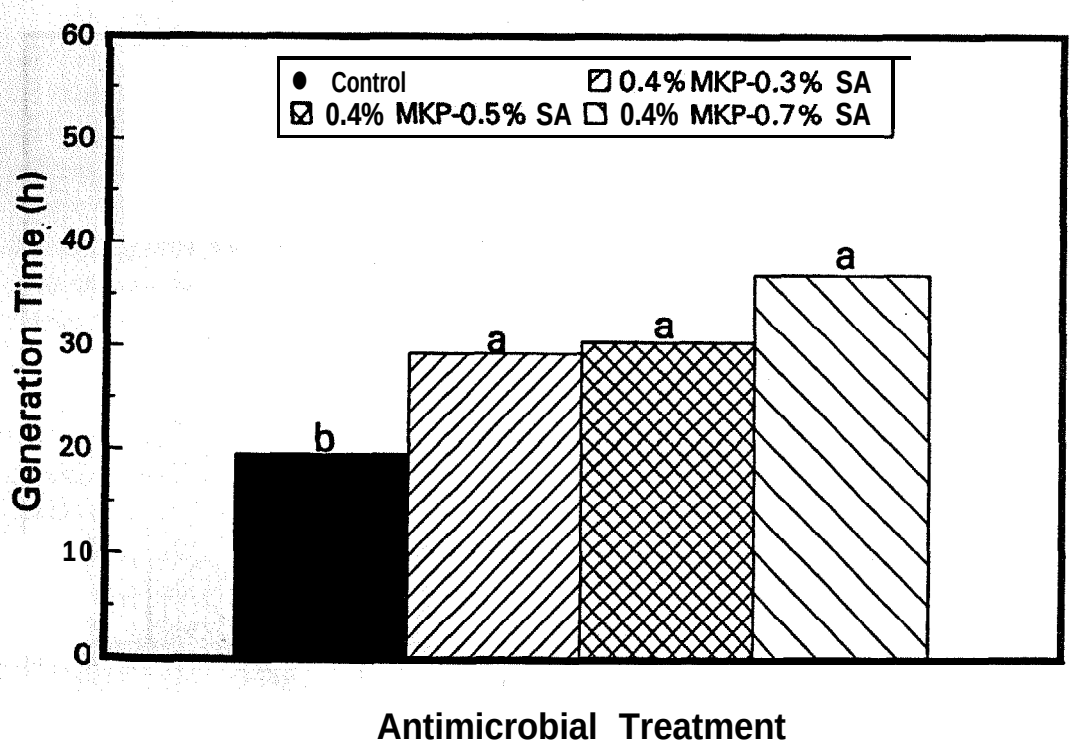


Fig. 3. Generation times of aerobic bacteria on catfish fillets treated with different concentrations of monopotassium phosphate (MKP) combined with 0.5% sodium acetate (SA) and stored at 4°C. Means with the same letter are not significantly different (P>0.05).



Pig. 4. Generation times of aerobic bacteria on catfish filets treated with different concentrations of sodium acetate (SA) combined with 0.4% monopotassium phosphate (MKP) and stored at 4°C. Means with the same letter are not significantly different (P>0.05).

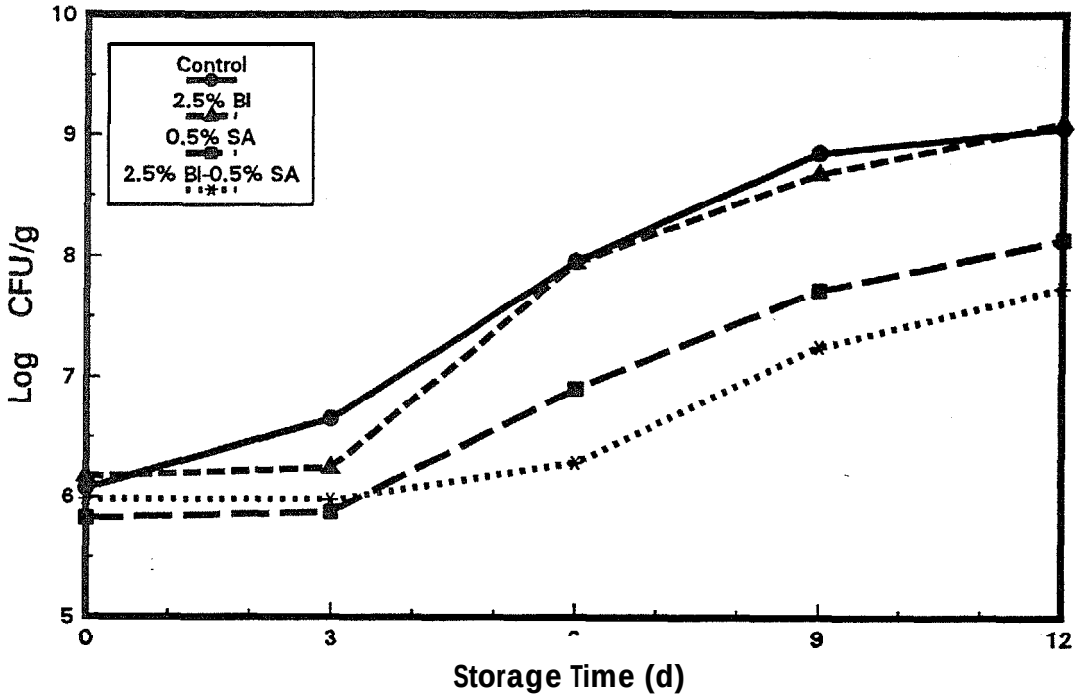


Fig. 5. Aerobic plate counts of catfish fillets treated with Bifidobacterium infantis culture (BI) and sodium acetate (SA), either alone or combined, during storage at 4°C.

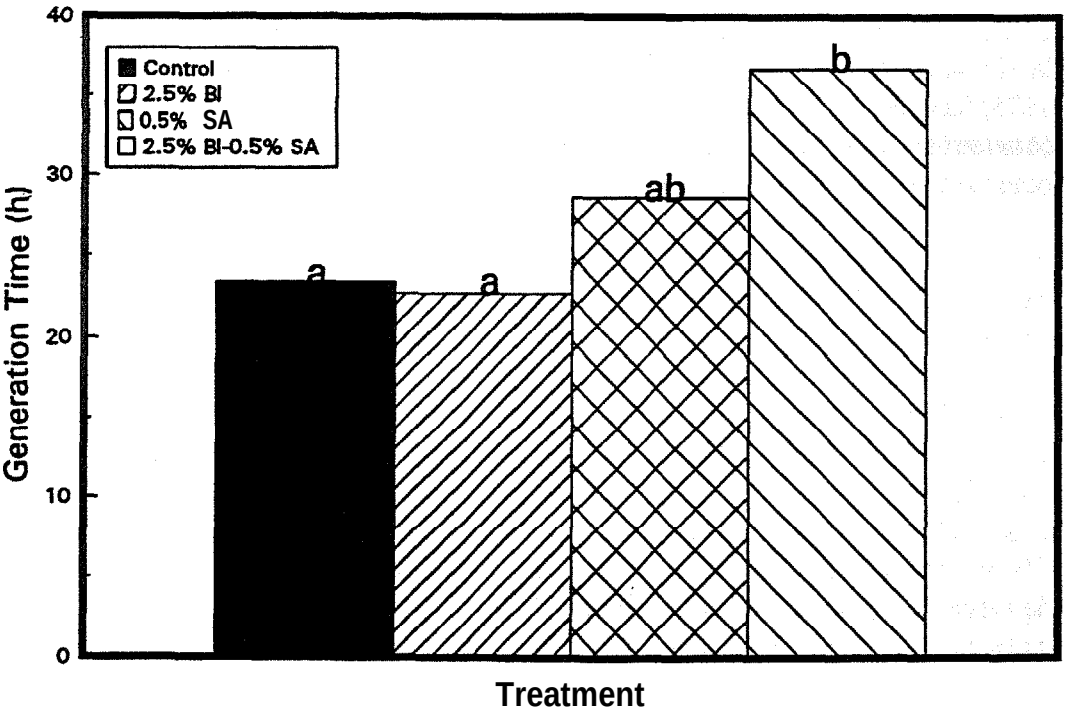


Fig. 6. Generation times of aerobic bacteria at 4°C on catfish fillets treated with Bifidobacterium infantis Culture (BI) and sodium acetate (SA), either alone or combined. Means with the same letter are not significantly different ($p>0.05$).

negative bacteria such as Pseudomonas spp. (Herbert et al., 1971; Herbert and Shewan, 1976). SA inhibits growth of gram-negative bacteria on refrigerated (4°C) catfish fillets (Kim and Hearnsberger, 1994), while gram-positive bacteria are more susceptible to inhibition by MKP than are gram-negative bacteria (Molins, 1991). This microbial specificity demonstrates that SA is a better chemical treatment than MKP to extend shelf-life of catfish. However, results indicate that a combination of SA and MKP may prolong the microbiological shelf life of catfish at 4°C by keeping counts below 10^7 CFU/g.

Combined effect of sodium acetate and bifidobacteria

The effects of various concentrations and species of BIF and SA on the shelf-life of refrigerated catfish fillets were studied on the basis of aerobic plate counts (APC) and sensory data. When a 2.5% culture of *B. infantis* was used alone, little impact on APC was noted with the exception of a significant ($P<0.05$) extension of the lag phase (Fig. 5). Once growth began, GT of aerobic microorganisms on fillets treated with *B. infantis* were not significantly different ($P>0.05$) from control fillets (Fig. 6). Similar ($P>0.05$) extended lag phases and GT were noted for fillets treated with 2.5% cultures of *B. adolescentis* or *B. longum* (results not shown). Hence, further results only will report data from *B. infantis* treatments. Lag phase increases of psychrotrophic populations were observed in ground poultry treated with *Lactobacillus plantarum or Pediococcus cerevisiae* (Raccach et al., 1979). It was assumed that inoculated BIF do not grow on refrigerated fish fillets and the extension of lag phase may be due to compounds produced during culturing, i.e. acetic and/or lactic acid, that were inhibitory to aerobic spoilage bacteria (Gilliland and Speck, 1975; Ray, 1992). Other reasons for inhibited aerobic growth might include nutrient competition, unfavorable changes in oxidation reduction potential, or production of other antimicrobial compounds.

Fillets treated with 0.5% SA had APC significantly lower and GT significantly higher ($P<0.05$) than control or BE-treated fillets (Fig. 5 & 6). The microbiological shelf-life (i.e. time to reach APC of 10^7 CFU/g or greater (Ayers, 1960)) of SA-treated fillets was prolonged by 3 d compared to untreated and BIF-treated fillets. Combining 2.5% BIF with 0.3% SA resulted in significant ($P<0.05$) lag phase extension and increased GT values approximately 8 h higher than controls (results not shown). No differences ($P>0.05$) among species was noted. When 2.5% *B. infantis* was combined with 0.5% SA, significant ($P<0.05$) decreases in APC and increases in GT were observed (Fig. 5 & 6). No significant difference ($P>0.05$) in GT was seen between this treatment and 0.5% SA alone, resulting in a 3d microbiological shelf-life extension. *B. infantis* behaved similarly ($P>0.05$) as the other two cultures when combined with 0.5% SA (results not shown). The combined treatments could increase GT by approximately 20 h. A previous report on SA combined with potassium sorbate or lactic acid culture has attributed antimicrobial effects primarily to SA (Kim and Hearnsberger, 1994). The present study shows that an additive interaction occurs when BIF are combined with SA.

Efforts to increase antimicrobial activity of the BIF-SA treatment proved unsuccessful. Increasing the amount of *B. infantis* culture had no influence ($P>0.05$) on APC (results not shown). Likewise, increasing the amount of time the *B. infantis* culture was incubated prior to application had no influence ($P>0.05$) on APC (results not shown). Reddy et al. (1970) reported that addition of 10% cultures of *Leuconostoc citrovurum was* effective in inhibiting the growth of aerobic spoilage bacteria in ground beef stored at 7°C for 7 days. In a later study, Reddy et al. (1983) found that when cultivation was prolonged from 24 to 48 h, growth and antibiotic production of *Lactobacillus bulgaricus* in skim milk was enhanced.

Catfish pH and sensory scores

Addition of SA decreased pH of fillets by 0.2-0.3 units (results not shown). Fillets treated with both SA and MKP had initial pH values 0.25-0.3 units lower than the control (Table 1).

Table 1. Mean pH values of catfish fillets treated with combinations of sodium acetate (SA) and monopotassium phosphate (MKP) during storage at 4°C.

	Storage Time (d)				
Treatment	0	3	6	9	12
Control	6.45[a]	6.40[a]	6.45[a]	6.30[a]	6.40[a]
0.3% SA-0.4% MKP	6.15[b]	6.25[a]	6.25[a]	6.25[a]	6.30[a]
0.5% SA-0.4% MKP	6. 15[b]	6.25[a]	6.30[a]	6.30[a]	6.35[a]
0.7% SA-0.4% MKP	6.20[b]	6.25[a]	6.30[a]	6.35[a]	6.40[a]

[a,b] Means in the same column with different superscripts are significantly different ($P<0.05$).

This decline was attributed to SA. After 3 d of storage, pH values changed little and did not differ ($P>0.05$) from the control Initial pH values of fillets treated with 0.5% SA and 2.5% BIF, either alone or combined, were 0.4-0.5 units lower than untreated control fillets (results not shown). Other work has shown that SA-treated catfish fillets do not necessarily have lower pH values than controls, but remain inhibitory to growth of aerobes (Kim and Heamsberger, 1994). Hence, inhibitory results seen in the present study are likely due to changes in pH and to the action of SA itself.

Sensory results of SA-MKP experiments clearly showed panel preference for treated samples over stored untreated controls (Table 2). Sensory assessment of untreated controls was halted after 3 d of storage because of excessive off odor. Fillets treated with SA alone or combined with MKP had odor scores that were statistically indistinguishable ($P>0.05$) from fresh controls for up to 9 d (Table 2). However, appearance scores of fillets were significantly lower ($P<0.05$) after 3 d, likely due to a brownish and watery appearance (Table 2). Microbial spoilage of fish muscle is due to production of volatile nitrogen compounds, volatile acids, H_2S, and mercaptans (Miller et al, 1973; Reineccius, 1977). Sensory data indicate that SA

concentrations up to 1% could be used on catfish without contributing a typical acetic acid (vinegar) odor.

Table 2. Mean sensory evaluation scores of catfish fillets treated with sodium acetate (SA) and monopotassium phosphate (MKP), either alone or combined, during storage at 4°C.

	STORAGE TIME (d)									
	Odor Score					Appearance Score				
Treatment	0	3	6	9	12	0	3	6	9	12
Fresh Control	5.0[a]	5.0[a]	5.0[a]	5.0[a]	5.0[a]	5.0[ab]	5.0[a]	5.0[a]	5.0[a]	5.0[a]
1.0% SA	5.2[a]	4.5[a]	4.4[a]	4.0[a]	3.7[b]	5.4[a]	5.2[a]	4.3[b]	3.4[b]	3.6[b]
0.3% SA-0.4% MKP	4.8[a]	5.0[a]	4.0[a]	3.8[b]	3.0b	4.6[b]	4.8[a]	4.4[b]	3.3[b]	3.9[b]
0.5% SA-0.4% MKP	4.4[a]	4.6[a]	4.7[a]	5.2[a]	2.9[b]	5.3[a]	4.6[a]	4.1[c]	4.0[a]	3.5[b]
Untreated Control	5.0[a]	1.5[b]	ND	ND	ND	5.0[ab]	2.9[b]	ND	ND	ND

[a-c] Means within the same column with different superscripts are significantly different ($P<0.05$).

ND: Not determined.

Note: Fresh control assigned 5, with samples liked less than the control scored 1 to 4 (1 = most disliked) and samples liked more than the control scored 6 to 9 (9 = most liked).

Sensory scores of SA-BIF experiments indicate that treated fillets were in the "liked less" to "typical" category in appearance and odor compared to fresh control fillets (Table 3). Stored untreated control fillets were considered organoleptically unacceptable (associated with spoiling fish) by 6 d of storage and were discontinued from sensory evaluation. Odor and appearance of fillets treated with 0.5% SA and 2.5% *B. infantis* either alone or combined, was the same ($p>0.05$) as fresh fillets up to 6 d. Fillets treated with SA and BIF or SA alone were rated higher than fillets with BIF alone after 9 d. APC of fillets treated with BIF alone rapidly increased after 3 d of storage, yet the fillets were not considered spoiled by the panel until after 6 d.

CONCLUSIONS

SA alone or combined with MKP or BIF inhibited growth of aerobic spoilage bacteria on refrigerated (4°C) catfish fillets MKP or BIF had little activity alone, but could increase effectiveness of SA. Therefore, use of MKP or BIF alone has trivial value in extending shelf-life of refrigerated cattish fillets SA alone or combined With MKP or BIF is recommended to extend the microbiological shelf-life of refrigerated catfish fillets with high (10^6 CFU/g) initial microbial loads.

Table 3. Mean sensory evaluation scores of catfish fillets treated with sodium acetate (SA) and *Bifidobacterium infantis* culture (BI), either alone or combined, during storage at 4°C.

	STORAGE TIME (d)									
	Odor Score					Appearance Score				
Treatment	0	3	6	9	12	0	3	6	9	12
Fresh Control	5.0[a]	5.0[a]	5.0[ab]	5.0[a]	5.0[a]	5.0[a]	5.0[a]	5.0[a]	5.0[a]	5.0[a]
2.5% BI	4.7[a]	4.8[a]	4.5[b]	3.1[bc]	1.4[c]	4.5[a]	4.5[a]	5.1[a]	3.2[b]	1.5[c]
0.5% SA	4.6[a]	4.4[a]	5.4[a]	3.7[b]	2.2[b]	4.6[a]	4.5[a]	5.3[a]	3.3[b]	2.5[b]
2.5% BI-0.5% SA	5.4[a]	5.0[a]	5.0[ab]	3.4[bc]	2.4[b]	4.5[a]	4.8[a]	5.2[a]	3.3[b]	2.6[b]
Untreated Control	5.0[a]	5.1[a]	ND	ND	ND	5.0[a]	5.3[a]	ND	ND	ND

[a-c] Means within the same column with different superscripts are significantly different ($P<0.05$).

ND: Not determined.

Note: Fresh control assigned 5, with samples liked less than the control scored 1 to 4 **(1 =** most disliked) and samples liked more than the control scored 6 to 9 (9 = most liked).

ACKNOWLEDGMENTS

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Effect of Tray Design and Packaging Type on Microbial Growth, Surface pH and Sensory Ratings of Refrigerated Channel Catfish Fillets

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INTRODUCTION

Gram negative spoilage bacteria such as ***Pseudomonas, Moraxella, Acinetobacter, Flavobacterium,*** and ***Cytophaga*** species are the major contributors to deterioration of fish and seafood products (Farber, 1991). Modified atmosphere packaging (MAP) using mixtures of carbon dioxide, oxygen, nitrogen, or other gases has been studied extensively to prolong shelf-life of fishery products (Silva et al, 1993; Wang and Ogrydziak, 1986; Stenstrom, 1985; Gray et al., 1983; Parkin et al., 1981; Wolfe, 1980) and thus, extend marketable life of the products. Studies by Gray et al. (1983) in fish products and Sander and Soo (1978) in chicken products, showed that MAP systems were better in extending shelf-life than ice or vacuum packaging systems.

Carbon dioxide is the major gas used in MAP because of its effectiveness in retarding microbial growth in refrigerated, perishable foods. It is especially effective against gram negative spoilage microorganisms by extending lag phase and decreasing growth rate during log phase (Farber, 1991). To achieve the greatest extension of shelf-life, low temperature, close to 0°C (Farber, 1991; Gray et al, 1983), low initial microbial load (Lannelongue, 1982), and high concentrations of carbon dioxide (Blickstad and Molin, 1983) are necessary.

Shelf-life and keeping quality of raw channel catfish fillets have been reported to be 19 d for tray packed/chilled (2°C) products, 7 d for ice-packed fillets (Reed et al., 1983), up to 28 d under 80% CO_2 in barrier bags at 2°C (Silva and White, 1994), and about 16 d for fillets exposed to CO_2 for 6 d and transfered to trays wrapped with HDPE and held at 2°C (Silva et al., 1993). Initial counts on fish for those studies were 3.9 log CFU/cm^2, 3.9 log CFU/cm^2, 6 log CFU/g, and 5.8 log CFU/g, respectively. One MAP technology is the Master-Pack System. It consists of placing small product units (prepackaged or not) into a larger package units (Brody, 1989) which could then be evacuated and backflushed with a gas mixture. The products remain in this system until strict temperature control is not feasible or until ready for consumer display or use.

The objective of the present study was to compare storage life of channel catfish fillets held in different environments (air, vacuum and MAP - Master-Pack) and tray types (single or double-bed) at 2°C by measuring anaerobic, psychrotrophic, and Lactobacilli plate counts, pH, moisture and sensory scores (odor and appearance rating) every 4 d for 28 d.

MATERIALS AND METHODS

Materials

Live channel catfish *Ictalurus punctatus* were processed into fillets at a commercial catfish processing operation and packed in a walk-in cooler at 2°C in single-(S) or double-(D) bed Styrofoam trays with a moisture absorbant pad. The tray-packed fillets (2 per tray) were wrapped with a 2.7 mil polyethylene (HDPE) film and heat-sealed. The trays were packed in ice chests and covered with ice until further treatment at the Mississippi State University Food Science laboratories (not more than 6 h).

One third of the trays were stored as packed (AIR); one third were unwrapped and placed individually in permeable E™ bags (Cryovac Corp., Duncan, SC), vacuumized, and heat-sealed (VAC); and the other third were packed 5 trays to a bag in a barrier B700™ Master-Bag, air evacuated, and back flushed with CO_2 to reach 90% CO_2, 3% 0, and 7%N_2 (MAP). The properties of each of the films are given in Table 1. All products were held at 2°C for up to 28 d and sampled periodically.

Each treatment was replicated twice and analyses were performed in duplicates. Treatments were tray-type (D or S), gas environment (AIR ,VAC, or MAP), and storage time (up to 28 d) at 2°C.

Methodology

Anaerobic (AnPC), psychrotrophic (PPC) and lactobacillii (LAC) plate counts were performed following AOAC methods (FDA, 1992). Standard plate count agar (DIFCO Labs.) was used for AnPC (pour plates) and PPC (spread plates) with plates incubated at 25°C for 72 h in an anaerobic chamber and 21 °C for 72 h in a temperature controlled chamber for AnPC and PPC, respectively. Rogosa agar (DIFC O Labs) was used for LAC, using pour plating technique and incubating aerobically at 25°C for 72 h.

Surface pH was measured using a flat-head electrode on the fish flesh as outlined by Silva and white (1994). Moisture was measured by shredding 5 g of fillet and heating at 100°C for 18 h (AOAC, 1990).

Appearance and odor ratings were conducted on the packaged products after sampling for microbial counts, by seven trained panelists. A 5-point rating scale for appearance and odor was used as follows: Appearance: 5 - fresh appearance 3 - slightly dry or slimy surface, 1 - slimy and off-colored surface, Odor: 5 - fresh, sweet odor, 3 - slightly spoiled (spoilage threshold rating), and 1 - totally spoiled/putrid.

RESULTS AND DISCUSSION

Initial anaerobic counts (AnPC) of fillets in double-(D) bed trays were lower than in single-(S) bed trays regardless of environment (Fig. 1). By the eighth day, AnPC were above 7 log CFU/g for all treatments except S/MAP, D/VAC, and D/MAP. By the 12 d, D/VAC products exceeded 7 log CFU/g, thus the product had spoiled (Handumrongkul and Silva, 1993; Martin and Hearnsberger, 1993; Anonymous, 1992). Products packed under 90% CO_2 in single-bed trays (S/MAP) exceeded 7 log CFU/g after 16 d, whereas those packed in double-bed trays (D/MAP) did not exceed 7 log CFU/g until after 24 d.

Table 1. Properties of films used for packaging study.

Film/Bag Type	Source	Thickness (mm)	WVTR	OTR	CO_2TR
			($cm^3/m^2/24h/atm$)		
HDPE	Dow Chem Co.	0.069	10.08 (0.65°C 100% RH)	1,070	3,350
E™ bag	Cryovac Corp.	---	—	4,000 (22.8°C)	16 - 20,000
B700 bag	Cjovac Corp.	---	0.5-0.6	3-6	15-30

WVTR - water vapor transmission rate, OTR - oxygen transmission rate, CO_2TR - carbon dioxide transmission rate

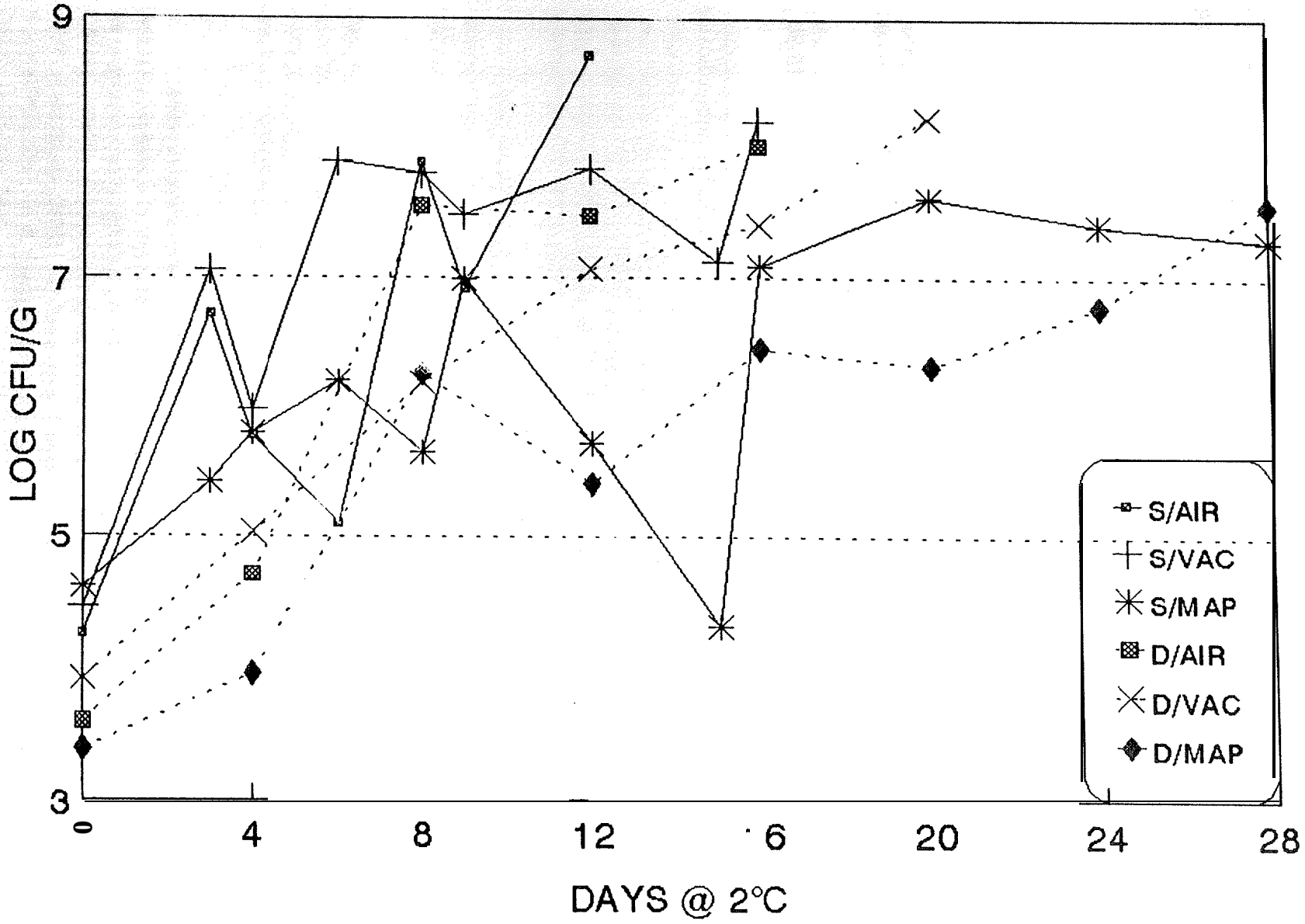

FIGURE 1. ANAEROBIC PLATE COUNTS IN CHANNEL CAT FISH FILLETS AS AFFECTED BY TRAY TYPE (SINGLE OR DOUBLE BED) AND ENVIRONMENT.

FIGURE 1. ANAEROBIC PLATE COUNT IN CHANNEL CATFISH FILLETS AS AFFECTED

Psychrotrophic plate counts (PPC) were below 7 log CFU/g for single-bed or double-bed tray packed products in MAP (Fig. 2) for over 12 d. The PPC of fish packed under other treatments exceeded 7 log CFU/g after 4 d except for S/AIR which took about 6 d.

Lactobacilli counts (LAC) were lower in fillets packed in double-bed trays (Fig. 3), but reached the same levels as for other treatments after the 16th day, between 4-5 log CFU/g. It is well known that gram negative spoilage bacteria population is replaced by gram positive *Lactobacillus* in meat products under anaerobic or modified atmospheres (Farber, 1991; Gray et al., 1983).

Surface pH of fillets tended to be lower for products in double-bed trays for the first 12 d (Fig. 4). After the 16th day, pH of products packed under VAC or MAP in single-bed trays was near 5.0. This is probably due to carbonic acid and lactic acid formation (Brody, 1989).

Initial moisture content of fillets ranged from 72 to 81% (Fig. 5). Even though there was more visual drip in vacuum-packed products (data not shown), moisture content was not significantly affected. However, D/MAP fillets had lower moisture than S/MAP fillets after 28 d. This may be the result of the pad not being in direct contact with the fish in D trays.

Odor ratings of fillets in D/MAP and S/MAP were above spoilage level, 3 for at least 24 d (Fig. 6). Vacuum and air packed products were rated below 3 after 6 to 8 d at 2°C except for D/VAC, which were rated below 3 by the 12th day.

Appearance ratings of fillets followed closely the trends of odor ratings. Ratings for MAP fillets did not reach below 3 until the 28th day (Fig. 7), whereas the other treatments reached 3 between the 6th and 16th day.

Results show that fillets packed under 90% CO_2 (MAP) will have lower bacterial counts than those packed under vacuum or air. *Lactobacillus* counts show that spoilage bacteria are not rapidly replaced by *Lactobacillus* under MAP in double-bed trays but the latter grow rapidly in single-bed trays under MAP or VAC. The double-bed trays have a foam, porous layer between the product and the pad, thus isolating the product from the drip, and the bacterial flora accumulated away from the product. Lower pH is thought to be from the conversion of CO_2 to carbonic acid and the production of lactic acid by lactobacilli (Daniels et al., 1985; Lannelongue et al., 1982). Odor (Fig. 6) and appearance (Fig. 7) ratings of fillets followed anaerobic (Fig. 1) counts (AnPC) very closely, and correlated somewhat with PPC (Fig. 2). A slimy, whitish appearance of the fish surface and an ammonia, putrid smell were the signs of fish spoilage, although MAP products also had a sour smell towards the end of their storage (24-28 d).

This work shows that not only products packed under 90% CO_2 in a Master-Bag will have a longer shelf-life, but also products packed in double-bed trays may have longer keeping quality. Also it shows that AnPC may follow better the microflora of fish fillets held at 2°C as compared to PPC.

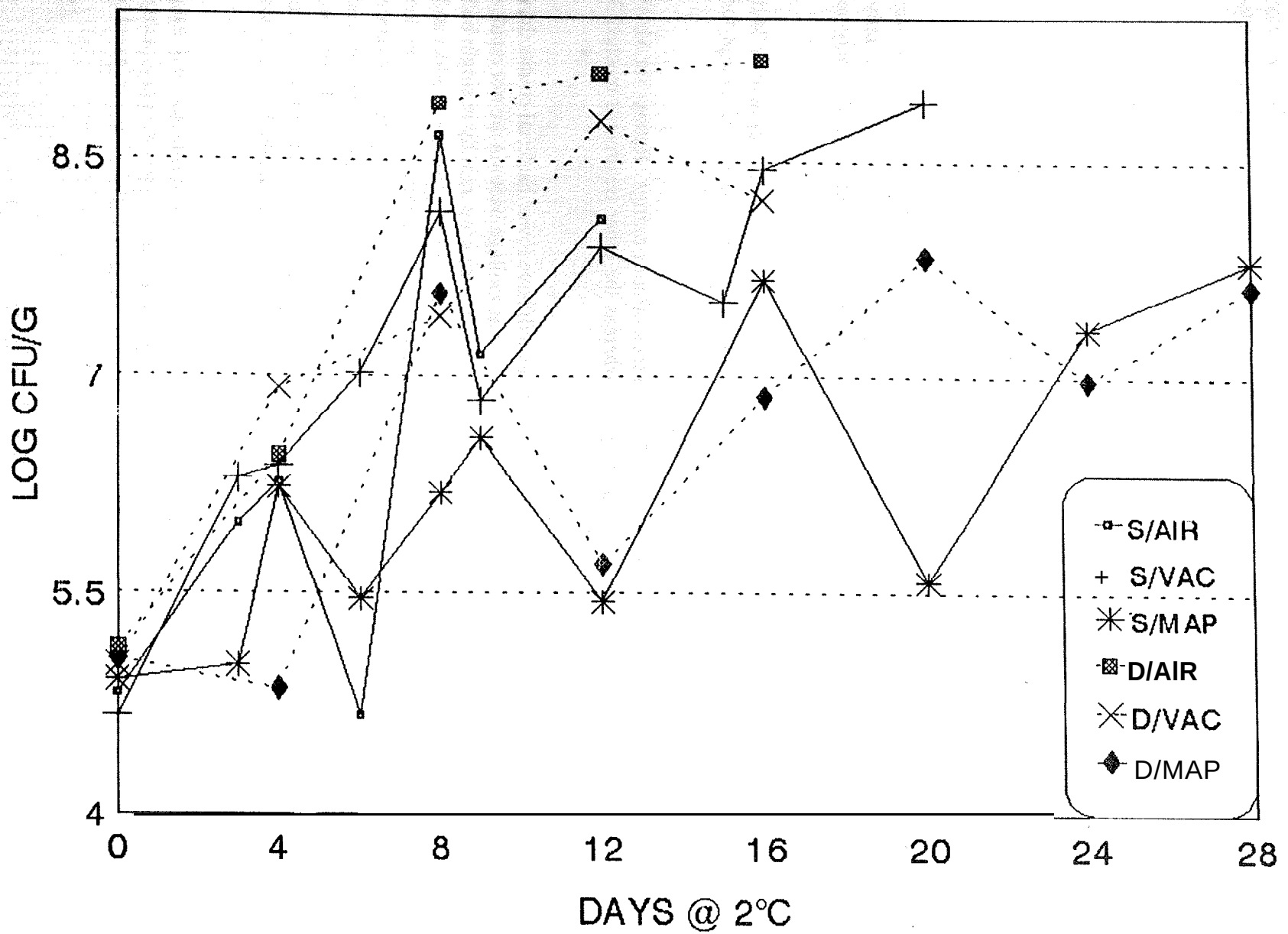

FIGURE 2. PSYCHROTROPHIC PLATE COUNTS IN CHANNEL CATFISH FILLETS AS AFFECTED BY TRAY TYPE (Single or Double bed) AND ENVIRONMENT.

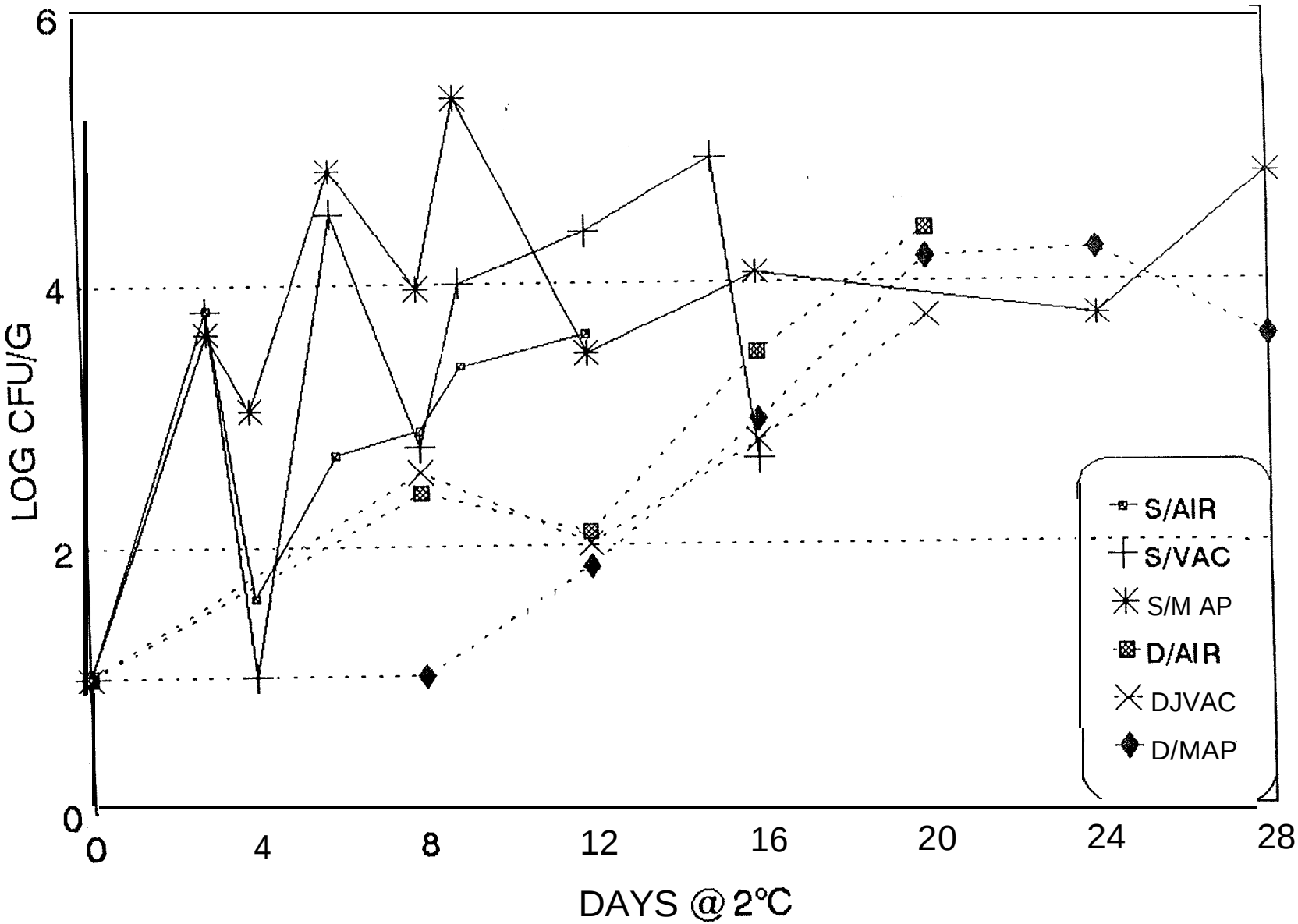

FIGURE 3, LACTOBACILLI PLATE COUNTS IN CHANNEL CATFISH FILLETS AS AFFECTED BY TRAY TYPE (Single or Double bed) AND ENVIRONMENT.

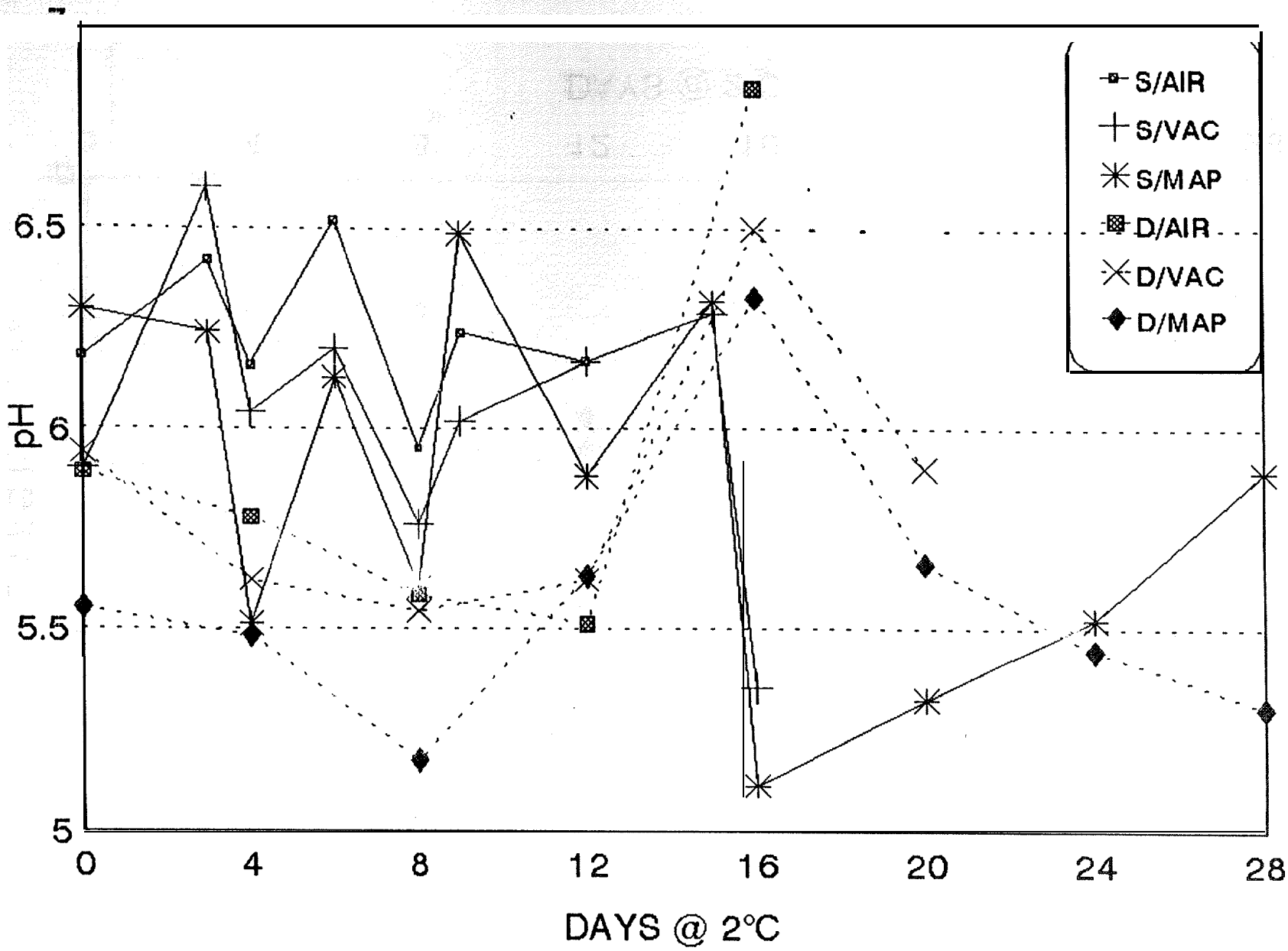

FIGURE 4. CHANGES IN pH OF CHANNEL CATFISH FILLETS AS AFFECTED BY TRAY TYPE (Single or Double bed) AND ENVIRONMENT.

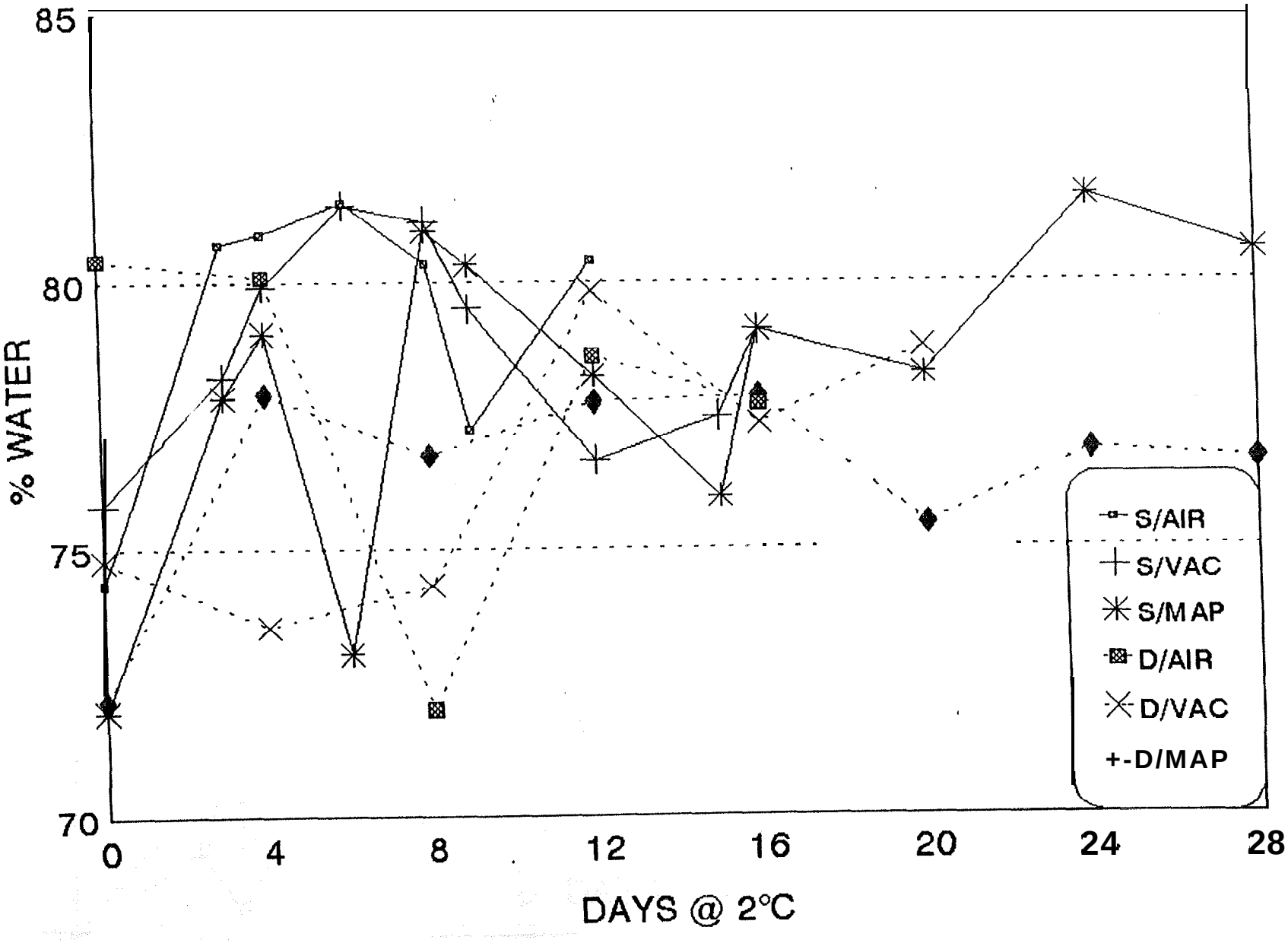

FIGURE 5. CHANGES IN MOISTURE CONTENT OF CHANNEL CATFISH FILLETS AS AFFECTED BY TRAY TYPE (Single or Double bed) AND ENVIRONMENT.

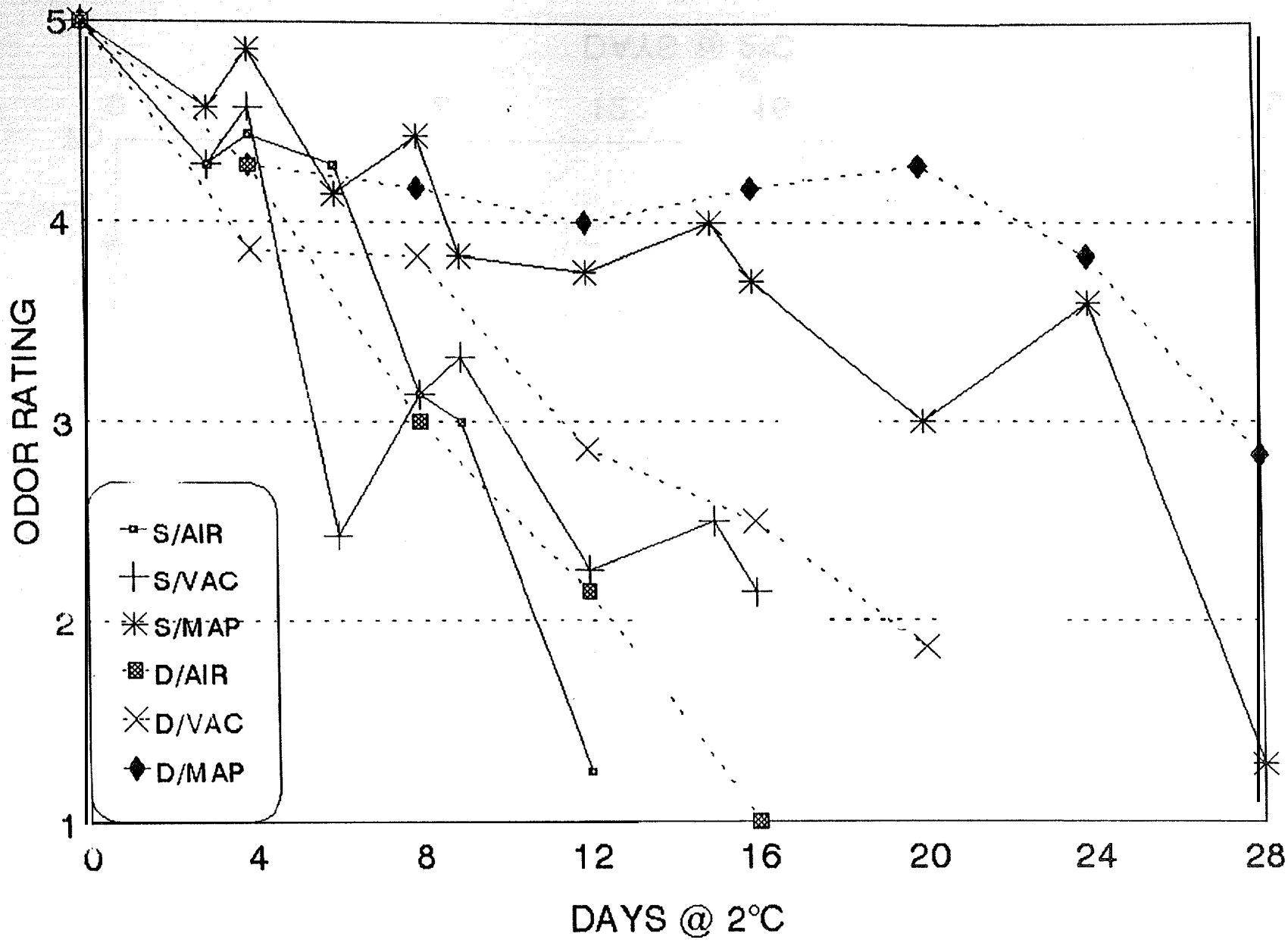

FIGURE 6. ODOR RATING OF CHANNEL CATFISH FILLETS AS AFFECTED BY TRAY TYPE (Single or Double bed) AND ENVIRONMENT.

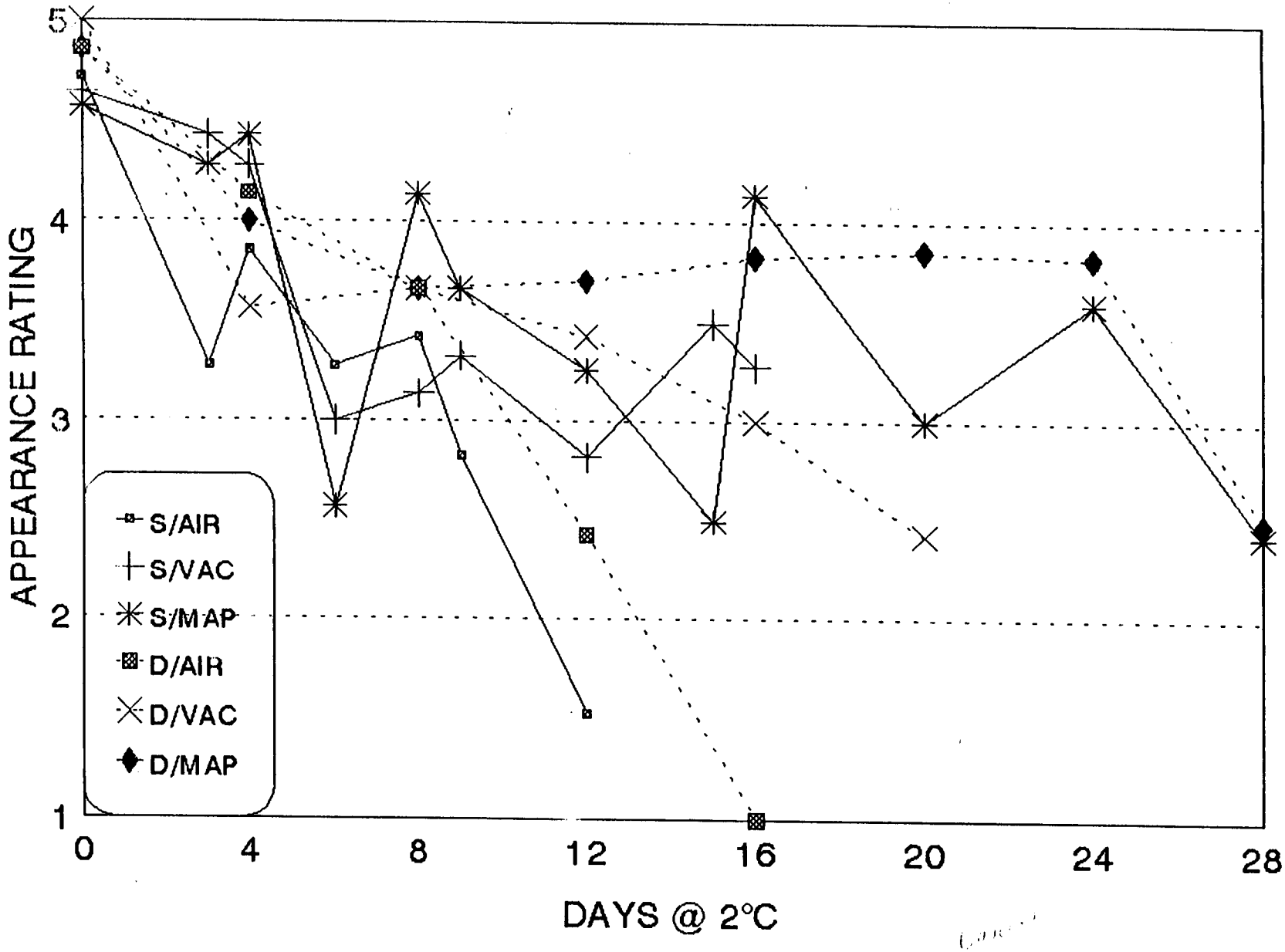

FIGURE 7. APPEARANCE RATING OF CHANNEL CATFISH FILLETS AS AFFECTED BY TRAY TYPE (Single or Double bed) AND ENVIRONMENT.

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(No figures submitted with text)

STABILITY OF FROZEN MINCE FROM CHANNEL CATFISH FRAMES

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INTRODUCTION

The demand for farm-raised channel catfish (*Ictalurus punctatus*) has grown tremendously in recent years due to year round availability, consistent quality, and healthy attributes of catfish. In 1993, over 459 million tons of catfish were produced in the United States (Anon., 1994). As this industry has grown, so has the amount of offal, trimmings, and other solid waste generated and the need to provide effective means of waste removal and disposal.

According to the USDA (Anon., 1992) 47% of catfish sold is in the form of fillets, 32% is whole fish, and the remaining 21% includes steaks, nuggets, and value added products. Only 68% of the fish is sold for food with the remainder being waste, including skeletons (frames), which account for 18% of the fish (Woodruff, 1984).

Currently, most catfish producers do not have the necessary equipment to use fish frames other than converting them into fish meal Income processors receive for catfish offal, about $.05lb, just covers the cost of transporting the waste materials to rendering plants.

These frames could be utilized more effectively. About 50% of frames is meat, which can be recovered by various means (deboning, cooking, and separating). There are several advantages to finding better uses for even a portion of this waste. Less waste and more useable product means less cost for discharging waste into waste treatment systems, reduces water pollution and strain on the environment. **A** profit could also be made on the additional muscle meat recovered (McAlpin et al., 1994).

By passing the catfish frames through a mechanical deboner, approximately 25-50% of the weight of the frame is removable meat (Ammerman, 1985). Once removed, methods to process and preserve this mince become important

Antioxidants can be added to increase shelf-life. Several food-grade antioxidants and metal chelators such as sodium citrate, phosphates and sodium erythorbate or in combination may be used to maintain quality of fish mince. Sodium citrate protected ocean trout against lipid oxidation (Shenouda et al., 1979). Citric acid can inactivate enzymes and sequester trace metals (Anon., 1985). Brifisol™, a phosphate mix, can be a useful antioxidant in finely ground meats (Anon., 1990).

Phosphates have been shown to prevent rancidity in fish (Gordon, 1971), and sodium erythorbate has been found to have protective effects on various fish species (Bilinski et al., 1979, Hwang and Regenstein, 1988, Santos and Regenstein, 1990, Licciardello et al., 1977, 1980, 1982). Erythorbates are comparable to ascorbates in antioxidant effectiveness, but cost less (Anon., 1982).

The present research was conducted (1) to explore the feasibility of producing a mince from channel catfish frames and, (2) to study methods of maintaining the overall quality of the frame mince during frozen storage.

MATERIALS AND METHODS

Sample preparation

Catfish frames were transported from a commercial processor in Mississippi to the Mississippi State University/National Marine Fisheries Experimental Seafood Processing Laboratory in Pascagoula, MS. Frames were packed on ice (1 to 3 days) prior to transportation and kept on ice until used. Once unloaded, frames were rinsed with filtered water using a rotary fish washer (Model GL300 Ryan Engineering Inc., Seattle, WA) that allowed them to drain as they tumbled. Frames were then passed through a deboner with 5mm holes in the cylinder (Model NDX13 Bibun Machine Construction Co. Ltd., Japan).

Mince used in unwashed treatments (U) was covered with plastic and stored at 3 ± 1 °C until used. Washed mince (W) was prepared by placing mince in wash tanks containing 1 part mince to 4 parts water (5°C). This slurry was stirred for 10 min and allowed to settle for 5 min before water was decanted. This procedure was repeated 3 times to remove lipids, some pigments, and water soluble proteins. Mince slurry was pumped to a rotary screen rinser (Model F32LW, Bibun Machine Construction Co. Ltd., Japan) to remove loose water and then transferred to a screw press (Model YS200, Bibun Machine Construction Co., Ltd., Japan) to remove remaining excess water and reduce the moisture content to 78%.

Treatments

The mince was held in a refrigerated room (3°C ± 1 "C) until used. There were 5 treatments for both washed and unwashed mince, consisting of four antioxidant combinations and one control. The following antioxidants and combinations of antioxidants were added to the mince: (1) 0.15% sodium citrate - CI (Haarman and Reimer Corp., Elkhart, IN); (2) 0.15% sodium erythorbate - ER Pfizer New York, NY); (3) 0.15% sodium citrate and 0.15% sodium erythorbate (CE); (4) 0.15% sodium citrate, 0.15% sodium erythorbate, and 0.4% Brifisol™ 414 (BR) - a mixture of sodium acid pyrophosphate, sodium pyrophosphate, and sodium polyphosphate, glassy (BK Ladenburg, Cresskill, NJ); and, (5) mince alone or control (CO). Sodium citrate and sodium erythorbate were calculated (w/w) based on acid equivalents.

Antioxidants were dissolved in a small amount of water added to unwashed mince and were added to the washed mince in powdered form. All treatments including controls were mixed for 3 min to evenly disperse the antioxidants. Replication 1 used a household mixer (ModeK45 Kitchen-aid Division, Hobart Manufacturing Co., Troy OH) and replications 2 and 3 used an industrial size mixer (Model A200, Hobart Manufacturing Co.). Mince was then placed in 20 0.45 kg wax coated cardboard boxes (Packaging Production Corp., New Bedford, MA) and frozen to -40°C in a plate freezer (Dole Freeze-Cell Model 2735-6A, Dole Refrigerating Co., Lewisburg, TN). Frozen mince was stored in a storage freezer overnight (- 20°C), packed in dry ice, and transported to Mississippi State University for chemical analyses. Samples were stored at - 14°C ± 2°C for the duration of the study.

Analyses

Oxidative rancidity was determined by the 2-thiobarbituric acid reactive substances (TBARS) test (Tarladgis et al, 1960). Optical density was multiplied by a factor of 7.8 to express the results as mg malonaldehyde/kg mince according to Sinnhuber and Yu (1958). Enzymatic rancidity (free fatty acids, FFA) was determined by the method described by Woyewoda et al (1986). Color was determined by a Hunterlab Model D25 Color Meter (Hunter Associates Laboratory, Inc., Reston, **VA),** standardized with a white plate standard no. LS-13601. All preceding tests were performed in duplicate after 0, 1, 2, 3, and 4 mo. of frozen storage.

Moisture, fat, protein, ash, copper, iron, phosphorus, and erythorbate contents were determined at time 0 for each experimental observations by the Mississippi State Chemical Laboratory. All analyses, with the exception of erythorbate, used AOAC (1990) methods. A standard curve was set up to evaluate erythorbate results using Vitamin C (ascorbate) procedures (Strohecker and Henning, 1966). In addition to AOAC methods, the phosphate analysis used procedures from Clesceri et al. (1989).

Statistical Analysis

The data were analyzed using analyses of variance for a split-plot in a radomized complete block design. Wash treatment (w and u), whole plot and antioxidant treatment (5), and storage time (0 to 4 mo.) were the subplots. Data was analyzed using PROC GLM of the Statistical Analysis System (SAS, 1985). If significant differences were found, means were separated using Fisher's Protected least significant difference (LSD) (Steel and Torrie, 1980) at the 5% level of significance.

RESULTS AND DISCUSSION

washed mince had lower ($P<0.05$) fat, phosphorus, and ash, but higher ($P<0.05$) moisture and Protein (Table 1). Washing removes lipids, blood, and other prooxidants along with water soluble proteins (Miyauchi and Steinberg, 1970). Proximate composition of the unwashed mince was similar to that reported by Silva and Ammerman (1993) for catfish muscle and by Freeman and Hearnsberger (1993) in the skin side and lateral portion of catfish fillets; washed mince was in composition to the internal portion and visceral side of fillets.

Table 1. Effect of wash treatment on proximate composition and selected nutrients (wet basis) in catfish mince averaged over antioxidant treatment.

Nutrient	Washed	Unwashed
Phosphorous (%)	0.22 b	0.30 a
Copper (mg/kg)	0.65 a	0.58 a
Iron (mg/kg)	5.29 a	5.21 a
Erythorbate (mg/g)_	0.57 a	0.58 a
Moisture (%)	82.20 a	74.25 b
Fat (%)	3.24 b	14.09 a
Ash (%)	0.51 b	0.66 a
Protein (%)	14.20 a	11.22 b

ab - Means within row not followed by same letter differ ($P<0.05$).

There were differences ($P<0.05$) between washed and unwashed minces in phosphorus, iron, erythorbate, and ash due to antioxidant treatment (Table 2). Mince containing Brifisol™ (BR) was higher in phosphorus and ash, due to Brifisol" phosphates. Erythorbate levels were higher ($P<0.05$) in minces containing added erythorbate (ER, CE, BR), as would be expected. Iron levels were higher ($P<0.05$) in minces containing citrate (CI, CE, BR) and was lower ($P<0.05$) in the control (CO) and erythorbate (ER) treated minces.

Color

Washed minces had higher ($P<0.05$) "L" (lighter product) and lower ($P<0.05$) "a" and "b" values than the unwashed mince (Table 3). Miyauchi and Steinberg (1970) noted that washing leads to improved color and flavor stability of mince by removing blood and heme pigments.

Though "b" values differed ($P<0.05$) between wash treatments, both washed and unwashed mince were more Yellow in hue than blue. There was also a difference ($P<0.05$) in "b" values among antioxidant treatments, regardless of wash treatment or storage time (data not shown). Mince containing Brifisol" had higher ($P<0.05$) "b" value than the other treated minces.

There was a gradual ($P<0.05$) decrease but significant shift in "b" values over storage time, regardless of wash or antioxidant treatment (Fig. 1). Nakayama and Yamamoto (1977) noted a shift toward yellowness in unwashed minces made from unskinned short spine thornyhead, turbot and dogfish Oxidation of dark muscle lipids can result in yellow-to-brownish discoloration in fish known as rusting (Licciardello, et al.,1982). Gordon (1971) reported that phosphate treatments in fish retards rancidity and prevents development of yellow appearance.

There were no changes in Hunter "L" values over time. Moledina et al (1977) found "L" values to decrease over time in mechanically deboned flounder meat, and found that preservatives could greatly minimize this. Minced turbot darkens while minced pollock lightens during storage (Nalkayama and Yamamoto, 1977). Fish should have an off white to cream color, a whiter, less red color is desired (Jahncke et al. 1992).

TBARs

Washed mince had lower ($P<0.05$) TBARs than unwashed mince regardless of antioxidant treatment or storage time (Fig. 2). This was probably due to lower lipid and heme pigment contents (Table 3). Fischer and Deng (1977) indicated heme iron as the major catalyst of lipid oxidation in mullet dark muscle. Hiltz et al. (1976) attributed variations in TBARs of silver hake to varying amounts of the lipid-rich red muscle. Silberstein and Lillard (1978) found hemoprotein content to influence prooxidant activity in extracts of minced mullet.

Freeman and Hearnsberger (1993) found the flesh located along the lateral line of catfish fillets to have higher TBARs than flesh from other parts of the fillets. Absence of the lateral line in catfish frames would produce mince with less heme pigments and could partially explain the relatively low TBARs reported here for both washed and unwashed mince.

There was an increase ($P\leq0.05$) in TBARs during storage (Fig. 2). Woodruff (1987) reported slightly higher TBARs values in catfish fillets during frozen storage as did Silva and Ammerman

Table 2. Effect of antioxidant treatment on proximate composition and selected nutrients in catfish mince averaged over wash treatment.

	TREATMENT[1]				
Nutrient	**CO**	**CI**	**ER**	**CE**	**BR**
Phosphorous (%)	0.20a	0.20a	0.22a	0.18a	0.50b
Copper (mg/kg)[NS]	0.47	0.62	0.54	0.49	0.97
Iron (mg/kg)	3.67a	7.05b	3.12a	5.23ab	7.18b
Erythorbate (mg/g)	0.03a	0.03a	0.94b	0.95b	0.94b
Moisture (%)[NS]	78.32	78.53	78.22	78.16	77.89
Fat (%)[NS]	8.68	8.39	8.49	9.03	8.73
Ash (%)	0.48a	0.53a	0.51a	0.49a	0.93b
Protein (%)[NS]	12.70	12.46	12.80	12.96	12.65

ab-Values within row not followed by the same letter differ (P<05).

NS - No significant differences.

[1]CO = Control, CI = sodium citrate, ER = erythorbate, CE = citrate + erythorbate, BR = brifisol™.

Table 3. Effect of wash treatment on TBARs and Hunter Color of catfish mince averaged over antioxidant treatment and storage time.

Variable	Washed	Unwashed
TBARs (mg malonaldehyde/kg fish)	0.20b	0.30a
Hunter L value	66.42a	61.35b
Hunter a value	-0.25a	1.87b
Hunter b value	8.36b	9.48a

ab - Means within row not followed by the same letter differ ($P<0.05$).

(1993) for whole dressed catfish. Ciarlo et al. (1985) found initial TBARs values to be lower in minced hake than hake fillets There were no differences (Pr0.05) in TBARs due to antioxidant treatment (Table 4). TBARs reactive material is generally produced in substantial amounts only from fatty acids containing three or more double bonds (Nawar, 1985) found in relatively low concentrations in channel catfish (Silva et al., 1993; Worthington et al., 1972).

FFA

Among antioxidant treatments, BR offered more protection ($P \leq 0.05$) against the formation of free fatty acids than CO regardless of storage time or wash treatment (Table 4).

There was an interaction ($P \leq 0.05$) between wash treatment and storage time (Fig. 3) for free fatty acids. In both washed and unwashed mince, free fatty acids increased ($P \leq 0.05$) over storage time. After the first month there was also a difference ($P \leq 0.05$) between washed and unwashed mince each month. These increases in free fatty acids are very similar to those found in catfish fillets over the same storage time (Nguessan, 1992). Hiltz et al. (1976) found the rate and extent of free fatty acid increase to be the same for fillets and minced silver hake.

Silva et al. (1993) found oxidation to occur primarily in phospholipids but not neutral lipids of catfish fillets Eun et al. (1993) reported an active system of peroxidases in catfish microsomes, and concluded that phenolic antioxidants, followed by phosphates and finally natural antioxidants were more effective in preventing oxidation of catfish muscle during storage.

Both the unwashed and washed mince made from catfish frames bad acceptable color and low TBARs and FFA values during frozen storage. In future experiments, other quality parameters such as sensory and microbiological evaluations would be necessary to determine overall acceptability.

ACKNOWLEDGMENTS

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Table 4. Effect of antioxidant treatment on TBARs and free fatty acid (FFA) values in catfish mince averaged over wash treatment and storage time.

Antioxidant	TBARs Value (mg malonaldehyde/kg fish)	FFA Value (µmol/log fish)
Control (CO)	0.33[NS]	3.39 a
Citrate (CI)	0.28	3.33 a
Erythorbate (ER)	0.25	3.53 a
Citrate + Erythorbate (CE)	0.19	3.40 a
Brifisol + Citrate + Erythorbate (BR)	0.20	3.05 b

ab - Means within column followed by different letter differ ($P<0.05$).

NS - No significant differences.

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GAS CHROMATOGRAPHIC/OLFACTOMETRIC EVALUATION OF FLAVOR EXTRACTS PRODUCED FROM CRAWFISH PROCESSING BY-PRODUCTS

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Crawfish processing plants produce over 85 million pounds of picking table scraps annually, with recovery of only 15% (by weight) of edible tail meat (Meyers et al., 1990). Potential exists for the utilization of these by-products for the production of marketable flavor extracts. This approach may generate additional revenue for crawfish processors, while at the same time significantly reduce their waste volume.

Enzymatic hydrolysis of protein followed by heat treatment can be applied to produce Maillard reaction flavor. The production of certain meat and savory flavors employs this technology (Dziezak, 1986). Many recoverable volatile flavor compounds and precursors are present in crawfish processing by-products (PBs). Baek (1994) selected a suitable protease for hydrolysis of PBs and optimized the processing conditions.

Gas chromatography/olfactometry (GC/O) involves the use of human subjects as GC detectors. The most commonly used GC/O techniques are dilution analysis methods such as CharmAnalysis™ (Acree et al., 1984) and aroma extract dilution analysis (AEDA) (Schieberle and Grosch, 1987). However, it takes a long time to complete the analysis in dilution analysis. Osme developed by Miranda-Lopez et al. (1992) needs only one run to obtain osmegram, reducing analysis time.

The objective of this research was to evaluate and compare aroma properties of flavor concentrates prepared with and without protease treatment using gas chromatography/olfactometry (GC/O).

MATERIALS AND METHODS

1. Materials

Live crayfish ***(Procambarus clarkii),*** hereafter referred to as crawfish, were purchased from a seafood processor in Baton Rouge, LA, and temporarily stored in a 4°C walk-in cooler, After being washed in tap water, live crawfish were boiled for 7 min at 100°C (Marshall et al., 1987). Tailrneat was removed from the boiled crawfish by hand. Composite crawfish processing by-products (PBs) and claws (CPBs) were collected separately. PBs was composed of claw, viscera, and shell. Both PBs and CPBs were ground with addition of distilled water to make final concentrations of 75 % (PBs) and 60 % (CPBs). These were the highest concentrations that could be effectively mixed. The ground PBs and CPBs were vacuum-packaged in polyethylene bags (Koch Supplies, Inc., Kansas City, MO) and then stored at -20°C.

2. Enzymatic Hydrolysis of PBs and CPBs

After thawing at 4°C overnight, one kg of PBs (or CPBs) was placed into a 1-L jacketed reaction vessel (Cat. No. 991780, Wheaton, Millville, NJ). Stirrer was attached to thoroughly mix PBs(or CPBs) during reaction. Reaction was performed under optimal reaction conditions (pH 8-9, 65 °C, and 2.5 hr reaction time) with 0.3% of alkaline protease APL-440 (Solvay Enzymes, Inc.). The pH was not adjusted because intrinsic pHs of both PBs and CPBs were in the optimum range. A control was run under the same conditions except for absence of APL-440 Each hydrolysis was performed in duplicate.

3. Aqueous Extraction of Flavor Compounds

After hydrolysis, aqueous extraction using boiling water was carried out. This is the most common method for obtaining seafood extracts (Ochi, 1980). Flavor compounds were extracted in a closed system consisting of a water-cooled condenser attached to a S-L round bottom flask. A heating mantle was used to heat the flask As soon as the hydrolysis was completed, PBs (or CPBs) hydrolysate was put into the flask containing boiling water (1 L) to inactivate protease, and an additional 1 L of distilled water was added to the flask Extraction was carried out for 2 hr after returning to boil. For control, APL-440 was added to boiling water 20 mm before PBs (or CPBs) hydrolysate was placed into the flask, and the extraction procedure was similar to that described previously. Filtration was performed using two consecutive steps after cooling the aqueous extract in the walk-in cooler (4°C): 1) cheese cloth (2-layers) and 2) filter paper (Whatman #41).

4. Atmospheric Evaporation and Vacuum Evaporation

Filtrates were concentrated to ≈ 25-30°Brix using two evaporation methods, atmospheric evaporation and vacuum evaporation. For atmospheric evaporation, an externally heated stainless steel container was used to concentrate the filtrate. Vacuum evaporation was performed at 60°C using a Rotavapor (Büchi, Switzerland). **It** took approximately 1 hr for atmospheric evaporation and 6 hr for vacuum evaporation to concentrate to 25-30°Brix, which was measured using a hand refractometer (Cambridge Instruments Inc, Buffalo, NY).

5. Vacuum Simultaneous Steam Distillation/Solvent Extraction (SDE)

The SDE apparatus was modified to perform under vacuum (~30 in. Hg; b.p. 60-65°C) in order to minimize artifact formation during extraction. The standard **SDE** apparatus (Cat. No. K-5230101-0000, Kontes, Vineland, NJ) was modified as described by Cadwallader et al (1994). Each concentrate was placed into a 5-L round bottom flask and brought up to a total volume of 2 L with distilled water. Glass beads and 45.4 µg of 2,4,6-trimethylpyridine (TMP) as the internal standard were added to the flask. Extraction was carried out for 4 hr using 100 mL of redistilled dichloromethane as solvent. Air was evacuated for 30 min prior to heating of the sample flask. SDE extracts were kept at -20°C overnight to facilitate water removal. The volume of SDE extract was reduced to 10 mL under a gentle stream of nitrogen, dried over 3 g of anhydrous sodium sulfate, and then further reduced to 100 µL prior to analysis.

6. Gas Chromatography/Mass Spectrometry (GC/MS)

A Hewlett-Packard (Palo Alto, CA) GC/mass selective detector (HP5790 GC/5970B MSD) was used for analysis of SDE extracts. A 5 µL aliquot of each SDE extract was injected in the splitless mode. Volatile components were separated using a fused silica capillary column (Supelcowax l0, 60 m x 0.25 mm i.d. x 0.25 pm film thickness; Supelco, Inc., Bellefonte, PA). The other conditions are the same as mentioned by Cadwal lader et al. (1994).

7. Gas Chromatography/Olfactometry (GC/O)

Sensory properties of individual volatile compounds in SDE extracts of flavor concentrates were evaluated by sniffing the GC effluent. Prior to sniffing, each extract was diluted to possess the same intensity of the internal standard peak. Each sample was evaluated by an expert panelist who was asked to record the description and intensity (strong, medium, and weak) of each odorant detected. This may be classified as a type of osme analysis (Miranda-Lopez et al., 1992). GC/O conditions are the same as described by Cadwallader et al. (1994).

RESULTS AND DISCUSSION

Aroma profiles of flavor concentrates of PBs and CPBs were evaluated and compared using GC/O. Table 1 lists the odor active compounds and their description in flavor concentrates of PBs and CPBs. Considerable sulfury, nutty and baked potato, crabby and grainy, and raw marine-like notes were detected. A larger number of odor active compounds were present in hydrolysate of PBs than in control (Figure 1). This may be due to the improved flavor extractability or increased precursors by enzymatic hydrolysis. Kim et al. (1994) reported that the flavor extractability was improved by proteolysis in crab processing by-products. Of these odor active compounds, dimethyl trisulfide (peak no. 14, cooked cabbage), unknowns having sulfury and crabby odors, and an unknown (peak no. 54, raw marine-like) had strong odors. Naphthalenes may contribute to raw marine-like odors, which play an important role in crawfish meat (Chen, unpublished data). The unknown peak no. 54 was thought to be a naphthalene derivative based on high RI value and odor description. 2-Acetyl-I-pyrroline (2-AP, peak no. 12, popcorn) and 2-ethyl-3, 5-dimethylpyrazine (peak no. 18, baked potato) also are thought to contribute to overall aroma quality.

After evaporation, a large number of odor active compounds disappeared (Figure 2). The most intense compounds after atmospheric evaporation were 2-AP dimethyl trisulfide and an unknown (peak no. 54). 2,3-Butanedione (peak no. 1, buttery), (Z)-4-heptenal (peak no. 8, fishy and rancid), and an unknown (peak no. 20, nutty and baked potato) were detected after atmospheric evaporation of PBs hydrolysate. Flavor concentrates after vacuum evaporation exhibited lower odor intensity.

Figure 3 indicates that there was no difference in the aroma profiles between hydrolysate and control in CPBs. However, 2-ethyl-3,5-dimethylpyrazine (peak no. 18) was detected only in hydrolysate. 2-AP was perceived as the only strong aroma in hydrolysate. After evaporation, another nutty and baked potato aroma (peak no. 20) was detected (Figure 4). These nutty and baked potato aroma notes may play an important role in flavor concentrates of CPBs.

When compared with CPBs, number and intensity of odor-ants in the flavor concentrate of PBs were higher. This may be due to the small amount of lipid in CPBs. Lipid plays an important role in production of volatiles. Lipid decomposition products can react with Maillard reaction intermediates to form heterocyclic compounds (Ho et al., 1989).

CONCLUSION

Aroma properties of flavor concentrates prepared with and without protease treatment from composite crawfish processing by-products (PBs) and from separated claws (CPBs) were evaluated and compared. More odorants were present in PBs

Table 1. Odor active compounds in flavor concentrates of PBs and CPBs

Peak no.	Compounds name	RI[a]	Odor description
1	2,3-Butanedione	985	Buttery
2	Unknown	1015	Sour, Onion
3	Dimethyl disulfide	1070	Sour, Sulfury
4	Unknown	1092	Sour, Sulfury
5	Unknown	1148	Chocolate
6	Unknown	1156	Chocolate
7	Unknown	1167	Rancid, Pungent
8	(Z)-4-Heptenal	1239	Baked potato, Rancid
9	Unknown	1258	Sulfury, Onion
10	Unknown	1293	Mushroom
11	Unknown	1302	Nutty, Peanut
12	2-Acetyl-1-pyrroline	1337	Popcorn
13	Unknown	1349	Nutty, Peanut
14	Dimethyl trisulfide	1380	Cooked cabbage, Sour
15	(E)-2-Octenal (?)	1421	Raw peanut skin
16	Unknown	1429	Mushroom
17	Unknown	1445	Nutty, Peanut skin
18	2-Ethyl-3,5-dimethylpyrazine	1455	Nutty, Baked potato
19	Unknown	1469	Nutty, Stale
20	Unknown	1483	Nutty, Baked potato
21	Unknown	1520	Sulfury, Sour
22	Unknown	1570	Sweet, Grainy
23	(E,Z)-2,6-Nonadienal	1585	Cucumber
24	Unknown	1601	Nutty, Peanut
25	Unknown	1605	Sour
26	Unknown	1633	Burnt
27	Unknown	1639	Sulfury, Sour
28	2-Acetylthiazole	1646	Popcorn, Chocolate
29	Unknown	1662	Sweet, Grainy
30	Unknown	1668	Grainy, Nutty, Crabby
31	Unknown	1673	Nutty, Meaty
32	Unknown	1691	Nutty, Crabby
33	Unknown	1717	Fishy, Fresh fish
34	Unknown	1731	Crabby, Grainy
35	Unknown	1747	Cucumber
36	Unknown	1751	Fishy, Fresh fish
37	Unknown	1768	Sweet, Grainy
38	A Schiff base (?)	1800	Plastic, Sweet
39	Unknown	1814	Burnt, Sulfury
40	Unknown	1857	Catty
41	Unknown	1881	Mushroom
42	Unknown	1897	Sulfury, Vegetable-like
43	Unknown	1935	Sulfury, Sour
44	Unknown	1994	Sweet, Crabby, Grainy
45	Unknown	2004	Sulfury, Sour
46	Unknown	2014	Skunky
47	Unknown	2025	Sweet
48	Unknown	2057	Sweet, Grainy
49	Unknown	2079	Sewage
50	A naphthalene (?)	2135	Mothball
51	Unknown	2170	Sweet, Melon
52	Unknown	2201	Sweet, Floral
53	Unknown	2214	Cooked mushroom
54	A naphthalene (?)	2220	Naphthalene, Raw marine

[a] Retention Index

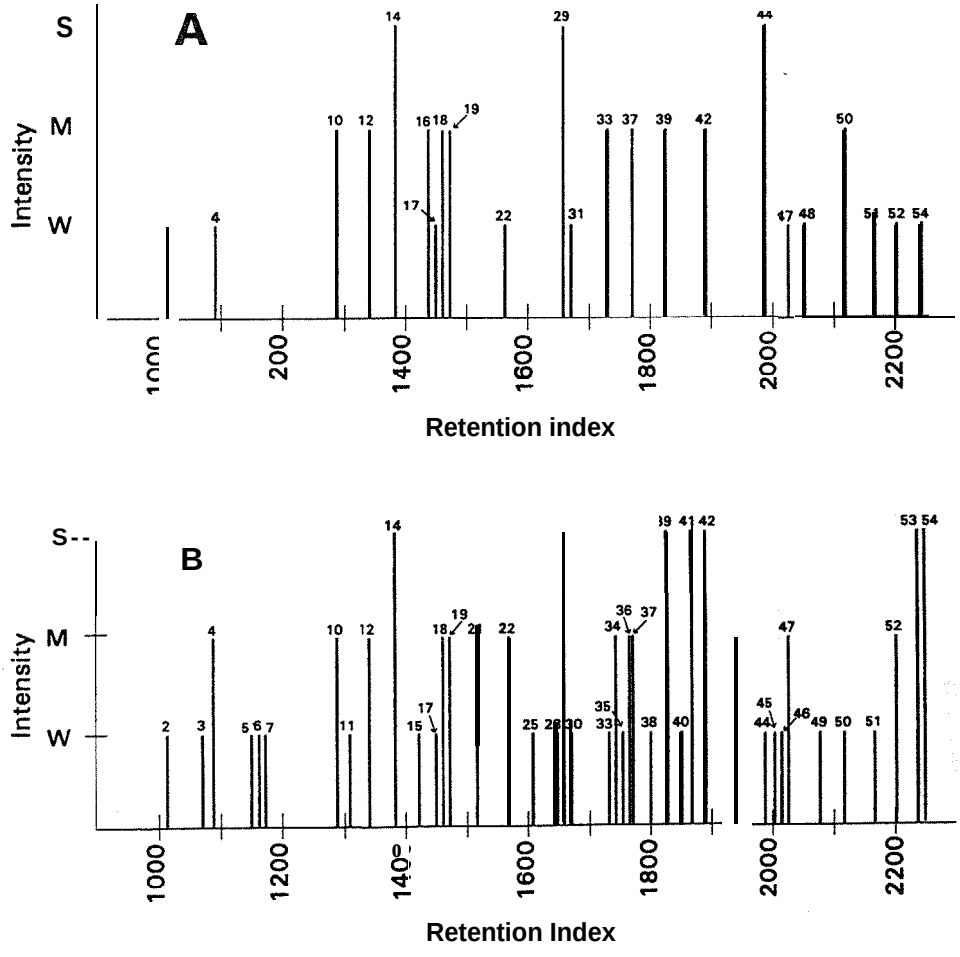

Figure 1. Aromagrams of volatile flavor components in PBs control (A) and PBs hydrolysate (B) before concentration (Peak numbers correspond to those in Table 1, S: Strong, M: Medium, W: Weak).

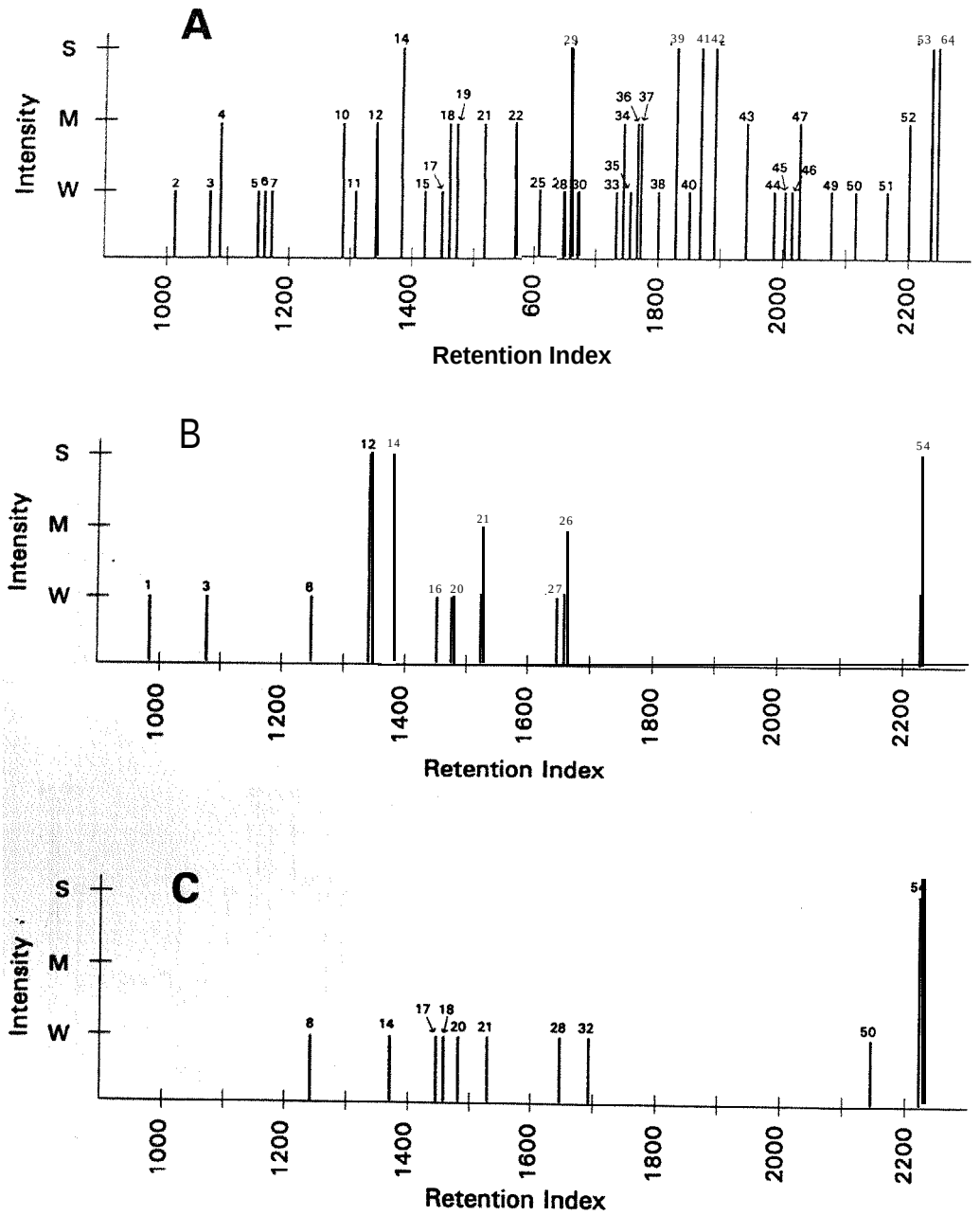

Figure 2. Aromagrams of volatile flavor components in flavor concentrates of PBs hydrolysate. A: Before concentration; B: Prepared by atmospheric evaporation; C: Prepared by vacuum evaporation (Peak numbers correspond to those in Table 1, S: Strong, M: Medium, W: Weak).

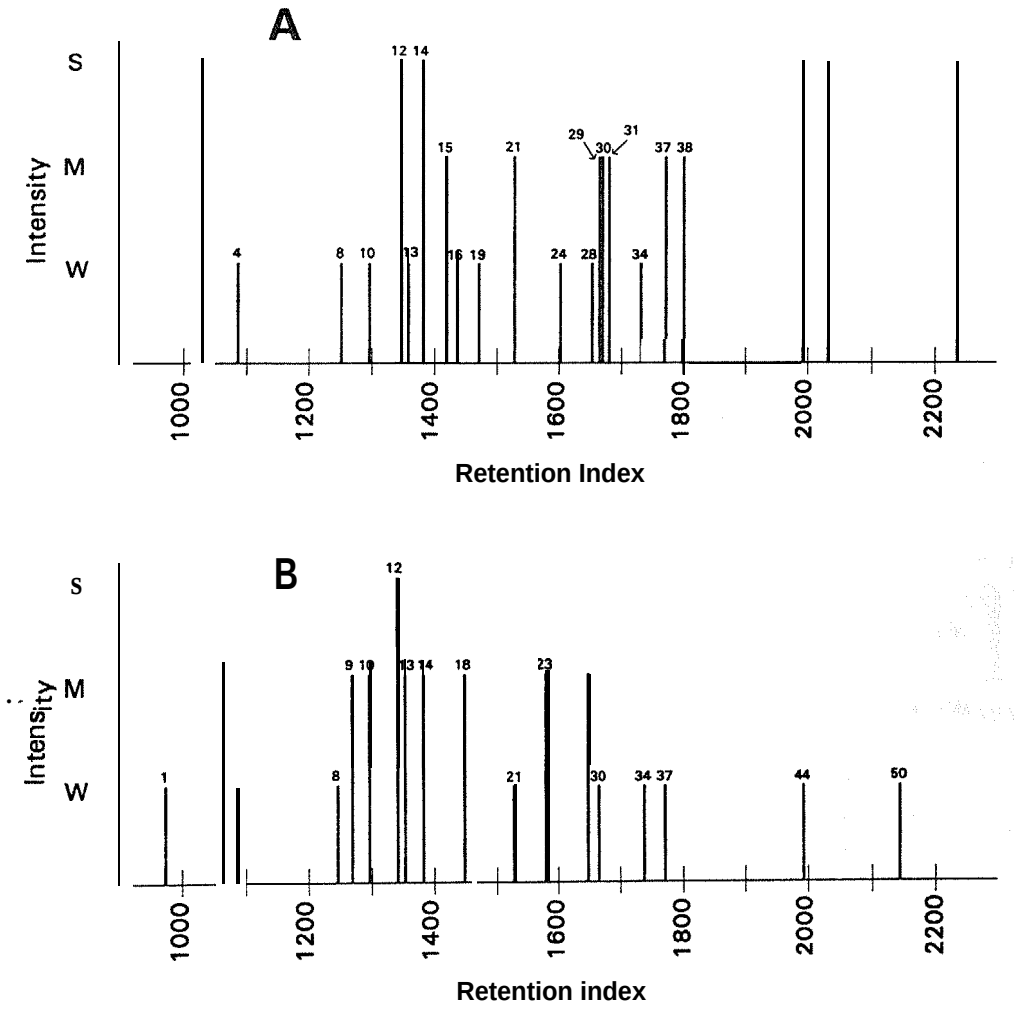

Figure 3. Aromagrams of volatile flavor components in CPBs control (A) and CPBs hydrolysate (B) before concentration (Peak numbers correspond to those in Table 1, S: Strong, M: Medium, W: Weak

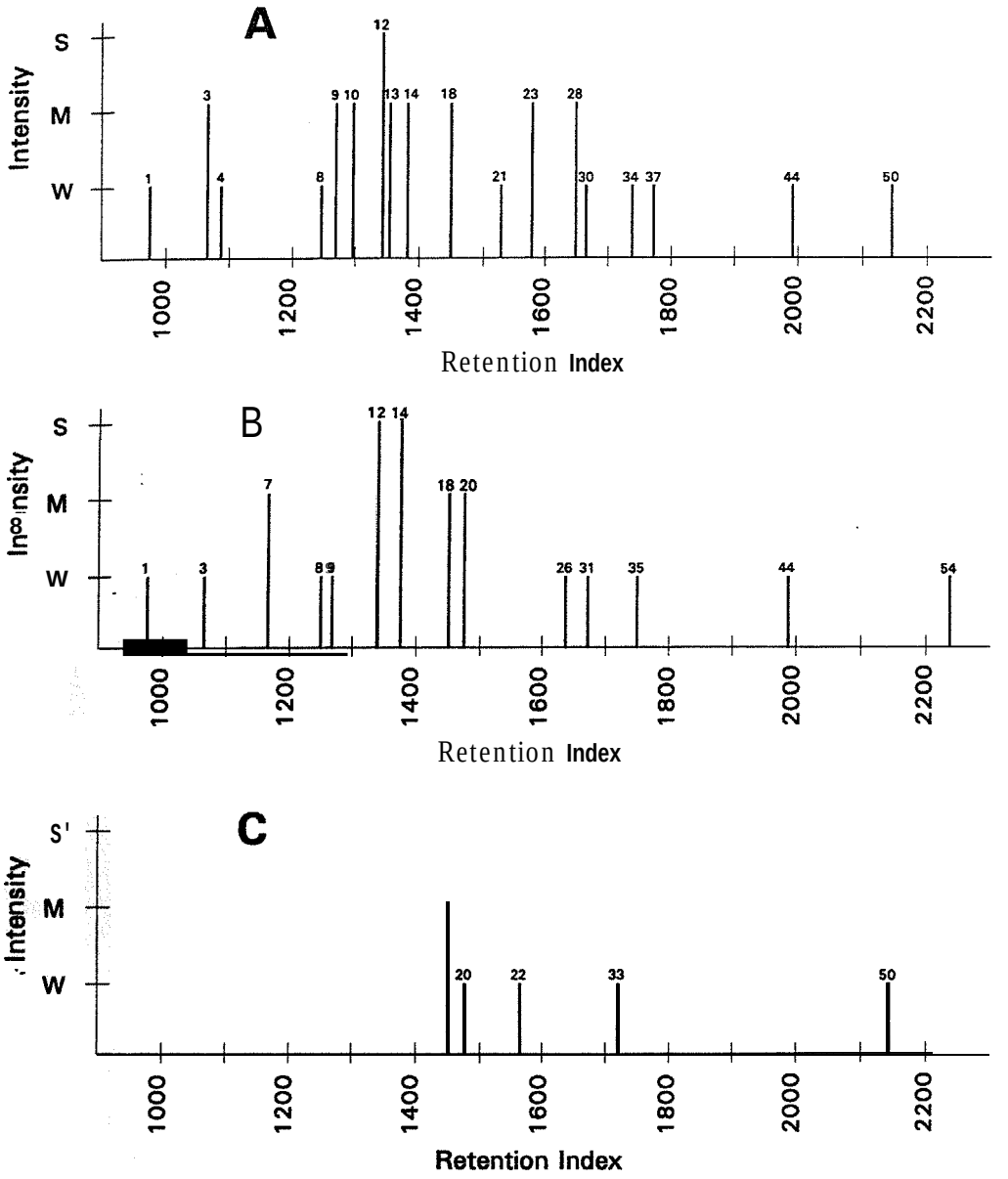

Figure 4. Aromagrams of volatile flavor components in flavor concentrates of CPBs hydrolysate. A: Before concentration; B: Prepared by atmospheric evaporation; C: Prepared by vacuum evaporation (Peak numbers correspond to those in Table 1, S: Strong, M: Medium, W: Weak).

hydrolysates than non-enzyme treated PBs flavor concentrates. Number and intensity of odorants in PBs flavor concentrates were higher than for CPBs. Evaporation (either atmospheric or vacuum) of flavor concentrates decreased the number and intensity of the odorants. Predominant odorants were identified as 2-acetyl-l-pyrroline (popcorn), 2-ethyl-3,5-dimethylpyrazine (baked potato), 2,3-butanedione (buttery), (Z)-4-heptenal (fishy and rancid), and dimethyl trisulfide (cooked cabbage).

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ABSTRACTS

CAN A HAZARD BE BOTH SAFETY AND NON-SAFETY IN A HACCP PLAN?

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In the Food and Drug Administration's proposed mandatory HACCP for seafood processor and importers, parasites are considered as both a safety and non-safety hazard. If a supplier produces products that are sold as sushi grade; he should handle parasites as a safety hazard. The control measure suggested by FDA for parasites in seafood that are intended for raw consumption is freezing.

The Model Food Code requires that fish to be served raw be frozen to at least -20°C for seven days or blast frozen to -35°C for 35 hours. This recommendation is derived from data gathered from anisakine nematodes in a variety of hosts. The literature contains data that indicates that this treatment is adequate for other parasites and data that indicates the process may not be adequate for all platyhelminthes.

FDA'S GULF COAST SEAFOOD LABORATORY: HISTORY, MISSION AND CURRENT RESEARCH

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This Laboratory was established in 1963 as the Gulf Coast Shellfish Sanitation Research Center under the U.S. Public Health Service and focused on shellfish research. In 1973, it's name was changed to Gulf Coast Technical Services Unit with a new mission of providing technical assistance to support the National Shellfish Sanitation Program. In the early 1980's the role of the Laboratory in technical assistance was reduced and it's responsibility for finfish and shellfish safety research broadened. The Laboratory was named the Fishery Research Branch until it was placed under the newly organized FDA Office of Seafood in 1992. The Gulf Coast Seafood Laboratory's present mission is to conduct research that will support the implementation of the FDA HACCP program for seafoods. Focus is on identification, source and significance of hazards and on developing methods to detect hazards.

AN OVERVIEW OF THE NMFS PRODUCT QUALITY AND SAFETY PROGRAM

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NMFS has four major laboratories concerned with seafood product quality and safety. These facilities are located in Gloucester, MA, Charleston, SC, Pascagoula, MS and Seattle, WA. With the exception of the NSIL facility in Pascagoula, MS which is solely dedicated to the National NMFS Inspection Program, the other facilities are fully integrated in the NMFS Fishery System of Habitat Protection, Environmental Contaminant Monitoring, Biotoxins, New Methods Development;Population Dynamics, and Aquaculture. Such Product Quality and Safety Laboratories bring an integrated fishery system approach to consumer protection and serve as available resource base to NSIL and the NMFS Inspection Program. The programs of each facility are integrated into supporting seafood inspection needs. To insure program integration, a National Seafood Product Quality and Safety "Tiger Team" coordinating is composed of each Product Quality and Safety Laboratory Director and the Directors of the Environmental Conservation Division and Inspection Services Division. The purpose of the "Tiger Team" is to annually plan, update, and implement a coordinated program of scientific research and services to meet both short and long term needs to the NMFS Inspection Program. All NSIL activities are integrated into this Product Quality Safety fishery support system. An overview of this program will be presented.

NATIONAL MARINE FISHERIES SERVICE AQUACULTURE RESEARCH PLANS

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The National Marine Fisheries Service (NMFS) is preparing plans for new research on marine aquaculture at both the national and southern regional levels. Increases in research are dependent upon policy decisions and allocation of resources by the Agency. Within the region, impediment to aquaculture development have been identified by three mechanisms: (1) review on recently published analyses of the industry; (2) informal discussions with researchers and entrepreneurs in all southeastern states and the U.S. Caribbean; and (3) a workshop that included representatives from throughout the region covering a broad range of technical disciplines and experiences.

Workshop participants described a progressive industry and identified major obstacles to full realization of industry goals within ten years. Recommendations for NMFS involvement have been prepared that consider priority needs for the industry, the strengths and mission of the Agency, and close coordination with other agencies and industry.

RECENT CHANGES IN OYSTER CONSUMPTION AMONG FLORIDA RESIDENTS

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A telephone survey was conducted during May and June of 1994 to determine current consumption levels of oysters and to ascertain changes in consumption behavior. The survey included 1,012 adults in seven metropolitan areas in north and central Florida. The study documents a continuing erosion of public confidence in the safety of oysters, largely fueled by adverse media publicity and consumption advisory notices.

EVALUATION OF ON-BOARD HANDLING TECHNIQUES ON THE QUALITY OF VARIOUS COASTAL HERRING SPECIES

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The Gulf of Mexico contains one of the two major latent fishery resources in the U.S. This resource includes many species of fish which are small in size and have not been readily accessible by traditional fish locating and harvesting methods. As a result, very little information is available concerning proper methods to handle, hold, and process these fish. Information is also lacking on the effect of season and capture site on yield, sensory characteristics, proximate and fatty acid compositions. Data are presented on the effects of on-board storage on the quality of rough scad stored in ice, refrigerated seawater (RSW), or a 3% salt-ice mixture. The results of proximate, chemical and fatty acid analyses are also shown for rough scad, round scad, chub mackerel, silver rag, round herring, and harvest fish that were collected during two different seasons over a three-year period. This information supports development of expanded fisheries and value-added products utilizing the coastal herrings and associated species complex.

STORAGE CHARACTERISTICS OF FROZEN BREADED POPCORN SHRIMP PACKAGED IN MODIFIED ATMOSPHERIC THERMOFORMED CONTAINERS AND HELD ON AN UPRIGHT DISPLAY CASE - A PRELIMINARY REPORT

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Previous work by the authors has shown that temperature cycling in retail display freezers causes rapid deterioration in the quality of frozen breaded shrimp. The authors are investigating the effects of modified atmosphere packaging on the quality and shelf-life of frozen breaded popcorn shrimp held in an upright retail display freezer, Chemical, microbiological, physical and sensory changes in the shrimp were monitored during frozen storage between 3and -18°C. The freezer has two defrost cycles per day and the doors are automatically opened every 15 minutes to simulate retail traffic. Popcorn shrimp were held in the following containers: (1) the current retail pack, a waxed carton containing a polyethylene bag; (2) a medium barrier clear thermoformed container with an air atmosphere; (3) high barrier clear thermoformed container with a CO_2 atmosphere; and (4) a medium barrier clean thermoformed container with a CO_2 atmosphere; and (5) a high barrier clear thermoformed container with a CO_2 atmosphere. Results from the first three months of retail storage will be presented. The following analyses were completed: (1) oxygen and CO_2 levels in the packages; (2) Hunter L, a, b, color values; (3) aerobic plate counts; (4) TBA levels; (5) free fatty acid levels and (6) odor, taste, and appearance analyses by a trained sensory panel.

SEAFOOD HACCP ALLIANCE FOR EDUCATION AND TRAINING

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The National Sea Grant Program has recently funded a two-year proposal to support plans for a "Seafood HACCP Alliance" for training and education. This Alliance was initiated by the Association of Food and Drug Officials (AFDO) and their regional affiliate of Southern States (AFDOSS) in conjunction with a cadre of Sea Grant Specialist which originally assisted the National Fisheries Institute (NFI) with their initial HACCP training programs. The first formal alliance meeting, June 22-23, 1994 in Portland, ME, established a project "Steering Committee" largely based on the contributing authors for the original proposal. This Committee includes members representing the three principal federal agencies, Food & Drug Administration, U.S. Department of Agriculture, and the National Marine Fisheries Services, the various state agency organizations through AFDO regional affiliates and the Interstate Shellfish Sanitation Conference, and the industry trade associations, NFI and National Food Processors Association.

The proposed approach recognizes the essential role of state regulatory authorities, the educational network of Sea Grant and Cooperative Extension Services, and the need for regional attention per seafood diversity. The Alliance does not plan to set or recommend policy. Their primary role will rest with education and communication for a more uniform and technical assistance program for the seafood industry and federal, state and local food inspectors. They plan to instruct this duel audience in a more coordinated and mutual manner. Their plan is not intended to be exclusive. They recognize, encourage and plan to assist any related educational efforts be they private, institutional and/or of government base. Specific tasks proposed by the Alliance include preparing a Core Curriculum, preparing instructors, maintaining a 'Compendium' of methods and conducting some pilot-tests in commercial settings.

PILOT-TESTING HACCP FOR OYSTER PROCESSING

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In response to the FDA's pending mandatory Hazard Analysis and Critical Control Point inspection program, HACCP pilot-tests are under investigation for the processing of oysters, (Crassostera .virginica) in Apalachicola, FL and Houma, LA. The initial USDA extension service/aquaculture project combined State regulatory and industry experience by which to judge the most appropriate HACCP plan relative to product safety, and practical monitoring and recordkeeping procedure.

The pilot HACCP plans include two critical control points, receiving and storage; plus a routine sanitation program with periodic and continuous monitoring procedures. After initial in-plant trial to refine the recordkeeping requirements, the commercial operation has continued conducting actual processing under the recommended HACCP program. During this period the firm was inspected by the pertinent State authorities utilizing both the HACCP and the traditional inspection modes. The combined results reflect on the commercial and regulatory perspectives on HACCP for oyster processing.

OPTIMUM CONDITIONS FOR THE PREPARATION OF DEHYDRATED QUICK SALTED FISH CAKE (DQSFC)

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A modified quick salting technique, consisting of filleting, grinding, quick salting, mixing, presenting at 150 Kg/cm^2 for 1.35 minutes and sun drying was carried out to preserve Bolti and Karmout fish in a form of a product which would have longer shelf-life at ambient temperature. To evaluate the benefits of this process, physical properties and sensory quality of the finished product were assessed. Chilled fish samples (3 days at +4°C) frozen samples (one month at -18°C) were used.

Minimum salt-to-fish ratios for optimum fish cake forming were 90%, 85%, 75% and 70% for chilled bolti, frozen bolti, chilled karmout and frozen karmout, respectively. Pressing was found to be the most important step which determined the optimum fish cake forming. Salting process increased the yield of the ground fish by approximately 2 folds in a samples. The initial values of the fresh fillets were attained by desalting in water for about 24 hrs. Drying period required for optimum moisture content were 17, 16, 13, and 8 hrs. for chilled bolti, frozen bolti, chilled karrnout and frozen karrnout respectively. Sensory evaluation showed that the finished product was completely coherent, compact and stable at room temperature. Desalted fish cake was quite acceptable with various cooking methods.

USE OF CHLORINE DIOXIDE, PEROXIDES, ALKALINE AGENTS AND ORGANIC ACIDS ON A CHANNEL CATFISH PRODUCT

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Psychotropic plate counts (PPC) of channel catfish (CC) steaks treated with 25 to 50 ppm chlorine (CL) or chlorine dioxide (CLO) rose from 4.45 log CFU/g to 6.75 log CFU/g after 10 d at 3 °C. CC nuggets dipped in 50-100 ppm CL or l-2% NaOH, sodium percarbonate (SPC), peroxyacetic acid (PAA), and NaOH/Acetic acid (NAA) for 20 min. at 2°C. NaOH at 2% showed the highest inhibition while PAA and NAA were equally effective at 1 or 2%, and SPC was as effective as CL. SPC and CL reduced PPC by one log CFU/g whereas the others reduced it by 2-2.5 logCFU/g. Most treatments increased Hunter L values of the products by four to eight units, but these tended to equilibrate over storage. Hunter a values did not change but Hunter b values decreased by one or two units on NaOH and PAA treated samples. Sensory Panelist rated all samples equally in appearance, and no different than the control. It was found that the sanitizers tested were effective in reducing PPC in CC products and did not have significant effect on the product' s appearance.

PACU - AN AQUACULTURE ALTERNATE

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The Amazonian Pacu is a fast growing, heartyfish that may be a good candidate for aquaculture. Pacu eat a variety of foods, display rapid growth rates and can thrive in conditions unsuitable for other fish. Pacu obtain weights of about 20 Kg in the wild and seem to be disease resistant. There are three species of genus Colossoma native to South America. The primary candidate species for aquaculture is C. Brachvnomum. This-species is dark grey to silver dorsally with hues of an orange to pink breast. They were originally transported to southern Florida by ornamental fish culturist, but interest in the fish as a food source is growing.

Several entrepreneurs are experimenting with growing Pacu and are finding the fish easy to grow, cheap to feed, and delicious to eat. Researchers have found the fish to be very meaty, much easier to fillet than tilapia and one does not have to scale the skin. It is compared favorably to opal (moonfish): firm textured, white meat and lean.

Some concerns have evolved with farming pacu They are not cold-hearty and require water temperatures above 24°C for best growth. Females in the genus Collosmid will not propagate naturally in Florida and must be given a hormone that causes them to release eggs. The edible portion of the pacu fillets contain small forked, intramuscular bones that must be removed or softened. Despite these concerns, it **is** believed by many that his large, fast-growing and excellent eating freshwater fish can be successfully **marketed** in the U.S.

BUILDING STRATEGIC PARTNERSHIPS FOR AQUATIC FOOD INITIATIVES

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The challenges facing the U.S. aquatic foods industry from production to processing to consumer points of purchase are complex and diverse. Consumers are vigilant of the safety and quality of all food products and the applications of biotechnological sciences. With changes in global trade policies and the creation of multilateral trade agreements, export opportunities and constraints are continuing to develop for U.S. aquatic products. Imports continue to dominate our seafood supply and aquaculture products are becoming more commonplace.

Domestic market pressures and associated world prices from imported seafood products presently impacts several aquaculture commodities. The surveillance and regulation by Federal agencies over aquatic foods production and processing are also increasing in response to the public's growing concerns for good health and nutrition. Because of the lack of Federally approved therapeutants, numerous aquacultural sectors are constrained by the access to critically needed drugs and chemicals.

The advent of HACCP voluntary inspection and certification programs has created inequities between large companies and those operated by small businesses. With approval of a mandatory Federal Seafood inspection regulation, training needs will develop for firms of all sizes and complexities found throughout the nation. The success of addressing these and other challenges will influence the ability of U.S. firms and farms to remain competitive and profitable in a global marketplace. The critical element for long-term sustainability for our nation's aquatic foods sector is building collaborative partnerships between federal, state, academic, consumer and industry stakeholders who can effectively and efficiently direct and coordinate limited fmancial, human and facility resources. This paper provides examples of successful partnerships and coalitions and evidence for the continuation and strengthening of such approaches to national aquatic foods related issues.

MATERIALS AND METHODS

The information presented in this paper is not based on scientific studies or experiments but rather on personal knowledge of various Federal programs and initiatives relating to aquatic foods safety and quality issues.

RESULTS AND DISCUSSIONS

The U. S . aquatic foods sector is extremely diverse by regions, stakeholders, products, issues, species and organizational strength of various constituencies. The economic value of commodities varies considerably with no single product dominating. There is also a strong mix of both domestic and imported products available for consumers from commercial fishery and aquaculture sources, with a growing contribution from the later. Funding is limiting for development of improved production and harvesting related activities and research related to food safety and quality concerns. The U.S. Congress is becoming dominated by an urban constituency which is less familiar with commercial food production and harvesting and is leaning toward consumer driven issues relating to food safety and quality. Federal agency programs have responded to this priority with various funding authorities for research and education projects. International trade is being guided by new policies and regulations affecting the importation of products. Improved transportation systems and capabilities for perishable goods now enable fresh salmon harvested from net pens in Chile, South America to reach restaurants in Louisville, Kentucky in less than 30 hours.

Because of these matters, the most effective strategy for addressing many aquatic foods related issues involves coalition building associated with public and private sector participants. Strategic partnerships enable leveraging of financial resources, pooling of human capacities and talents, sharing infrastructures and organizational networks, and pursuing development of legislation, policies and regulations that address concerns of a broad-based coalition.

There has been a dramatic change in the Federal surveillance and regulatory oversight of the U.S. aquaculture industry and seafood processing sector. In 1989 the Food and Drug Administration's (FDA) Center for Veterinary Medicine (CVM) conducted on-farm surveys of different types of aquaculture operations and followed this with an extensive review of existing aquaculture literature pertaining to drug and chemical use in 1990. Also in 1990, FDA's Center for Food Safety and Applied Nutrition (CFSAN) initiated the pesticide monitoring program to include aquatic food products of domestic and foreign origin. The FDA Office of Seafood was established in 1992 and that same year the National Marine Fisheries Service offered a voluntary HACCP-based seafood inspection certification program. Finally, in 1994 FDA CFSAN proposed a mandatory seafood HACCP inspection regulation which was approved in 1995 for implementation in 1997.

These developments lead to the creation of two examples of public-private sector coalitions that have been instrumental in providing critically needed national forums. The coalitions are the Working Group on Quality Assurance in Aquaculture Production under the auspices of the Federal Joint Subcommittee on Aquaculture (JSA) formed in 1990 and the Seafood HACCP Alliance for Education and Training established formally in 1993.

The JSA reports to the National Science and Technology Council's Committee on Health, Safety and Food. The Council is associated with the Office of Science and Technology Policy of the Executive Office of the President. The JSA serves as a Federal interagency coordinating body and has several national working groups and task forces one of which is the Working Group on Quality Assurance in Aquaculture Production. The Working Group solicits participation from any individual, agency or company with an interest in pursuing additional new animal drug approvals

for aquaculture supporting development and implementation of producer-based quality assurance programs, developing educational materials and programs for the U.S. aquaculture community and fostering public and private sector collaboration. There are presently 100 persons involved with this effort including private sector (42), academia (13), government (39) and international (6). The Working Group is co-chaired by a representative from FDA and U.S. Department of Agriculture (USDA).

The mission of the Working Croup is "to increase understanding of and compliance with Federal requirements regarding drug and chemical use inaquaculture production through education and coordination of related efforts of government, industry and academia."

The coalition has developed two publications (1,2) for national distribution and has been instrumental in promoting the development of producer-based quality assurance programs (3,4,5,6,7) which have been funded by public and private sectors. The implementation and refinement of these quality assurance programs is an ongoing effort. With the deadline for compliance with FDA's mandatory HACCP seafood inspection regulation approaching in December 1997, there will be more interaction between aquaculture producers and processors as the source of the product is the first critical control point (CCP). Some processors are already referring producers to the industry quality assurance programs as a means of addressing recordkeeping and other reporting requirements.

Another project supported has been a software program and manual on aquatic pesticides approved for use in aquaculture and aquatic sites (8). This aquatic pesticides database allows the monitoring and tracking of pesticides that are registered by the U.S. Environmental Protection Agency (EPA) for legal and proper use in aquatic environments. Another accomplishment has been the creation of a National Aquaculture New Animal Drug Application(NADA) Coordinator's position This person facilitates the process and coordination of new animal drug approvals and liaisons with FDA's Center for Veterinary Medicine on behalf of producers, drug and chemical companies and others interested in developing data for new animal drug approvals. This function has been extremely valuable in making progress with drug-related matters which is evidenced by contributions of more than $100,000 annually from public and private sources to support this position.

The collaborative, innovative approaches developed by this Working Croup are serving as models for other minor use animal species which need approvals of new animal drugs. Priority drug needs of industry have been identified and a new category of Low Regulatory Priority (LRP) Drugs was created by FDA CVM to address the legal use of innocuous, commonly used materials.

Future plans include assisting drug companies with industry assessments, broadening educational outreach, expanding the coalition to include processors, strengthening international linkages and expanding use of computer-based applications.

The issue of mandatory versus voluntary compliance to regulations or policies is complex and far reaching. The HACCP concept is site and firm specific and involves some level of knowledge to develop and implement an acceptable "plan" and recordkeeping system. Yet to be detected is the public trust and confidence in such an approach to assuring seafood safety.

Implementation will require a learning period and development of education and training programs that will in fact change behaviors in many cases. The need for public education about HACCP is also critical. The Seafood HACCP Alliance for Education and Training was formed in 1993 as a public-private sector coalition to establish a national forum to coordinate an effort to train the nation's seafood processing sector. The National Sea Grant College Program has provided critical funding to realize the objectives of the Alliance. Objectives include development of a core curriculum training manual and compendium of processing methods, organization of a train-the-trainers program, creation of public education materials and identification of research priorities related to seafood HACCP. The Association of Food and Drug Officials and their regional affiliates have been instrumental in this coalition for organizing and certifying training under the Alliance initiative. Alliance members have been successful in acquiring funds from a variety of Federal agencies to further Alliance objectives. Association with the Alliance is an attribute for funding consideration by competitive grants programs. Several Total Quality Assurance (TQA) and HACCP implementation manuals have been developed for processors of various aquaculture products (9,10,11,12,13,14,15). The partnerships associated with this Alliance are also critical in assuring that needed training is within economic reach of all seafood processing firms regardless of size and economic stature.

CONCLUSIONS AND RECOMMENDATIONS

The two examples cited in this paper, the JSA Working Group on Quality Assurance in Aquaculture Production and Seafood HACCP Alliance for Education and Training demonstrate the effectiveness of building strategic partnerships to address issues of national importance and complexity. The public-private sector coalitions leverage the resources both human and financial that are required for broad-based consensus building and meaningful strategic planning.

With a trend of declining resources, there is a growing need for collaboration and working across state and institutional boundaries for the best interest of a region or the nation. Many issues are complex and far reaching and cannot be adequately addressed by a single government agency or academic institution. The pooling of resources overcomes any single institution or agency weakness and activates a much broader network of stakeholders who may become important participants, may be impacted by coalition actions or may in turn directly extend benefits to others.

A coalition affiliation including a process for prioritization and consensus building provides an advantage in acquiring funds from competitive grants programs. The success of the coalition also depends on the good faith participation of agencies, institutions and individuals who are recognized leaders in their fields and professions which lends credibility and accountability.

Another element of importance is the vision to the future and being able to proactively address issues as they emerge or are likely to emerge. This requires strong leadership and a willingness to react before a critical need develops. The issues of seafood safety and quality are likely to persist for some time and the introduction of HACCP to our diverse food sectors is also expected to elicit considerable public scrutiny. The efforts of the two coalition models should continue as the journey is long for each as new issues and needs emerge. These approaches to

problem solving of complex regional and national issues should be supported by those who can contribute to identifying priorities and developing effective strategic, collaborative action plans. The accomplishments of each coalition initiative demonstrate clearly the advantages of viable public-private sector partnerships.

There is a growing body of information and knowledge being developed to aid processors of various aquaculture products. These materials linked to educational programs at the state and local levels should prove helpful in facilitating HACCP compliance requirements of processors. The next challenge is further integrating and coordinating food safety and quality issues at the aquaculture producer-processor interface. The linkage of producer-based quality assurance programs to processor HACCP plans is a logical action and should be encouraged by all stakeholders. This will require an interdisciplinary approach and thoughtful development of educational programs of mutual benefit to both producers and processors and ultimately the consumer public.

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DESIGN OF AN AUTOMATED QUALITY EVALUATION DEVICE FOR SHRIMP

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INTRODUCTION

The current evaluation of shrimp involves inspectors who judge a sample based on its visual, texture and smell attributes. Also, parameters such as count and uniformity ratio are manually determined This is subjective, hard to repeat and to relate to others. It is desirable to automate this process and to make it objective and repeatable. Use of machine vision, texture, or smell to evaluate quality have been studied individually in the literature.

Fish and prawns have been automatically graded by size and packaged in one layer with uniform orientation by combining computer vision and robotics (Kassler et al., 1993). Morphological and spectral features of shrimp have been determined to find the optimum location for shrimp head removal (Ling and Seamy, 1989). Machine vision was used to calculate the weight, the uniformity ratio and the count of shrimp (Balaban et al., 1994). Fish species have also been sorted according to shape, length and orientation in a processing line (Strachan, 1993; Strachan et al., 1990). However, melanosis has not been measured by machine vision, and correlation of objective and sensory results in visual analysis is lacking.

Ammonia (NH_3) is a major component of decomposed odor of seafood and has been used as an objective quality index (Cobb et al., 1973a, 1973b; Ruiter and Weseman, 1976, Finne, 1982). Its concentration in shrimp was shown to increase during storage with a good correlation between concentration and traditional spoilage indicators (Cheuk and Finne, 1984; Finne, 1982). Ward et al. (1979) used an NH_3-electrode to show the relationship between microbial numbers and NH_3 concentration during refrigerated storage of fresh shrimp. NH_3 analysis is seldom used by the shrimp industry due to the complexity and length of the methods. Therefore, a simple and rapid method of objective NH_3 analysis is necessary.

In high quality seafood, the tissue resumes its original shape when pressure is removed. A soft texture or a slimy feel are indicators of enzymatic and/or microbial deterioration (Gorga and Ronsivalli, 1988). Few researchers have analyzed the relationship between textural properties and quality of raw seafood. Studies of simple sensory analysis were not correlated with instrumental

measurements. Two methods that could be used to measure texture of shrimp are elasticity and texture profile analysis (TPA).

The objectives of this study were : 1) to design and build an automated quality evaluation device for shrimp including hardware and computer software, 2) to measure objective visual, NH_3 and texture attributes of shrimp aged on ice for up to 14 days, and 3) to correlate visual and smell indices of shrimp with sensory attributes.

MATERIALS AND METHODS

A light box was built of acrylic sheet with top and bottom lighting boxes, and a chamber. **A** color CCD Video Camera with an S-video output was connected to a color frame grabber. The Red-Green-Blue (RGB) color space had 256 units per axis, resulting in 16 million separate color combinations. To reduce the number of colors to be analyzed, each axis was divided into 4 sections, resulting in 64 cubes covering the color space. Color combinations in a given cube were lumped into the center color of that cube. Software was developed to analyze view area and color of individual objects in an acquired image using Microsoft Visual Basic Professional 3.0. Details of the experimental setup are described by Luzuriaga et al(l996a).

Ammonia was measured with an ion-selective electrode (Model 95-12, Orion Research, Inc., Boston, MA). Intact shrimp samples were placed in an air-tight box and sprayed with 5N NaOH to volatilize ammonia Extensive experiments were conducted to determine proper amounts of sample, NaOH to be sprayed, and the effects of temperature, sample preparation and equilibration time on NH_3 readings. It was found that ammonia readings could be completed within 2 mins (Luzuriaga et al., 1996b) with minimum sample preparation.

Texture was measured with a force sensing mechanism with a miniature load cell (max.capacity 9 kg), and a stepper motor driven probe. Details of the experimental setup are described by Luzuriaga et al. (1996c).

Visual Experiments

One hundred head-on, non-frozen white shrimp (Penaeus setiferus), from 90 count to 30 count per lb were used. Intact shrimp were placed individually in the light box, an image was acquired, and the view area of the shrimp was determined in pixels. The shrimp was then weighed. For the same shrimp batch, heads were removed manually, and they were processed as described above; the shrimp were then peeled, leaving the shell in the last segment and the tail, and processed as before, and the last segment shell and the tail were removed and same procedure was followed. The four sets of view area vs. weight data were analyzed by fitting 6 types of equations (Luzuriaga et al., 1996a). The three best fitting equations are shown in Table 1. This allowed for prediction of weight based on view area. Count and uniformity ratio could be estimated solely from the image.

The degree of melanosis in shrimp samples stored on ice for up to 17 days was evaluated by a trained inspector and by the automated system at days 0,3,7,9,13,15, and 17. Melanosis of both

sides of the shrimp were averaged. The trained inspector identified as melanotic areas that were black and dark. RGB colors of these dark areas were obtained. Nine color blocks from the 64 color blocks used in this system were included into melanosis calculations. At the same time that the shrimp was being evaluated for melanosis by the automated system, the color information was extracted and presented in a histogram of 64 colors. The variation in these histograms represented the change in color of the shrimp during storage on ice. The most representative colors were selected for this specie of shrimp and plotted vs time.

Ammonia measurements

Frozen, headless, shell-on, white shrimp Penaeus setiferus (40-50 count per lb) were thawed under running water, separated into 9 samples of 200 g each, stored in plastic bags and kept on ice for 0,2,4,6,8,10,14 and 21 days. Levels of NH_3 were measured by the procedure by Ward et al. (1979) and plotted against time. To correlate sensory odor data with NH_3 probe readings, headless, peeled white shrimp (Penaeus setiferus) 26-30 count per lb was separated into 400 g samples, aged on ice to obtain different NH_3 levels, and their odor evaluated by a 16 member sensory panel in two sessions, Grading was done on a scale from 0 (poor) to 10 (good). The samples were then analyzed for NH_3 and an equation to predict sensory odor grade from machine-read NH_3 levels was developed.

Texture measurements

Elasticity of raw shrimp stored on ice was measured with the Instron equipped with a 50 kg load cell and a spherical indenter, and with our device. The yield point of raw shrimp were measured. The effects of shrimp size, cross head speed, sample temperature, degree of deformation on the measured elasticity values were determined. To measure the TPA parameters, 2 successive compression decompression cycles at the center of the first segment at various deformation levels was performed at different crosshead speeds. Results were analyzed statistically to evaluate diferences in results at different treatments

RESULTS AND DISCUSSION

Visual attributes

Table 1 shows that estimation of weight by the view area is reliable, and that count and uniformity ratio can be accurately measured by machine vision. The error encountered during area reading was the highest when shrimp had legs present. In the case of peeled shrimp, this would not be a problem, and the error associated with measurement of view area would be much less than 2%.

Table 1. Experimentally determined and estimated total weight, count and uniformity ratio values for different forms of white shrimp.

	Total weight (g)	count / lb	Uniformity Ratio
Intact			
Experimental	867.0	50.9	3.11
$y = a+b^x$	867.5	50.8	2.98
$y = a+b^{1.5}$	865.7	50.9	2.95
$y = a+be^{x/c}$	867.0	50.9	2.96
Headless			
Experimental	593.6	75.8	3.17
$y = a+b^x$	592.2	76.0	2.89
$y = a+b^{1.5}$	593.5	75.8	3.08
$y = a+be^{x/c}$	593.7	75.8	3.12
Peeled			
Experimental	509.4	90.1	3.23
$y = a+b^x$	505.6	90.8	3.12
$y = a+b^{1.5}$	508.7	90.3	3.22
$y = a+be^{x/c}$	509.6	90.1	3.19
Tail off			
Experimental	461.1	98.6	3.32
$y = a+b^x$	460.2	98.8	2.93
$y = a+b^{1.5}$	462.0	98.4	3.22
$y = a+be^{x/c}$	462.1	98.4	3.17

Melanosis and Color

Melanosis scores of shrimp stored on ice increased with time up to 15 days, but was lower on day 17. Both the trained inspector and machine readings detected this decrease (Luzuriaga et al., 1996a). Figure 1 shows the color change in shrimp stored on ice for up to 17 days. Melanotic colors (a) showed an increase in color 2 (R =96 , G = 32, B = 32) and 4 (160,96,32). Others stayed the same or fluctuated without a defmite pattern.

Non-melanotic colors (b) showed drastic decreases in colors 61 (224, 224, 160) and 57 (160, 160, 96), and an increase in color 6 (224, 160, 32). The change in these colors could be monitored, and quality related information extracted.

The computer program also evaluated the color spectrum of the samples and compared it to the minimum and maximum values of colors considered "normal" for that species, stored in a database. These values would be different for different species, but the evaluation method would be identical.

Ammonia Measurements

Figure 2 shows the change in the ammonia levels of shrimp stored on ice, and the grades given to samples by sensory panelists. The variability in the sensory panel data is expected. An exponential equation was fitted to the data:

$$y = 0.3338 + 13.363\, e^{-x/229.93}$$

where: y = grade (from 0 to 10) and x = NH_3 level. The fitted curve showed an exponential decrease of grade with an increase in the NH_3 levels. Based on these results a grade of 10 could be given to samples with 75 ppm of NH_3 or less. An intermediate score of 5 was represented by 230 ppm of NH_3. During sensory- evaluation, panelists expressed that they considered a grade of 5 the limit between accepting or rejecting a sample. Therefore it could be concluded that the ammonia level for rejecting shrimp is around 210-230 ppm. This correlated with the data reported by Cheuk and Finne (1984) where spoiled shrimp was considered to have ammonia levels above 230 ppm.

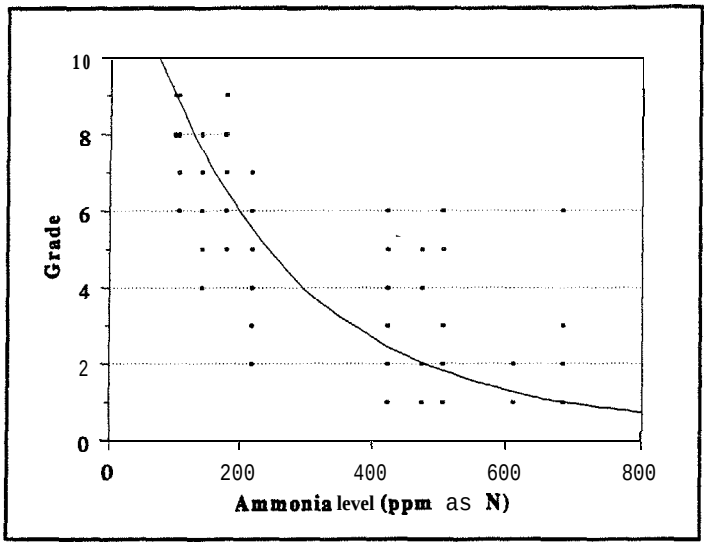

Figure 1. Correlation of sensory results with machine-read ammonia levels in shrimp stored on ice.

Texture Measurement

Table 2 shows the elasticity measurement results for shrimp stored on ice. Elasticity did not change significantly during the first 6 days. Between day 6 and 9, there was a significant decrease in elasticity. This corresponded well with the onset of melanosis, and the ammonia levels that the panelists considered as unacceptable. Therefore elasticity could be used as a texture attribute in evaluating overall quality. Elasticity values did not change significantly after day 9.

Table 2. Measurement of elasticity of peeled white shrimp during storage on ice Deformation level = 27%, crosshead speed = 100 mm/mm.

Storage Time (days)	n (shrimp)	Degree of Elasticity (%)	Std. Deviation (%)	Difference[a] p<0.05
0	20	45.6	4.90	a
3	20	45.3	7.67	a
6	20	44.7	4.51	a
9	20	40.8	5.39	b
12	20	40.3	4.86	b
15	20	41.0	4.11	b
18	20	38.5	4.46	b

a : Same letter indicates no significant difference at $p<0.05$.

Texture Profile Analysis (TPA) was also performed (Bourne, 1978). Data obtained were not of great value for the purpose of correlation with the quality of shrimp. Most of the TPA parameters showed little difference over time, and in most cases no significant differences were observed. The cohesiveness, hardness, fiacturability, gumminess and chewiness parameters did not show any trend or significant change during storage. Springiness and adhesiveness showed some significant differences. Springiness at day 0 was higher and significantly different from that at the other storage times. After day 3 the value of springiness remained constant. Similar behavior was observed for adhesiveness. At day 0 the adhesiveness was low compared to that at the rest of the storage times. After day 3 adhesiveness increased four times compared to day 0, and remained constant up to day 18. This'increase was confirmed subjectively: shrimp were sticky on and after the third day.

ACKNOWLEDGMENTS

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QUALITIES OF FRESH AND PREVIOUSLY FROZEN MARINATED SHRIMP

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INTRODUCTION

Oysters, Frozen-Thawed-Refrigerated(FTR) was studied by Hackney et al. (1987). Frozen oysters were thawed and repacked, then stored at 4°C for up to 12 days for fresh markets. Aerobic bacterial count of was about 2 log CFU/ml lower than that of the freshly packed oysters throughout the entire refrigeration storage, indicating freezing and frozen process resulted in inactivation and injuries of bacterial. Both previously frozen and freshly packed oysters were acceptable by sensory evaluation of raw and cooked oysters. Many works have been reported on the refrigeration, icing or partial frozen storage of fresh shrimp for different species captured from different regions (Cobb III et al., 1973, 1977; Novak, 1973; Fatima et al., 1981,1988; Shamshad et al., 1990). However, few efforts have been done on quality changes of previously frozen then thawed shrimp during refrigeration storage.

The previous study in the second chapter of this desertation demonstrated that marination increased weight gain, moisture content and product yield and sensory scores (juiciness, springiness and taste desirability). However, no information regarding microbiological, textural and sensory qualities of marinated and unmarinated shrimp during frozen and refrigeration storage is available.

The objective of the study was to evaluate the effects of marination, freezing, and frozen storage on the microbiological, chemical, textural and sensory qualities of frozen thawed refrigerated shrimp.

MATERIALS AND METHODS

Shrimp Samples

Shrimp (*Penaeus* spp.), harvested from off the coast of Brunswick, Georgia, in May, 1995, were headed and washed with sea water. The samples were kept on ice and transported to the Food Science Building in The University of Georgia, within 24 hours. The size of headed shell-on shrimp sample was 3 f-35 counts per pound.

Experimental Design

Fresh (iced) shell-on shrimp were divided into two portions. The first portion was unmarinated shrimp, which was directly packed on styrene-foam trays and over-wrapped with PVC film, with ten pieces in each package. The other portion of shrimp was peeled and deveined, and marinated with 2: 1 ratio of shrimp to marinade. The marinade was composed of 1.75% of Lemon Pepper Marinade Seasoning (A.C. Legg Pack Inc., Birmingham, Alabama), 1% of commercial food grade polyphosphates (BK Ladenburg Corp., Cresskill, NJ), 1.5% of whey protein concentrate 600 (AMPC, Inc.. Ames, Iowa), and water. After tumbled at 4°C for 30 min and holding for 30 min, marinated portion was packed as same as first portion. Before refrigeration storage at 4°C for 10 days (sampled at 1,4, 7, or 10 days respectively), shrimp were treated as the following.

Trial A: unmarinated; fresh.
Trial B: marinated; fresh.
Trial C: unmarinated; freezing-(four hours)-thawed.
Trial D: marinated; freezing-(four hours)-thawed.
Trial E: unmarinated; frozen-(three months)-thawed.
Trial F: marinated; frozen-(three months)-thawed).
Trial G: unmarinated; frozen-(six months)-thawed).
Trial H: marinated; frozen-(six months)-thawed).
Trial I: frozen-(six months)-thawed; then marinated.

All samples were thawed at 4°C for 24 hours. This was counted as one day of refrigeration storage.

Microbiological Analysis

Psychrotrophic bacterial count: 100 ml sterile distilled water was added into sterile bag with approximate 1.5 grams shrimp sample inside, then homogenized with stomacher for 30 seconds. Serial dilutions were plated on plate count agar (PCA) and incubated at 20°C for 3 days.

Mesophilic bacterial count: 100 ml sterile distilled water was added into sterile bag with approximate 10 grams shrimp sample inside, then homogenized with stomacher for 30 seconds. Serial dilutions were plated on plate count agar (PCA) and incubated at 37°C for 2 days.

Sensory Evaluation

The panelists were trained specifically for this sensory evaluation. First, different reference foods were used to train panelists for each attribute (juiciness, springiness, and cohesiveness) according to the methods described by Meilgaard (1987). The reference shrimp was unmarinated shrimp frozen by dry ice (CO_2) and was stored at -60°C for less than seven days before being baked. Panelists were asked to score on a 15 cm structured scale according to its juiciness, springiness, cohesiveness and odor of freshness The scores from ten panelists were averaged. Averaged scores were discussed by panel members, and final agreement was made by modifying averaged scores into reference scores for reference shrimps. Results showed that 4.5 was for juiciness, 5.5 was for springiness, 7.5 was for cohesiveness and 12 was for odor of freshness.

For the samples stored for six months at -27°C, the attributes juiciness, springiness and hardness were used in the panelists training and in sample evaluation. The reference foods used during sample evaluation were unpeeled fresh carrot (3.0), unpeeled red delicious apple (7.5) and orange (12.0) for juiciness, Hebrew National frankfurter (5.0) and marshmallow (9.5) for springiness, and Yellow-American pasteurized process cheese (4.5) and Hebrew National frankfurter (7.0) for hardness.

Forty pieces of shrimp samples for each treatment were placed on aluminum foil, baked at 177°C (350°F) for 15 min. Four pieces of shrimp for each treatment were placed into a 4 oz. plastic cup with lid to keep sample warm. The cups were labeled with random three-digit code. All treatments (cups) were served at the same time to panelists. Red lights were used to mask color to prevent bias on scoring of other attributes. Panelists were asked to score on a 15 cm universal scale using frozen-(several days)-thawed unmarinated shrimp as reference.

Free Fatty Acids (FFA) and Fat Content

The method of Woyewoda et al. (1986) was used to determine free fatty acid and fat content.

Textural Analysis

Maximum shear force on individual shrimp sample was determined using Warner-Bratzler (WB) blade fitted on Instron Universal Testing Machine (Instron model no. 1122, Instron, Inc., MA), to cut the area between second and third segments. Instrument setting included full scale load of 10 kg, cross head speed of 50 mm/min and chart speed of 100 mm/min Peak force recorded was used to represent shear force required to cut the shrimp sample. Ten measurements were evaluated for each treatment.

Hunter Color Value

Hunter L, a, b value were measured using Chroma Meter CR-200 (Minolta Camera Co., Ltd, Japan). Each mean value was the average of 20 measurement on four baked unmarinated shrimp samples. The Hunter value L represents the lightness, value a represents redness ($a>0$) or greenness ($a<0$), and value b represents the yellowness of sample.

Water Holding Capacity (WHC)

The modified method of Foegeding and Ramsey (1987) was used to determine WHC: 2.5 grams sliced baked shrimp was wrapped with nylon cloth first. Four layers of Whatman No. 1 filter paper (Whatman International Ltd., Springfield Mill, Maidstone, Kent, England) were wrapped outside. The wrapped samples were placed in the centrifuge tube and were centrifuged at 3000 rpm for 30 min. Relative weight loss after centrifuged was used to calculate the percentage of centrifuged tissue fluid (CTF). Ten measurements were used for every treatment.

Moisture Content

Moisture contents of shrimp sample were monitored before and after frozen storage according to the method in AOAC (1992).

Statistical Analysis

Data was analyzed using Statistics Analysis System (SAS, 1987). Duncan's multiple range test was used to compare and determine the significant difference of the mean logarithm bacterial counts, sensory evaluation results and other attributes among the shrimp with different treatments.

RESULTS AND DISCUSSIONS

Bacterial Growth on Shrimp

At day 1, marinated shrimp had significantly higher psychrotrophic bacterial populations than unmarinated shrimp (Table 1). The shrimp was possibly contaminated during peeling and deveining by hands. After 4 or 7 days refrigeration storage, marinated shrimp had lower psychrotrophic bacterial populations than unmarinated shrimp. The frozen shrimp had lower populations than fresh sample. On the day 7, both the fresh unrnarinated shrimp and frozen unmarinated shrimp had populations higher than 10^7 CFU/g. After 10 days storage, all shrimp were spoiled. Psychrotrophic bacteria are the main spoilage bacteria during refrigeration storage.

Table 2 shows mesophilic bacterial populations of Trials A, B, C and D during refrigeration storage. Similarly to psychrotrophic bacteria, marinated shrimp had higher initial populations at day one. After 4 days storage, frozen thawed shrimp had lower populations than freshly refrigerated shrimp. After 7 days storage, either marinated, frozen, or marinated and frozen shrimp had lower populations than fresh unmarinated shrimp.

After three months frozen storage at -27°C, unmarinated shrimp (Trial E) and marinated shrimp (Trial F) were thawed at 4°C for 24 hours, then stored either at 4°C, at 0°C or in ice. Psychrotrophic bacterial populations and mesophilic bacterial populations are shown in Tables 3 and 4. Marinated shrimp still had higher initial populations (at day 1) than unmarinated shrimp. After 4 days at 4°C and 4 or 7 days at 0°C or in ice, marinated shrimp had the same level of bacterial counts as unmarinated shrimp. After 7 days storage at 4°C or after 10 days storage at 0°C or in ice, marinated shrimp had significantly lower populations than unmarinated shrimp. This implies that marination had an inhibitory effect on bacteria in refrigerated shrimp. Even considering initial higher population, marinated shrimp still has longer microbiological storage life than unrnarinated shrimp. Generally, shrimp stored at 0°C or in ice had lower bacterial population than at 4°C. Lower temperature extended the storage life of shrimp.

After six months frozen storage at -27°C, unmarinated shrimp (Trial G) and marinated shrimp (Trial H) were thawed at 4°C for 24 hours. Trial I was the unrnarinated shrimp frozen-stored-(six month)-thawed then marinated. Psychrotrophic bacterial population and mesophilic bacterial population are shown in the Tables 5 and 6. For comparison, the bacterial populations of shrimp in Trial B are also shown in Tables 5 and 6. The unmarinated shrimp, which has been frozen

stored for six month, had lower bacterial population than freshly refrigerated unmarinated shrimp (Trial A) in Tables 1 and 2. Nevertheless, no significant difference of bacterial population was found between freshly marinated shrimp and frozen-(six months)-thawedthen marinated shrimp during storage at 4°C. These implies that six months frozen storage did not affect bacterial population in marinated shrimp in the succeeding storage period at 4 °C.

Sensory Qualities of Shrimp

It has been established that increased cooking time reduced tenderness of shrimp (Ahmed et al., 1972). Therefore, the baking temperature and time were fixed in this study, in order to observe only treatment and storage effects.

Sensory attributes of baked marinated (Trials A, C) and non-marinated (Trials B, D) shrimp during seven days refrigeration storage are shown in Tables 7 and 8. Marinated shrimp had significant higher juiciness than umnarinated (either fresh or frozen-thawed) after either 1,4 or 7 days storage. The springiness of umnarinated shrimp became gradually lower than marinated shrimp after 4 or 7 days storage. This implies that marination helped shrimp to maintain springiness during storage at 4°C. No significant difference was found for cohesiveness between marinated and unmarinated shrimp, between fresh and frozen-thawed refrigerated shrimp as shown in Table 12. Odor of freshness decreased during storage, but no significant difference was found between marinated and unmarinated shrimp (Table 13).

Juiciness, springiness and hardness of six month frozen stored shrimp (Trials G, H and I) were shown in Tables 9,10 and 11. Both marinated shrimps (Trials H and I) had higher juiciness than unmarinated shrimp (Trial G) after six months at -27°C and one or four day(s) at 4 °C The marinated shrimp had higher springiness after six month of frozen storage, however, the springiness of all samples decreased after four days refrigerated storage. On the other hand, the hardness of marinated shrimp (Trial H and I) were lower than unmarinated shrimp (Trial G) after stored at -27°C for six months and at 4°C for 4 days.

Color and FFA of Stored Shrimp

Hunter color values of baked unmarinated shrimp are shown in Table 14. Frozen-(four hours)-thawed shrimp had lower lightness and lower yellowness at day one, and lower redness after four or seven days storage at 4°C, comparing with freshly refrigerated shrimp. No difference was found for the lightness and yellowness between fresh and frozen-(four hours)-thawed shrimp after four or seven days storage. This implies that freezing procedure resulted in lower redness.

Free fatty acids of shrimp increased during refrigeration storage, as shown in Table 15. However, there was no significant difference between fresh and frozen-thawed umnarinated shrimp. Fat content of shrimp remained unchanged during storage at 4 °C (Table 15).

Textural Properties and Water-Holding Capacity

Warner-Bratzler maximum shear force, baking loss, moisture content and centrifuged tissue fluid of baked shrimp are shown in Table 16. Marinated shrimp had significantly lower shear force than unmarinated shrimp. Shear force represented the hardness. Reduced hardness implies that marination resulted in shrimp tissue tenderization. These results could be related to the sensory hardness mentioned previously in this report. Love (1968) and Ahmed et al. (1973) theorized that the tenderizing effect of polyphosphates on shrimp muscle was due to the weakening of the fibers to give a protein gel system which increased the water holding-capacity of the muscle proteins. Webb et al. (1975) found that raw shrimp presoaked in 6% phosphate had significantly lower shear values than untreated raw shrimp; and phosphate treated then cooked shrimp also had lower shear value than untreated cooked shrimp, even though the increase in shear force was significant due to frozen storage. However, under the conditions of a relatively high acid medium (Creole sauce), the pretreatment of shrimp with phosphates did not sufficiently buffer the muscle tissue to prevent toughening during frozen storage as indicated by shear force measurements (Webb et al., 1975).

Marinated shrimp has significantly-higher moisture contents than unmarinated shrimp after frozen stored at -27°C. After frozen storage and baking, marinated shrimp still contained higher ($\alpha<0.05$) moisture content than unmarinated shrimp. However, the moisture contents of unmarinated and marinated shrimp after frozen storage did not change significantly in this study. Since marinated shrimp had lower baking loss, more moisture was held in the baked sample, and thus resulted in higher ($\alpha<0.05$) centrifuged tissue fluid in the marinated baked shrimp (Table 5). This means marinated shrimp had higher water-holding capacity. Love and Abel (1966) stated that STPP interacted with proteins to produce a surface film on treated fish fillets. They theorized that such a film would seal in fluids and thus not only reduce thaw drip but also minimize moisture loss during frozen storage.

CONCLUSIONS

Marination inhibited the growth of psychrotrophic bacteria and mesophilic bacteria during refrigeration storage, and extend microbiological shelf life of shrimp. Frozen shrimp had lower bacteria population than fresh shrimp. Frozen-stored-six-month also resulted in lower bacterial counts compared with fresh or frozen-(4 hours)-thawed refrigerated shrimp during stored at 4°C.

Marinated shrimp had higher juiciness than unmarinated shrimp during refrigeration storage. Marination helped shrimp maintain springiness during refrigeration storage. Marinated shrimp had lower hardness than unmarinated shrimp.

No significant difference was found on sensory, textural and microbiological qualities of freshly marinated and frozen-six-month-thawed marinated shrimp during storage at 4°C. The data supported practice of Frozen-Thawed-Refrigerated approach to the storage of marinated shrimp.

Table 1. Psychrotrophic bacterial populations of refrigerated shrimp previously marinated and/or frozen

	unmarinated		marinated	
days	fresh	frozen	fresh	frozen
1	3.96c*	4.20b	4.95a	4.61ab
4	6.81a	6.10b	5.43c	4.61d
7	8.44a	7.81b	7.12c	7.02c
10	9.46ab	9.64a	9.29ab	9.08b

*: (1) n=3; (2) means with same letter abcd in the same raw have no difference (α<0.05); (3) the unit is in CFU/g.

Table 2. Mesophilic bacterial populations of refrigerated shrimp previously marinated and/or frozen

	unmarinated		marinated	
days	fresh	frozen	fresh	frozen
1	3.66c*	3.75c	4.89a	4.50b
4	5.49a	4.87b	5.27a	4.58b
7	7.59a	6.78b	6.8533	6.98b
10	9.16a	9.15a	9.29a	9.15a

*: (1) n=3; (2) means with same letter in the same raw have no difference (~0.05); (3) the unit is in CFU/g.

Table 3. Psychrotrophic bacterial populations of refrigerated shrimp previously frozen-(three-months)-thawed

	at 4 °C		at 0 °C		in ice	
marin.	no	yes	no	yes	no	yes
1	3.45b*	4.24a	3.45b	4.24a	3.45b	4.24a
4	5.56a	5.43a	4.06c	5.31a	4.66b	5.18a
7	7.96a	6.83b	5.24c	5.32c	5.32c	5.29c
10	8.77a	8.89a	7.85b	6.70c	7.82b	6.34d
13	10.38a	10.20b	9.45c	8.87d	8.70d	8.23e

*: (l)n=3; (2) means with same letter in the same raw have no difference (α<0.05); (3) the unit is in CFU/g.

Table 4. Mesophilic bacterial populations of refrigerated shrimp previously frozen-(three-months)-thawed

	at 4 ℃		at 0 ℃		in ice	
marin.	no	yes	no	yes	no	yes
	3.47b*	4.17a	3.47b	4.17a	3.4713	4.17a
4	4.62b	5.35a	4.33c	5.10a	4.39b	5.12a
7	6.54a	6.57a	4.42c	5.29b	5.14b	5.13b
10	7.32b	8.30a	6.27c	6.29c	6.26c	6.15c
13	10.33a	10.13b	8.59c	8.16d	8.20d	7.85e

*: (1) n=3; (2) means with same letter in the same raw have no difference ($\alpha<0.05$); (3) the unit is in CFU/g.

Table 5. Psychrotrophic bacterial populations of refrigerated shrimp previously frozzen-(six-months)-thawed

days	unmarin. frozen	marinated frozen	fresh marinated	frozen marinated
1	3.67c*	4.25b	4.95a	4.98a
4	4.64b	4.22c	5.43a	5.18a
7	6.48c	6.60bc	7.12ab	7.34a
10	8.15b	7.66c	9.29a	9.52a

*: (1) n=3; (2) means with same letter in the same raw have no difference ($\alpha<0.05$); (3) the unit is in CFU/g.

Table 6. Mesophilic bacterial populations of refrigerated shrimp previously frozen-(six-months)-thawed

days	unmarin. frozen(6)	marinated frozen(6)	fresh marinated	frozen(6) marinated
1	2.91d*	4.27c	4.89b	5.23a
4	3.78c	4.17b	5.27a	5.20a
7	5.50c	6.15b	6.85a	7.24a
10	7.47c	7.67c	9.27a	8.61b

*: (1) n=3; (2) means with same letter in the same raw have no difference ($\alpha<0.05$); (3) the unit is in CFU/g.

Table 7. Juiciness of refrigerated shrimp previously marinated or frozen-thawed judged by trained panel

	unmarinated		marinated	
days	fresh	frozen	fresh	frozen
1	4.93b*	5.04b	6.48a	6.84a
4	4.52b	4.7433	6.79a	6.08a
7	4.40b	3.91b	6.68a	6.72a

*: (1) n=10; (2) means with same letter in the same raw have no difference ($\alpha<0.05$).

Table 8. Springiness of refrigerated shrimp previously marinated or frozen-thawed judged by trained panel

	unmarinated		marinated	
days	fresh	frozen	fresh	frozen
1	7.47a*	7.86a	7.91a	8.27a
4	7.09b	6.04b	8.22a	8.07ab
7	5.97b	7.06ab	8.37a	7.82a

*: (1) n=10; (2) means with same letter in the same raw have no difference ($\alpha<0.05$).

Table 9. Juiciness of refrigerated shrimp prevously frozen-six-month-thawed judged by trained panel

day(s)	unmarinated frozen-6-mons	marinated frozen-6-mons	frozen-6-mons marinated
1	3.2b*	5.2a	4.9a
4	2.9b	5.4a	4.8a

*: (1) n=10; (2) means with same letter at the same day have no difference ($\alpha<0.05$).

Table 10. Springiness of refrigerated shrimp previously frozen-six-month-thawed judged by trained panel

day(s)	unmarinated frozen-6-mons	marinated frozen-6-mons	frozen-6-mons marinated
1	6.0b*	6.9a	7.3a
4	5.5a	5.8a	5.6a

*: (1) n=10; (2) means with same letter at the same day have no difference ($\alpha<0.05$).

Table 11. Hardness of refrigerated shrimp prevously frozen-six-month-thawed judged by trained panel

day(s)	unmarinated frozen-6-mons	marinated frozen-6-mons	frozen-6-mons marinated
1	6.9a*	6.0b	5.8b
4	8.2a	6.8b	6.6b

*: (1) n=10; (2) means with same letter at the same day have no difference ($\alpha<0.05$).

Table 12. Cohesiveness of baked refrigerated shrimp

	fresh		frozen(4 hours)	
days	unmarinated	marinated	unmarinated	marinated
1.	6.02*	5.56	6.32	6.04
4	5.20	5.88	5.23	5.34
7	5.21	6.10	5.89	5.42

*: (1) n=10; (2) means in the same row have no difference ($\alpha<0.05$); (3) means in the same column have no difference ($\alpha<0.05$).

Table 13. Odor of freshness of baked refrigerated shrimp

	fresh		froen(4 hours)	
days	unmarinated	marinated	unmarinated	marinated
1	11.17a*	9.28a	10.63a	9.34a
4	10.54a	8.64ab	7.58b	8.67ab
7	5.14b	8.43a	5.62b	7.81a

*: (1) n=10; (2) means in the same row with same letter have no difference ($\alpha<0.05$);

Table 14. Hunter color vaues (L, a, b) of baked refrigerated shrimp

	L		a		b	
Days	fresh	frozen	fresh	frozen	fresh	frozen
1	70.50a*	68.21b	13.71a	12.62a	23.41a	21.61b
4	67.52a	66.27a	12.23a	10.45b	21.93a	20.66a
7	63.56a	64.34a	10.75a	7.48b	22.02a	20.20a

*: (1) n=20; (2) means in the same row with same letter for same value have no difference ($\alpha<0.05$);

Table 15. Free fatty acid and fat content of refrigerated shrimp tissue

	free fatty acids		fat content	
days	fresh	frozen	fresh	frozen
1	3.47a*	3.73a	1.48**	1.48
4	4.09a	3.82a	1.15	1.16
7	5.07a	4.89a	l-20-	1.29
10	5.16b	6.12a	1.16	1.47

*: (1) n=3; (2) means of free fatty acids in the same row with same letter have no difference ($\alpha<0.05$).
**: No difference ($\alpha<0.05$) on fat content between fresh and frozen-thawed shrimp during whole refrigerated storage.

Table 16. **Shear force** and centrifuged tissue fluid of baked shrimp

treatment**	unmarinated shrimp				marihated shrimp				frozen(6)
	fresh	froz0	froz3	froz6	fresh	froz0	froz3	froz6	marinated
Moisture (%) before baked	76.8b*	76.3b	76.6b	77.0b	80.0a	80.5a	79.6a	79.3a	79.8a
Baking loss	29.1a	28.0a	26.6a	28.5a	23.2b	23.0b	22.8b	22.9b	23.1b
Moisture (%) after baked	65.6b	66.1b	67.5b	66.9b	72.0a	70.5a	71.3a	73.0a	72.7a
Shear force (Kgf)	1.51a	1.55a	1.49a	1.50a	0.96b	0:81b	0.79b	0.84b	0.82b
Centrifuged fluid (%)	16.1b	17.4b	16.8b	17.3b	25.6a	25.4a	25.9a	26.0a	26.1a

*: (1) n=10. (2) means with same letter in the same row have no difference (~0.05).
**: fresh: means fresh shrimp; froz0, froz3, or froz6: means frozen stored at -27 °C for 0, 3, or 6 months respectively; frozen(6) & marinated: means unmarinated shrimp frozen stored 6 month, thawed, peeled and deveined, then marinated.

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DIPSTICK TESTS FOR BIOGENIC AMINES

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ABSTRACT

A rapid colorimetric dipstick test for histamine in tuna was developed based on the coupling of diamine oxidase to a peroxidase/dye system. The assay was linear to 1.0 mM histamine and the minimum detectable concentration was 0.07 mM corresponding to 2.3 mg% in tuna extracts. Histidine and cadaverine did not interfere with the assay whereas putrescine reacted slightly with the dipstick. Histamine determinations in fresh and spoiled tuna gave good agreement between the dipstick method and the AOAC fluorometric method. The dipstick assay technique should be readily adaptable to putrescine or cadaverine.

INTRODUCTION

A variety of assay methods exist for the determination of histamine in seafood including spectrophotometric (1), chromatographic (2,3), and chemical chelation (4). However, they are generally slow and require considerable training on the part of the user. Enzymatic colorimetric assays for histamine using diamine oxidase (histaminase) have also been reported (5,6). In the presence of oxygen, diamine oxidase (DAO) deaminates histamine to form hydrogen peroxide, ammonia, and imidazolacetaldehyde.

$$\text{Histamine} + O_2 \xrightarrow{\text{DAO}} H_2O_2 + NH_3 + \text{imidazolacetaldehyde}$$

The liberated hydrogen peroxide, coupled with horseradish peroxidase (HRP), converts a reduced dye to its oxidized form with accompanying color development.

$$H_2O_2 + \text{reduced dye} \xrightarrow{\text{HRP}} H_2O + \text{oxidized dye}$$

Dry chemistry tests exist for glucose (7) and cholesterol (8) using the appropriate oxidase, HRP, a compatible dye, buffers and stabilizers embedded in a solid matrix such as filter paper or silica. In combination with reflectance photometry, they give simple, rapid, quantitative results. Alternatively, color development may be read visually and compared

with a color chart. In this paper, we report the production of a dry chemistry test (dipstick) for histamine in tuna.

MATERIALS

Pig kidneys were obtained from Cascio's Abatoir (Hattiesburg, MS) and used immediately or frozen at -20°C until use. Bluefin tuna loins were obtained on ice within 24 hrs of harvest from the Gulf of Mexico by J&R Seafood (Hattiesburg, MS). All tuna samples were stored at -80°C until use. Serim Peroxide Reagent Strips were obtained from Serim Research Corporation (Elkhart, IN). Horseradish peroxidase (HRP) (2000 U/ml) was obtained from Sigma Chemical Company (St. Louis, MO). Histidine, putrescine, cadaverine, and histamine dihydrochlorides were obtained from Sigma Chemical Company. Stabilcoat™ was obtained from Biometric Systems, Inc. (Eden Prairie, MN).

METHODS

Enzyme Purification

Diamine oxidase (DAO) was purified through the hydroxylapatite step of Rinaldi (9) with the following modifications: potassium phosphate buffer (0.1M, pH 6.5) was used throughout, and entire defatted pig kidneys were used. The final active fractions were pooled, concentrated using 70% ammonium sulfate, redissolved and dialyzed against potassium phosphate buffer. The specific activity of the final preparation was comparable to that found by Rinaldi (1-2 U/mg protein).

Preparation of Dipsticks

The following reagents were mixed on ice: 2.5 ml HRP (2000 U/ml>, 2.5 ml Stabilcoat, and 5.0 ml of DAO (1.5-2.5 U/ml). Serim Peroxide Reagent Strips were dipped in this mixture, dried at 37°C for 30 minutes, and stored in a desiccator at room temperature. This produced a lot of approximately 750 dipsticks with the same activity.

Preparation of Decomposed Tuna

Fresh tuna on ice was cut into approximately 100 g chunks and portioned into plastic freezer bags. Control samples were frozen immediately at -20°C. Test samples were maintained at 8°C, and aerated every 48 hrs by opening the bags and allowing the air to exchange, then resealing the bags. Samples were removed after various time intervals, split into equal portions, and frozen at -20°C. Subsequently, one portion was thawed and tested for histamine using the Association of Official Analytical Chemists (AOAC) reference method (10). The other portion was thawed and homogenized in 2 volumes of potassium phosphate buffer (0.1 M, pH 6.5) for 2 minutes, centrifuged, heated at 75°C for 1 min to decolorize, and clarified by brief centrifugation. The supematant was then tested with the histamine dipsticks and the modified AOAC fluorometric test (4).

Histamine Determination

To determine the histamine concentration, dipsticks were immersed briefly in samples to be tested and then observed visually. Color was allowed to develop for 10 minutes unless otherwise stated, and the reflectance was determined at 660 nm. A standard curve was

performed with each experiment using fresh tuna extract (zero histamine tuna) spiked with known concentrations (0-l.0 mM) of histamine, and the concentrations of the unknown samples were determined using this curve. Samples with elevated concentrations of histamine were diluted in fresh tuna extract and reassayed.

RESULTS AND DISCUSSION

Dipstick Assav

A time course was performed using histamine (0.8 mM) spiked into buffer, fresh tuna extract, and heat-treated tuna extract (heated at 75°C for 1 min), in order to determine the optimal duration for the dipstick assay (Fig 1). At 10 min there was maximal reproducible color development and therefore this time was selected as the assay time.

To determine the sensitivity of the assay, 20 samples of fresh tuna were analyzed and the mean plus two standard deviations calculated. The minimum detectable concentration for the assay, i.e. the threshold level distinguishable above random noise, was thus observed to be 0.07 mM. (One mg% corresponds to 0.03 mM.)

The linearity of the assay was- determined using fresh tuna spiked with varying concentrations of histamine. To illustrate reproducibility, figure 2 shows the means (and their standard errors) of data obtained from three separate experiments and two lots of dipsticks (n= 6). For an individual experiment using a single lot of dipsticks the assay was found to be linear up to 1.0 mM, with a correlation coefficient of 0.994. This was repeated substituting potassium phosphate buffer (0.1 M, pH 6.5) for the fresh tuna. Using the buffer-based calibrators, the assay was found to be linear to 5 mM. To determine the extent of interference from other diamines, putrescine and cadaverine were analyzed in parallel with histamine (Fig 3). Cadaverine did not react with the dipstick whereas putrescine gave some response at 10 fold higher concentrations. Similarly, histidine did not react with the assay (Fig 3). In order to test the longevity of the dipsticks, four out of sixteen different lots of dipsticks-stored desiccated at room temperature were tested weekly with histamine (0.1 to 1.0 mM). All lots of dipsticks were stable (average 70% of initial color response) for at least 2 months. The longest lot tested was stable for 4 months (87% of initial color response). Although not quantitated, it was clear that both response and stability was increased with increased specific activity of the diamine oxidase preparation.

Method Comparison

Samples of tuna in various stages of decomposition were split and assayed by the dipstick test, the AOAC reference method, and a modified flurometric procedure based on the AOAC method. The results and the corresponding standard deviations are presented below.

TABLE 1
Comparison of Histamine Test Methods in Decomposed Tuna Samples.

#	Reference AOAC (mg%)	n	Modified AOAC (mg%)	n	Dipstick (mg%)	n
1	3.3 ± 1.1	4	2.7 ± 0.7	10	2.7 ± 1.7	9
2	6.7 ± 1.2	6	4.5 ± 0.5	8	4.8 ± 2.4	9
3	8.5 ± 1.3	4	8.3 ± 0.8	8	5.9 ± 1.2	9
4	10.3 ± 0.4	4	13.2 ± 4.5	14	11.7 ± 2.8	9
5	15.2 ± 1.5	6	14.4 ± 2.0	14	5.9 ± 3.3	20
6	20.9 ± 1.6	6	20.7 ± 2.1	13	20.2 ± 4.3	20
7	20.4 ± 1.6	6	20.5 ± 0.6	13	11.5 ± 2.6	20
8	22.7 ± 0.8	4	22.9 ± 2.8	9	23.6 ± 2.9	9
9	56.0 ± 3.4	4	56.0 ± 4.3	8	47.4 ± 11.2	9

Direct Testing of Decomposed Tuna Loin

In an effort to determine the suitability of using the dipstick test directly on the tuna, dipsticks were rubbed at various places on the outside of decomposed tuna loins and inserted into slits cut in the fish (one location/dipstick). The tuna loins had a dry pasty consistency and in both cases there was no color development. In one case the tuna loin was cut into chunks and the dipstick rubbed over the cut flesh with slight color development. Next tuna loins were slit at various places and the slits were filled with potassium phosphate buffer (0.1 M, pH 6.5). Dipsticks were then dipped into the buffer at various time intervals and increasing color development was observed. Chunks of tuna flesh were also placed in 5 volumes of buffer, incubated for 15 minutes, and the dipsticks were dipped into this solution with subsequent color development. In an effort to determine if the degree of decomposition was reflected by the color development, tuna loins with minimal, moderate, and advanced decomposition were tested by the addition of buffer to cut slits and then tested with the dipsticks because the fish proved too dry to obtain sufficient fluid for testing when rubbing the sticks directly on the fish. However, when chunks of tuna were incubated in phosphate buffer, or buffer was added to a crevice sliced in a loin and then subsequently tested with the dipstick, there was slow (approximately 30 minutes) blue-green color formation. Repeated measurements on a single small chunk gave consistent results but measurements taken on different chunks from the same fish did not. There appeared to be "hotspots" in the tuna loins where the histamine levels were 2-3 fold higher than in other areas of the same loin Thus it was impossible to determine a single value for the decomposed loin regardless of the degree of decomposition. These findings are similar to those reported by Lerke (1) who postulated that histamine formation is not uniform throughout the fish.

Future improvements to the current histamine assay should include improvements in enzyme preparation to increase assay sensitivity and stability, possibly producing DAO by recombinant techniques (11) or substituting a more stable histamine-oxidizing enzyme for such as the fungal amine oxidase used in a recently published histamine electrode

A more complete description of the histamine dipstick has been published (13).

It is probable that a similar protocol may be useful for producing dipsticks reactive solely with either putrescine or cadaverine. Preliminary results in our laboratory using partially purified bacterial putrescine oxidase in place of the diamine oxidase resulted in a stick that was reactive with putrescine, but not histamine or cadaverine.

ACKNOWLEDGEMENTS

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FIGURE 2

Linearity of the Dipstick Assay.

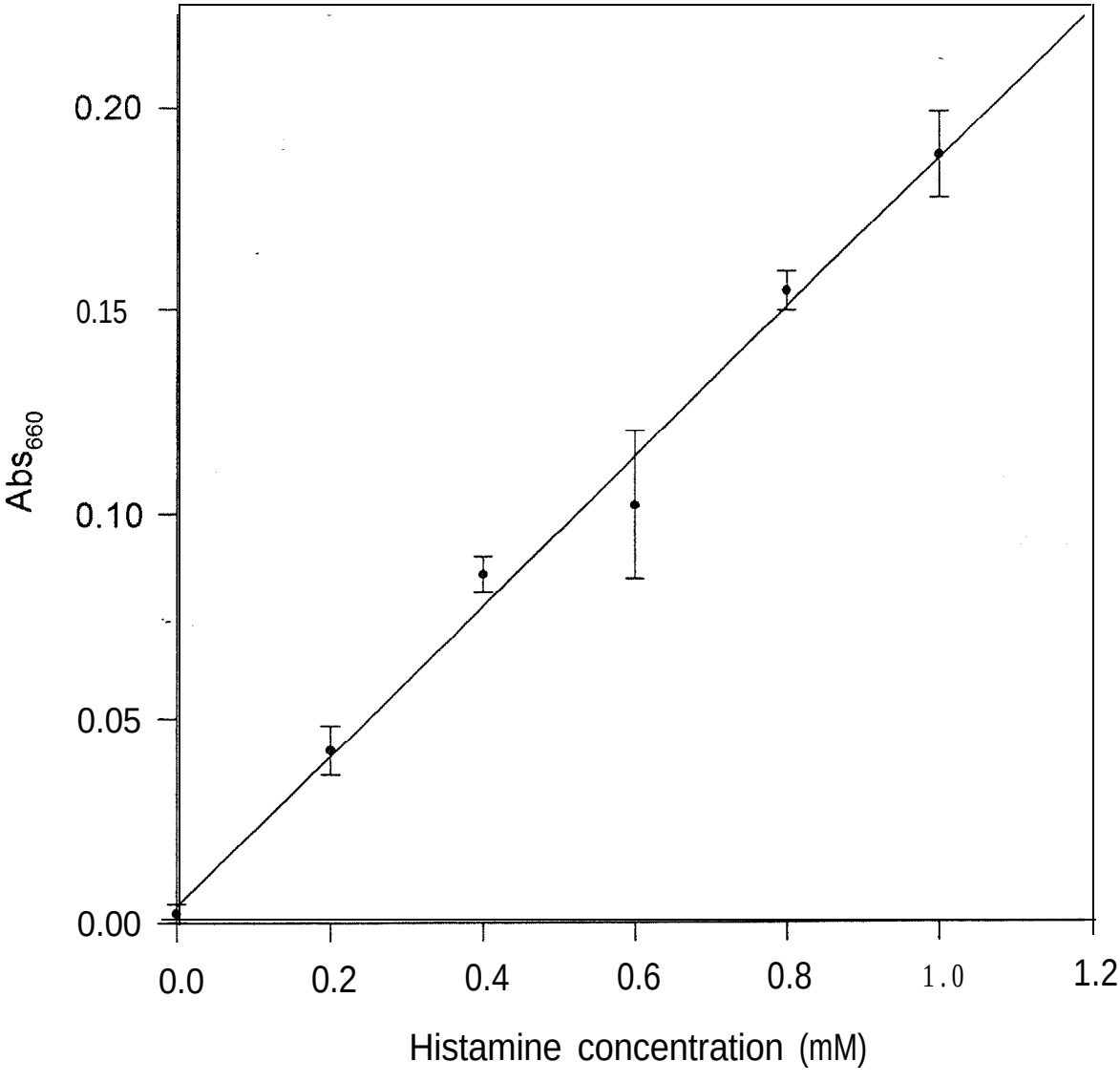


FIGURE 1

Time Course for Histamine in Buffer, Raw Tuna Extract, and Meat-Treated Tuna Extract.

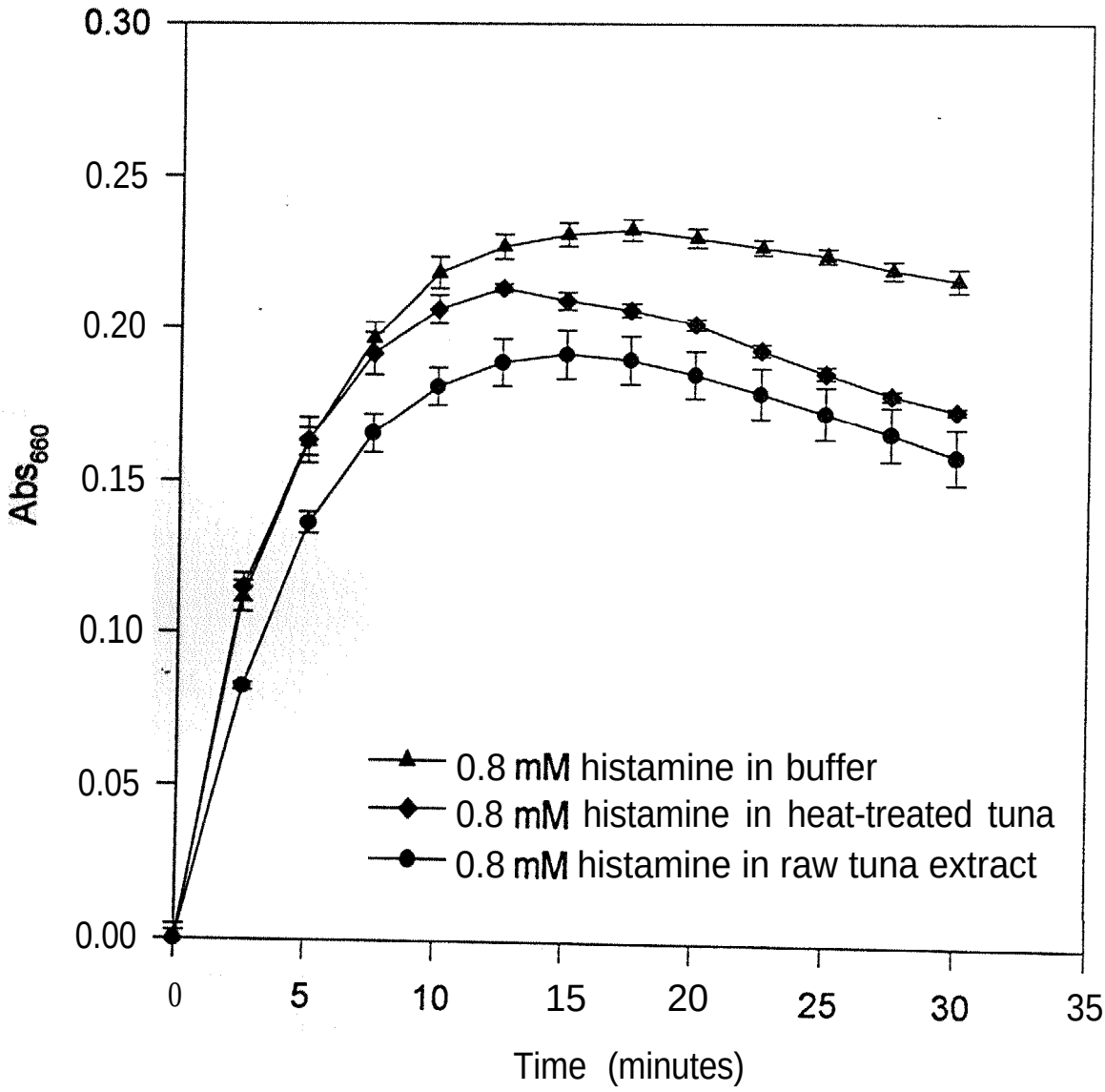
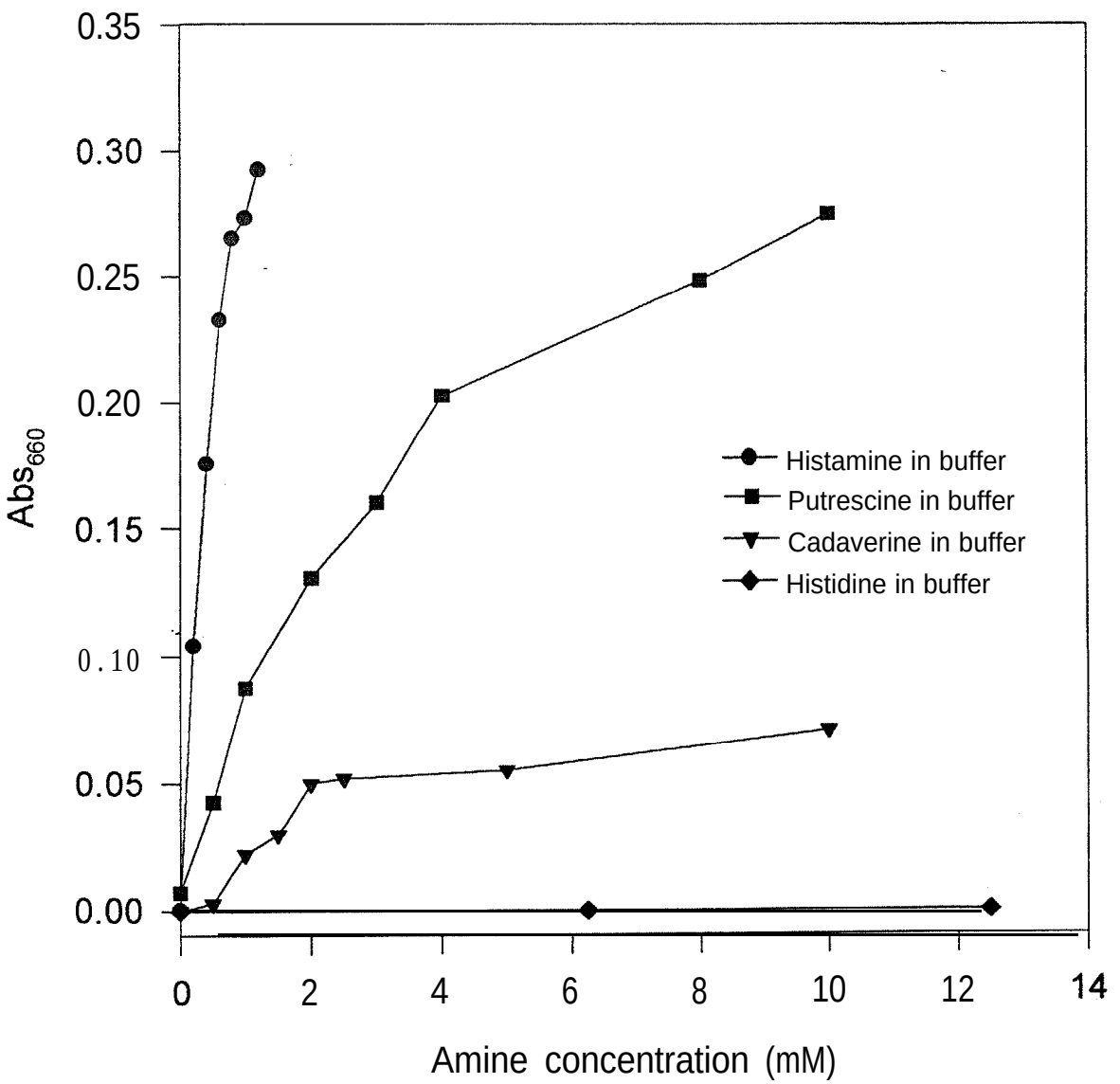


FIGURE 3

Comparison of Dipstick Test with Putrescine, Cadaverine, and Histidine with Histamine.



A Rapid, Easily Used Test Kit To Determine Histamine Concentrations in Fish

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BACKGROUND

Recently the U.S. Food and Drug Administration announced it was improving its histamine policy in the revised Compliance Policy Guide 7108.24 Decomposition and Histamine "Raw, Frozen Tuna and Mahi-Mahi; Canned Tuna; and Related Species" (Fed. Reg. v. 601149, August 3, 1995; pp. 39754-39756). In summary the FDA:

- lowered the Defect Action Level (DAL) to 50 ppm for decomposition.
- eliminated the requirement that findings of < 200 ppm had to be confirmed by organoleptic tests [i.e., histamine determination alone is sufficient].
- application of the revised DAL applies to raw and frozen tuna and mahi-mahi; and furthermore, on a case-by-case basis histamine levels ≥ 50 ppm to < 500 ppm may be used as evidence of decomposition in other species.
- the Action Level (AL) of 500 ppm will apply to species of fish that have been implicated in histamine poisoning outbreaks.

The FDA notice calls our attention to the fact that ". ..nonvolatile spoilage compounds such as histamine remain in the product (the fish) and can be determined reliably by chemical analysis." The most commonly used method for histamine determination is AOAC Official Method 977.13--the fluorometric method, which received Final Action approval in 1987. This method has three phases:

First - extraction of histamine from it matrix (fish). This phase consists of a methanol extraction of histamine from (blended) fish followed by heating (60 °C) and filtrations (Fig. 1).

Second - purification of the histamine from the "extract." This phase involves ion exchange chromatography whereby histamine passes through the column but interferences, such as histidine and other free amino acids are retained.

Third - detection of histamine in the column effluent. This phase involves adjusting the sample to alkaline pH, reaction with OPT (o-phthalic dicarboxaldehyde a.k.a. OPA), sample neutralization followed by fluorescent detection (λ ex = 350 nm and λ em = 444 nm) (Fig. 2).

Quantitative histamine concentrations are determined by comparing sample fluorescence values to a standard curve generated daily or more often. Standards are prepared in a fashion that they are subjected only to the detection phase of the method.

THE NEW "OLD" ASSAY

Our assay utilizes an AOAC approved chemical method (spectrophotometric) in a new, user-friendly format. The first phase, the extraction procedure is identical to the fluorometric AOAC method.

The second phase uses an anionic exchange resin to remove interfering substances only in a batch-wise fashion by means of a simple filtration device. The filtrate is pH adjusted by means of dilution and a 100 μL aliquot is added to a detector cup. Then, captured histamine is reacted with diazotized p-nitroaniline (previously activated by reacting sodium nitrite in a crushable ampoule within a tube of p-nitroaniline). The reflectance value of the reaction product (diazo dye), which is reddish colored, is measured in a simple reflectometer, the AgriMeter (Fig. 3, 4).

Table 1 shows the reflectance ranges for important levels of histamine including the Defect Action Level of 50 ppm.

Figure 5 shows a standard curve with error bars derived from values like those in Table 1.

Figure 6 is a "scattergram" comparing the fluorometric AOAC method values for 85 samples of canned and fresh frozen tuna to the ranges of the Alert™ for Histamine test with these same samples. Please note the single high sample (ca. 53 ppm) in the > 5 < 19 range. Upon examination, that particular sample was found to have a high salt content.

Figure 7 shows the effects of salt on the Alert assay; i.e. at > 2.0 % (w/w) salt a standard curve is changed markedly. However, canned tuna should have ≤ 1 .0% salt, a specification that is determined separately in Good Manufacturing Practices (QA) procedures.

Table 2 lists histamine levels in nine samples of fresh/frozen mahi-mahi as determined by the AOAC fluorometric method and the Alert for Histamine test both of which used standard curves. The classification column was the conclusion drawn by the technologist conducting the test. It should be noted that extractions with 75% methanol were used.

ACKNOWLEDGMENTS

We acknowledge with thanks the interests of Drs. Walter Staruszkiewicz, Patricia Rogers and **George Hoskin** of the FDA's Office of Seafood. In addition, we would like to acknowledge the support of the tuna canning industry which supplied samples of fresh/frozen and canned tuna for the **evaluation** of the Alert for Histamine test.

Fig. 1

AOAC Method of Extraction

Homogenize Sample (AOAC 937.07)

↓

Extract 10 g in
50 ml 100% MeOH in
Blender 2 min.

↓

Heat Sample to 60^0C
in H_2O Bath, Incubate
15 Mill.

|

Cool to 25^0 and
Dilute to 100 ml

|

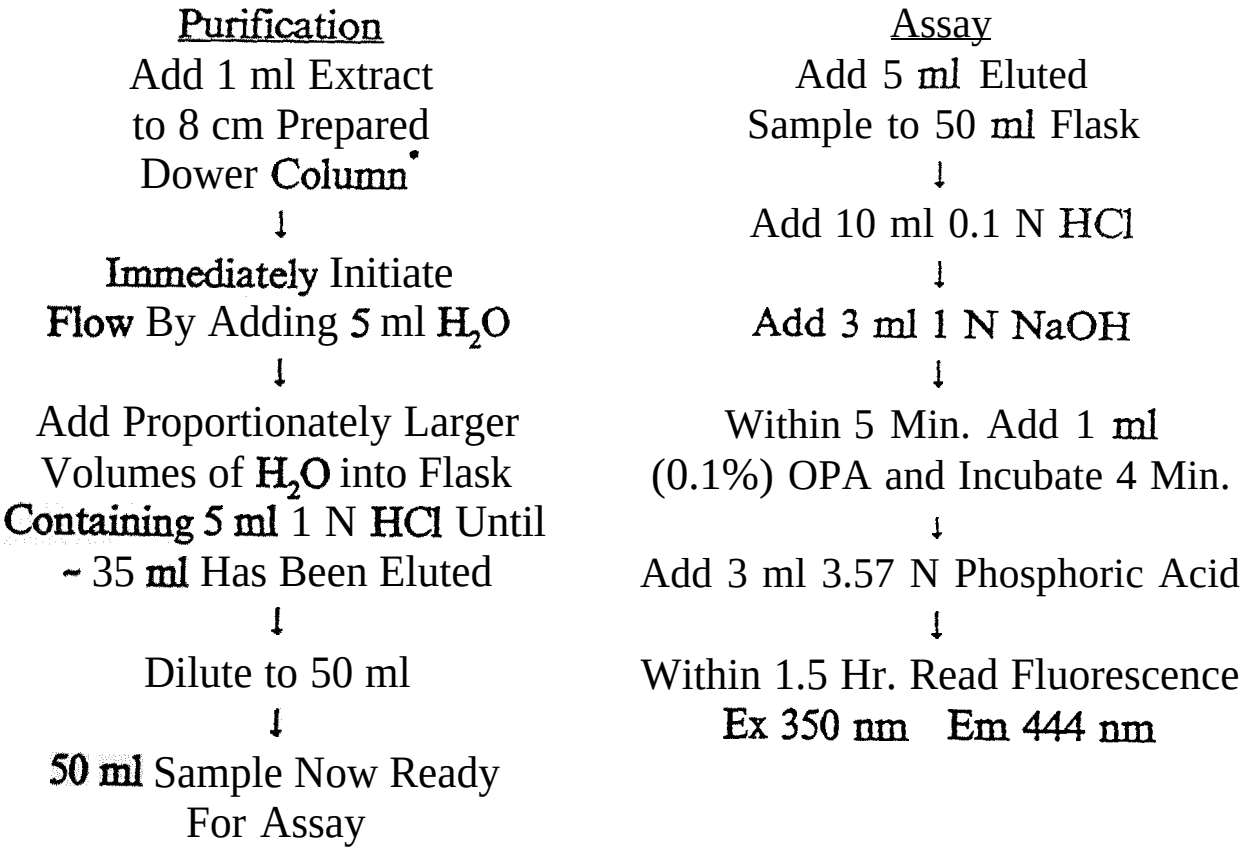
Filter Through Folded
Filter Paper

|

Sample Now Ready
For Purification

Fig. 2

AOAC Fluorometric Method



*Prepare columns before test by (preparing weekly):
1. treating with 1N NaOH for approximately 1 hour (15 ml/g resin);
2. washing extensively;
3. transferring resin to columns 8 cm in height;
4. washing column with 10 ml of H_2O prior to purification.

Fig. 3

ALERT® HISTAMINE ASSAY

Purification and Dilution
↓
Add 1 mL Filtered Extract to 0.5 g Dowex AG 1x8-OH, Mix ~ 15 Sec. 100-200 Mesh
↓
Using Serum Separator Push Extract Through Filter Separating From Dowex
↓
Transfer 0.5 mL Eluate to Clean Test Tube
↓
Add 0.20 mL Neutralization Buffer (0.05 M Phosphate 6.5 pH)
↓
Sample Now Ready For Testing

Assay
Add 0.1 mL Diluted Sample to Detector
↓
Squeeze on 2 Drops of Wash Buffer (0.05 M Phosphate 6.5 pH)
↓
Squeeze on 2 Drops of Recently Prepared Diazo Reagent[1]
↓
Squeeze on 1 Drop of 10% Carbonate 3.5% Borate Buffer
↓
Read Color with AgriMeter™

[1]The diazo reagent is made by reacting p-nitroaniline (0.1 g/100 mL 0.1 N HCl) with $NaNo_2$ (4 g/100 mL H_2O). This is done in a dropper bottle by crushing a glass ampoule containing the $NaNO_2$; allowing it to mix with the solution of p-nitroaniline. This activated reagent is immediately ready for use and is stable for up to 8 hours.

Fig. 4

HISTAMINE CAPTURE AND DETECTION

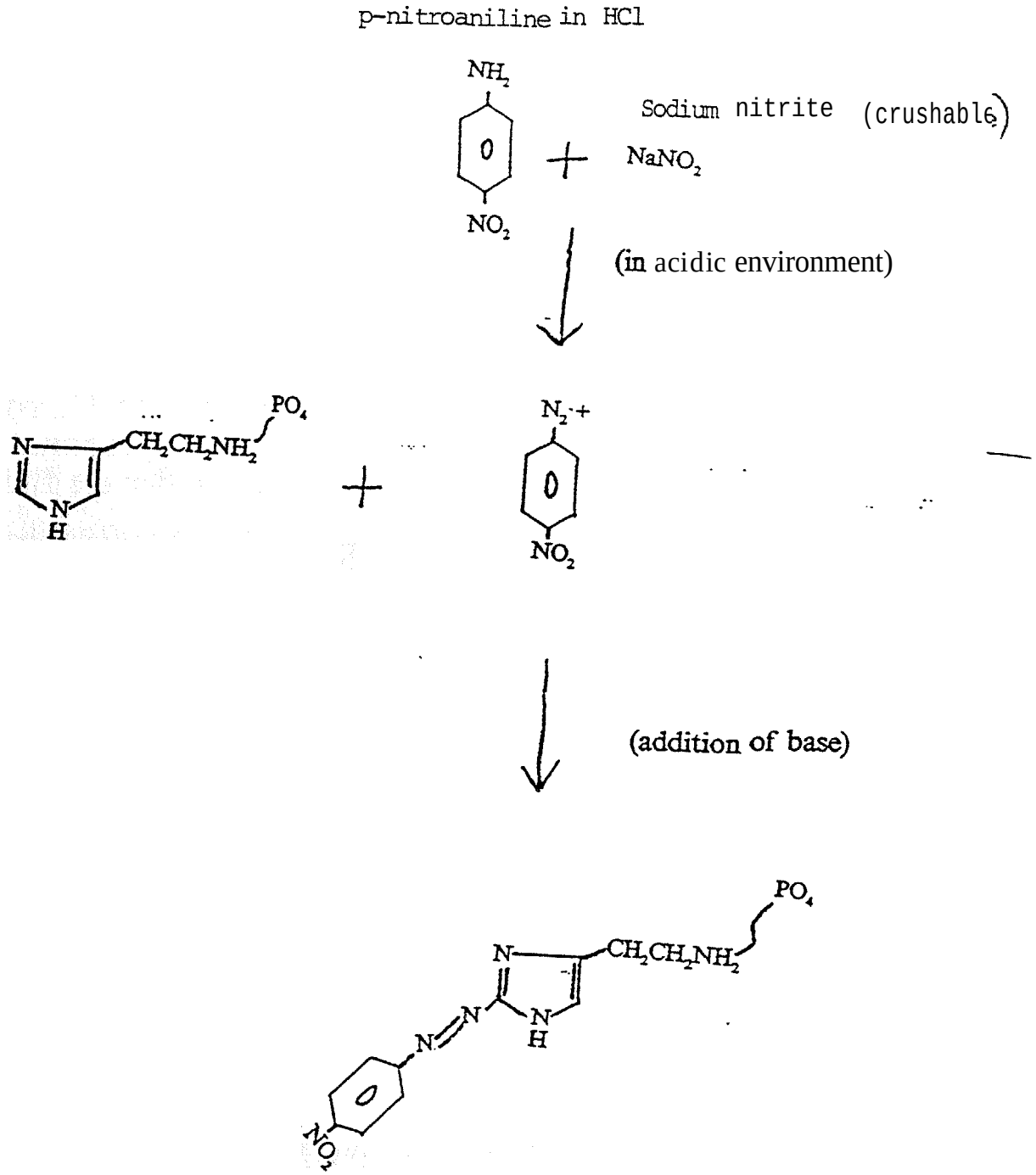


Table 1

Critical Control Levels of the Alert® for Histamine Test

Histamine Level (μg/mL)	Reflectance (Arbitrary Units) X ± 2 S.D.[1]	Range
0	515 ± 40	475 - 555
5	465 ± 40	425 - 505
20	365 ± 20	345 - 385
50	225 ± 35	190 -260

[1]Critical control levels were established using 10 runs/meter and 3 meters (n = 30).

Fig. 5

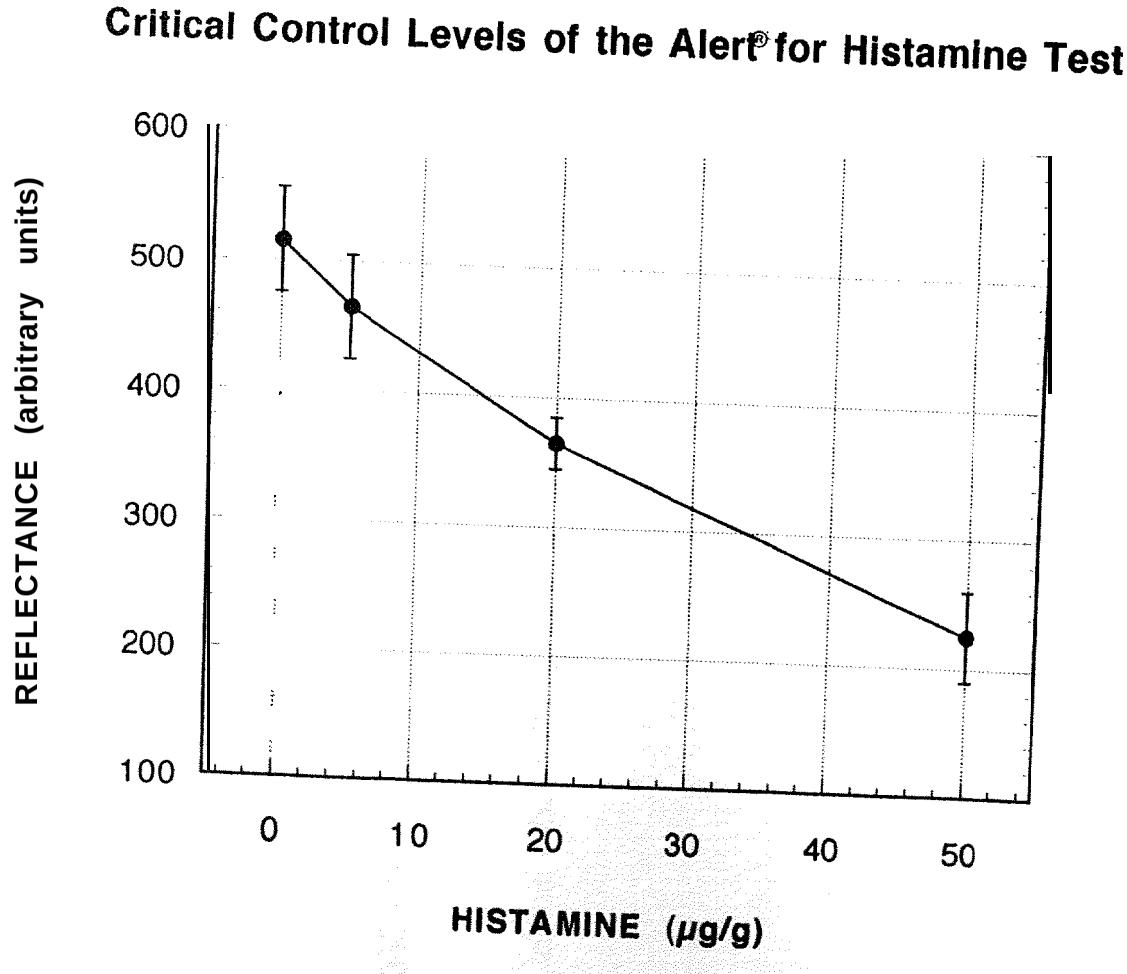
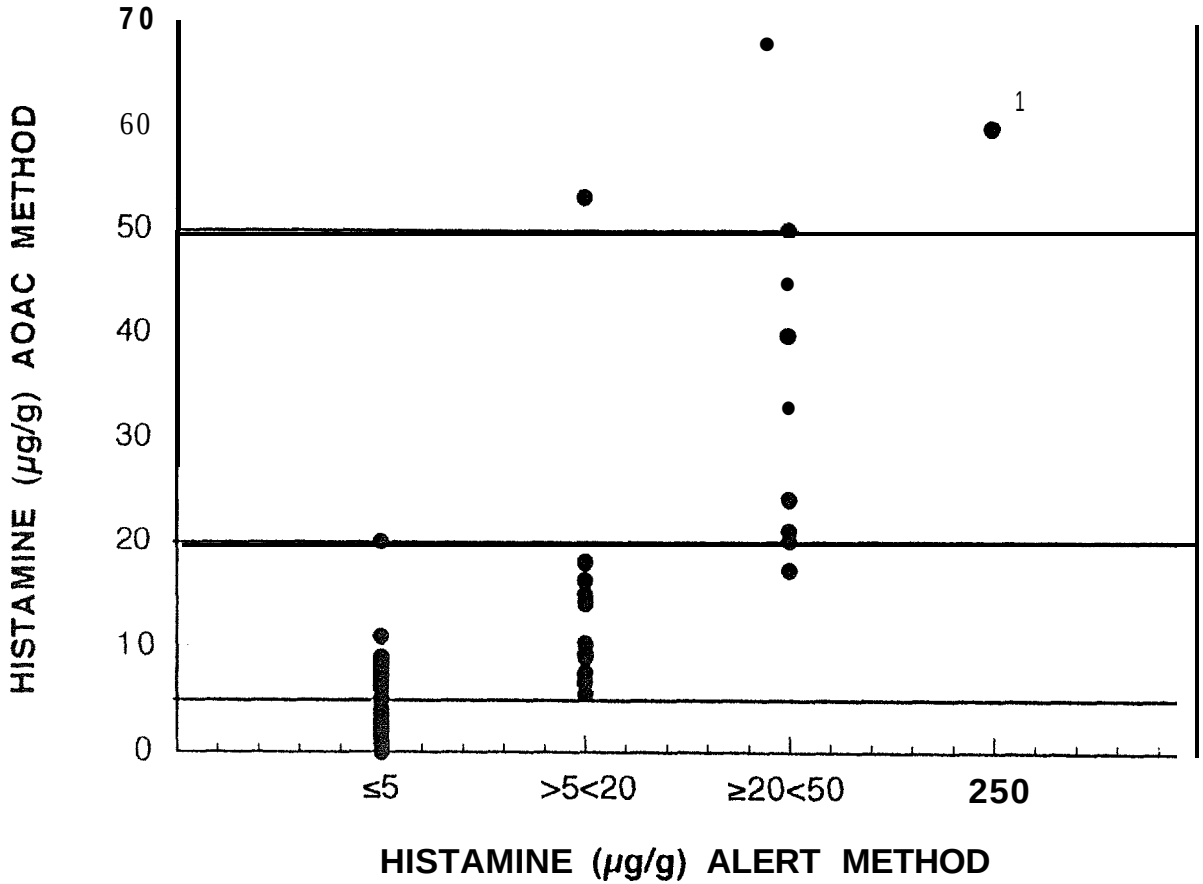

Critical Control Levels of the Alert® for Histamine Test
REFLECTANCE (arbitrary units)
600
500
400
300
200
100
0
10
20
30
40
50
HISTAMINE (µg/g)

Fig. 6

Comparison of the Alert for liistamine Test and the AOAC Fluorometric Method in Canned and Fresh Tuna



'Seven additional samples fell into this range that exceeded 70 μg/g.

Fig. 7

Salt Effects in the Alert^{R} for Histamine Test

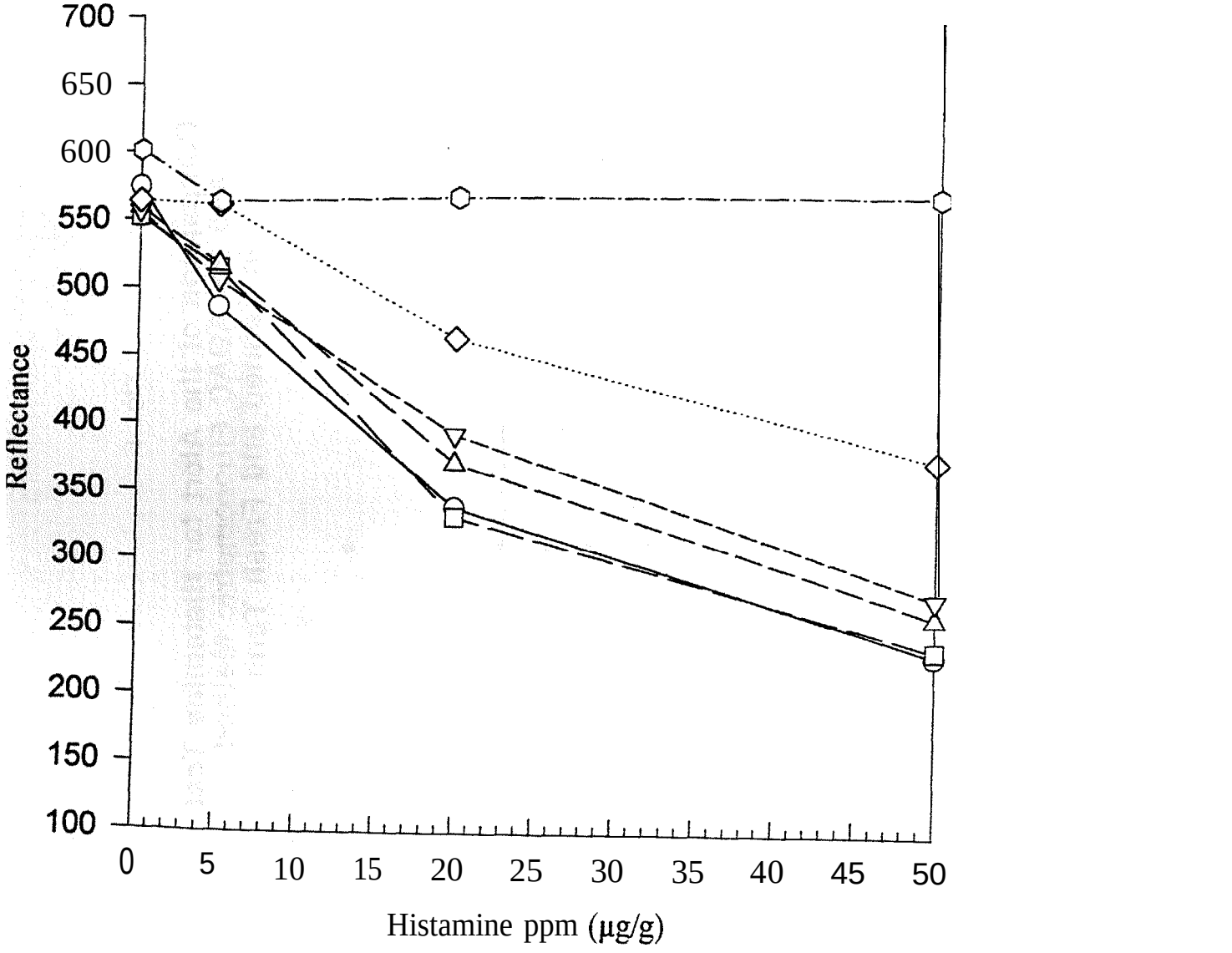


Table 2

Detection of Histamine Levels in Mahi Mahi using AOAC Fluorometric Method and the Alert® for Histamine Test: Prepared by FDA Office of Seafood

Fish #[1]	AOAC Fluorometric Method	Alert for Histamine[2]	Classification
1	24	37	P
6	106	> 50	F
7	1466	Hi (> 750)	Toxic
10	2	5	P
14	1138	Hi	Toxic
16	10	13	P
17	191	> 50	F
22	162	81	F
22-2	148	175	F

[1] Extracted in 75% MeOH.

[2] Levels determined using a standard curve in the Alert for Histamine Format.

IMMUNOLOGIC APPROACHES TO THE IDENTIFICATION OF FISH PROTEINS: TOWARD SPECIES-SPECIFIC "FIELD KITS" FOR STUDIES IN ECOLOGY AND SEAFOOD TECHNOLOGY

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Introduction The use of immunological methods to investigate basic problems in oceanographic research and marine or freshwater ecology provides sensitive and specific tools with wide versatility and applicability (I, 11,12). Polyclonal and monoclonal antibody probes can be labeled with radioisotopes, fluorochromes, enzymes, metals such as colloidal gold, and by biotin-avidin technology for immunoassays; the probes are detected microscopically, with automatic flow cytometry devices, by electrophoretic methods, scintillation counters, and automated microplate readers (6). Although formerly used almost exclusively in clinical and basic medical research, increasing numbers of investigators are adapting these methods for studies in both applied and theoretical marine biology, and also seafood technology.

In this report we have used immunological techniques to investigate predator-prey relationships with recently released red drum fingerlings *(Sciaenops ocellatus,* Linnaeus), and to distinguish early life history specimens of specific species of the family Lutjanidae. The immunological detection methods described here are also applicable for problems associated with identification of commercial seafood products and safety, and are valuable for preventing deliberate or unintentional substitution of inferior products for valuable fish species (4).

Immunologic methods to assess predation of hatchery-reared, juvenile red drum (*S. ocellatus*).

During the last seven years, the University of Miami has been part of a cooperative stock enhancement program with Florida International University (Miami, FL), and the Florida Department of Environmental Protection. The purpose of the program has been to test the feasibility

of reestablishing and enhancing red drum populations in Biscayne Bay, Florida, because of the virtual disappearance of the species from coastal waters in this area. Since 1988, hundreds of thousands of hatchery-reared red drum fingerlings have been released at mainly two sites, Richenbacker Causeway (Hobie Beach) in north-central Biscayne Bay, and Matheson Hammock, located on the mangrove-lined western shore of central Biscayne Bay[4].

Our role in the red drum stock enhancement program has been to investigate predation of newly released fingerlings. The program appeared to be ideal for predation studies because after each release, the red drum fingerlings remained inshore in shallow aquatic vegetation close to the release site for at least 10 hours, facilitating the capture with a center-bag seine of a number of potential predators attracted to the site.

Visual examination limited the identification of many of the specimens found in gut contents of the two major predators seined at both of the release sites, *Sphyraena barracuda* (Walbaum) (great barracuda) and *Strongylura notata* (Poey) (redfin needlefish). Many of the ingested fish were partially proteolysed and could not be identified as to the species. Therefore, we have used immunological methods to develop reagents for assays to detect red drum in the gut contents of predators.

Initially, a goat polyvalent antiserum was produced to soluble red drurn whole fish extract (anti-RDE serum) for use as the primary antiserum in Western blots. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out with soluble extracts of 12 different species of fish found in Biscayne Bay (Table 1), followed by Western blots with the anti-RDE serum. A red drum 80 kDa protein was selected that was not found in soluble extracts of the other fish, The protein was purified to homogeneity by sequential steps of solid ammonium sulfate fractionation and anionic and cationic exchange resins using the Fast Protein Liquid Chromatography system (FPLC) (11). The highly purified 80 kDa protein was a single chain polypeptide that reacted positively with periodic acid-Schiff reagent, indicating a glycoprotein. It was found in soluble extracts as two isoforms in small fish *(ca.* 1.5 cm.), but as a single protein from fish greater than 17 cm in length. The amino terminus of the 80 kDa protein was not blocked, and structural information is shown in Table 2. Further structural work for the purpose of developing assays of the protein (e.g., ELISA) was not carried out at this time. A goat antiserum was produced (anti-80 kDa gp) which was used as the primary antiserum in Western blots to test red drum fingerling extracts. Although both preimmune and immune goat sera reacted nonspecifically with extracts of many of the fish (e.g., silver jenny), adsorption of the antiserum with glutardehyde-insolubilized silver jenny extract resulted in an antiserum reactive only with the 80 kDa red drum protein and not with any proteins from the 12 species of fish and three invertebrate species selected for these studies (11).

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[4]Rickenbacker Causeway (Hobie Beach): [25°44'50" (Lat.); 80°13'20" (Long.)]; Matheson Hammock: [25°44'15"(Lat.)]; 80°15'20" (Long.)].

Table 1. Fish collected in Biscayne Bay, Florida, and tested with goat anti-red drum (*Sciaenops ocellatus)* extract serum. Soluble extracts of each fish did not contain a 80 kDa protein found in soluble extracts of red drum.

Name	Rank in Abundance*
Silver jenny (*Eucinostomus gula)*	1
Gulf toad fish (Opsanus beta)	2
Bluestriped grun *(Haemulon sciurus)*	4
Gray snapper (*Lutjanus griseus)*	5
White grunt (Haemulon plumieri)	6
Fringe filefish (Monacanthus ciliatus)	8
Tomtate (Haemulon aurolineatum)	14
Bronze cardinal fish (*Astrapogon alutus*)	16
Silver perch *(Bairdiella ch rysoura*)	20
Sea trout (Cynoscion nebulosus)	30
Spotfin mojarra (*Eucinostomus argenteus)*	not listed
Bar jack *(Caranx ruber)*	not listed

*RSMAS, rollerframe trawl, July-December, 1993, Biscayne Bay, Florida.

Table 2. The N-terminal sequence analysis of the red drum 80 kDa glycoprotein*.

Ala Pro Ala Asn Lys Val Glu/Lys Lys Ala Val/Pro Lys/Glu Ser Asn Gln Glu His Gln Lys Lys Met Asp Leu Ala Ala Lys

*Analyzed at the University of Florida, Dept. of Biochem & Mol. Biol. (ICBR Protein Chemistry, Core Laboratory), Gainesville, FL.

Red drum fingerlings were fed to both adult sea trout and bar jack in controlled laboratory feeding experiments. The red drum protein was detected in stomach contents by Western blots after 1 and 2 h, but was not found after 4 h using the anti-80 kDa gp IgG fraction as the primary antibody. Three breakdown products of the 80 kDa protein were detected in prey after 2 h using Western blots: 58, 37, and 28 kDa. In order to facilitate testing multiple samples of red drum soluble extracts, we also developed a highly sensitive and specific Enzyme-linked Immunosorbent Assay (ELISA) for the 80 kDa protein for an automated microplate reader (1).

Fish sampling was started the morning after the release of batches of several thousand red drum fingerlings into the sites on Biscayne Bay. Both newly released red drum fingerlings and indigenous predators were captured by seining and the predators were immediately frozen with dry ice to prevent proteolysis of englutted fish. Visual examination of the guts of S. *barracuda* and S. *notata in the* laboratory showed both identifiable and unidentifiable fish remains. Soluble extracts of the latter were tested in indirect ELISA, and from standardized linear plots of the average $O.D._{490}$ *versus the* 80 kDa red drum protein concentration, it was possible to detect ng quantities of the 80 kDa red drum protein in the fish extracts (1). Thus, it was possible to detect unrecognizable red drum fingerlings in gut contents of predators in nature by both Western blots and ELISA. Positive identification of the 80 kDa protein depended on the degree of decomposition in the gut, which was influenced by such factors as the species of predator, its feeding habits and metabolic rate, and the amount of time elapsed between ingestion of prey and capture of the predator. Since the predators are poikilothermic, the water temperature is also important for digestion rates.

Immunologic methods to assess early life history specimens of Family Lutjanidae.

The second major project is to produce immunologic reagents for the specific identification of eggs and early life history specimens of western Atlantic snappers (Family Lutjanidae). Specific species identification of early life history stages (especially eggs and young larvae) and the source of recruits of lutjanids are important research pursuits because 1) early life history stages are known for only a few species in their complex recruitment strategies, 2) overfishing occurs for several species, 3) habitat loss occurs because of pollution and development, and 4) they are of high commercial and recreational importance (8). Overfishing and habitat loss contribute to the unbalance of the fragile equilibrium of the reef community, and lutjanids constitute one of the major predators of coral grazing species. The innovative use of immunological methods may provide answers to identification problems for early life stages. These stages must be identified to species in order to understand recruitment mechanisms which determine adult population sizes. Currently eggs and early larvae cannot be identified because of morphological similarities. Only biochemical/immunological techniques offer promise to solve identification problems.

Based on unpublished data comparing the proteins in soluble extracts of different lutjanid species in SDS-PAGE in our laboratory, it may be possible to isolate a few species-specific proteins to produce polyclonal antisera for identification of individual life history stages of Lutjanidae. For example, soluble extracts of *Lutjanus griseus* (Linnaeus) contain a 66 kDa protein not detected in *L. vivanus* (Cuvier), *L. mahogoni* (Cuvier), *L. cyanopterus* (Cuvier), *Etelis oculatus* (Valenciennes) and a hybrid of *O. chrysurus x L. synagris = L. ambiguus.* In addition, *L. apodus* (Walbaum) soluble extracts contain at least one protein with a molecular weight greater than 66 kDa and two proteins less than 66 kDa that are not detected in *L. griseus.* Individual antisera to these proteins could be incorporated into portable, field-usable kits, such as a dotblot assay (3). However, there are 18 species of lutjanids in the western Atlantic distributed within five genera (9). The necessary time and costs would be prohibitive for purifying specific proteins from each of the 14 lutjanid species and one hybrid that are currently available to us, and for the animals required for immunization, antibody production, and testing.

The approach we have taken is to select a protein from SDS-PAGE gel profiles of soluble extracts of *L. griseus.* A 66 kDa protein was purified by FPLC utilizing successive steps of solid ammonium sulfate fractionation, cationic and anionic resins, and a molecular sieve gel. The highly purified *L. griseus* protein was injected into a adult female goat to produce a polyclonal antiserum that reacted with not only soluble extracts of *L. griseus,* but also reacted strongly with soluble extracts of *L. apodus* and *L. jocu* (Schneider). Additionally, the antiserum reacted weakly with soluble extracts of *L. buccanella* (Cuvier), O. *chrysurus* (Bloch), *Pristipomoides aquilonaris* (Goode & Bean), *L. synagris* (Linnaeus), *L. analis* (Cuvier), *Apsilus dentatus* (Guichenot), and *L. campechanus* (Poey) *in* Western blots. The anti-66 kDa antiserum reacted strongly with soluble extracts of oocytes, juveniles, and adults of *L. griseus* in Western blots. Most experiments have been with the 66 kDa protein of *L. griseus, L. apodus,* and *L. jocu.* In spite of the fact that the anti-66 kDa antiserum reacted strongly with the protein from the three lutjanid species in Western blots, evidence was obtained that each protein had both interspecies and species-specific determinants. For example, adsorption of the IgG fraction of the anti-66 kDa antiserum with glutaraldehyde-insolubilized *L. apodus* extract resulted in an antiserum that remained strongly reactive with *L. griseus extracts* but was weakly reactive with *L. apodus,* and negative with *L. jocu in* Western blots. In addition, the *L. griseus* protein was slightly heavier than the *L. apodus* and *L. jocu* proteins in SDS-PAGE and Western blots, although collectively the proteins from the three species are referred to as 66 kDa proteins. This information indicated that it will be possible to produce monoclonal antibodies using the 66 kDa protein, and the resulting antisera should selectively react with the three different species. Monoclonal antibodies have been used to identify a variety of other marine species (7, 5, 2). The next step is to produce immunofluorescent and immunoblot assays in order to identify specifically the eggs of each species in the plankton. Currently the 66 kDa protein from *L. griseus, L. apodus,* and *L. jocu* has been purified separately to homogeneity for comparative studies of the three proteins after amino acid sequencing. This will confirm the feasibility of monoclonal antibodies which are reactive with these three lutjanid species.

A dot-blot assay with the specific monoclonal antibody entails preparation of a diluted solution or suspension of a mixture of the life cycle form(s) of the lutjanid of interest. This is "dotted" onto nitrocellulose paper and incubated first with the specific mouse monoclonal antibody and second with a peroxidase-conjugated second antibody directed against the first mouse antibody. The peroxidase activity is assayed with H_2O_2 and 4-chloro-1 -naphthol color development reagent. A positive reaction is visualized as a colored dot against the white nitrocellulose paper, and a negative reaction is colorless. The protein-dotted filter may be stored dry for several weeks without any loss of activity. Thus, specimens collected from many different sites could be stored and screened at the same time. Positive reactions are confirmed later in the laboratory on field- collected specimens using immunofluorescence in which the primary antibody is conjugated with a fluorochrome such as fluorescein and the bound antibody is visualized with a fluorescence microscope. A second procedure is to further test the diluted solution or suspension in an ELISA (1). Sarver *et al.* (10) has used the polymerase chain reaction to amplify portions of two mitochondrial genes to examine phylogenetic relationships of species of the Family Lutjanidae, but the-relationships of some of the members are still unclear.

In summary, immunological methods were employed for two major research projects currently under investigation in our laboratories: 1) predation of hatchery-reared, juvenile red drum (*S. ocellatus)* following a program of reestablishing and enhancing the populations in Biscayne Bay, Florida, and 2) specific species identification of early life history stages (eggs and larvae) and the source of recruits of western Atlantic snappers (family Lutjanidae). After the development of immunoassays for these studies, the specificity and sensitivity of the methods have resulted in significant progress.

Immunological detection methods have also been valuable for many aspects of seafood science. Recently, Huang *et al* (4) distinguished the commercially valued red snapper (L. *campechanus)* from less valuable substitutes using ELISA with two monoclonal antibodies raised to a red snapper protein. To ensure quality and safety of seafood products, immunology offers powerful methods for these goals.

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HOW CONSUMER EDUCATION AFFECTS ATTITUDES TOWARD FOOD IRRADIATION

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A major obstacle facing the food irradiation industry is the lack of understanding by consumers as to the purpose for irradiation processing of perishable food items. Consumers have been exposed to much misinformation as to the safety and wholesomeness of irradiated food products. Research work over the past 50 years has proven low dose gamma irradiation(<10 kGY) to be effective in reducing pathogenic bacteria and extending shelf-life of such perishable food items as seafood, spices, fruits, vegetables, meat, and poultry. Extensive studies have shown that low dose gamma irradiation does not alter the wholesomeness of fresh products any more than thermal processing. Another misconception is that food producers will try to use irradiation to extend shelf-life of partially spoiled foods. This is exactly opposite of the irradiation processors purpose, in that they only submit the freshest products to irradiation. Another misconception is that irradiated products become radioactive. The effective gamma rays emitted from radiation sources in fact are much the same as X-rays. The rays pass through the food and dissipate into harmless lower energy particles. The purpose of this study was to determine the effect of food irradiation education on attitudes of a group of teens and young adults.

MATERIALS AND METHODS

Students, aged 16-24, were evaluated for their attitudes about food irradiation. Students aged 16- 17 were from a local public parish high school enrolled in second semester chemistry. Students aged 18-24 were University students, non food science majors enrolled in either basic Food Science or Food and Drug Law course.

Students were given an attitudinal PreTest in which they were asked general questions about the purpose and safety of food irradiation. After a period of 2-3 weeks, students were presented a general lecture and video, prepared by the Purdue Cooperative Extension Service, (1994). In this lecture, students were given general information about all food processes and where irradiation processing fit into the overall picture of food processing. The video also in picture form presented the general topic of food irradiation, showing how an irradiation plant is designed and what

irradiated foods look like. After the lecture and video, the students were given the same attitudinal test (PostTest).

Thirteen attitudinal questions were scored as Strongly Agree, Agree, Uncertain, Disagree, Strongly Disagree. One question on whether they would buy irradiated food products was scored as Yes or No. Analysis of mean variance were evaluated by SAS, 1987.

RESULTS AND DISCUSSION

Attitudinal questions follow with the pre and post test results. In all instances the mean attitudes about the benefit, safety, and wholesomeness of irradiated foods showed significant improvement ($p<0.01$). Students were less concerned about the potential of irradiated foods causing cancer (74% to 27 %); 84% said they would consider buying irradiated foods if available, up from 47% on pretest, and attitudes about the ability of irradiation to reduce the threat of foodborne illness were improved from 3 1% to 75%.

Evaluation of older consumers is currently under study to determine if consumer education, in general, will alter attitudes and affect buying behaviors.

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BIGHEAD CARP: ASSESSMENT OF TWO POTENTIAL CANNED PRODUCTS

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INTRODUCTION

The catfish, bait minnow and Chinese carp industries in the U.S. were all born in Arkansas. Arkansas has over 65,000 acres of water devoted to aquaculture production (McNulty, 1996). Arkansas today trails only Mississippi in acreage and sales of catfish and leads the nation in bait minnow production and Chinese-carp-hatchery production (USDA, 1995). Commercial fish farming has a significant impact on the economy of the state and surrounding Delta region and has a potential for growth that far exceeds its present economic importance.

Alternative fish products and alternative species would allow Delta fish farmers to diversify production in their ponds as a risk management strategy. However, little information is available on the marketing potential of alternative species such as bighead carp (Hypophthalmichthys nobilis). Carp, as a group, make up the greatest production of fish by weight from worldwide aquaculture (Billard and Perchec, 1993) with bighead carp ranking third in total aquaculture output (Engle, 1992). Bighead carp have been raised in Arkansas since the 1970's as a polyculture species in efforts to improve water quality in catfish ponds. Although other countries have established markets for this freshwater fish, bighead carp have not been marketed on a wide scale in the U.S. Arkansas fish farmers have sold bighead for resale in Asian ethnic markets as a live product. This market is easily saturated resulting in wide fluctuations in supply and price. Therefore, a higher-volume market outlet, such as a cannery, would provide stability and a constant market for bighead. Consumer acceptance of potential, canned bighead carp product forms is relatively unknown.

Regardless that the U.S. has not been a very sophisticated marketplace for canned fish products, with the exception of specific ethnic consumer demands, there may be room in the marketplace for new canned products made from non-traditional fish species (Regenstein and Regenstein, 199 1). Despite the emergence of frozen, convenience products and the more ready availability of fresh seafood, canned seafood is still consumed by more households than any other fish product form (Thomas and Engle, 1995). The most popular canned fish in the U.S. are salmon, tuna, and sardines in oil or water packs (Regenstein and Regenstein, 1991). In 1987, the U.S. imported 10.8 million cases of (105,000 tons) of canned tuna (Parks et al., 1990).

silver carp (Hypophthalmichthys molitrix), closely related to bighead carp, was evaluated in the fresh and canned form. In market studies conducted at Auburn University, silver carp marketed as a fresh product was not well accepted (Crawford et al., 1978; Engle, 1978). Engle (1978) reported that only 14% of people who purchased silver carp would repurchase it. The foremost reason for consumer dislike of this fish was the abundance of small intramuscular bones. However, silver carp were more accepted by consumers when canned (Woodruff, 1978).

Early market studies indicated that fresh bighead carp was readily accepted for its taste, but, as with silver carp, the fresh bighead was deemed too bony (Engle, 1992). Since canning softens bones, a canned bighead product was evaluated in a limited 1992 study by researchers at UAPB (Thomas and Engle, 1995). They assessed the attitudes of 471 Arkansas consumers and concluded the canned product had market potential. Respondents to the informal product evaluations suggested that the low-fat content of the product and its taste appeal were positive attributes (Engle and Kouka, 1995).

The primary god of this on-going study is the development and evaluation of a value-added, canned fish product from the alternative freshwater fish, bighead carp. The specific objective of this phase of the study was to determine sensory quality, acceptability and market potential of two products prepared from either steam or oven cooked carp prior to canning.

MATERIAL AND METHODS

Carp

Six hundred, seventeen kg of bighead carp ranging in size from 3.18 to 4.99 kg each were harvested in November, 1994. The carp had been grown for approximately a year in polyculture with channel catfish in experimental ponds at the University of Arkansas at Pine Bluff, Pine Bluff, AR. The carp were immediately transported alive to a nearby fish-dressing area where they were stunned, beheaded, eviscerated, skinned and fins and tails removed to yield primal loin cuts. The loins were washed to remove extraneous materials and packed in ice for transport to the University of Arkansas Food Science Department in Fayetteville, AR, for processing into hybrid-type canned products having properties that fall between those of a totally boneless product, like tuna, and those of a fully boned product, like salmon.

Precooking Treatments and Flesh Removal

For Purposes of this study, the term "precook" means heated/cooked by some means prior to flesh removal and can filling for subsequent thermal processing (retorting). Approximately 106 kg of loins (approximately 2.2 kg ea) were removed from the ice and divided into two equal lots representing the following precooking treatments: steam-cooked and oven-cooked. For both precooking treatments, loins were placed belly-side down on perforated stainless steel trays and heated either in an atmospheric steam cabinet for 30 min or convection oven at 177°C for 60 min. The duration of heating for the two precooking methods was determined as the amount time required for approximately 85% of the loins to reach a backbone temperature of 77°C. After precooking, loins were placed in a walk-in cooler at 5°C for 7 hr to firm the muscle tissue. After

firming, the flesh was separated into halves from both sides of the backbone. The portions were again divided horizontally and the red meat removed completely. The loins were "polished" by scrapping off the remaining flesh from the ribs and the backbone. Edible flesh was removed from ribs and backbones in chunks as large as possible. Weights of the various meat and waste portions from each treatment were determined to enable yield calculations.

Thermal Processing

Three hundred, thirty grams of meat was packed into 300 X 407 C-enamel cans. The cans were filled with a hot (93°C) 2% NaCl brine, exhausted for 5 min in a steam tunnel and then immediately seamed with a commercial can closer. Two retort cooks were carried out - one for each of the two precook treatments. Approximately 40 cans from each treatment were thermally processed in a stationary retort. Heat penetration tests were conducted in duplicate for each product according to the procedure described by Kattan et al. (1989). All cans were processed at 121.8°C for 85 min (F_o values 30 to 45).

Proximate Comnosition

The contents of the canned samples were drained through a 0.60 mm stainless steel sieve for 10 min. Raw loin chunks (approximately 20 g ea) from several loins and drained samples were homogenized in a blender and then analyzed for moisture, protein (%N X 6.25), crude lipid, and ash following standard AOAC methods for meat analyses.

Consumer Perception Testing

Sample preparation. The contents of the canned samples were drained for 2 mm on a U.S. No. 8 sieve. Samples were separated gently with a fork to provide pieces of similar size for presentation to panelists. Portions of approximately 60 g each were placed into cups for presentation to the panelists.

Consumer trials. The two products were evaluated by 90 consumers, all of whom had indicated that they like salmon and tuna-style canned fish products. Sensory and acceptance testing was conducted by the Sensory Analysis Group, Food Science Department, University of Arkansas. Panelists were recruited through parent-teacher organizations in the northwest portion of Arkansas. Prior to evaluation, the ballot and the evaluation procedures were discussed with each panelist to familiarize them with the evaluation process. To avoid any bias toward carp, panelists were not told they would be evaluating bighead carp. Panelists were only told the product was a freshwater fish with commercial potential. Each panelist was then seated in a sensory booth or an individually partitioned area to evaluate the products. The products were presented together in balanced random order and evaluated under GE Chroma 50 lamps that provide natural daylight illumination. The samples were served at room temperature. After the evaluations were completed, panelists were asked to return to the lobby area of the facility until their ballots could be checked to ensure all questions had been answered.

Sensory Analysis. Sensory data were analyzed as described in Mailgaard, et al. (199 1). Analyses of Variance were performed for attribute liking rated on 7-point hedonic scales for appearance, overall liking, and flavor.

Acceptability and Marketing Attitudes. Panelists were asked to compare the carp products with similar canned fish products and rank them as better than, equal to, or not as good as the other products. Purchase intentions were estimated by asking open-ended questions related to willingness to pay as much as for the carp as other similar products.

Attribute Diagnostics. To determine product attribute direction for change, frequency distributions were calculated for 5-point Just Right scales for color, aroma, salt, firmness and moistness (Mailgaard, et al., 1991). Categories of not enough and too much were compared to determine direction for change.

RESULTS AND DISCUSSION

Proximate Analysis

The proximate composition of raw carp loins and the canned products are presented in Table 1. Heating causes various physical and chemical changes in canned fish products (Lazos, 1995). There was a decrease in moisture after canning which probably could be attributed to the moisture loss experienced during the precooking step as well as to protein denaturation during thermal processing (Table 1). The precooking treatment affected the water content of the canned products with moisture loss being more profound in the oven-cooked samples compared to the steam-cooked samples. During precooking, loins lost approximately 20% weight in the dry conditions of the oven compared to losing approximately 13% in the wet environment of the steam cabinet (data not shown). Obviously, the meat rehydrated, to some extent, in the can since moisture contents of the two canned products didn't vary more than 4.5% (Table 1). Percent lipid, protein and ash of the canned carp varied little from the raw carp. Protein and lipid contents were slightly greater in the two canned products which most likely can be attributed to moisture loss during the precooking step prior to canning.

Table 1. Proximate compostion of raw and canned carp.

	Percent			
Carp Form	H_2O	Protein	Lipid	Ash
Raw	83.0	16.5	0.7	1.7
Oven-Baked	78.6	16.9	ct.8	1.7
Steam-Baked	80.9	16.6	0.7	1.7

Liking/Acceptability

Hedonic sensory scores, with the percentage of panelists who rated the canned carp as like very much or like moderately, for appearance, flavor and overall liking are presented in Table 2. Method of precook significantly affected the hedonic scoring of the two products, but overall acceptability was extremely good for both canned carp products. The worst-case, average hedonic score for either product was only 3.08 (7=dislike very much) for the oven-cooked product category of appearance. Panelists consistently preferred steam-cooked carp over the oven-cooked carp. Sixty-four percent of panelists judged the appearance of the steam-cooked carp superior to the oven-baked carp (Table 2). Likewise, over 50% of panelists consistently ranked the

Table 2. Liking/Acceptability - Mean hedonic scores[C] and percentages ( ) of panelists who rated canned bighead carp as like very much or like moderately.

Precook	Appearance	Flavor	Overall Liking
Oven	3.08[a] (43%)	3.02[a] (47%)	3.07[a] (48%)
Steam	2.47[b] (64%)	2.47[b] (58%)	2.52[b] (54%)

[a-b]Means within columns followed by the same superscript are not different (P>0.05) by LSD test.
[C]Hedonic scale, 1 =like very much; 7=dislike very much.

steam-cooked carp as like very much or like moderately for flavor and overall liking compared to the oven-cooked products. Even though steam-cooked carp was more preferred, Table 2 reveals that the oven-cooked carp also was well liked since it ranked only 6 percentage points below steam-cooked carp for the attribute overall liking.

Comparisons to Other Canned Fish Products

Panelists were asked to compare the two carp products with canned mackerel, salmon and tuna. As shown in Table 3, panelists indicated that the bighead carp compared most favorably with tuna and least favorably with mackerel. Over 60% of panelists indicated that either carp product was better than or equal to tuna compared to 45 and 38 % for salmon when compared to steam and oven-cooked carp, respectively. The low comparability for mackerel may not be meaningful since 77% of the panelists indicated that they were not familiar (don't know) with canned mackerel. Steam-cooked carp garnered higher "better than or equal to" scores compared to oven-cooked carp when compared to either salmon or tuna. Exit interviews with panelists provided insight into why many felt that the carp did not compare favorably with salmon. The carp products, it seems, were deemed too bland or too mild for valid comparison with salmon. Panelists expected a salmon-type product to have strong, fishy odors and flavors.

Table 3. Percentage of panelists who indicated that canned bighead carp was better than or equal to other products - Comparison of precooking treatments.

Comparison Product and Precook	Better Than or Equal to	Not as Good	Don't Know
Mackerel			
Oven	20	3	77
Steam	18	4	77
Salmon			
Oven	38	39	24
steam	45	32	24
Tuna			
Oven	62	38	0
steam	67	33	0

Purchase Intentions

Attitudes about products obviously dictate purchase intentions. For similar-sized cans, panelists were asked whether they would be willing to pay as much for the bighead carp as other canned fish products. Responses to such bottom-line type questions provide insight into overall acceptance and attitude. Willingness to pay as much for a new product as traditional canned products is viewed as an overall positive for the product concept. Table 4 presents the percentages of panelists willing to pay as much for the two canned carp products as canned mackerel, salmon and tuna. Fifty-two percent indicated they would not pay as much for the oven-cooked carp as mackerel while 55% would pay as much as mackerel for the steam-cooked carp (Table 4). It should be noted that only 50 panelists answered the mackerel section of the willingness to buy questionnaire. With that and the fact that 70% of the panelists indicated they were unfamiliar with mackerel (Table 3), any comparisons with mackerel may not be valid. Obviously, some panelists unfamiliar with mackerel chose to guess rather than base their answer on experience. Comparisons with salmon and tuna should be more meaningful since 76% and 100% of panelists were familiar with salmon and tuna, respectively (Table 3). As Table 4 presents, 58 and 54% were not willing to pay as much for oven or steam-cooked carp as salmon, respectively. The strongest response on willingness to pay was the comparison with tuna. Sixty-three and 67% said they would pay as much for oven or steam-cooked carp as tuna, respectively. These results are encouraging since tuna is the highest-volume canned fish product sold in the U.S. Table 4 data indicate there may be market potential for either of the canned carp products if either could be priced competively with canned tuna.

Table 4. Percentage of panelists willing (unwilling) to pay as much for canned bighead carp as for other canned fish products- Comparison of carp precooking treatments.

Comparison Product and Precook	Yes	No
Mackerel		
Oven	48	52
Steam	55	45
Salmon		
Oven	42	58
Steam	46	54
Tuna		
Oven	63	37
Steam	67	33

Directional Attribute Diagnostics

Because canned bighead carp likely would be in direct competition with canned tuna, a well liked product, it's important that the potential "gold standard" carp product has the quality and sensory attributes most desired by consumers. Improvements to the carp products must be based on the reasons panelists may have preferred or rejected certain sensory attributes. Therefore, attitudes about product attributes were determined using just right scales as described by Meilgaard et al. (199 1). Just right scales allow assessment of the intensity of a particular attribute relative to some mental criterion of the panelist's ideal for that attribute.

Panel responses to the just right scales as applied to the attributes, color, aroma, salt level and firmness are presented in Table 5. Responses were mixed between the two carp products with respect to suggested directions for change for the attribute color.

Table 5. Percent response of panelists in each category for color, aroma, salt level and firmness - Comparison of carp precooking treatments.

Attribute and Precook	Much too Little	Somewhat too Little	Just Right	Somewhat too Much	Much too Much
Color					
Oven	2	11	54	31	1
Steam	10	21	67	1	1
Aroma					
Oven	1	11	51	30	7
Steam	3	16	69	10	2
Saltiness					
Oven	13	29	44	12	1
Steam	22	32	42	3	0
Flavor					
Oven	4	24	53	14	3
Steam	14	28	56	2	0

"just right scale used, too light to too dark
'just right scale used, too weak to too strong
'just right scale used, not salty to too salty
'just right scale used, too soft to too firm

Fifty-four percent of the panelists indicated the oven-cooked carp was just right in color intensity while 67% felt the steam-cooked carp was just right in color. Interestingly, panelists felt the oven-cooked carp was too dark (3 1% somewhat too dark and 1% much too dark) and the steam-cooked too light (2 1% somewhat too light and 10% much too light). Table 5 presents that panelists believed the oven-cooked carp smelled somewhat too strong (30%) while a majority of panelists deemed the steam-cooked carp just right (69%) to somewhat too weak (16%) in odor. Directional responses further indicate that panelists would have preferred saltier and firmer products regardless of cooking method (Table 5).

CONCLUSIONS

Canned bighead carp appears to have potential as a canned, freshwater fish product. Overall consumer acceptance of the product concept was positive. Canned bighead carp has several desirable characteristics which include a low fat content (<1%), a very mild taste and a white

appearance similar to albacore (white) tuna. Acceptance varied with method of precook. Sensory scores indicated that the steam-cooked carp were consistently preferred over the oven-cooked carp. Sixty-four percent of panelists judged the appearance of the steam-cooked carp superior to the oven-baked carp. Likewise, over 50% of panelists consistently ranked the steam-cooked carp as like very much or like moderately for flavor and overall liking compared to the oven-cooked products. Over 60% indicated that either canned carp product (steam-cooked or oven-cooked) was better than or equal to canned tuna. Likewise, over 60% of panelists indicated a willingness to pay as much for either carp product as canned tuna. Directional responses using just right scales demonstrated that steam-cooking produced the most desirable color and aroma, but panelists indicated both products should have more salt and be firmer. Further product development efforts will focus on increasing product firmness, evaluating a "smoked" line extension, determining processing costs and conducting break-even analyses.

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PROPERTIES OF SURIMI MADE FROM TILAPIA

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INTRODUCTION

Surimi is a Japanese Term for mechanically deboned fish meat washed with water and mixed with cryoprotectants. Washing step removes water-soluble proteins, fats and undesirable materials such as blood, pigments, and odorous substances, enzymes, and trimethylamine oxide (TMAO). It increases the concentration of myofibrilla proteins (actomyosin), which improve gel-forming ability and elasticity (Lee, 1984; Okada, 1985).

Surimi is an intermediate product for further production of fabricated seafood products such as imitation crab legs, scallops, and shrimp. It can be substituted for a variety of traditional animal and vegetable proteins (Lee, 1984; Wu, 1992). The overall amino acid score of surimi is similar to those of beef and turkey (AFDF, 1987).

At present time, 90% surimi is made from Alaska pollack with small amounts made from croakers and sharks (Piggot, 1986). Little efforts have been made to produce surimi from freshwater species. Tilapia is the most important freshwater aquaculture species worldwide. In Egypt, this fish is abundant and low market value. Consumption of fish in Egypt is not very high. Processed fish products are very limited. To use these species, the objectives of this study were (1) to determine the yield and chemical quality and (2) to exam the functional properties of surimi made from tilapia.

MATERIALS AND METHODS

Preparation of tilapia surimi

Forty-five kg of life tilapia *(Oreochromis niloticus),* averaging 30.85 cm in length and 4564.00g in weight, were obtained from Dekalb Farmers Market, Atlanta, Georgia. Fish were removed from a water tank and filleted. Both fillets and frames were packed on ice and transported to Department of Food Science and Technology, University of Georgia, Athens, GA. Surimi was prepared from fillets and frames using the method of Park et al.(1990) with some modification (Figs. 1 and 3). Minced meat was recovered from fillets and frames using a deboner (Yanagiya Machinery Works, Ltd. Japan) with a 4-mm hole drum. The minced fish were immediately washed four times

in a stainless steel tank (50L) with iced water at a ratio of 1 part flesh to 3 parts water (w/w). Water was used for the fourth wash cycle contained 0.15% (w/w) NaCl to facilitate de-watering .Hand whipper was used to stir the slurry for 5 min and excess water was removed between washes using cheesecloth. A basket centrifuge (Wastem States, Hamilton,OH) was used for the final de-watering to the extent that water was no longer visibly being extracted from the washed mince. Raw surimi was either directly vacuum-packaged or chopped with cryoprotectants (4% sucrose, 4% sorbitol and 0.25% sodium tripolyphosphate, STPP) for 2 min using a Hobart silent cutter and then vacuum-packaged. Oxygen barrier-bags (Surlyn, oxygen transmission rate = 5cc/m^2/24hr/atm, at 4.4 °C and 0% Rh, Cryovac Co. Duncan, SC) and a Multivac AG 900 vacuum packaging machine (Multivac, Sepp Haggenenmuller KG, Germany) were used to pack all samples.

Praximate Composition

Moisture, crude protein, fat and ash content were determined according to AOAC (1984) methods.

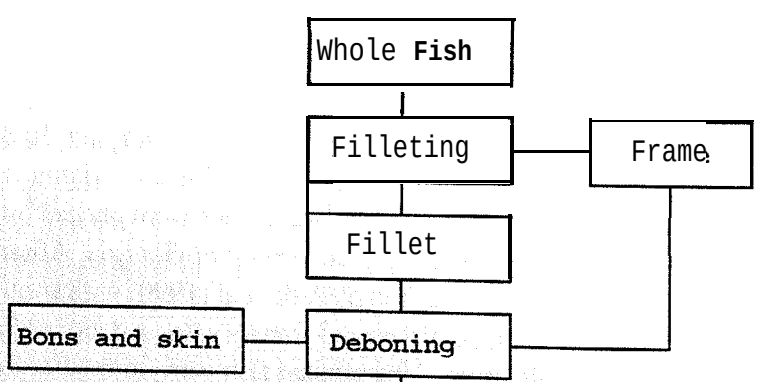
Protein in Washing Water

Washing water in different washing cycles were filtrated using Whatman No.2 filter papers. The filtrate was used for protein analysis (Douglas-schwarz and Lee, 1988).

Expressible Water

A 3mm thick and 20mm diameter slice of surimi gel was pressed between four sheets of Whatman No. 1 filter paper under a weight of 10.8kg for 1 min. The weight loss of a gel slice after pressing was expressed as percent of the original sample weight (Hennigar et al., 1988).

Expressible water for a surimi sample was determined according to Alvarez et al. (1992). A 2g of a surimi sample was folded with one sheet of filter paper Watman No. 1 and centrifuged at 3000 rpm for 15 min at room temperature (Servall angel centrifuge type SPX., Ivan Servall Inc. Norwalk, Corn.). Preweighed nylon screen was placed between the surimi sample and the filter paper sheet. This was used to aid in removing the sticky "cake" rapidly from the filter paper after centrifugation (Weinberg et al., 1984). Values reported represent an average of six samples per treatment.



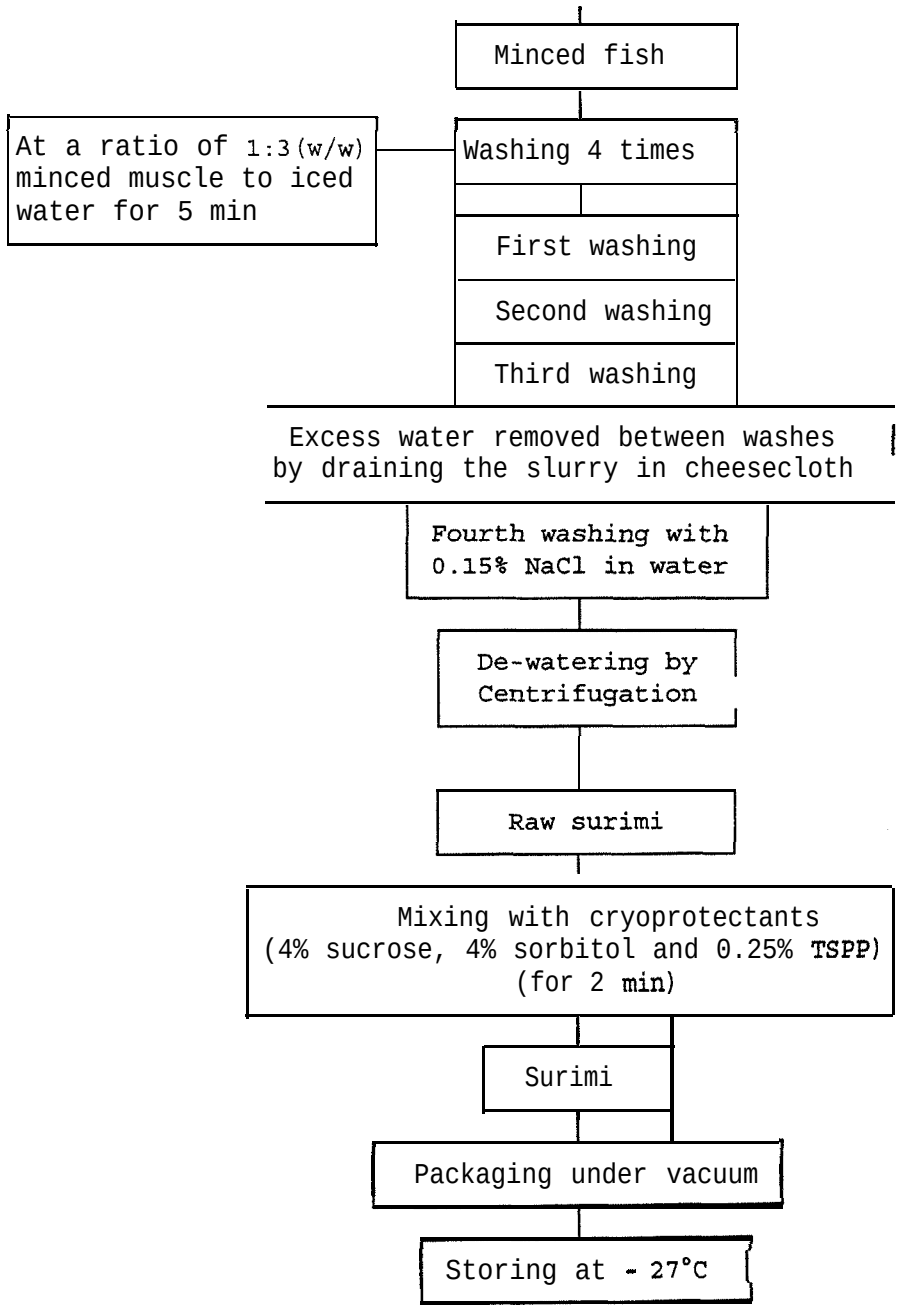


Fig. 1 A flowchart of processing surimi from tilapia

Hunter Color Values

Hunter color values (L, “a”, “b”) of fish mince during washing steps and final surimi products were measured using a colorimeter (Minolta Chroma Meter CR-200, Minolta Camera, Ltd., Osaka, Japan). The value L was the lightness ranged from 0 to 100; "a" was chromaticity where positive value indicating redness and negative value indicating greenness; while positive value of “b” indicating yellowness and negative value indicating blueness. Three random spots on each sample were measured and the average data were recorded.

Mineral Content

Five macro-elements (Na, Ca, Mg, P, and K) and fourteen micro-elements (Zn, Cu, Fe, Si, Co, Mn, Mo, Ni, Pb, Sr, Ba, B, Al, and Cr) in fish mince and surimi were determined. One g of homogenized sample was weighed into a 100-ml beaker and 5 ml concentrated nitric acid was added, After the sample was dried on a hot plate, the beakers were placed in a muffle furnace at 500 °C for 4 hrs. The ash was then taken up in 10 ml 20% aqua regia. The resulting solution was assayed by inductively coupled plasma (ICP) emission spectrometry using a Thermo Jarrel-Ash 955 AtomCounter ICP Spectrometer.

Amino Acid Content

A 0.2g sample (contained 30 mg crude protein) was hydrolyzed by adding 180 ml 6N HCI under nitrogen for 24hr in a 500ml flat bottom flask connected with a condenser. The hydrolysate was filtered through Whatman No. 1 filter paper and distilled water was added to adjust the volume to 200 ml.

20 ml of hydrolysate was placed into a 500 ml round bottom flask evaporated under a vacuum at 60 to 65 °C, 15 ml of diluting buffer(Sodium citrate; pH 2.20) was added to dried content and sufficient volume was injected in the Beckman high performance amino acid analyzer system 6300, (Beckman Instruments Inc., Palo Alto, CA)

Measurement of Gel Strength

The gel was prepared by comminuting surimi with 2% salt in a food processor (Regal Lamachinei, VB., VA) at 0°C for 10 min (Lee and Chung, 1989). The Moisture content of surimi was adjusted to 78% by adding ice during chopping. The resulting paste was immediately vacuum packaged in oxygen barrier-bags. The paste was stuffed into both stainless steel tubes (1.87cm id and 16.5cm length) and plastic casing (3cm diam) with minimum incorporation of air bubbles and sealed at both ends. The interior of the tubes was coated with a thin film of PAM (American Home Food Inc,, Madison, NJ). The tubes and plastic casings were submerged vertically in 40 °C water bath for 20 min and then in 90°C water bath for 15 min. The tubes and plastic casings were kept at 40°C for 12 min and 17 min, respectively before calculating the setting time. The tubes and plastic casings were kept at 90°C for 10 min and 13 min, respectively before calculating the cooking time. The temperature in the center of the gels was monitored in the tubes and casings using small thermocouple probes. Immediately after heated the tubes and casings were cooled in a mixture of ice and water for 10 min. Gels were removed from tubes and casings and kept in sealed plastic bags at 4°C for overnight until testing (Fig. 4).

Measurement of Strain and Stress at Failure

Surimi gels (1.87cm dia.) were removed from the refrigerator and warmed to the room temperature. Plastic disks were glued onto the ends of each sample in 2.87cm length with cyanoacrylate glue. Samples were cut into dumbbell-shaped specimens with a final minimum diameter 1cm using a machine (Accu-tool corp, cary, NC).

The dumbbell-shaped samples were placed in a modified Brookfield viscometer (Model DV-I) with

a torsion fixture attached and twisted at 2.5 rpm until failure (Fig. 5). Stress and strain at failure were calculated from the data using the equation given by Hamann (1983).

Penetration Test ("Punch" Test)

Surimi gels (3cm dia.) were brought up to room temperature and cut into 4cm length. The sample was placed on a texture analyzer (Voland Texture Analyzer TA- 1000, Voland Corporation, Hawthorne, NY) and a 5mm ball probe was driven into the flat top of the surimi sample. The speed of the probe was 1 .0mm/sec, the depth was 23mm and the chart speed was 10.0cm/min. Breaking force (g) and breaking distance (cm) were recorded by using the flat-bed record (Linseis L6512, Linseis Inc., Princeton-Jet., NJ) (Fig. 6).
Gel strength (gxcm) = force (g) x breaking distance (cm).

Folding Test

A surimi gel slice (3cm diameter and 3mm thick) was folded by thumb and forefinger. Five stages were used to evaluate the sample (Kudo et al., 1973):
AA=5 (no cracks on folding twice), A=4 (no cracks showing after folding in half), B=3 (crack gradually when folding in half), C=2 (crack immediately when folding in half), and D=l (breaking by finger pressure).

Data Analysis

Data were analyzed with the GLM (General Linear Model) program using statistical analysis system (SAS, 1987). Mean values were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Surimi Yield

Table 1 showed that the yields of surimi from tilapia (18.99%) and grass carp (18.26%) were lower than that of Alaska pollack (24.00%). Hustings (1989) reported that lower surimi yield may be due to the loss of water soluble protein during wash steps and of some insoluble protein during the draining of slurry. Park et al. (1990) found that a higher yield of protein recovery (5 1.59%) and ease dewatering due to the pre-rigor tilapia.

The yield of minced flesh from tilapia frames without head was 42.50% and that from the whole fish was 5.63% (Table 2). The yield of surimi from the frame without head was 10.82% and that from whole fish was 1.43% that equal 6.965 of the surimi processed from fillets.

Protein Loss During Washing

Most of the water-soluble proteins were removed in the first washing cycle (Table 3). This data agrees with the report of Yean (1993) indicating that most soluble protein loss occurred in the first wash (2.7g protein/L^{-1}) during processing surirni from fresh Threadfin bream (*Nemipterus tolu*). Eid et al. (1991) also reported that the leached out protein percentage in six fish species (catfish,

Table 1 - Yield of surimi made from tilapia and grass carp

	Tilapia		Grass Carp	Alaska pollack[a]
	flesh	frame	%	
Whole fish	100	-	100	100
Fillets to whole fish	39.74	-	47.19	47.00
Minced flesh to whole fish	30.90	5.63	35.21	-
Minced flesh to fillet	77.78	-	74.61	-
Washed mince to whole fish	18.99	1.32	18.26	22.00
Washed mince to fillet	47.78	-	38.71	-
Washed mince to minced flesh	61.43	-	51.88	-
Surimi[b] to whole fish	20.54	1.43	19.76	24.00
Surimi to fillet	51.69	-	41.88	-
Surimi to minced flesh	66.46	-	56.13	-

a from AFDF, (1987)
b Washed minced flesh mixed with cryoprotectants (4.00% sorbitol, 4.00% sucrose and 0.25% sodium triplyphosphate (STPP)

Table 2 - Yield of surimi made from tilapia frames

	Tilapia Frame %
Frames with head to whole fish	36.20
Frame without head to whole fish	13.25
Minced flesh to whole fish	5.63
Minced flesh to frame with head	15.55
Minced flesh to frame without head	42.50
Washed mince to whole fish	1.32
Washed mince to frame with head	3.66
Washed mince to frame without head	10.00
Washed mince to minced flesh	23.53
Surimi* to whole fish	1.43
Surimi to frame with head	3.95
Surimi to frame without head	10.82
Surimi to minced flesh	25.46

* Washed minced flesh mixed with cxyoprotectants (4.00% sorbitol, 4.00% sucrose and 0.25% sodium tripolyphosphate (STPP)

Table 3 - Protein content in washing water during surimi processing from minced fillet and frame of tilapia and fillet of grass carp

Samples	Tilapia meat"	Tilapia frame[a]	Carp meat[b]
Number of washing		Protein g/100ml	
First wash	0.81	0.95	0.54
second wash	0.29	0.26	0.21
Third wash	0.11	0.07	0.10
Fourth wash	0.05	0.06	0.05

n=2
a = Water:minced flesh (3: 1 W/W)
b = Waterminced flesh (8:1 V/V)

Table 4 - Proximate composition of tilapia and grass carp surimi

Samples	Moisture %	Protein %	Fat %	Ash %	Carboh-ydrate %	Express - ible water %
Minced fillet*	77.87d ±0.18	16.23ab ±0.94	3.87c ± 0.34	1.20a ±0.03	0.00	31.95b ±0.66
Minced frame*	79.5lc ±0.23	14.32cd ±0.39	5.15b ±0.08	0.99b ±0.03	0.00	42.69a ±0.87
Washed meat	81.08b ±0.52	16.27ab ±0.79	2.56d ±0.06	0.58c ±0.04	0.00	26.50c ±0.20
Surimi fillet*	73.91f ±0.89	14.81cd ±0.54	2.31d ± 0.27	0.55c ±0.02	8.00	20.64d ±1.20
Surimi frame*	72.94g ±0.76	15.36cb ±0.92	2.46d ±0.19	0.53c ±0.02	8.00	14.54e ±1.46
Carp meat	74.68f ±0.18	17.33a ±1.13	6.60a ± 0.25	1.07b ±0.03	0.00	31.37b ±1.87
Washed mince	82.64a ±0.19	14.84cd ±0.59	1.18e ±0.12	0.23d ±0.02	0.00	27.07c ±2.27
Carp surimi	76.63e ±0.15	13.45d ±0.07	0.78f ±0.04	0.56c ±0.19	8.00	20.63d k2.44
Alaska pollack surimi[a]	76.00	16.00	0.20	0.34	7.50	NA

a from AFDF, (1987).
n=3
* Samples prepared from tilapia.
abcdefg Means in a column with different letters are different (p< 0.05).

Table 5 - Change of Tilapia and Grass Carp meat color during surimi processing

Samples	"L"			"a"			"b"		
	minced fillet*	minced frame*	minced carp	minced fillet*	minced frame*	minced carp	minced fillet*	minced frame*	minced carp
Minced flesh	52.05a ±0.29	49.11b ±0.65	52.62a ±1.44	+2.3 lc ±0.52	+7.33b ±0.91	+9.36a ±0.69	+2.89b ±0.58	+5.52a ±0.38	+6.03a ±1.07
First wash	59.09b ±1.57	54.50c ±0.39	62.29a ±1.08	+0.69c ±0.19	+1.45b ±0.13	2.31a ±0.18	+1.53c ±0.38	+2.81b ±0.41+	+4.05a ±0.71
Second wash	61.13b ±1.34	55.87c ±1.18	66.18a ±0.65	-0.70b ±0.25	-0.29a ±0.09	-0.88b ±0.13	-1.21b ±0.63	-0.28a ±0.18	+0.33a ±0.12
Third wash	61.73b ±1.09	58.24c ±1.47	66.20a ±0.77	-0.99a ±0.20	-0.87a ±0.41	-1.23a ±0.19	-1.40a ±0.93	-1.62a ±0.32	-0.53a ±0.42
Fourth wash	64.12b ±0.68	65.78b ±1.48	70.27a ±1.77	-0.62a ±0.17	-0.82a ±0.24	-1.07a ±0.46	+1.44a ±0.86	-1.86b ±0.57	+1.56a ±0.60
Surimi	70.68a ±1.27	70.46a ±1.99	71.40a ±0.83	-0.83a ±0.11	-0.68a ±0.15	-1.24b ±0.12	+2.68a ±0.54	+3.07a ±0.94	+2.41a ±0.48

n = 3

* Samples prepared from tilapia

abc Means in a row for each group of L, a, and b with different letters are different ($p < 0.05$).

goatfish lizardfish, ponyfish, therapon, and trevally) catch from Arabian Gulf was ranged from 30% to 58% during surimi processing.

Proximate Composition

The composition of flesh though processing surimi was changed by removed of water-soluble protein components, lipids and minerals (Table 4). Surimi from Tilapia fillet and frame were lower ($p < 0.05$) moisture content and higher fat content than that of Carp and Alaska pollack surimi. Tilapia and Carp surimi have lower protein content than Alaska pollack, but tilapia surimi has higher protein content than that of Carp surimi. Park et al. (1990) stated that the moisture and protein contents of surimi processed from tilapia in pre-rigor, in-rigor and post-rigor stages were 70.53% and 20.27%, 75.63% and 15.42%, and 75.03% and 16.57%, respectively. The lipid content of minced flesh from tilapia fillet and frame and grass carp was reduced by 40.31%, 52.23% and 88.18%, respectively during processing surimi. Ash content also reduced by 54.17%, 46.46% and 47.66%, respectively. Chang-Lee et al.(1990) reported that lipid and ash contents of Pacific whiting flesh were reduced by 40.60% and 77.40%, respectively from minced flesh to surimi. Expressible water of all surimi samples was decreased significantly ($p < 0.05$) after processing into surimi (Table 4).

Hunter Color Values

"L" values of all minced flesh samples were increased significantly ($p < 0.05$) after washing steps but, "a" and "b" values were significantly decreased (Table 5). The increasing of "L" value (lightness) and the decreasing of "a" value indicated that most of myoglobin and hemoglobin that responsibly for the red hue of fish meat were removed during washing (Park, 1995). There was no significant difference between "L" and "b" values of surimi fillet and frame from tilapia and grass carp surimi, but grass carp surimi had higher value of blueness. Eid et al. (1991) stated that "L", "a" and "b" values (52.20, 3.00 and 9.6) of unwashed lizardfish mince were changed to L(67.50), a(-2.60) and b(4.80) after washing. Cooked surimi gel of Alaska pollack have higher greenness and lower Yellowness than that of Tilapia and Grass carp gels (Table 6).

Amino Acids

Amino acid profile of surimi fillet and frame from tilapia and grass carp surimi were similar to that of Alaska pollack surimi (Table 7). The overall amino acids score of Alaska pollack surimi is similar to those of land-animal meats (AFDF, 1987).

Minerals

Grass carp surimi had lower level of Ca, Mg, P, K, Zn, Cu, Si, pb, Ba, B, Al and Cr and higher level of Na, Fe, Co, Mn, Mo and Ni than that of surimi from tilapia fillets (Table 8). Frame surimi from Tilapia has the highest level of Fe, Cu, Mo, Ni, Co, Ba, Sr and Cr. Nettleton (1985) reported that Pollack surimi has 143mg/100g sodium, 78mg/100g potassium and 0.26mg/100g iron. The high level of sodiurn content in a surimi sample attributed to adding sodium tripolyphosphate (Nettleton, 1985).

Table 6 - Hunter color values (L, a and b) of surimi gels

Samples	L	a	b
Tilapia surimi	82.44a ±0.69	-1.38b ±0.23	4.63b ±0.10
Tilapia frame surimi	79.03c ±0.20	-1.62a ±0.12	4.42b ±0.15
Grass carp surimi	81.48b ±0.27	1.63a ±0.18	5.97a ±0.24
Alaska pollack surimi*	79.64 ±0.62	-3.84 ±0.03	1.78 ±0.22

* from Kim et al.(1993).
n = 3
abc Means in a column with different letters are different
($P < 0.05$)

Table 7 - Amino acid composition of tilapia and grass carp surimi

	Tilapiaminced fillets	Tilapiasurimi Fillets	Tilapia minced frames	Tilapia surimi frames	Carp minced fillets	Carp Surimi fillets	Alaska[a] pollack
	g amino acid/l 00g protein						
Asp.	10.99	10.75	9.34	11.04	10.99	11.47	11.60
Thr.	4.67	4.78	4.17	5.07	4.79	5.37	4.60
Ser.	3.73	3.35	3.33	3.86	3.99	4.37	5.37
Glu.	17.00	17.31	14.55	18.73	16.09	18.93	18.90
Pro.	3.61	3.00	3.49	3.87	3.91	4.08	3.60
Gly.	4.79	4.92	4.42	4.29	4.17	3.91	4.50
Ala.	5.41	5.70	5.09	5.48	5.48	5.58	6.50
Cys.	0.56	1.08	1.12	0.95	0.68	0.62	1.00
Val.	4.27	4.50	3.80	4.6	4.51	4.94	4.90
Met.	2.88	3.11	2.42	3.47	2.76	3.27	3.10
Iso.	5.02	5.12	4.18	5.69	4.91	5.58	6.20
Leu.	8.38	8.50	7.09	9.35	8.17	9.23	10.60
Tyr.	3.40	3.52	2.83	3.96	3.42	3.72	3.60
Phe.	4.05	3.75	3.36	4.09	3.93	4.13	3.80
His.	2.32	2.22	2.00	2.47	2.28	2.36	2.50
Lys.	9.74	9.74	8.29	10.93	9.62	10.71	11.70
Arg.	6.13	6.21	5.02	6.91	5.67	6.80	7.00
Total	96.96	97.57	84.49	104.77	95.37	105.07	109.30

a From AFDF, (1987).

Table 8 Macro-elements (Na, Ca, Mg, P, and K mg/100g minced fish) and micro-elements (ppm) content of flesh and surimi processed from tilapia and grass carp

Elements	Tilapia minced fillets	Tilapiasurimi fillets	Tilapiaminced frames	Tilapia surimi frames	Carp minced fillets	Carp surimi fillets
Na	47.76	107.37	61.62	106.61	44.12	119.04
Ca	41.31	71.49	49.84	64.44	14.82	25.17
Mg	23.16	13.08	18.68	9.68	21.79	7.65
P	175.82	87.39	133.73	84.89	202.05	84.41
K	342.48	22.81	222.39	9.63	331.79	5.72
Zn	37.68	49.41	40.58	35.20	9.27	5.31
Cu	0.59	0.69	1.80	7.52	0.79	0.47
Fe	17.58	0.00	146.68	55.15	71.62	22.77
Si	8.54	23.10	11.97	17.29	9.77	7.99
CO	0.04	0.00	0.21	0.08	0.07	0.05
Mn	0.00	0.00	0.00	0.00	1.17	0.51
Mo	0.01	0.00	0.31	0.14	0.03	0.02
Ni	2.08	0.56	16.87	5.64	5.43	2.27
Pb	1.22	0.55	0.72	0.26	0.09	0.08
Sr	0.29	0.51	0.31	0.58	0.09	0.56
Ba	0.07	0.19	0.85	0.26	0.03	0.07
B	0.87	0.27	0.45	0.25	2.75	0.04
Al	5.31	3.77	3.51	1.49	4.56	2.30
Cr	3.56	0.94	26.18	9.30	8.87	3.04

Gel Strength

For surimi gels, stress is an indicator for gel strength that affected by the moisture content and heating temperature. True shear strain at failure is used to measure of the protein quality of fish gel and its indicator of gel cohesiveness (NFI,1991). Stress and true shear strain of tilapia and carp surimi were higher than that of Alaska pollack but, tilapia surimi has higher stress and strain than carp surimi (Table 9). Park et al.(1990) stated that stress of tilapia surimi gel prepared in pre-rigor, in-rigor, and post-rigor were 98.16kPa, 81 .51kPa and 70.89kPa, respectively. The strains of that were 2.47, 2.38, and 2.21, respectively. No difference between folding test score for tilapia and carp surimi that received the highest score (AA = 5 extremely elastic) as the same of Alaska pollack surimi. The results agree with the data reported by Somboonyarithia,(1990), surimi processed from tilapia stored in ice (0±2°C) for 0, 1 and 4 days has the highest score AA extremely elastic. No significant difference was observed between the expressible water of Tilapia and Carp surimi gels. Gel strength (g.cm) of Tilapia surimi was higher than that of carp and Alaska pollack surimi (Table 10). Gel strength of tilapia (1,061 gxcm) was higher than that data for tilapia (562 gxcm) reported by Somboonyarithia (1990).

CONCLUSIONS

This study showed that the yield of surimi from tilapia and grass carp was lower than Alaska pollack. The yield of surimi from frames was 6.96% of total surimi weight. Most of the water-soluble protein was removed in the first washing cycle. No significant difference between "L", "a" and "b" values of tilapia and grass carp surimi. Tilapia surimi has higher fat and lower moisture content than Carp and Alaska pollack surimi, but carp surimi has lower protein content than Tilapia and Alaska pollack Grass Carp surimi has lower level of Ca, Mg, P, K and Zn and higher level of Ni, Fe, and Na than Tilapia fillet surimi. Tilapia frame surimi has the highest level of Fe, Ni and Cr. Tilapia and carp surimi produced very elastic gels that have high gel strength. Tilapia and grass carp may be being suitable materials to processed surimi.

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Table 9 - Gel strength of surimi processed from tilapia and grass carp

Samples	Shear stress kPa	True shear strain	Rigidity KPa	Folding test	Expressible water%
Surimi fillet*	86.81a ±8.93	2.69a ±0.13	32.33b ±2.83	AA=5	6.1 lb ±0.67
Washed mince*	57.20b ±10.73	1.88d ±0.23	30.15b ±2.94	AA=5	6.05b ±1.02
Surimi frame*	84.59a ±12.76	2.21c ±0.22	38.13a ±1.94	AA=5	5.95b ±0.54
Carp surimi	52.32b ±4.53	2.47b ±0.15	21.299d ±2.51	AA = 5	6.33b ±0.93
Washed mince	40.395c ±3.89	1.67e ±0.08	24.23c ±1.79	B = 3	9.50a ±0.96
Alaska pollack surimi[a]	49.40 ±11.5	2.19 ±0.31	22.90 ±5.60	AA=5	NA

a from Reppond, et al., (1987).
n = 6
* Samples prepared from tilapia.
abcde Means in a column with different letters are different($p < 0.05$).

Table 10 - Gel strength (gxcm) of tiiapia and grass carp surimi

Samples	Breaking Force(g)	Breaking distance (cm)	Gel strength (gxcm)
Surimi fillet*	683.71a ±82.37	1.55a ±0.17	1061.46a ±216.87
Washed mince*	380.00b ±22.17	1.04c ±0.05	395.20c ±31.03
Frame surimi*	625.00a ±71.89	1.38b ±0.16	862.50b zt196.67
Carp surimi	306.43c ±19.12	1.07c ±0.08	327.73c ±42.11
Washed mince	211.00d ±12.49	0.71d ±0.05	149.81d ±16.46
Alaska pollack surimi[a]	440.00 ±87.00	1.39 ±0.14	617.00 ±153.00

a from Reppond, et al., (1987).
n = 6
* Samples prepared from tilapia.
abcd Means in a column with different letters are different($p < 0.05$).

CONSUMER SURVEY OF POND RAISED CATFISH TO ESTABLISH A STANDARD LEVEL OF FLAVOR ACCEPTABILITY.

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Catfish off-flavors have frequently prevented the transfer of live caffish from the farm to the processor. Traditionally, the acceptability of fish from the farmer is based on the opinion of "taste testers" trained to recognize the variety of off-flavor bouquets that may be present in amounts from trace or threshold to very strong. The sensitivity of the "taste testers" palates increase with experience and are presumed to be more sensitive than that of the average consumer. As much as 90% of fish taste tested by some processors has been rejected due to off-flavor detection. With this high rejection rate based on off-flavor, processors have not been operating at peak production and consequently have lost man hours and money. To address this concern, it was the objective of this research to determine whether trained caffish "taste testers" have been overly sensitive to off-flavors, rejecting catfish when the fish flavor would readily have been very acceptable to the average consumer.

MATERIALS AND METHODS

SENSORY PANEL

Catfish consumers (persons who consume catfish at least once/month) were recruited from the Louisiana State university community of faculty, staff(including food service managers), and students and personnel from a local nuclear power plant(engineers, managers, trainers, secretarial staff). The total consumer panel, consisted of 56 persons, with each person evaluating duplicate samples of catfish provided by Cargill.

CATFISH SAMPLES AND PREPARATION

Catfish samples were provided by a local catfish processor and included fish containing no off-flavor(O) to varying amounts of off-flavor graded from threshold (th-lowest) to one(l), two(2) and three(3-highest) as determined by the "taste testers" at the processing plant. Fish fillet samples were baked from a frozen state at 325°F in pyrex dishes with lids for approximately 20-25 minutes until they lost opacity and were determined to be completely cooked but not over cooked. Samples were assigned random numbers and cut into approximately 30 g portions and placed in 5 oz plastic souffle cups with lids for presentation to panel members. Duplicate samples were assigned different numbers before presentation to the panel.

SENSORY EVALUATION

Using a randomized complete block design, each panelist was presented every sample in duplicate for evaluation of acceptability. Panelists were asked to complete a brief survey of personal history (form inclosed) before evaluating the catfish.

RESULTS

SENSORY PANEL DESCRIPTION:

Thirty one(31) members of the Louisiana State University Community participated in consumer panel. Of these, 10 were managers of various food service facilities on campus, 17 were either faculty or graduate students, and the remaining 4 were members of the housekeeping staff at LSU. Ten members of the faculty and graduate students were experienced in participating in food sensory analysis and were classified as the expert panel. The remaining 25 panelists were personnel from a local nuclear power plant facility. Of these 25, 17 were managers or engineers and the remaining 8 were from the secretarial staff. The total "overall" panel was 48% male and 52% female and consisted of every member participating from all groups. The age variations were divided into three groups with the following breakdown: 22 were age 0-30(youngest-21), 27 were between 30-50 yr, and 7 were over 50 (oldest-62). Panelists were asked to designate a region of origin that they considered influenced their taste preferences with the following results: The largest group was local with 39 from South Central(Louisiana, Arkansas, and Texas). The other groups were 5-Southeast, 3-Northeast, 2-Midwest, 5-Southwest, 2-foreign countries, O-Northwest. Panelists were asked to give the number of times each month that they ate catfish with the following results: 11 - once/month, 11 - twice a month, 10 - three/month, and 24 - four or more times/month. Preference of cooking method was breaded and fried (68%), with other preferences including baked, broiled, or blackened.

ACCEPTABILITY SCORES:

The overall panel rated fish known to have no off flavor with 58% acceptability(Fig. 1). The fish with some off-flavor (th, 1,2) all received higher acceptability scores with 82, 78, and 64%, respectively. The lower acceptability of the catfish having no off-flavor could be explained by the lack of experience of the panelists with the corny/buttery flavor associated with grain fed pond reared catfish. Catfish with level 3 off-flavor were scored the lowest with a 21% acceptability. The nearly 25% acceptability rating for these fish was not expected due to the very strong muddy/musty odor and flavor exhibited by the fish in this group.

Fish evaluated by the expert panel alone were more in linear agreement with the company taste-testers evaluation with 100% acceptability for the fish with no or threshold off flavor(Fig.1). Level 2 and 3 fish were acceptable 70-75 % and level 3 was totally rejected by this group.

Acceptability scores of male and female panelists showed differences in preference for level of off-flavor(Fig.2). Male panelists preferred some off-flavor, rating the threshold,level 1, and level 2 off-flavor higher in acceptability than the samples with no off-flavor. The female panel however, showed a preference for samples with level 1 or less off-flavor.

When the panel was divided among professions it was interesting to note that the food service personnel rated the threshold off-flavor at 100% acceptability (Fig.3). The professional and academic panelists preferred some off flavor to no-off-flavor. Classified employees preferred samples with level 2 off flavor with level 1 and threshold coming in second and third in preference.

Acceptability scores based on region of food preference origin were somewhat different (Fig.4). However, several of the groups were of small sample size(midwest and foreign countries).Panelists from the Southeast and South Central areas rated the acceptability quite similarly with greater than 60% acceptability for catfish with no, threshold, level 1, and level 2 off-flavor. Panelists from the midwest preferred all the samples with off-flavor to samples with no off-flavor. Panelists from the northeast preferred the no or low off-flavor with complete rejection of samples at level 3 off-flavor. Southwest panelists had the lowest acceptability rating of any group with 50% acceptability of catfish with no to level 2 off-flavor.

When the panelists were divided by age (Fig.5), there was little difference in ratings of fish with no,threshold, and level 1 off flavor. However, samples of level 2 catfish off-flavor rated with a high acceptability by the two older groups of panelists. The oldest group of panelists even rated the level 3 off flavor sample with a near 60% acceptability. This could be explained first by the known fact that aging reduces sensitivity to flavor intensities with this group not perceiving the very strong off-flavor or secondlythat the older panelist prefer the wild catfish flavor over the pond raised corny flavor since the wild flavor is likely the way they expect catfish to taste.

PROBABILITY HISTOGRAMS

Probability histograms were constructed to predict consumer acceptability for varying levels of catfish off-flavor. The histograms with the largest sample base included the overall panel, the professional, tire academic, South Central region and the 30-50 yr. All of these panels showed similar histogram patterns with a density curve peaked at the threshold of off-flavor.

CONCLUSION

From the results of this experiment, it was evident that the current standards of this processor's taste-testers were much more stringent than the consumer panel required or even preferred The results of this study indicated that what has recently been rated as threshold and level 1 off-flavor were definitely acceptable by this consumer panel and could presumed to be acceptable for processing. Catfish with level 2 off-flavor were nearly as well received but may be considered borderline at this time. Level 3 off-flavor was definitely not acceptable to most of the panelist.

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Procedures

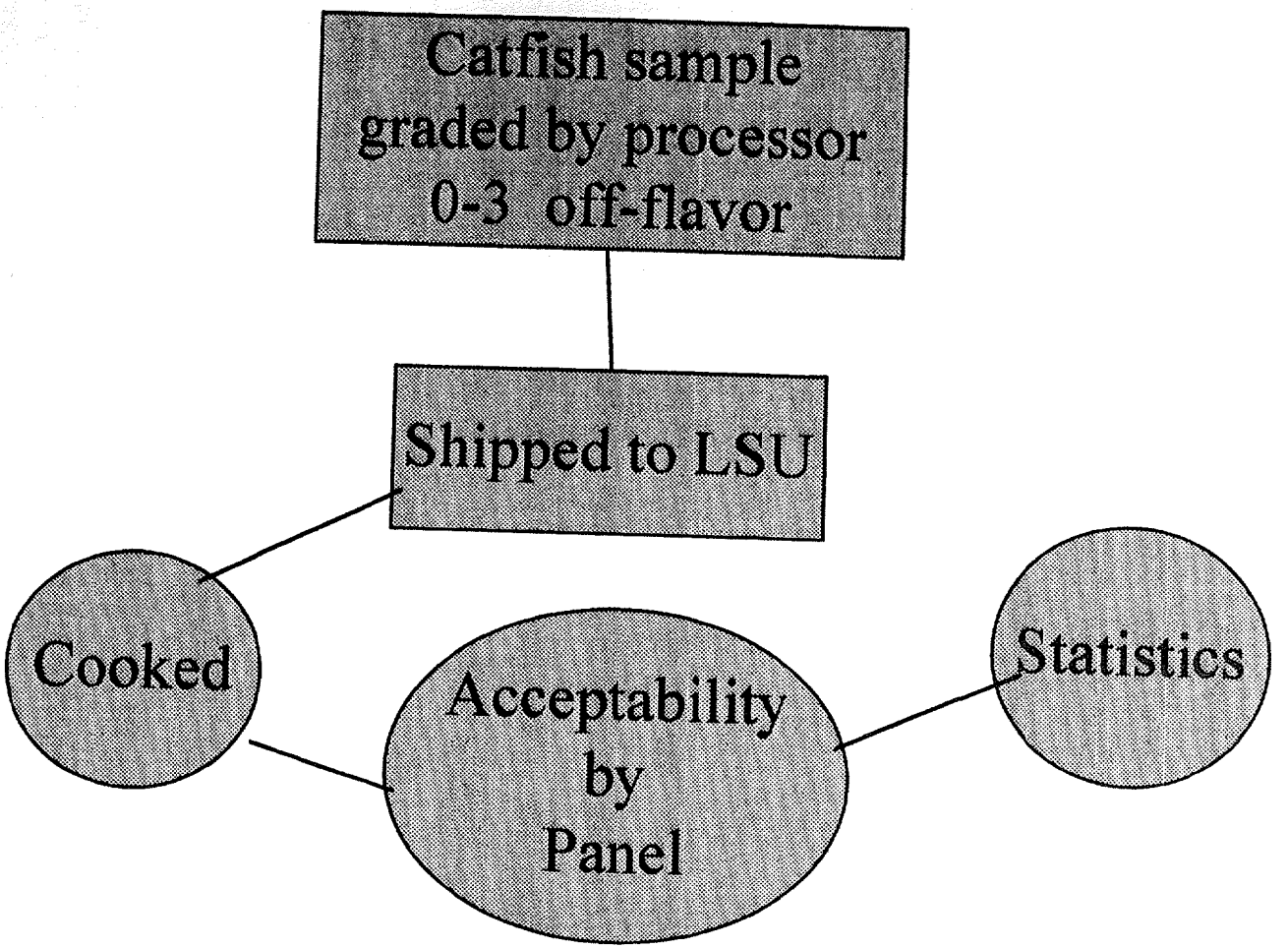

Catfish sample graded by processor 0-3 off-flavor
Shipped to LSU
Cooked
Acceptability by Panel
Statistics

Fig. 1 Catfish Acceptability Expert and Consumer Panels

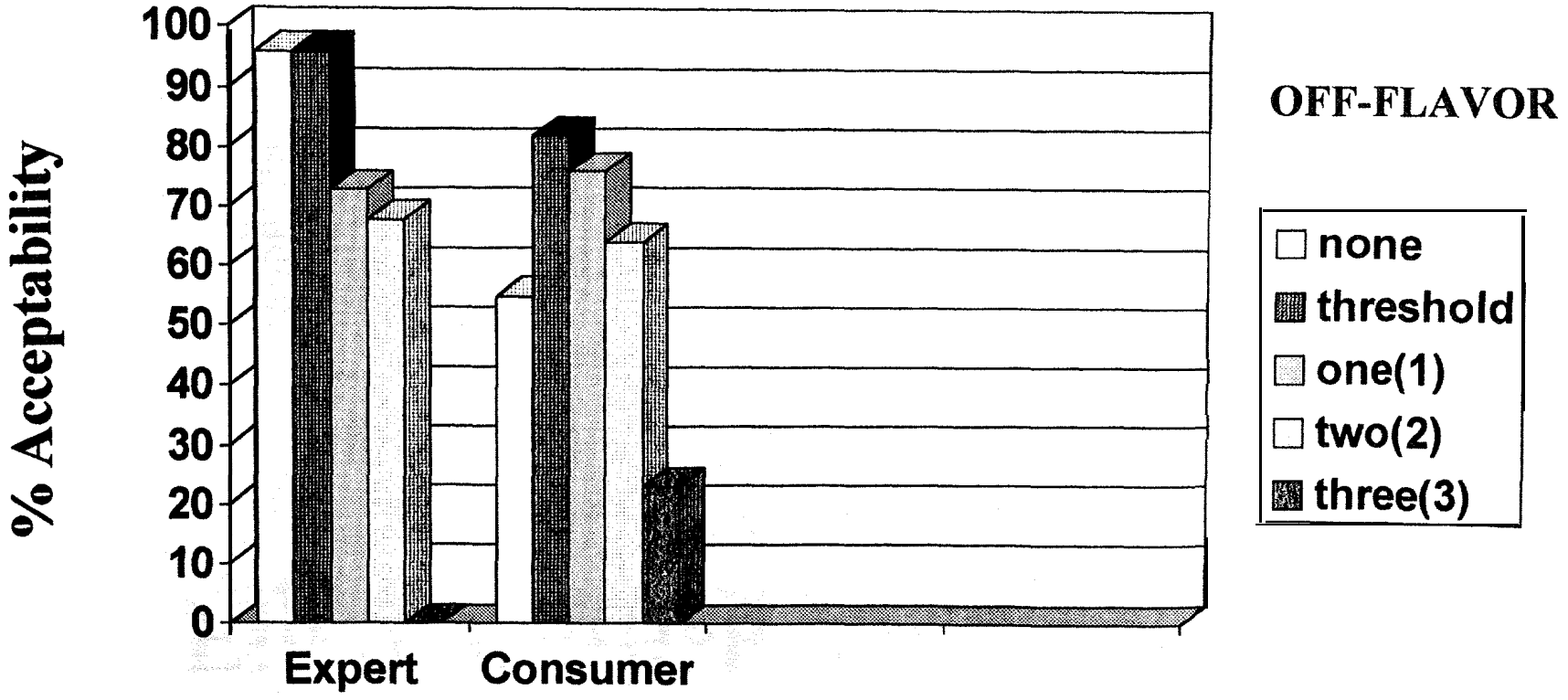


Fig.2 Catfish Acceptability by Gender

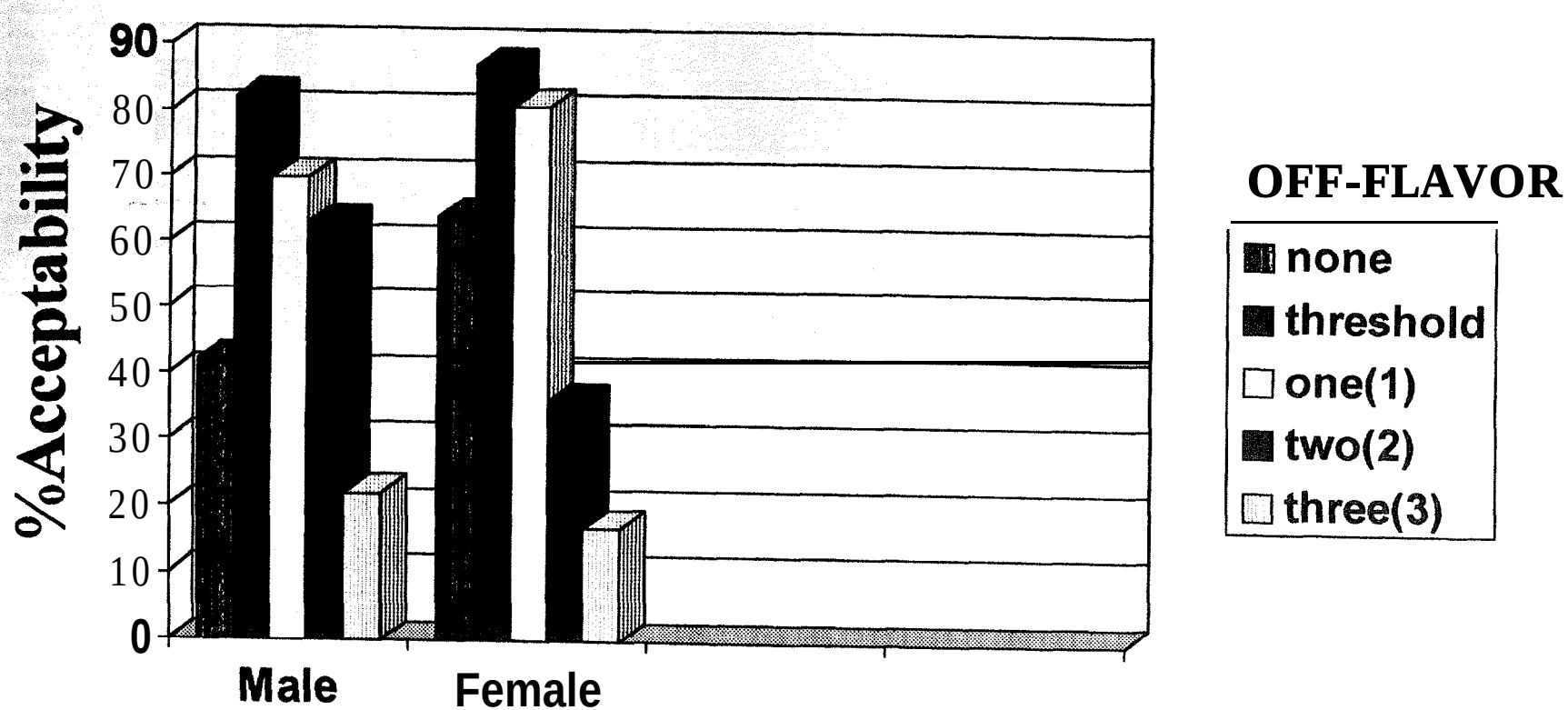


Fig. 3 Catfish Acceptability by Profession

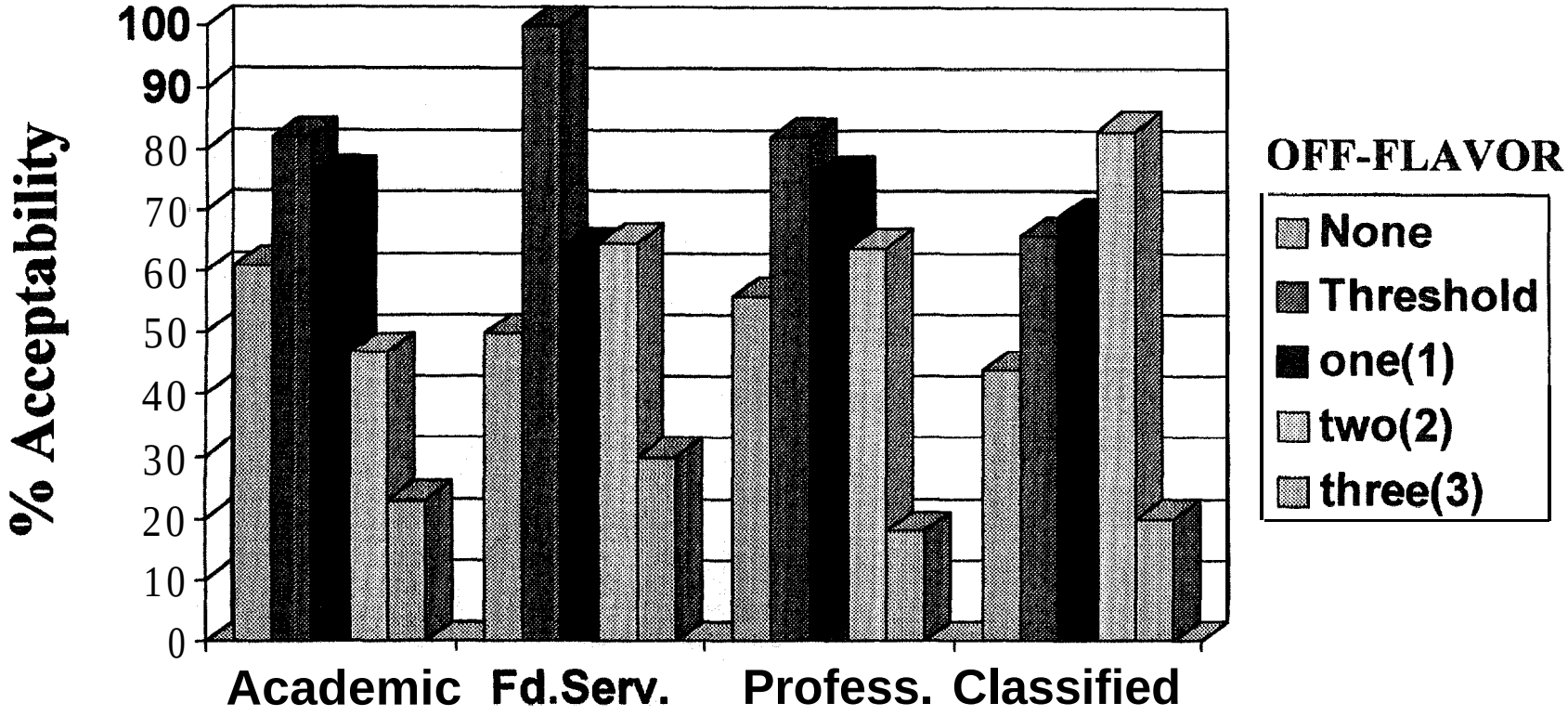


Fig. 4 Catfish Acceptability by Origin of Birth

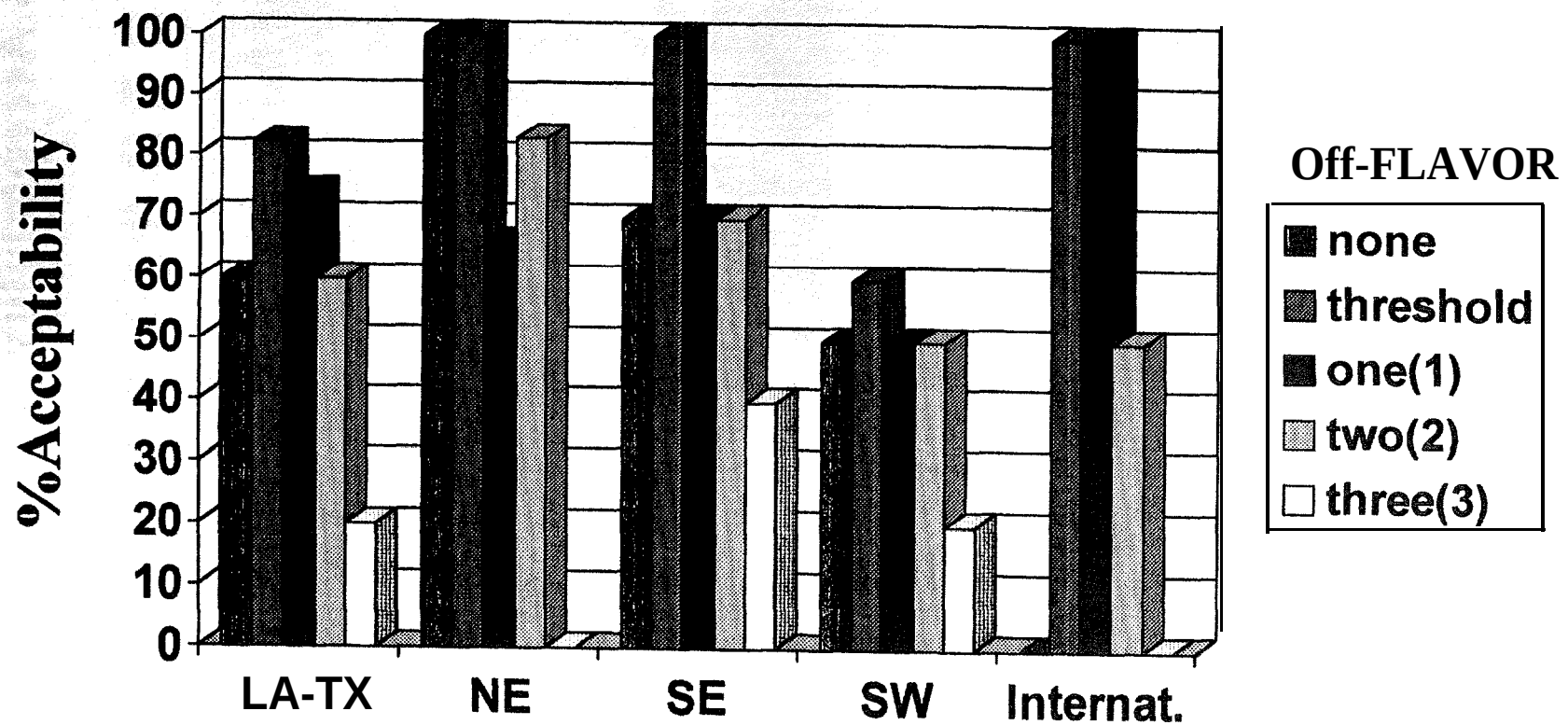
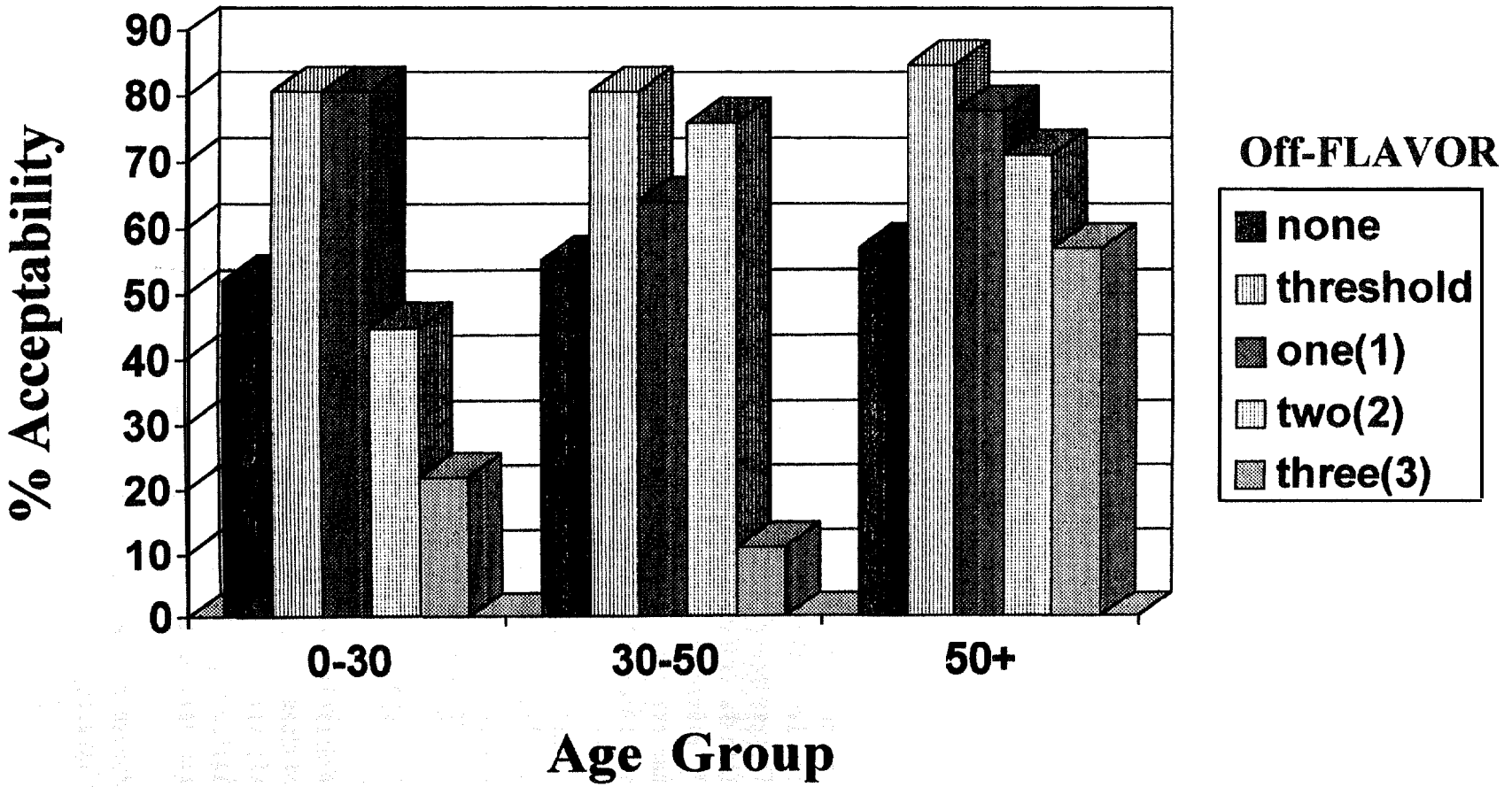


Fig.5 Catfish Acceptability by Age



Nicholls State University

Evaluation of Commercial Irradiation and Other Processing Methods for *Vibrio vulnificus* Control in Louisiana Oysters

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Oysters commercially harvested September 1994 and April 1995 from Black Bay, Louisiana were processed as live boxed, shucked 1202. (packed in ice), and frozen half-shell products, and shipped by commercial refrigerated truck for irradiation processing at Food Technology Services, Inc. Live shellstock were held at the recommended temperature of 40°F from harvest through sampling. The ambient levels of *V. vulnificus in the oysters* were 4.6 X 10^5 MPN/g in September 1994 (water 80°F) and 1.5 X 10^2 MPN/g in April 1995 (water 69°F). Two 60 lb. boxes of live oysters were each treated at minimum levels of 0.0 (controls), 0.5, 1.0, 1.5 and 2.0 KGy (400 animals per dose). The dose ratios were calculated to be about 2: 1. Levels of *V. vulnificus*, total aerobic plate counts and % cumulative mortality were enumerated through day 14 post harvest for the September 1994 sample, and through day 28 post harvest for the April 1995 sample. Oysters treated with doses of 0.5 KGy and 1.0 KGy did not show significantly higher mortalities than the unirradiated control group, but did show a very significant 4 log reduction in levels of *V. vulnificus* at 0.5 KGy and 5 log reduction at 1.0 KGy 2 days post irradiation (4 days post harvest) for the September 1994 samples. The April 1995 samples were reduced to below detectable levels at all doses. Commercial shucking, washing/blowing and packing or freezing in the half-shell also reduced levels of *V. vulnificus* 3-5 logs by 14 days post harvest. Additional irradiation processing of these products reduced levels to below detectable MPN/g. Freezing shucked non-irradiated oysters cryogenically in CO2 and in blast freezers also showed significant 3 log reductions in *V. vulnificus* levels one week post freezing and 4 log reduction by 8 weeks post freezing. There was no significant difference between the two freezing methods.

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Acknowledgements:
Michael Rabalais and Darren Duet for research technical assistance;
Motivatit Seafoods, Fox Seafoods, Leavins Seafoods, RD Seafoods, Morgan Seafoods, Cowart Seafoods and Food Technology Services, Inc. for commercial harvesting, processing, shipping and irradiation processing of Louisiana oysters.

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METHODS

This study evaluated the feasibility and effectiveness of actual commercial level harvesting, processing, packaging and shipping of live shellstock in 60 lb (200 count) boxes, cases of shucked 12 oz containers of fresh oysters (12 per case), and cases of l/2 shell frozen oysters for commercial irradiation processing.

- Shellstock oysters were commercially harvested in September 1994 (end of spawing season) from Louisiana, shipped by refrigerated truck (40°F) to Florida and Virginia for further processing and packaging, and from there to Food Technology Services, Inc. for irradiation processing.

- In Florida, ten 60 lb. boxes of 200 count live shellstock and 8 cases of 12 each 12 oz. shucked fresh oyster were commercially packaged.

- In Virginia, the oysters were shucked on the half shell, shrink-wrapped, on a Styrofoam tray for 6 half-shell oysters and cryogenically package quick frozen ***(PQF) with C02.***

- At ***Food Technology*** Services, Inc. two 60 lb. boxes of live shellstock oysters were each treated with minimum levels of 0.0 (controls), 0.5, 1.0, 1.5 and 2.0 KGy (400 animals per dose).

- Two cases of 12 oz. fresh shucked oysters were each treated with minimum dose levels of 0.0 (controls) 1.0, 1.5 and 2.0 KGy.

- Sixteen PQF trays of 6 half-shell oysters/tray were each treated with minimum dose levels of 0.0 (controls), 1.0, 2.0, 3.0 and 5.0 KGy.

- Eight samples of 12 washed and blown oysters were vacuum-packed in non-permeable Mylar bags and commercially package quick frozen (PQF) by two commercial methods: $\bar{C}\bar{O}\bar{2}$ cryogenic freezing at -600F and blast freezing at -200F. The were not subjected to further irradiation processing. One 12 oyster bag from each freezing method was sampled each week post freezing for 8 weeks.

- The processed oysters were returned to NSU by commercial refrigerated truck (4OoF) and analyzed for MPN/g *V. vulnificus,* total APC, and % cumulative mortality (liveshellstock only).

- This was repeated in April, 1995 for live shellstock and fresh shucked oysters to evaluate % mortality at the beginning of spawning season.

RESULTS

Live Shellstock Irradiated Oysters

- Doses of 0.5 and 1.0 KGy showed significant 5 log reduction in levels of *V. vulnificus* (4.6×10^5 to 4.6 and 2.3 MPN/g respectively) without causing significant increases in mortality as compared to the controls through 14 days post harvest (shelf life for live oysters).

Fresh **Shucked Irradiated Oysters**

- There were no detectable levels of *V. vulnificus* in irradiated fresh shucked oysters, even at the lowest dose of 1.0 KGy.

- Even non-irradiated controls dropped from 4.6×10^6 to < 5 MPN/g by 14 days post harvest on ice in cold storage at 40°F; by 17 days at 40°F, there were no detectable *V. vulnificus* in non-irradiated controls.

Half-Shell Frozen Irradiated Oysters

- There were no detectable levels of *V. vulnificus* in any of the irradiation-processed frozen half-shell oysters, even at the lowest dose of 1.0 KGy; non-irradiated controls dropped to <10 MPN/g after 2 months.

Shucked, Washed and Blown Oysters Vacuum-Packed in non-permeable Mylar Bags and PQF by CO2 Cyrogenic Freezing and Blast Freezing

- Levels of *V. vulnificus* dropped significantly by 3 logs from 4.6 X 10^5 MPN/g to 2.5 X 10^2 in CO2 frozen and 2.1 X 10^2 in blast frozen 5 days post freezing. By 8 weeks post freezing, levels of *V. vulnificus had* dropped 4 logs to 9 MPN/g in both freezing methods.

CONCLUSIONS/ RECOMMENDATIONS

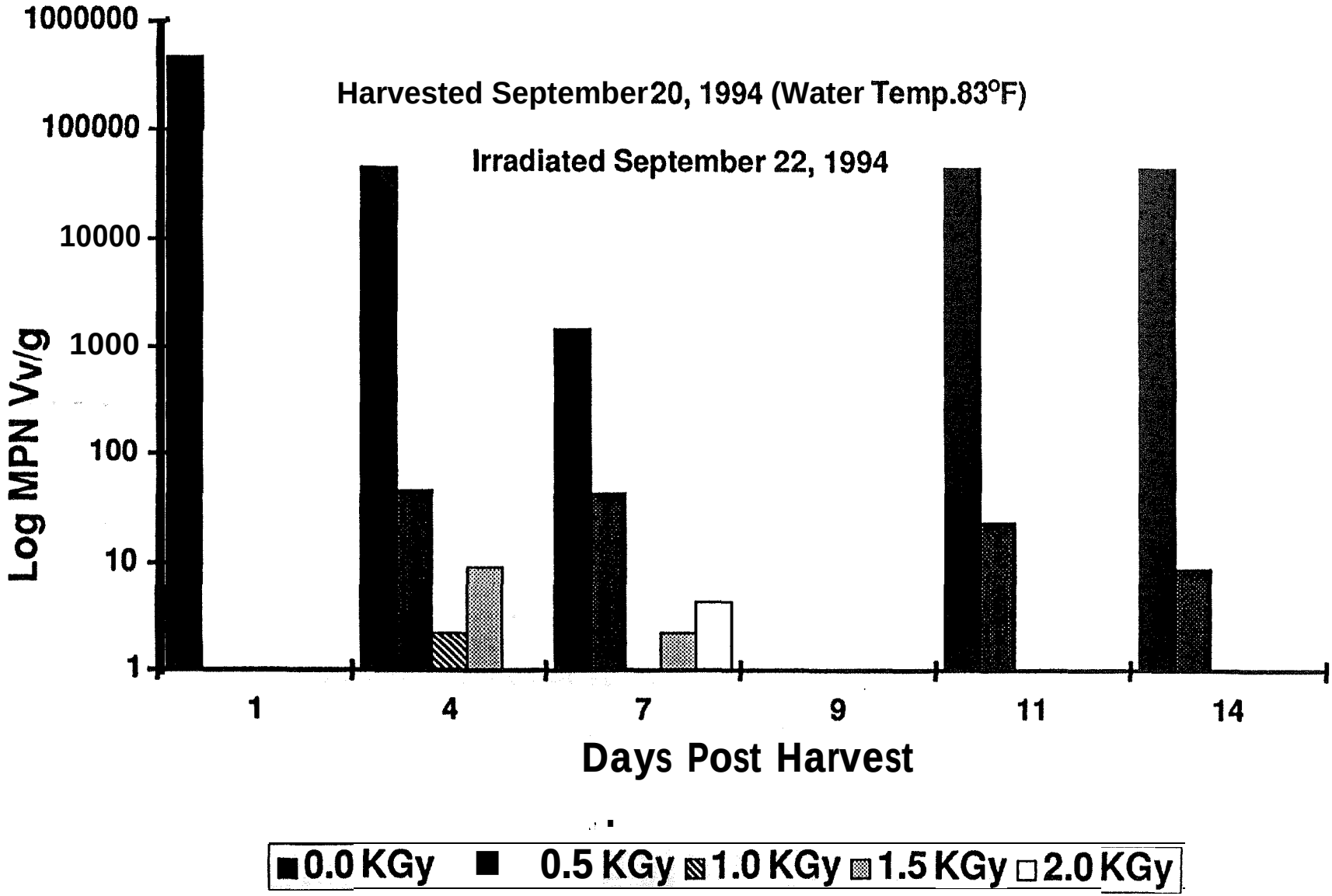
- 0.5 and 1.0 KGy are effective in greatly reducing or eliminating *V. vulnificus* from live, fresh shucked or frozen oysters commercially.

- High mortalities even in control live oysters were mainly attributed to the weakened physiological state of the oysters in September and April, and the cold storage temperature of 40°F.

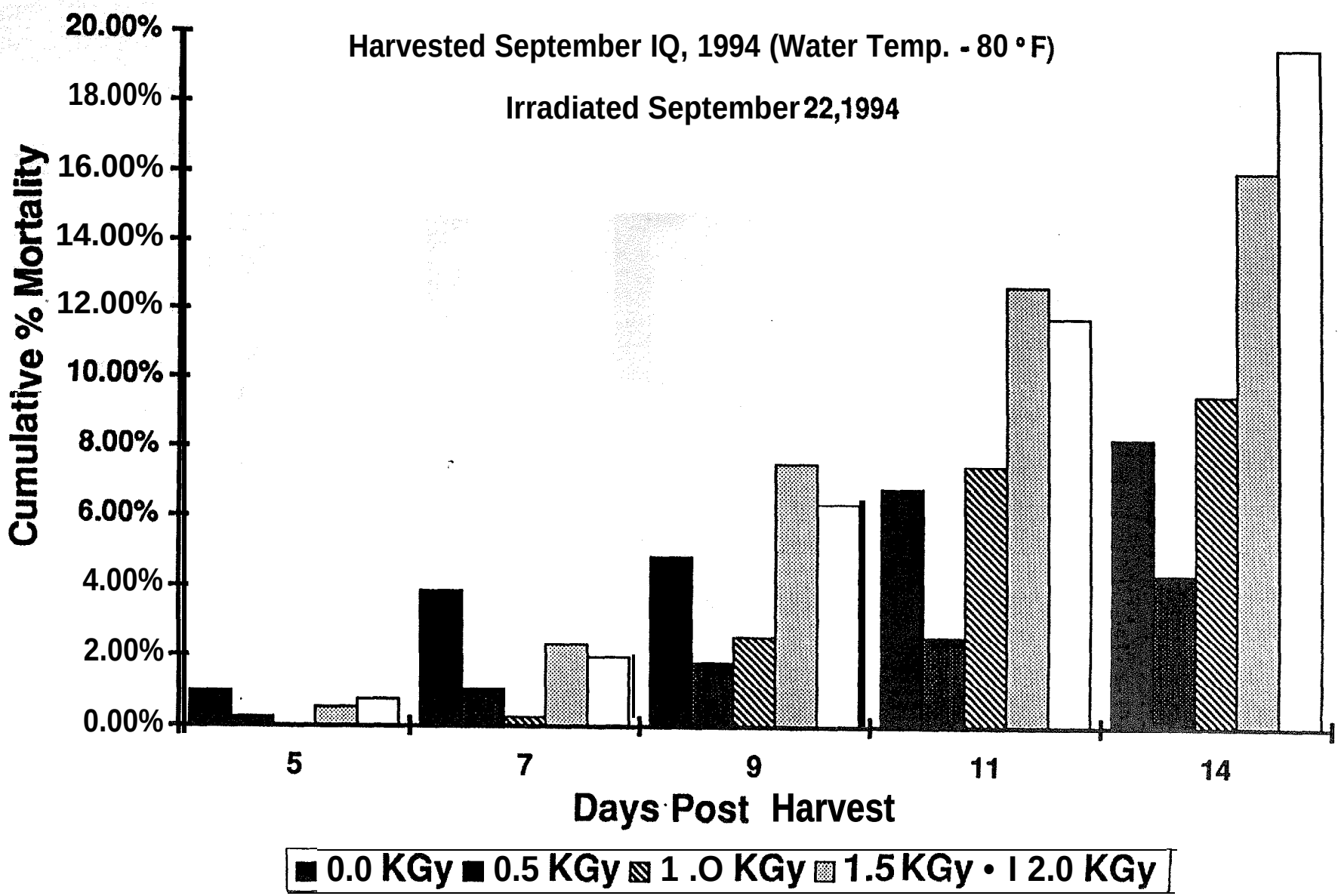
- Frozen shrink-wrapped half-shell oysters irradiated even at the lowest dost level of 1.0 ~~0.5~~ KGy show complete elimination of *V. vulnificus*, and have practically unlimited shelf-life. These Products have the greatest large retail market potential for a processed half-shell oyster.

- Freezing of processed shucked and washed oysters either cryogenically or in blast freezers without further irradiation processing also shows tremendous reduction of *V. vulnificus* levels from 10^5 *to* 10^0 within 2 months of frozen storage.

THE EFFECT OF IONIZING IRRADIATION ON *V.vulnificus* POPULATION IN LIVE SHELLSTOCK OYSTERS



CUMULATIVE MORTALITY FOR LIVE SHELLSTOCK OYSTERS TREATED WITH IONIZING IRRADIATION



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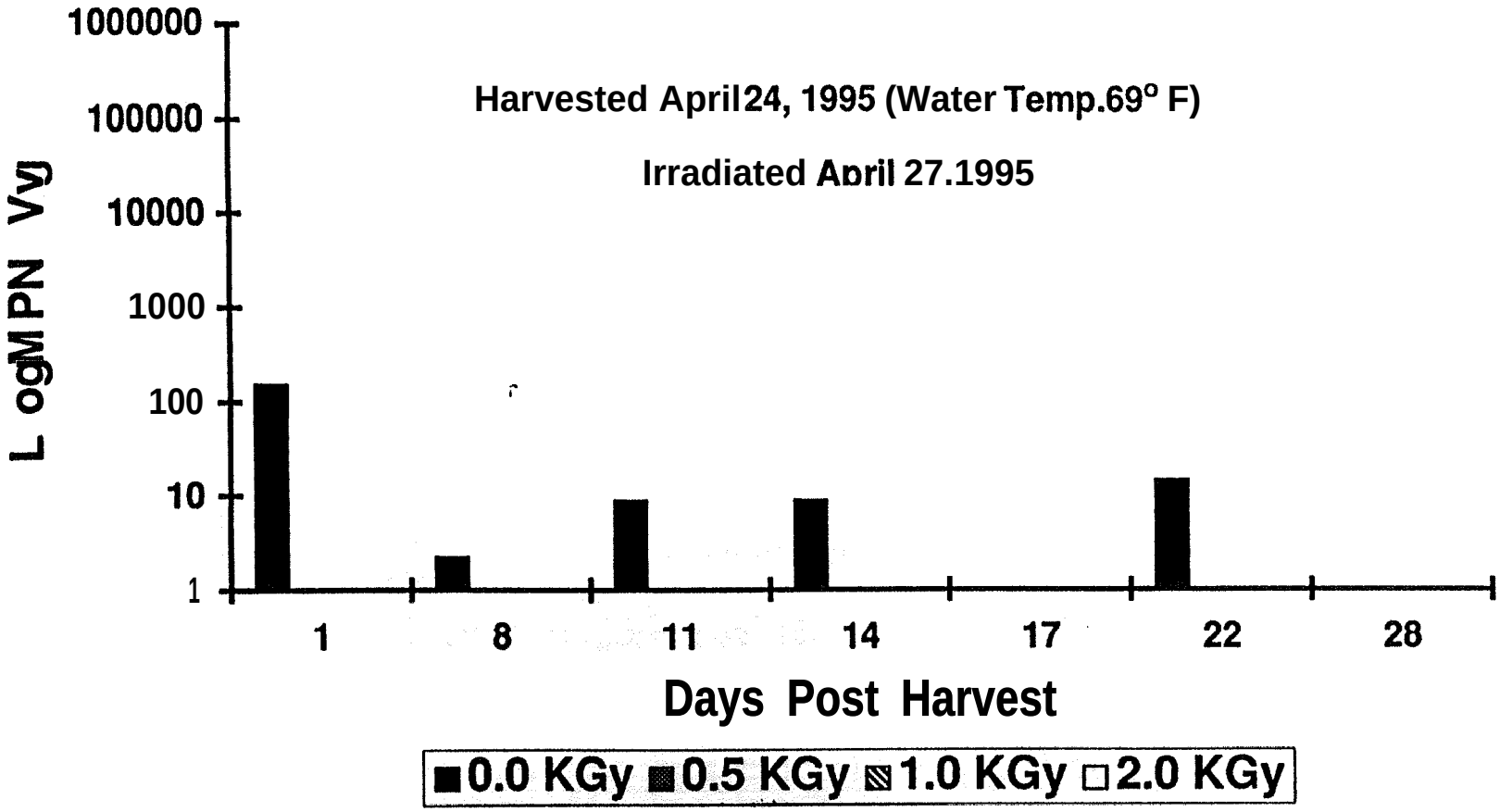

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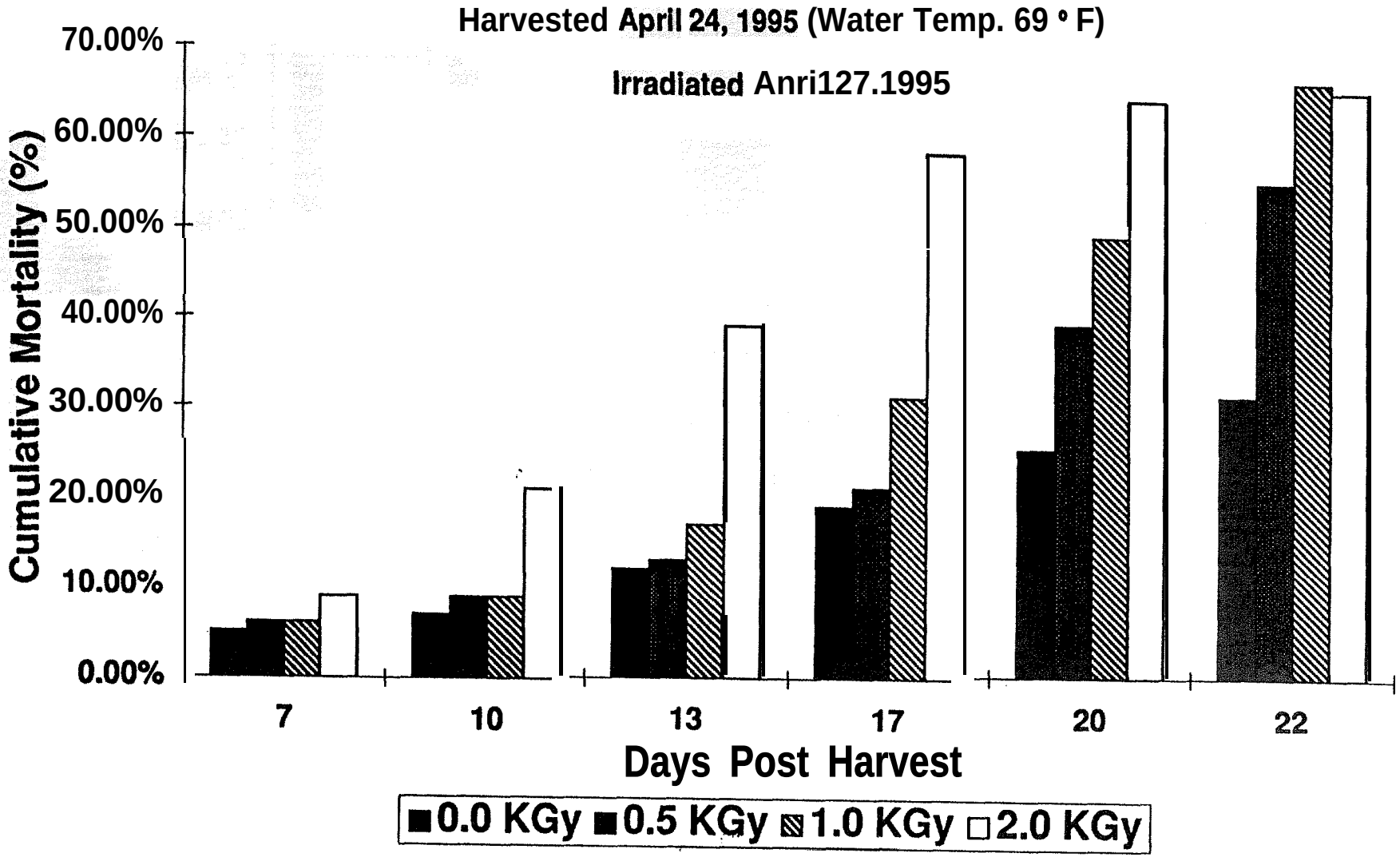
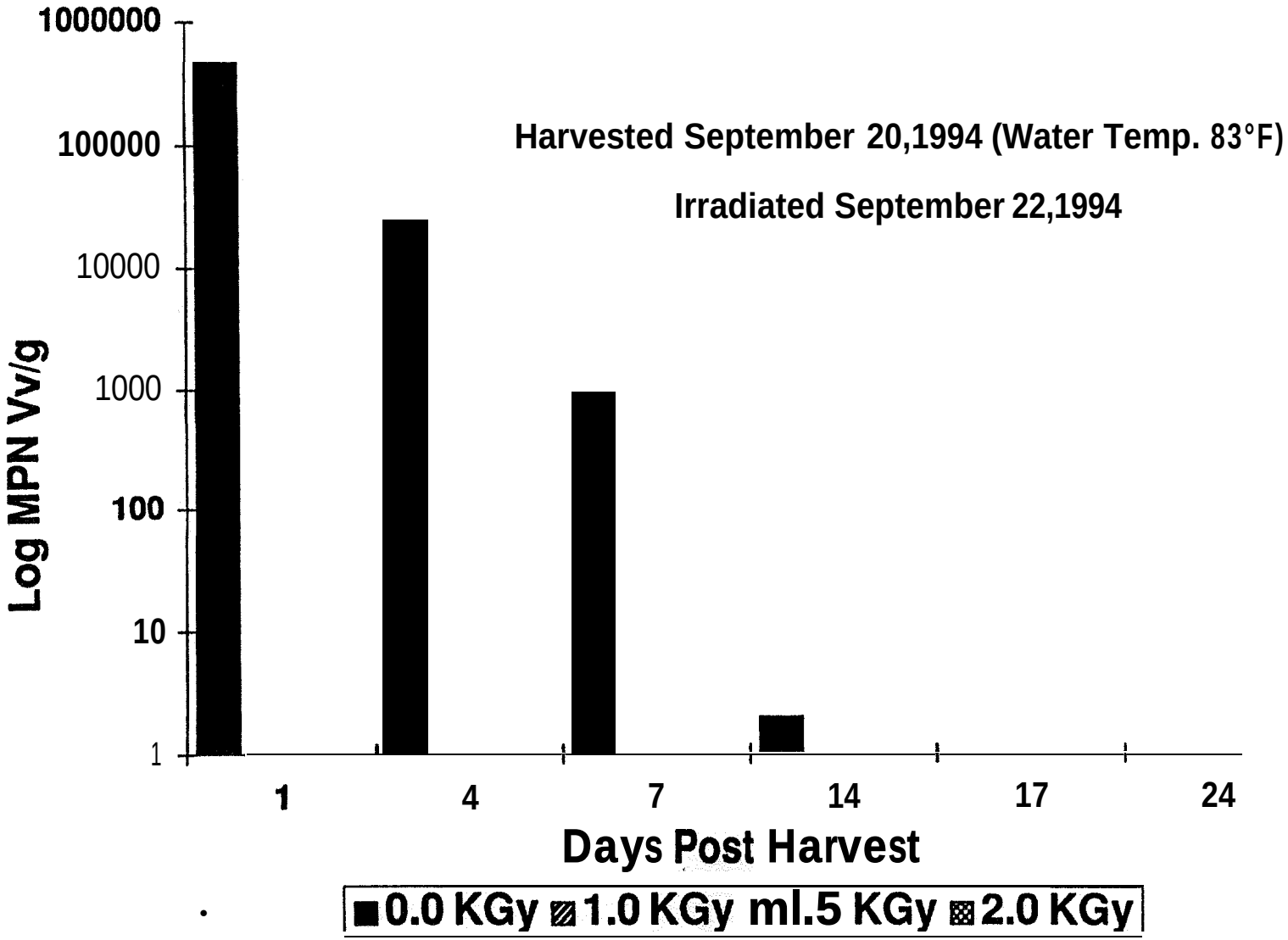
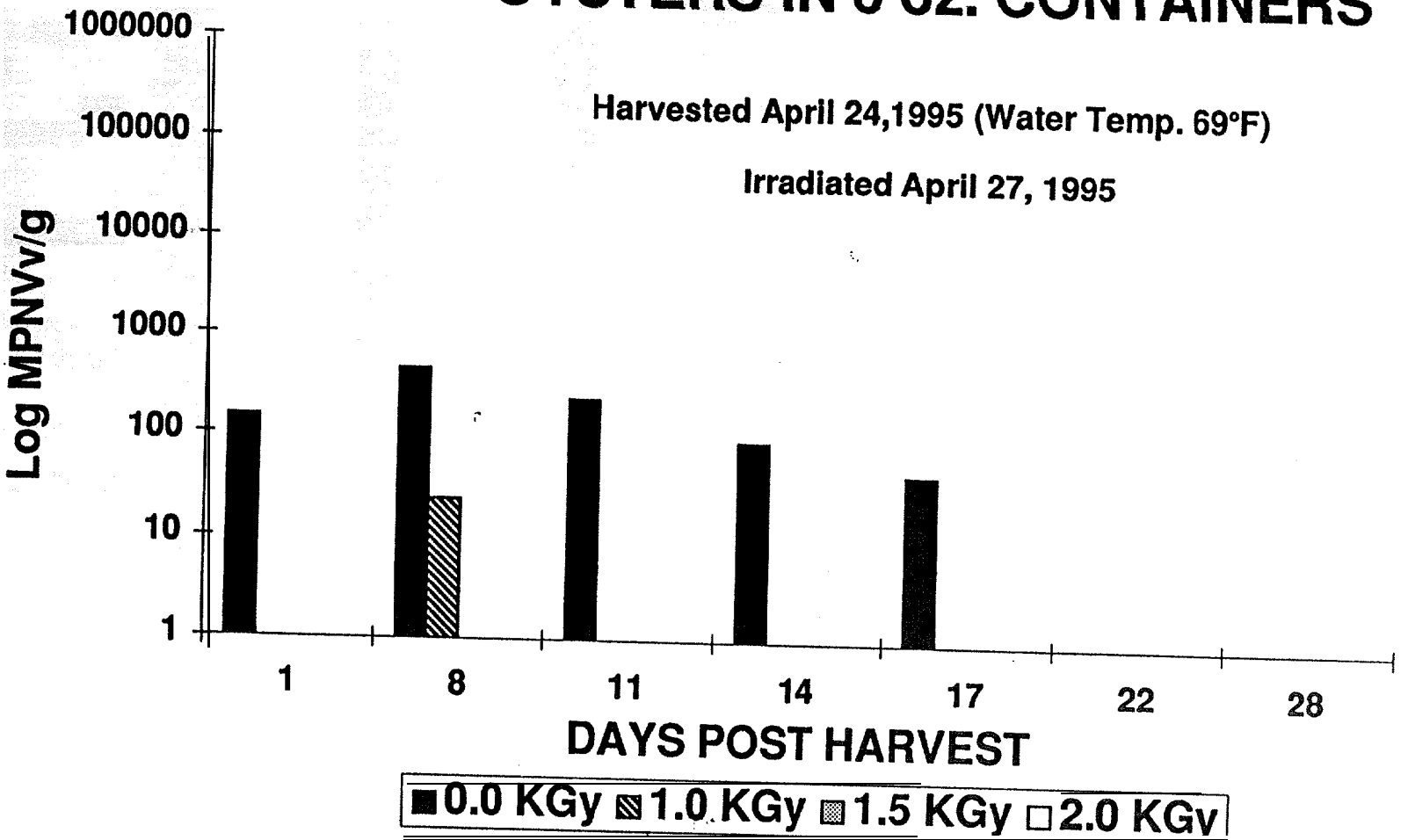


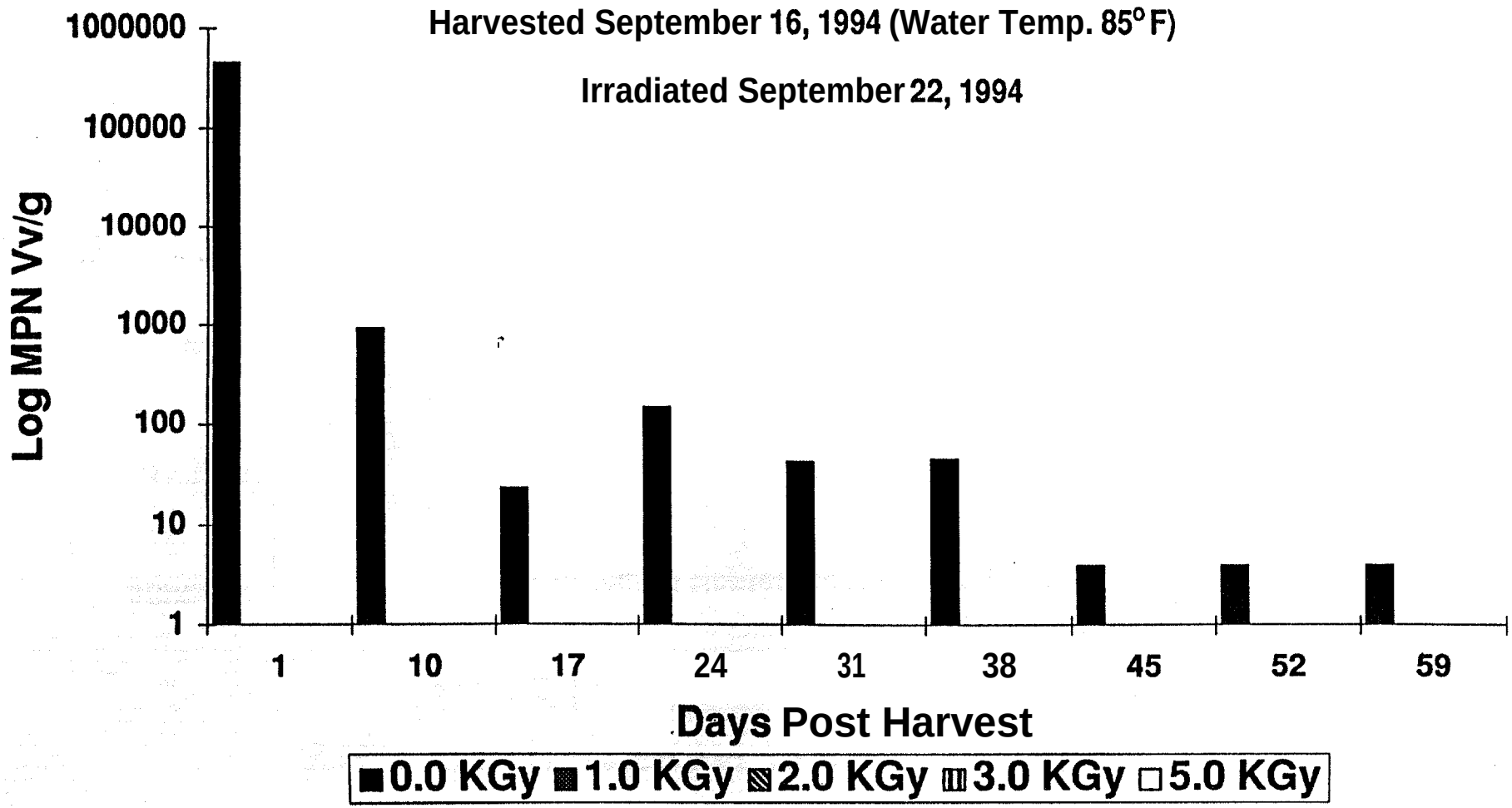
THE EFFECT 0F DIFFERENT DOSE IONIZING IRRADIATION ON *V.vulni* POPULATIONS IN SHUCKED OYSTE 12 oz. CONTAINERS



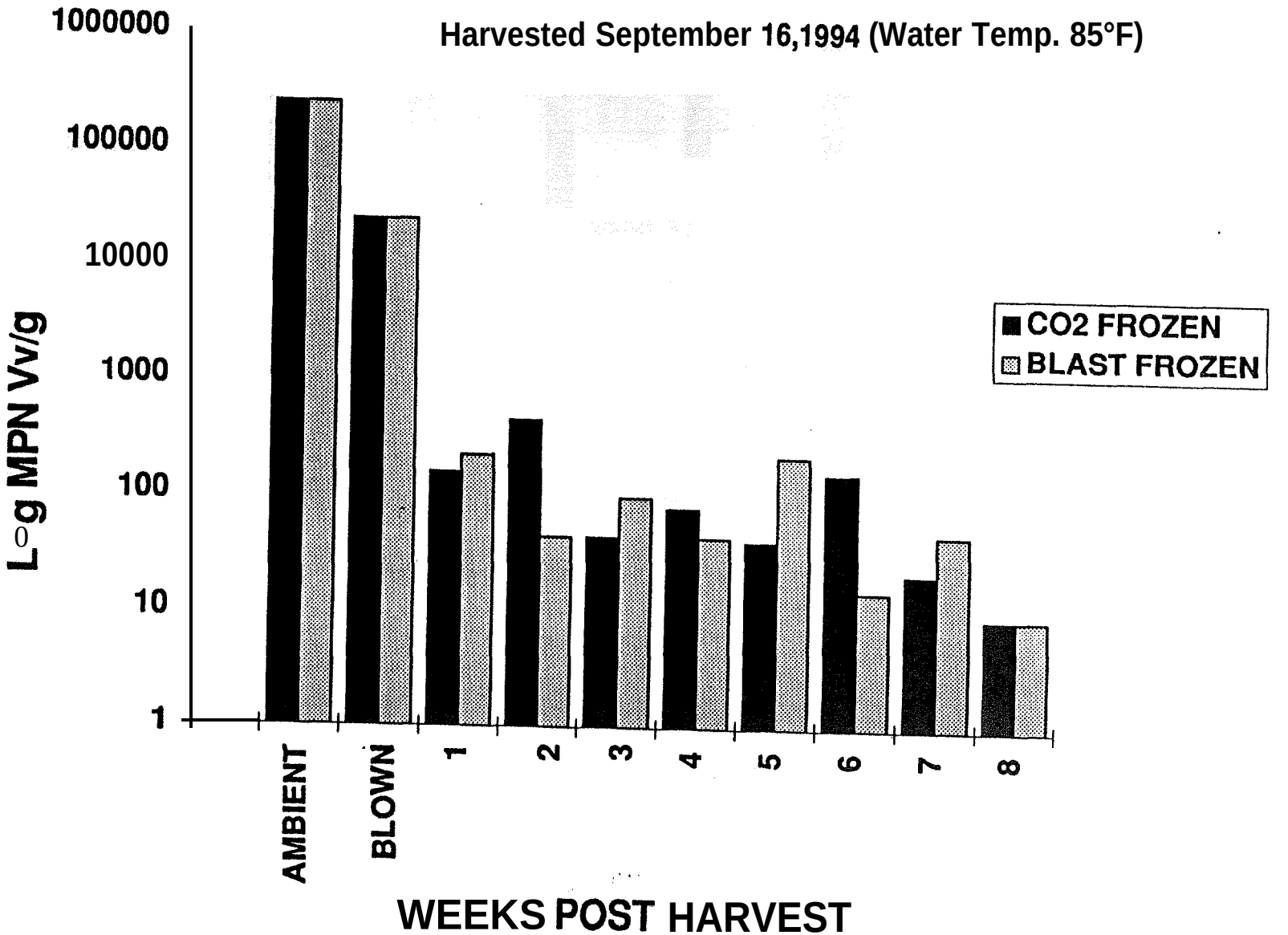
THE EFFECT OF IONIZING IRRADIATION ON *V. vulnificus* POPULATIONS IN SHUCKED OYSTERS IN 8 oz. CONTAINERS



THE EFFECT OF IONIZING IRRADIATION ON *V.vulnificus* POPULATIONS IN OYSTERS ON THE HALF SHELL (FROZEN)



THE REDUCTION OF *V.vulnificus IN CO2* AND BLAST FROZEN SHUCKED OYSTERS



Vibrio Vulnificus: Past, Present, and Future Control Strategies

Mark L. Collins, MPA

Florida Department of Environmental Protection

I would like to thank Dr. Otwell for inviting me to participate in the Tropical and Subtropical Seafood Sciences and Technology Society of the Americas 20th Annual Conference. As way of introduction, I am Mark Collins, Environmental Program Administrator, in the Bureau of Marine Resources Regulation and Development, Florida Department of Environmental Protection. The Bureau's activities include classification of the states shellfish waters, inspection of shellfish aud crab processing plants withiu the state, assessment and enhancement activities for the states shellfish resources, and aquaculture lease activities.

Most of my talk this afternoon will focus on past, present, and future control strategies to control illnesses caused by the marine bacteria Vibrio vulnificus.

Since 1925, the present shellfish sanitation program has operated as a cooperative program between state and local health agencies, and the U.S. Public Health Service. Individual state agencies classified growing waters, inspected processing plants, and patrolled closed growing waters. The U.S. Public Health Service provided technical support and evaluated individual state programs. In 1982, state agencies formed the Interstate Shellfish Sanitation Conference (ISSC) in order to provide input into changes for the National Shellfish Sanitation Program (NSSP)(FDA Compliance Policy Guide 7158.04, 1988).

Grover Starling (1988) quotes John W. Kingdon of the University of Michigan who said, " Conditions become defined as problems when we come to believe that we should do something about them." Starling states that we are surrounded by conditions--bad weather, illnesses, pestilence, fanaticism, highway congestion, and dirty air. We may or may not choose to consider this set, or parts of it, a problem. This is the situation I believe we are currently faced with by the marine bacteria Vibrio vulnificus.

Infection by Vibrio vulnificus is no recent occurrence.Koeng, Iklueller, and Rose (1991), suggest that Hippocrates described what may be the first case in the medical literature. A patient named Criton from the island of Thasos presented with "violent pain in foot," fever, delirium, and black blisters of his shin. Despite state-of-the-art therapy, be died on the second day after the onset of symptoms. As Criton was a fisherman, it is likely that he was exposed to the Vibrio vulnificus organism in seawater. The first recent report of clinical infection was in 1970, with the case of a previously healthy man in whom leg gangrene, a generalized hemorrhagic rash, thrombocytopenia, hypotension, and vomiting and diarrhea developed two days after he bathed and clammed in the seawater of Narragansett Bay in Rhode Island.

The historic study of Vibrio vulnificus as a pathogen dates to the late 1970's when the organism was named by CDC scientists.(Blake, Merson, Weaver, Hollis, and Heublin, 1979). In 1987 the shellfish specialists from the FDA Southeast Regional office identified Vibrio vulnificus as au emerging public health concern due to illness and death caused by the consumption of raw oysters (Casey, Omstead, and Herrington, 1987). This increased

concern led to a workshop on Vibrio vulnificus in 1988. Recommendations from this workshop iucluded educate the target at-risk population, educate the industry concerning good handling practices, collect illness data to determine the scope of the problem, conduct research to answer unknowns, and analyze the results of the data collection and research to determine reasonable controls (Richard Thompson, 1994). As we can see past actions concerning this organism centered on gathering information on the magnitude of the problem.

Actions by the Interstate Shellfish Sanitation Conference during the 1990% centered on the education of at-risk persons, ie., persons with liver disease, blood disorders, and innnune compromised conditions to avoid consumption of raw oysters. Incremental education efforts were initiated by individual states through the adoption of consumer messages on oyster packages and at retail locations, in an effort to educate high risk consumers. Messages were adopted by Louisiana in 1990, California in 1991, and Florida in 1993 and 1994 (Louisiana Register, 1990, Richardson, 1991, F.A.C. 1993, 1994). In 1994 the FDA and ISSC again sponsored a workshop in Washington D.C. to discuss the latest findings on Vibrio vulnificus. From the findings of this workshop, FDA submitted an issue at the 1994 ISSC conference requesting the conference adopt harvest controls which would restrict the harvest of oysters from the Gulf of Mexico during the months April-October if the oysters were for sale as raw oysters (ISSC issue 94257,1995) This issue was not adopted by the ISSC, however it was referred to a committee in order that manual language for adoption be drafted, and alternatives to this option be identified (Thompson, 1994). The Consumer Protection Committee met twice during 1994 to work

on it's charges. As a result of deliberation at the committee level, regulatory officialsand industry members in the states of Florida, Alabama, Mississippi, Louisiana, and Texas agreed to implement controls prior to action by the ISSC. Controls were initiated to voluntarily restricted harvest times during the summer of 1995 to 14 hours or less a day, place at-risk consumer information labeling on oysters,and require a SELL BY date on shellstock tags not to exceed 14 days post harvest. These common sense Good Manufacturing Practices reduced the time of harvest to refrigeration from 20 hours a day, initiated consumer information labeling on both finished products and endorsed labeling at retail for Gulf of Mexico oysters, and established for the first time a shelf life for oysters in the shell. The Consumer Protection Committee Report to theISSC executive board included these same immediate actions as alternatives to the FDA proposal to restrict the raw oyster sales of Gulf states during April-October (ISSC Issue 94-257, 1995). Final action on this issue was addressed at the 1995 ISSC meeting.

At the 1995 ISSC meeting, held in Orlando, Florida, a total of ten separate issues dealing with Vibrio vulnificus were considered. An alternative method of control was approved by the voting delegates of the States. This proposal called for the use of a temperature matrix to establish harvest controls in states where Vibrio vulnificus has been implicated. The matrix would call for decreasing the harvest time to refrigeration as water temperatures warm. This matrix inherently recognizes that as water temperatures warm, Vibrio vulnificus levels increase in both waters and meats. It also recognizes that reducing the time to refrigeration reduces Vibrio vulnificus levels. Table one below outlines the Matrix as adopted by the ISSC.

Table One.

ACTION LEVEL	WATER TEMPERATURE**	TIME TO REFRIGERATION*
LEVEL 1	November December January February March	Present manual requirements
LEVEL 2	65 - 74 degrees F	14 hours
LEVEL 3	75 - 84 degrees F	12 hours
LEVEL 4	> 84 degrees F	6 hours

* Product must be under ambient refrigeration at 45 degrees F within the hours specified above based upon the first shellfish harvested. During action levels periods 2,3, and 4, the product shall be shaded.

** The state shellfish control authority shall establish Average Monthly Maximum (AMMWT) Water Temperature for each growing area by averaging the previous 5 years maximum monthly water temperature (ISSC Task Force Two Report, 1995). Evaluation of exact implementation strategies are still in development by Gulf states regulators (Personal Communication Heil, 1995).

Performance measures to judge the success of this matrix have yet to be determined. This step will be addressed in 1995/96 by a committee of state/federal/industry representatives in order that the matrix can be evaluated when it is adopted (Personal Communication Moore, 1995). This future control should be able to be implemented by Gulf states during 1996.

What can be seen from this short chronology of events is that health officials, and the shellfish industry have been struggling with Vibrio vulnificus and raw shellfish consumption for nearly a decade. Debate has raged on whether this condition is a public health problem or a personal health problem, This debate will continue. What becomes evident, as Eldein (1988) states, is "The choice confronting politician and policymakers is not 'What are the right things to do?' but rather 'What might serve as a basis for common action of people with widely differing goals, values, and perceptions?'" Eldein continues that "Despite the fact that information is always incomplete, bad, or just not available , decisions have to be made: problems have to be confronted even though knowledge is imperfect."

It remains a question, whether or not the consumers of this nation are demanding protection from oysters in the raw form. What does appear from testimony at the 1994 Vibrio workshop, is that consumer advocates wish that consumers are given the information to make informed decisions themselves (1994 Vibrio vulnificus workshop transcript, page 229). This can best be done through both directed and general education materials to consumers.

Current efforts in this regard include the education of over 700 health professionals in the Gulf states during 1994 through funding by the EPA Gulf of Mexico Project. This education project will continue in 1995 by researchers at the University of Florida and Florida State University (Personal Communication Tamplin, 1995). $500,900 was provided by the National Marine Fisheries Service in 1994 for use by the U.S. Food and Drug Administration in an effort to educate high risk consumers about their risk of raw oyster consumption. FDA with the help of the ISSC is developing messages for distribution (ISSC Vibrio vulnificus Information Sheet, 1995).

A balanced approach to this problem will call for regulators, industry, and the consumers of shellfish to each take increased responsibilities to find a solution. Regulators will have to implement and enforce regulations which are balanced, which provide for an safer raw shellfish product, yet does not prevent through regulatory burden the industry operation. Industry must increase its efforts to provide safe products to the marketplace, to develop innovative practices which provide for improved shellfish products, and strive to educate their customers. Consumers must accept responsibility for their own health conditions and make informed choices about the risks they take, realizing that eating is a risky business but that not eating is fatal.

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USE OF "COOL PASTEURIZATION" TO CONTROL *Vibrio vulnificus IN* RAW SHELL-STOCK OYSTERS

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Vibrio vulnificus is a natural inhabitant of estuarine environments and may be transmitted to humans by the ingestion of raw seafood. This bacterium has been recovered from natural waters in high numbers during the warmer months (Ruple and Cook, 1992). Oysters from these waters are often incriminated in human diseases since the oysters are commonly consumed raw. Several methods have been used to control *V. vulnificus* in oysters for raw consumption. Depuration has proven largely unsuccessful even though such treatment is effective in removing "indicator" bacteria. Ionizing radiation is very effective but requires an expensive facility and is not yet federally approved. The use of additives such as organic acids, BHA, or diacetyl, although effective in reducing the vibrios, produces unacceptable flavor changes. This study focused on the use of a mild heat treatment to reduce *V. vulnificus* while preserving the sensory characteristics desired in raw oysters.

MATERIALS AND METHODS

Oyster Study. Artificial Inoculation: During the spring (March, April, May 1995) live shell stock oysters were artificially inoculated with *V. vulnificus* by placing them into *2-20* gal *aquariums* containing the pathogen. Concentrations were of either 10^4 or 1 0^6 cfu vibrio/ml seawater. Oysters were held for 24 hr for self inoculation of the pathogen by filtration and concentration.

Oysters Study. Environmental Contamination: During the summer (June, July, Aug, Sept. 1995) the oysters harvested contained a naturally high level of *V. vulnificus* of approximately 10^{5-6}cfu/g oyster meat.

Post Harvest Oyster Processing: In-shell *oysters* were sorted into 3-sizes prior to heat treatment. Oysters, with shell clamped to stay closed, were submerged in a 55°C 50 gal water kettle and brought to an internal temperature of 48-50°. Oysters in baskets were then transferred to a like kettle and held at 50°C for 0,5,10,15 min. Oysters were then transferred to a cold water bath and cooled to 2-4°C. The oysters were then enumerated for *V. vulnificus* at 0,4,7,10, and 14 days.

Microbial Analysis: A combination of 3-tube MPN and mCPC(modified Colistin-Polymyxin B-Cellobiose) agar with EIA (enzyme immunoassay) was used for the isolation and enumeration of *Vibrio vulnificus in* raw shell stock oysters(FDA-BAM 1992).

RESULTS AND DISCUSSION

Survival rate of *V. vulnificus* in aquarium water

The percent growth and survival of *V. vulnificus* in aquariums rose by 15% to 36% during the 48 hours inoculation period and then began to drop(Fig. 1). The cell density of *V. vulnificus* in the aquariums showed *a* slight increase during the initial inoculation period (2 days) and was then followed by a decrease. Since the growth rate of *V. vulnificus* during the inoculation period was slow, the change of cell density was not significant.

Survival of V. *vulnificus* in heat treated oysters

The results of the 50° C water heat treatment of inoculated shellstock oysters are presented in Table 1 and Table 2. The treatment was very effective in the reduction of *V. vulnificus* in both 1.5 x 10^5 MPN/g (high level) and 2.4 x 10^3 MPN/g (low level). Time exposure of 5 min was sufficiently effective to reduce the numbers of the pathogen by 99.9% Preliminary work on shucked oysters, which lead to our study was reported by Cook and Ruple (1992). They reported death of *V. vulnificus* at temperature above 45°C and that 50° C was sufficient to reduce the bacterium to undetectable levels.

Table 1. Effect of 50° C internal temperature heat treatment on the survival of *V. vulnificus* in the shellstock oysters treated with high level of contamination

Heating time	*V. vulnificus* MPN/g of oyster meat from high concentration level	% Reduction
0 min	1.5 x 10^5	0%
5 min	93	99.9 %
10 min	<3	100%
15 min	<3	100%

Table 2. Effect of 50° C internal temperature heat treatment on the survival of *V. vulnificus* in the shellstock oysters treated with low level of contamination

Heating time	*V. vulnificus* MPN/g of oyster meat from high concentration level	% Reduction
0 min	2.4 x 10^3	0%
5 min	<3	99.9 %
10 min	<3	100%
15 min	<3	100%

Survival rate of *V. vulnificus* in oysters during storage

The survival of inoculated *V. vulnificus* in non-heated oysters stored on ice is presented in Fig. 2. The data demonstrates the sensitivity of this bacterium to temperatures below 5°C with a significant decrease in the nurnber of *V. vulnificus* during the first 7 days and a further slow decrease in numbers from day 7 to 14. *Vibrio vulnificus* was not recovered from oysters stored at -20° C after 30 days.

Environmental Contamination

Naturally incurred *V. vulnificus* was found to exist in the shellstock oysters harvested from Adam bay, Louisiana from June through September. Results of heat treatment of these oysters gave similar results to the first experiment with no *V. vulnificus* detected after heat treatment (Table 3). The numbers of aerobic plate count of heat treated shellstock oysters were also significantly reduced (Table 4).

Table 3. Number of *V. vulnificus* in both heat treated and control shellstock oysters.

Heating time	*V. vulnificus* MPN/g of oyster meat	% Reduction
0 min	8.9×10^4	0%
3min	9	99.9%
5 min	<3	100%
10 min	<3	100%
15 min	<3	100%

Table 4. Aerobic plate count of both heat treated and control shellstock oysters.

Heating time	CFU/g of oyster meat	% Reduction
0 min	3.5×10^5	0%
3 min	2.7×10^3	99.2%
5 min	6.6×10^2	99.99%
10min	6.0×10^2	99.99%
15 min	4.6×10^2	99.99%

Effect of ice storage on the survival of naturally contaminated *V. vulnificus in* shellstock oysters: The numbers of naturally occurring *V. vulnificus* in shellstock oysters dropped dramatically during the first 4 days of ice storage before stabiliznig. No *V. vulnificus* were detected in heat treated samples

throughout the iced storage. (Table 5.) Cook and Ruple (1992) reported a reduction of *V. vulnificus* during iced storage of shucked oysters but at a slower rate than with the shellstock and/or heat treated oysters analyzed in this study.

Table 5. Survival numbers (MPN/g) of *V. vulnificus* in mild heat treated and non-treated shellstock oysters during ice storage

Storage time	Control	3 min	5 min	10 min	1.5 min
Day 0	8.9×10^4	9	<3	<3	<3
Day 4	1,100	<3	<3	<3	<3
Day 7	460	<3	<3	<3	<3
Day 10	240	<3	<3	<3	<3
Day 14	240	<3	<3	<3	<3

Effect of ice storage on the aerobic plate counts and psychrotroph counts

Aerobic plate counts of shellstock oysters showed a significant drop during the first 7 days of iced storage before beginning to show recovery (Fig. 3). Cold storage has proved effective in reducing numbers of viable bacteria cells. In this study, about 80% of the living cells presented in shellstock oysters were killed or inactivated when the temperature was lowered to about 0°C during the ice storage. It was believed that the later rise of aerobic plate counts were due to the growth of psychrotrophs (Fig. 4). As shown in Fig. 3 and Fig. 4, both aerobic plate count numbers and psychrotrophic numbers of mild heat treated oysters remained low and stable, which suggested that the mild heat treatment effectively inactivated most of the bacterial cells, preventing the growth of psychrotrophs even after 7 day of storage on ice.

CONCLUSION

The use of mild heat treatment (50°C for 5 min) was very effective in eliminating the pathogen *V. vulnificus* while not eliminating all microorganisms. Therefore it is appropriate for this treatment to be classified as a "cool pasteurization" method. This treatment is currently under review by the U.S.F.D.A for approval. With approval and use, the Gulf Coast Oyster Industry should be able to continue harvesting and selling raw in-shell oysters year round. Early sensory testing of the treated oysters have indicated that panelists detect no noticeable adverse effects of the treatment. Results of sensory studies will be reported in the future. Further work on the effect of the treatment on other microbial pathogens continues.

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SATISFYING PUERTO RICO DEMAND FOR SEAFOOD WITH A VISION TO THE FUTURE USING FOOD SCIENCES AND RELATED TECHNOLOGY: UNIVERSITY OF PUERTO RICO INITIATIVES

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Puerto Rico is the smallest of the islands that comprise the Greater Antilles. It has a tropical climate and ubiquitous water supply providing. an adequate environment for aquaculture production. The University of Puerto Rico, Mayagüez Campus has a long history of commitment to support the different programs involved in Seafood through the activities of the Marine Science Department, Sea Grant Program, the Cooperative extension Programs and the Aquaculture Research in the Experimental Station and the recently established, Food Science and Technology Graduate Program.

The Food Science and Technology Multidisciplinary Graduate Program was established in March 8,1991, by the Council of Higher Education Certification 91-118. This program was the first one of its kind in the University system due to the multidisciplinary approach. This multidisciplinary program is part of the College of Agricultural Sciences but involves the coordination of the activities of three additional colleges: College of Arts and Sciences, College of Engineering, and College of Business Administration. It also involves seven departments: Agricultural Engineering, Animal Industries, Biology, Chemistry, Chemical Engineering, Horticulture, Marine Sciences, and Marketing.

The Mission of the Program is to gather and coordinate already existing activities in the Food Science and Technology Area in Campus to offer a Master Degree in Food Science and Technology. The goals are:

- To develop the professional resources that Puerto Rico needs to ensure a diverse, safe and nutritious food supply for our society.

- To contribute to the development of the scientific and technological knowledge needed for the growth and improvement of the food industry.

- To promote the research and development of processed food products to help local agriculture by adding value to its production and the possible opening of new markets for such commodities.

- To promote the cooperation and a productive coordinated effort among the Departments involved in the Program, required for a successful Multidisciplinary Graduate Program.

- To provide a contact and forum for the efficient exchange of information and utilization of expertise between university, government agencies and the food sector,

The Food Science and Technology Program created in 1991 had been since involved in seafood science and related technology through the research and extension activities in the processing of seafood and seafood products. There are 10 faculty members and 27 graduate students in the program of which 6 of them are working in seafood products research. In addition, graduate students had been participating in Cooperative training and research with the largest Tuna Processing Industry, Star-Kist Caribe at Mayagüez, PR.

After looking through the goals established by the faculty members and students involved in the program, it is not suprising that it plays an important role in the development of seafood research. Last May of 1995, a research grant was approved by the Science and Technology Board of the Economic Development Administration (best known as Fomento) to establish and Aquaculture Institute to promote the development of commercial technologies needed for aquaculture. A total of 2.5 million dollars was approved, for a three years period, of which 33% of the money are matching funds from the University. However the expansion of the aquaculture industry can not stand alone in its development without the involvement of the safety, shelf life, new products development research activities. The Food Science and technology Program provides the multidisciplinary approach for the development of new sea food products as well as safety and shelf life studies.

There are research projects in the several seafood products: surimi, smoked tilapia, shrimps, mackerel, canned tilapia and fish fillets. The research projects are:

A. Development of commercial scale procedure for the small size Tilapia or the stunt ones.

B. Determination of shelf life of smoked tilapia packed under modified atmosphere and stored under refrigeration.

C. Development of traditional sausages' recipes made out of minced tilapia meat.

D. Study of the effect of antioxidants in the shelf life of frozen tilapia fillets.

E. Determination of histamine level in Mackerel.

F. Development of a database for the presence/ absence of metals in the aquaculture farms around the island.

Domestic aquaculture production of tilapia increased from twelve and one half million pounds in the eighty's to thirteen million pounds in 1994 and further expansion is expected. Imports increased from 32 million pounds in 1993 to forty nine- million pounds in 1994. The Food Science and Technology Program provides the scientific staff and students needed to coordinate the research required for the development of new and innovative products using the underutilized products as well as the safety, chemical and microbiological studies needed to support these activities.

SAFETY CONCERN IN THE USE OF MODIFIED ATMOSPHERE PACKAGING IN SEAFOOD PRODUCTS: A REVIEW

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INTRODUCTION

Modified atmosphere packaging (MAP) is a food packaging technique in which the air in a package or container is replaced by one or more gases, in various concentrations, before sealing. During storage, the initial atmosphere of the product, will be modified as a result of metabolic, chemical and microbiological activity and by the package's permeability. The choice of gas mixture used is influenced by the microbiological flora capable of grow on the product, the product's sensitivity to O_2 and CO_2, and color stabilizing requirements.

The use of MAP system has numerous advantages such as: extends product shelf life and quality, reduces economic loss, and allows the product to be transported longer distances for distribution and marketing. However, MAP also has some problems that also must be considered, since the technique needs strict temperature control, costs twice as much as vacuum packaging, and the container and packaging vary from product to product. Also, it may retard or inhibit spoilage microorganisms that might warn the consumers of problems, while allowing the growth of pathogens (Farber, 1991, and Reddy et al., 1992). But the major concern related to MAP is the growth and potential production of *Clostridium botulinum* type E in MAP fresh fish. The concern is well founded because of the pathogenesis significance of these organisms (Bristor and Hotchkiss, 1986, Farber, 1991, and Church, 1994).

LITERATURE REVIEW

The gases normally used in MAP are those found in the atmosphere: O_2, CO_2, and N_2 (Church, 1994). Each of these gases plays a specific role in extending the shelf life of fresh seafood. Oxygen (O_2) generally stimulates the growth of aerobic bacteria while inhibiting the growth of the strictly anaerobic, although there is a very wide variation in the sensitivity of anaerobes to O_2 (Church, 1994). Nitrogen (N_2) is an inert, tasteless gas with low solubility in both water and lipids. N_2 displays little or no antimicrobial activity on its own. Usually N_2 is used to balance a gas mixture or reduce the concentration of other gases in a mixture, to displace O_2 in packaging, delay oxidative rancidity and inhibit the growth of aerobic microorganisms. Also it is used as a filler to prevent pack collapse (snuffing), which can be a problem in atmospheres containing a high CO_2 concentration (Farber, 1991, and Church, 1994). Carbon dioxide (CO_2) is both water and lipid soluble and is

mainly responsible for the bacteriostatic effect on microorganisms in modified atmosphere. CO_2 inhibit aerobic bacterial, yeast and mold activity in foods. The overall effect on microorganisms is an extension of the lag phase and a decrease in the growth rate during the logarithmic phase.

This bacteriostatic effect is influenced by the CO_2 concentration, volume of headspace gas, acidity, water activity, the type of microorganisms, the growth phase and load of the initial bacterial population, the storage temperature and the type of product being packaged (Farber, 1991, Church,1994 and Reddy, et al. 1992). Although the bacteriostatic effect of CO_2 has been known for many years, the precise mechanism of its action is still a subject of much scientific interest. Since the bactericidal and bacteriostatic effects of CO_2 are temperature dependent, lack of refrigeration at any time during a product's life could allow the growth of organisms that had been inhibited by CO_2 during storage at a lower temperature. Pathogens that are resistant to the antimicrobial effects of CO_2 that cannot grow at low temperatures might grow during temperature abuse. MAP products do not represent a new or unique situation in that temperature abuse after processing and packaging is of serious concern. Under conditions of product temperature abuse, pathogens will grow in almost any atmosphere including air. Any atmosphere, then, must be considered as potentially dangerous (Bristor and Hotchkiss, 1986).

The incidence of *C. botulinum* in foods, although very serious, is low. C. *botulinum type* E has been isolated almost exclusively from aquatic sources. Fish may present a more significant problem because of the occurrence of C. *botulinum type* E in their natural habitat (Bristor and Hotchkiss, 1986). In order for foodbome botulism to occur, the following conditions must be met: First, the food must be contaminated with the spores or cells of toxigenic C. *botulinum.* Usually, contamination is due to the presence of C. *botulinum in the* environment where the food is produced, harvested, processed or stored. Second, the cells or spores must resist the food processing treatment. Alternatively, postprocessing contamination must occur. Next, the organism must multiply and produce toxin in the food. For this, the food must have an environment or microenvironment favorable for germination and outgrowth of the spores, and for growth and toxin production of the vegetative cells. Finally, the food must be consumed without sufficient cooking to destroy the heat labile toxin (Eklund, 1992).

Commercial use of modified atmosphere to extend the shelf life of fishery products has been limited by the potential of C. *botulinum growth* and toxin production in refrigerated, modified atmosphere packed fish. Despite these concerns, fillets of fresh fish packaged under modified atmospheres and stored continuously at temperatures below 3 °C have appeared in European supermarkets. No cases of botulism have been associated with the consumption of such products thus far MAP shows great promise for the extension of shelf life and control of the growth of food pathogens at refrigerated temperature. It is not, however, a substitute for refrigeration (Bristor and Hotchkiss, 1986). Overall, the majority of the studies reported in the literature indicate that the risks from foodbome pathogens in MAP are no greater and arefrequently less than those from aerobically stored foods. These findings are substantiated by the excellent safety record, to date of MAP (Church, 1994).

CONCLUSION

According to some researchers the success of MAP depends on: 1) good initial quality, 2) good hygiene from slaughter on, 3) maintenance of controlled temperature, 4) correct packaging material selection, 5) reliable packaging equipment, and 6) appropriate gas mixture. Also we have to keep in mind that there are some causes of concerns and that we have to take care of them such as: 1) under-processing, 2) post- process contamination, 3) temperature abuse, and 4) prolonged refrigerated storage.

RECOMMENDATIONS

1) To establish a Hazard Analysis of Critical Control Points (HACCP) that may include, among others' things, prevention of under-processed or postprocess contamination products and temperature abuse. 2) Educate the consumer. 3) Keep product below 3 °C at all times. 4) Factors that contributes to the incidence of botulism are often related to improper refrigeration, so the recommendation is that to prevent outgrowth, the fish must be heated sufficiently (SO-82 "C) to destroy spores of non proteolityc strains and refrigerated sufficiently (10 ° C) to prevent the growth of proteolytic strains of C. *botulinum* (Sperber, 1982). 5) In the future, the addition of lactic acid starter cultures. 6) The product should be placed in modified atmosphere immediately after harvesting and processing. 7) Most expert feel, that at this time, consumer sized packages of MAP fish should not be available for sale retail level. 8) If for some reason the product is for sale at a retail level, the addition of a time-temperature indicator (TTI) should be useful on each package. These indicators would provide warnings and messages to the consumer if the product was improperly handled at some point. 9) For products in which the removal of oxygen could permit the growth of anaerobic pathogens, it is recommended that one or more of the following criteria should be met: a) water activity < 0.92, b) pH < 4.5, and c) addition of sodium nitrite. 10) Use a pretreatment step such as radiation or antimicrobial treatment before storage.

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UTILIZATION OF WASTE TILAPIA IN THE PRODUCTION OF A SARDINE SUBSTITUTE

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Tilapia nilotica a fish native to Africa and the Levant, recently reclassified in the genus Oreochromis, is one of the most cultivated species in aquaculture systems in the world[1]. This is due in part, to its easy adaptability to tropical and subtropical areas, than are able to grow in waters that would kill many fishes, are able to utilize a wide variety of nutrient inputs for growth, are disease resistant, and are appetizing food items. [2] Tilapias have a firm, semi-moist, delicately flavored flesh with no intramuscular bones which, in part ,explains why in 1994. Tilapia was named as the restauranteurs fish of the year in the United States, in Latin America the development of Tilapia culture has begun as a mean of satilsfying local markets for high protein foods and as an export product to satisfy the growing demand in the Unilted States[3]. However, a major problem in tilapia culture has been that its proclivity for early reproduction, at just 3 months of age, which often leads to a stunted population. [4] Due to this, a large part of the harvest in mixed stocked systems are fishes too small to sell, resulting in a loss for the producer. [5] One viable solution to this problem, which can also help in feeding the wold world's expanding population, is to develop canning methods[6,7,8] that would permit small tilapia to be utilized as a substitute for sardines or other similar size products.

Puerto Rico's fishery resources have shown indications of overfishing. One indication is that landings of fish and shellfish have shown a consistent decline since 1979, when decrease in production from 7.2 millions of pounds, to 5.4 million pounds in 1982 . this is equivalent to a 25% reduction in the total fisheries production for the island over this time period and when in 1987 while fishing effort did not decreaase, only 2.1 millions of pounds were reported[9]. Another indication of overfishing is thet fish species that in the past did not have commercial value (e.g. Holocentrus ascensionis and Acanthurus spp.) are now easily being sold. Biostatistical data collected have also provided indications of overfishing. For example some important commercial species (e.g. panulirus arqus, Lutianus vivanus, Epinephelus quttatus and Ocyurus chrysurus) Were caught in large percentages before reaching minimum size of sexual maturation[10]. The total estimated value of reported landings for the years 1992 - 93 by 42 municipality and coast, are 2.5 pounds

MATERIAL AND METHODS

Product Preparation. Live commercially harvested tilapia were obtained from the Aquacultural Experimental Station of in Lajas. P. R. Their body weight and length was 90 - 100 g (average 92 g) and 17 - 18 cm long for tilapia. After the fish were chillkilled and rinsed with water, the scales, head, tail, viscera and backbone were discarded. The remaining was precooked in a conventional oven at 350°F. precooking times were 0 , 10, 20, or 30 min. The precooked tilapia were cooled and packed in 301 x 411 cans with sufficient patching to give a net weight of 535 ± 5 g , with 230 ± 5 milliliter portions of canned tomatoe juice seasoning , vegetable broth, or distilled water were then placed on top of the meat, leaving a headspace of about 10 mm. All cans were sealed (Dixie model 24 eletric set to close cans), immediately after being exhausted for 15 min. (Fisher scientific model 139, serial 115). The cans were then steam-retorted at 100°C for 87 min, allowed to cool under running water, and stored at room temperature for subsequent analysis. According to the food and Drug Agency, a manufacturer must thermally process acidified foods sufficiently to destroy all vegetative cells of microorganisms of public health concern[11].

CONCLUSION

Fisheries statistics indicate that the Puerto Rico fisheries resources are declining. Evidence of this are: a) decrease in number of pounds landed; b) the selling of species, that in the past did not have commercial value; c) large percentages of individuals of commercially important species being caught prior to obtaining minimum size of sexual maturation. Puerto Rico with its tropical climate has the advantage that it could develop aquaculture systems that produce the year round, that could satisfy part of the demand of the consumption of the fish to local level. Besides the fish that are highles marketable the possibility of beins able to turn small, unsellable fish into a product that would generate additional revenues for the producer would only improve the economic viability of tilapia production in Puerto Rico. By determinig consumer acceptabel and sensory characteristics of stunted and non-stunted tilapia it should be posible to determine the commercial valve of this unutilized resource.

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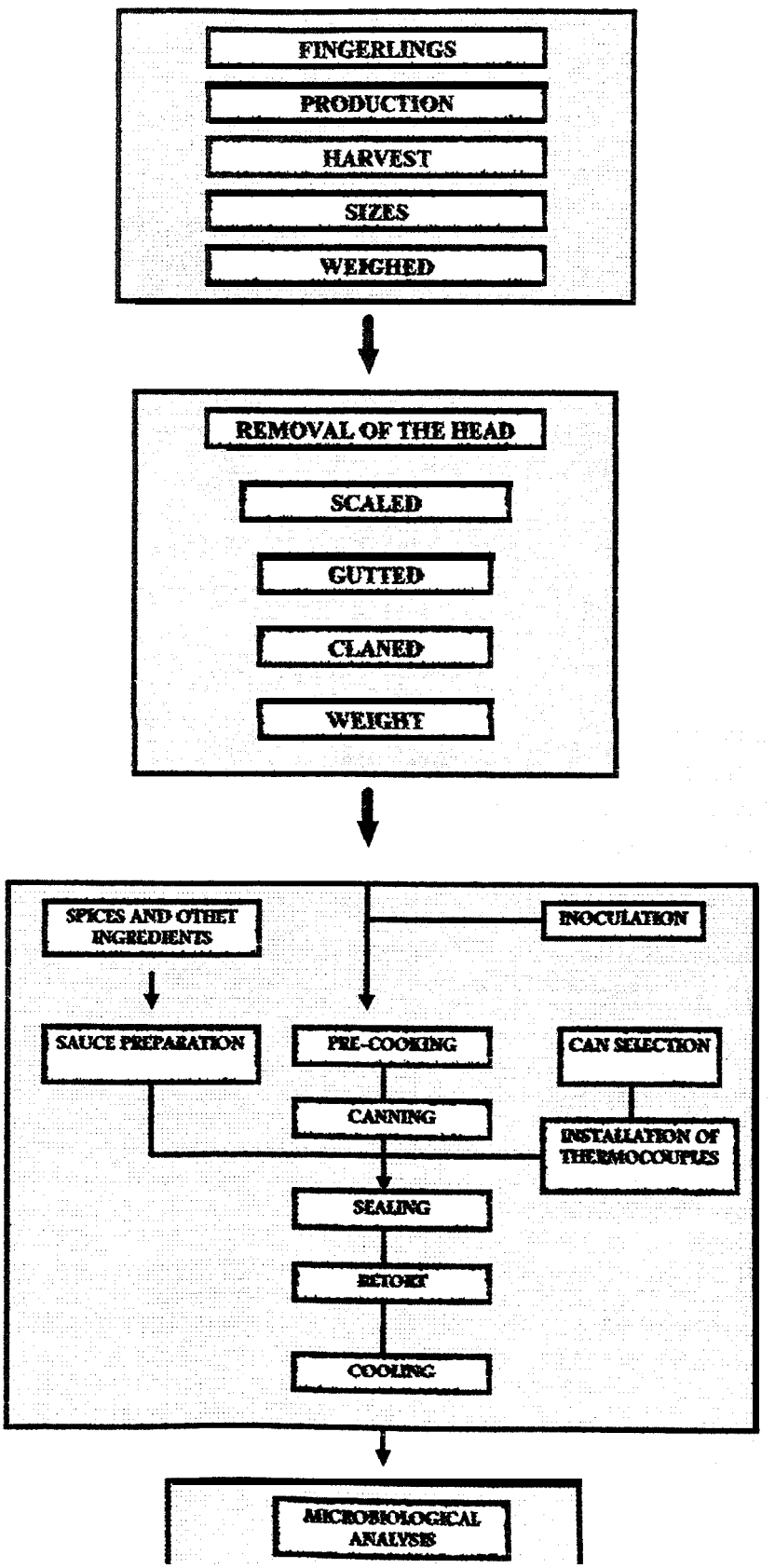
FLOWCHART DIAGRAM FOR THE CANNING PROCESS OF THE TILAPIA



FDA'S SEAFOOD HACCP INITIATIVE

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Good morning! Thank you. I appreciate the opportunity to talk to you today about FDA's Seafood HACCP Initiative. This is a time of great change in the world of seafood in **the** United States. It is a time of changes in how we regulate seafood domestically and internationally, as well as changes in how we participate in international trade. It is also an exciting time for the U.S. Food and Drug Administration, for consumers and for the seafood industry. I want to talk about these changes and explore further with you the impact they will have on us.

Side 1: Why HACCP (Environmental.. Species)
Wild Caught, Natural Hazards, Most Perishable

First and foremost, seafood is unique - for a several reasons.
Most of it is still predominantly wild-caught and therefore potentially exposed to a wide range of natural hazards, as well as hazards from human pollution. We must cope with a multitude of diverse environmental hazards, industrial pollutants near shore, and whatever problems or hazards may occur in the open ocean.

Seafood is the most perishable of all flesh foods; often resulting in decomposition, and occasionally illness from scombroid poisoning.

Consumed Raw, Many Species, Varied Regulatory Control
Seafood is consumed raw much more than any terrestrial flesh food.

Seafood consists of literally hundreds of species from all over the globe, many having little in common other than an aquatic origin.

No other flesh food is imported into the U.S. in such quantity (53% of the supply) or from so many places - over 135 countries Some of these countries have advanced regulatory structures for seafood safety, but many do not.

Widely distributed, Recreational to Commercial, Seasonal
The distribution system for seafood is so extensive that both safety and shelf life can be adversely affected.

Americans consume almost four pounds per capita of recreationally caught fish and shellfish. Some of the recreational harvest finds its way into commercial channels, which raises concerns about where it was harvested and how it was handled.

The seasonal nature of the business, sometimes at very remote locations, presents the industry with special challenges in terms of employee training, facility upkeep, etc.

New **Stresses** and **Challenges**
Besides the intrinsic characteristics of seafood, other aspects of the world of food production and consumption have changed drastically in the last decade. These include:

- New ways of processing and packaging food
- Our food is more widely distributed affecting safety and shelf life.
- Different consumption patterns - more food is eaten outside the home
- New pathogens affecting food safety, new concerns about chemical contaminants, new concerns about the health impact on small population groups
- HACCP is being adopted internationally, therefore the U.S. must adopt it we want to maintain our place in international trade.

Slides 2: Roles and Responsibilities

One of the greatest benefits of HACCP is its effect on the respective roles of government and industry.

- Under HACCP the food industry is responsible for ensuring the safety of the food it produces.
- Under HACCP the government is responsible for verifying that the industry is meeting its responsibility.

Slide 3: What is HACCP?

Now, I want to talk a little about what HACCP means to us in FDA.

HACCP is an internationally recognized, state-of-the-art system that was first used by the food industry in the production of safe food for the U.S. astronauts. It was adopted for and has been very successfully used by the canning industry for over 20 years.

HACCP has gained recognition throughout the developed world as the best safety assurance system developed to date. It has been recommended by the National Academy of Sciences (NAS) and by the CODEX. In addition, the European Community (EC) and many developed nations that export seafood have adopted HACCP for their seafood products or are in the process of doing so.

Slide 4: The HACCP System

Under our HACCP system, U.S. processors will develop plans for their operations. These plans will anticipate likely food safety hazards. These may be environmental hazards from the water, or processing hazards, such as cooking temperatures that are too low, or refrigeration temperatures that are too high. Each processor will then identify points where a hazard could be introduced or where a hazard already present may be eliminated. After identifying all the critical control points in a processing operation, the processor must then know how to measure whether these critical control points are operating the way they should. The first step is to establish critical limits for each CCP. A critical limit can be a cooking or refrigeration temperature. It can be the amount of detectable decomposition in a lot of fish.

Each individual HACCP plan will reflect the uniqueness of the seafood being produced, its method of processing and the facility in which it is prepared.

Slide 5: The HACCP Regulation

Under our proposal, all U.S. commercial processors of seafood products will be required to develop and operate under a HACCP plan. This includes packers, repackers and warehouses. We will not be requiring HACCP plans from retail stores, restaurants, common carriers and vessels that only harvest seafood. The HACCP plans must identify likely hazards, critical control points and critical limits, and indicate the monitoring procedures that will be employed at the critical control points and the records that will be maintained. These records and the HACCP plan itself will be available for review by our investigators.

I would like to point out that sanitation is a controled prerequisite program to HACCP and that it is unlikely that HACCP will be effective if sanitation is not controlled.

Slide 6: Safety Hazards

The hazards that we are requiring processors to control through HACCP are safety hazards. Poor quality and economic fraud will continue to be violations of the FD&C Act, of course, and we will continue to look for them and apply our traditional controls and remedies when we find them, but HACCP will be required only for safety.

Slide 7: Imports

Because 53 percent of the seafood consumed in the United States is imported, foreign processors also fall under this new regulation. That is, all foreign processors shipping product to the U.S. will have to meet all the requirements I have just described for U.S. processors. In addition, all U.S. importers will be required to ensure that foreign processors have met their obligations. There are a number of ways that an importer could do this. The easiest way will be to import products from a country with which the U.S. has entered into an agreement establishing that our regulatory systems are equivalent. We are aggressively

pursuing such agreements and expect to enter into several of them within the next few years. Other means include auditing foreign processors or maintaining copies of their HACCP plan.

Slide 8: Molluscan Shellfish

Finally, the molluscan shellfish industry which supplies clams, oysters and mussels, will be required to process only product that originates from growing waters approved for harvesting by a shellfish control authority. This means that processors can only receive and process shellstock that is appropriately tagged or labeled and sold by a licensed harvester or processor, licensed by a shellfish control authority. In the U.S., this means the producer is in full compliance with the requirements of the National Shellfish Sanitation Program. For foreign imports, the same requirements must be met under the auspices of a Memorandum of Understanding.

In addition, all records must be maintained for shell stock that document the date and location of harvest, the quantity and type of shellfish, the date of receipt by the processor, and the name and identification number of the harvester.

Slide 9: HAZARDS AND CONTROLS GUIDE

To help the industry prepare for HACCP we have developed an unprecedented package of guidelines - the FDA Fish and Fishery Products Hazards and Controls Guide - that, for virtually every species and commercial process, identifies hazards and the types of controls that should be in place to keep those hazards from actually occurring.

There is a safety hazards listed for tuna, as per example - histamine.
Histamine is produced in specific fish as a result of temperature abuse. The hazard statement describes how temperature control, especially on the harvest vessel is critical to controlling the hazard.

The critical control point for this hazard is receiving.

There are two options for control provided in the guide. One applies to the first processor, the other to subsequent processors. We will use the first processor as an example - this is option 1 which recommends, among other things that processors determine the temperature history of the fish on the vessel.

The control measure which is to determine the on-board temperature history of the fish should be done on every lot.

One of the critical control limits that applies to this control measure is that the internal fish temperature should be reduced to 40°F as soon as possible after capture. Other critical limits are also provided. (that pertain to iced fish, levels of histamine and decomposition.)

An example of a record that would be necessary to document that the fish temperature was dropped rapidly might be on-board refrigerated sea water temperature records, if refrigerated seawater is used to cool the fish upon capture. In other circumstances, other records may be appropriate. The guide addresses this (i.e., the results of histamine testing, calibration of thermometers and sufficiency of ice).

If the critical limit is not met the records would show that the temperature of the fish was not dropped as rapidly as necessary to prevent the development of histamine. The lot should be sampled and analyzed for histamine. If the results are high, the product must be frozen or cooked. If the results are very high, the product must be destroyed.

Slide 10: International

And finally, as I mentioned earlier, HACCP is the key to maintaining our position in world trade. HACCP will serve as the basis for bilateral agreements, since most of our key trading partners have switched to HACCP already or are planning to do so. Agreements based on HACCP will ensure that foreign processors are applying systems of preventive controls equivalent to those being required of domestic processors. We have already met with the EC and with Australia and New Zealand to discuss such agreements. Of course, in the case of Canada, the North American Free Trade Agreement (NAFTA) provides the framework for establishing equivalency and, therefore, a mutual agreement.

Slide 11: Training

I would like to say that training is critical to the successful implementation of HACCP systems in the seafood industry. Certain key functions such as reviewing monitoring records, developing and modifying HACCP plans which will require training in HACCP. The need for training exists not just for industry. Both state and local regulators, as well as FDA employees will need to be trained on how to evaluate HACCP plans and conduct HACCP inspections. For training, FDA is working closely with the Seafood Alliance to develop a HACCP training curriculum.

Slide 11: Final Rule

As you know, on January 28,1994, we published in the Federal Register, the proposed regulation. During the comment period, the Agency received approximately 260 submissions each containing many comments on every part of the proposal. After each of those comments were reviewed, the Agency developed a final rule that accommodated as many of the comments with merit as possible. The FDA Commissioner Dr. David Kessler and the Honorable Secretary of Health and Human Service Ms. Donna Shalala and the have signed the final rule. At this moment, the final rule is being reviewed for final approval by the Office of Management Branch (OMB). After OMB signs off on the final rule, it will be published in the Federal Register. We are quite confident that by January 1996 the final rule will be published in the Federal Register.

Thank you

ABSTRACTS

REPORT ON FOOD PROTECTION ACTIVITIES OF THE PAN AMERICAN HEALTH ORGANIZATION

Primo Arámbulo, III and Claudio R. Almeida
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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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The food protection activities of PAHO/WHO are 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.

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Delivery 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 of foodbome disease, and (4) Sanitary control of street foods and consumer protection.

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

As part of its efforts to implement Epidemiological Surveillance of Foodbome 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 studies 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 CO_2; and (5) a high barrier container under CO_2. The following analyses were **completed:** (1) percent O_2 and CO_2 (2) Hunter L, a, b; (3) aerobic plate counts; (4) TBA; (5) FFA, and (6) odor, taste and appearance sensory analyses. The CO_2 MAP high and medium barrier thermoformed packages reduced moisture loss when compared to control polyethylene-coated cartons and polyethylene bags. Shrimp packaged under a CO_2 atmosphere in both high and medium barrier thermoformed packages maintained significantly elevated CO_2 levels during five months of frozen storage. Oxygen levels remained low in CO_2 packaged shrimp. Fresh control moisture levels were not significantly different from percent moisture in CO_2 packaged shrimp at the end of five months. The FFA correlation coefficients for CO_2 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 CO_2 in high barrier thermoformed packages produced a strong correlation relationship between time and increasing TBA values. The high barrier CO_2 package reduced the rate of rancidity development as defined by the TBA value. Thermoformed packages with CO_2 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^{16}_{185} 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 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 CO_2 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 post considerable challenges. The methods must perform well on a wide varity 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 thecomplexity and variability to 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 posses 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 bind 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 throughout 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-CTXl provided estimates ranging from 0.7 to 1.3 ppb. The study documents improved capabilities for screening andconfirmation of ciguatoxins.

OXYGEN-DERIVED 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 O*lenticularis*. 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 0. *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 REALPTY

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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 resoure utilization. Tests based on eith 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 tasts, 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 incorrdiantional and inability to stand have all been reported. Hyporeflexia, areflexia, stocking and glove hypesthesia or numbness, malaiseor 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 and 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 been 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 poiyethers 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 if gastrointestinal, neuromuscular, cardiovascular, dermatological, genitourinary or emotional symptoms should arouse the clinical suspicion to inquire if fish had been ingested. The initial gastrointestinal phase 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 H_2S-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

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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 CLA USSENII AS A CONTRIBUTOR IN YELLOWFIN TUNA SPOILAGE

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The rule of yeasts in seafood spoilage is probably being underestimated. We have isolated several hidtidine 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 volatic 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. Protcolytic activity was also determined.

The threshold value of spoilage for TVB-N (30mg/l00 g) was reached between the second and fourth day of storage at 25 ° C an 3 7° C. Under the same conditions, TMA-N concentration was 10 mg/l00 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 0.5 19 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 measurementand 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, aspetic 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 theLaboratory'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 deveioped.