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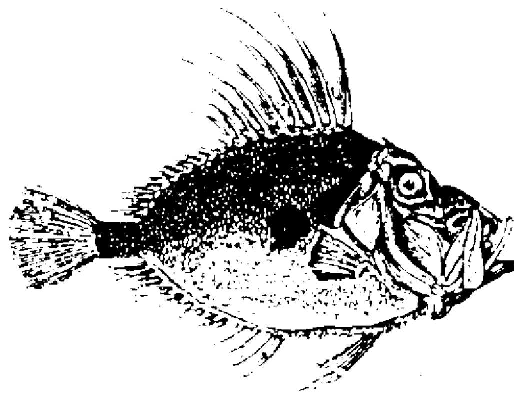
Seventeenth Annual Tropical and Subtropical Fisheries Technological Conference of the Americas

held jointly with:

Gulf and Caribbean Fisheries Institute, Inc.



Program and Abstracts

November 4-6, 1992
Merida, Yucatan, Mexico

Florida Sea Grant College Program
University of Florida
Building 803
Gainesville, FL 32611-0409

SEVENTEENTH ANNUAL TROPICAL AND SUBTROPICAL FISHERIES TECHNOLOGICAL CONFERENCE OF THE AMERICAS

held jointly with the
Gulf and Caribbean Fisheries Institute, Inc.
45th Annual Meeting

4-6 November, 1992
Mérida, Yucátan, México

Hosted by
Centro De Investigacion Y
Estudios Avanzados Del - IPN
UNIDAD MERIDA

Edited by
W. Steven Otwell
Food Science & Human Nutrition Dept.
University of Florida
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The Tropical and Subtropical Fisheries Technological Society of the Americas is a professional, educational association of aquatic food product technologists interested in the application of science to the unique problems of production, processing, packaging, distribution, storage and preparation of the tropical and subtropical fishery species be they harvested, cultured or imported. Their principle objective is to advance the use and understanding of applied and basic science as relates to product quality and safety. Their membership includes 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.

The first joint TSFT-GCFI meeting combines the technical and administrative expertise of fishery sciences, seafood technology and related marine sciences in presentations and discussions concerning fisheries, marine habitats, seafood production, aquaculture, processing and marketing in countries throughout the Gulf of Mexico and Caribbean Basin. The 45th annual GCFI meeting will continue their commitment to exchanging information on key issues affecting management and development of fishery resources in the Gulf and Caribbean regions. The 17th Annual TSFT conference is their international attempt to expand their commitments to all applied and basic aspects of aquatic product harvest, culturing, processing, packaging, distribution, storage and preparation as relates to product quality and safety.

La Sociedad Tropical y Subtropical de Tecnología Pesquera de las Americas es una asociación de tecnólogos de productos acuáticos interesados en la aplicación de la ciencia a los problemas de la producción, del procesamiento, del almacenamiento y de la preparación de las especies tropicales y subtropicales, ya sean cosechadas, cultivadas ó importadas. El objetivo principal de esta sociedad es el avance en el uso y entendiemiento de la ciencia básica y aplicada en relación con la calidad y seguridad del producto. Entre los miembros de esta sociedad se encuentran el interés comercial, agencias gubernamentales, la experiencia académica y estudiantes. Los temas tipicamente abarcan los productos y localidades del Golfo de México y las regiones del sur del Atlántico, desde Texas hasta Virginia incluyendo las regiones del Caribe y los países del golfo de México. Este grupo invita y fomenta la participación de otras regiones tropicales y subtropicales alrededor del mundo.

Esta primera reunión conjunta de la conferencia TSFT y del GCFI combina la experiencia tecnológica y administrativa en ciencias pesqueras, en tecnología de alimentos provenientes del mar y en ciencias relacionadas al ambiente marino, en presentaciones y discusiones relacionados con la pesca, el habitad marino, la producción de alimentos provenientes del mar, la aquacultura, el procesamiento y el mercadeo de países del golfo de México y del Caribe. La 45ava conferencia anual del GCFI continuará con su cometido de intercambiar información acerca de los aspectos claves que afectan el manejo y el desarrollo de los recursos pesqueros en el golfo y en las regiones del Caribe. La 17ava conferencia anual TSFT será el primer intento internacional de expandir el compromiso de esta conferencia a todos los aspectos básicos y aplicados a la cosecha de productos acuáticos, el cultivo, el procesamiento, el empaquetamiento, la distribución, el almacenamiento y la preparación, con relación a la calidad y la seguridad del producto.

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Colonia Roma
México D.F., México 06700

[Editors Note: For accuracy Dr. Garcia's paper is published in a condensed formate consistent with his presentation]

ANTECEDENTES

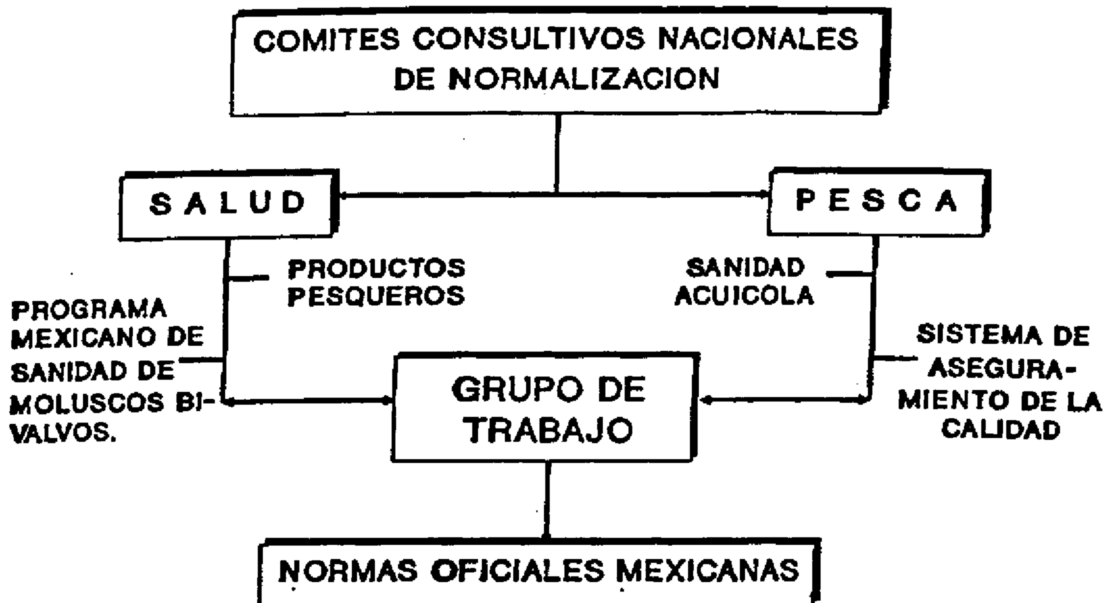
A PARTIR DE LA DESREGULACION NORMATIVA ESTABLECIDA EN EL PROGRAMA DE POLITICA COMERCIAL DEL GOBIERNO DE MEXICO, SE HA LLEGADO A LA MODIFICACION DE LA LEY FEDERAL SOBRE METROLOGIA Y NORMALIZACION CON EL OBJETO DE:

- ✓ **ESTABLECER UN PROCEDIMIENTO CLARO, UNIFORME Y COORDINADO PARA LA EXPEDICION DE NORMAS OBLIGATORIAS POR PARTE DE LAS DEPENDENCIAS DE LA ADMINISTRACION PUBLICA FEDERAL, EN LAS MATERIAS COMPRENDIDAS EN LA LEY.**

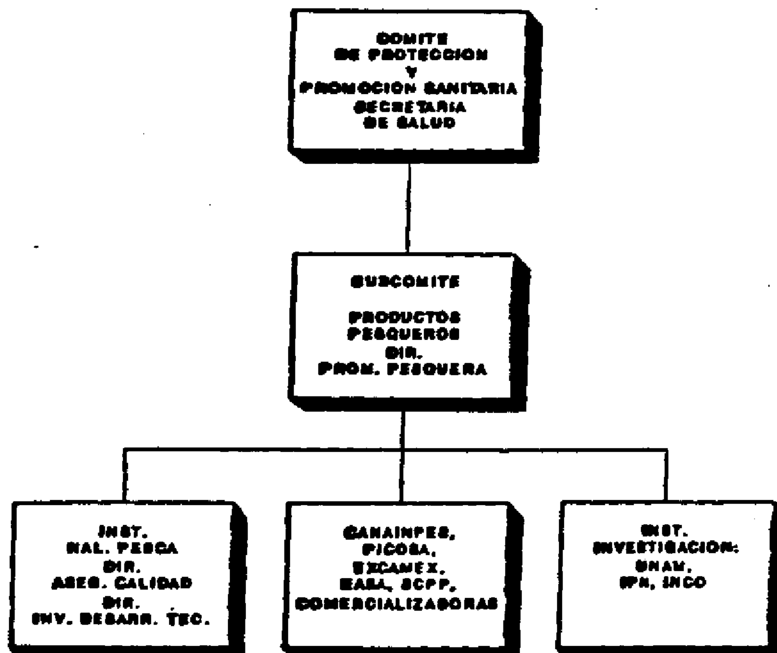
- ✓ **FORTALECER LOS MECANISMOS DE VERIFICACION DEL CUMPLIMIENTO DE LAS NORMAS.**

- ✓ **CREAR UNA CONCIENCIA ENTRE LOS SERVIDORES PUBLICOS DE LOS COSTOS SOCIALES DE LA REGULACION.**

ESTRATEGIA



ESTRUCTURA DEL SUBCOMITE PARA PRODUCTOS PESQUEROS



SUBCOMITE PARA PRODUCTOS PESQUEROS

OBJETIVOS

- ✓ ESTABLECER LAS CARACTERISTICAS Y ESPECIFICACIONES QUE DEBEN REUNIR LOS PRODUCTOS Y PROCESOS PESQUEROS PARA GARANTIZAR LA SEGURIDAD DE LA SALUD HUMANA, ANIMAL Y VEGETAL.
- ✓ COLABORAR PARA GARANTIZAR QUE LOS PRODUCTOS SEAN SANOS, GENUINOS, NO ADULTERADOS, QUE ESTEN DEBIDAMENTE ETIQUETADOS Y PRESENTADOS PARA PROTEGER LA SALUD DEL CONSUMIDOR.
- ✓ COLABORAR PARA ASEGURAR QUE LOS PRODUCTOS MEXICANOS CUMPLAN CON LAS NORMAS Y REQUERIMIENTOS SANITARIOS Y DE CALIDAD EN LOS PROCESOS DE CAPTURA, MANEJO. PROCESAMIENTO Y COMERCIALIZACION.
- ✓ PARTICIPAR EN LA INSPECCION, VERIFICACION Y CERTIFICACION DE LOS PRODUCTOS, PROCESOS, METODOS, INSTALACIONES, SERVICIOS Y ACTIVIDADES, PARA QUE CUMPLAN CON LAS NORMAS OFICIALES MEXICANAS EMITIDAS.

ESTRATEGIA

- ✓ PROPUESTAS PARA LA ELABORACION, REVISION, ACTUALIZACION Y EMISION DE NORMAS OFICIALES MEXICANAS.
- ✓ ACTUALIZACION DE LOS MECANISMOS DE INSPECCION, VERIFICACION Y CERTIFICACION DE LAS NORMAS OFICIALES MEXICANAS, QUE INCLUYEN:
  - LA CONTINUA ACTUALIZACION DE LA RED NACIONAL DE LABORATORIOS.
  - LA APROBACION Y VERIFICACION DE LOS ORGANISMOS DE CERTIFICACION, DE CONFORMIDAD CON LAS NORMAS OFICIALES MEXICANAS EMITIDAS.
  - LA INSPECCION DE LAS UNIDADES DE VERIFICACION, DE CONFORMIDAD CON LAS NORMAS OFICIALES MEXICANAS EMITIDAS.
  - LA PARTICIPACION ACTIVA EN LOS COMITES NACIONAL E INTERNACIONAL DE INSPECCION Y CERTIFICACION DE LAS IMPORTACIONES Y EXPORTACIONES DE ALIMENTOS DEL CODEX-ALIMENTARIUS.

PROGRAMA MEXICANO DE SANIDAD DE MOLUSCOS BIVALVOS

OBJETIVO GENERAL

✓ VIGILAR Y CERTIFICAR LA CALIDAD SANITARIA DE LOS MOLUSCOS BIVALVOS PARA GARANTIZAR SU CONSUMO SEGURO.

OBJETIVOS ESPECIFICOS

✓ DESARROLLAR ACCIONES Y MECANISMOS PARA LOGRAR QUE TANTO EL PRODUCTO DE CONSUMO INTERNO COMO EL DE EXPORTACION REUNAN LOS CRITERIOS SANITARIOS NACIONALES E INTERNACIONALES.

✓ ESTABLECER Y PONER EN PRACTICA UN PLAN DE PREVENCION, CONTROL Y ATENCION OPORTUNA DE INTOXICACIONES RELACIONADAS CON FENOMENOS DE MAREA ROJA.

ESTRATEGIAS

✓ ESTABLECIMIENTO DE BASES Y ACUERDOS DE COORDINACION.

- BASES DE COORDINACION INTERSECRETARIAL (FIRMADAS EN SEPTIEMBRE DE 1989)
- ACUERDOS DE COORDINACION CON AUTORIDADES FEDERALES Y ESTATALES (BAJA CALIFORNIA, BAJA CALIFORNIA SUR, SONORA, GUERRERO Y TAMAULIPAS).

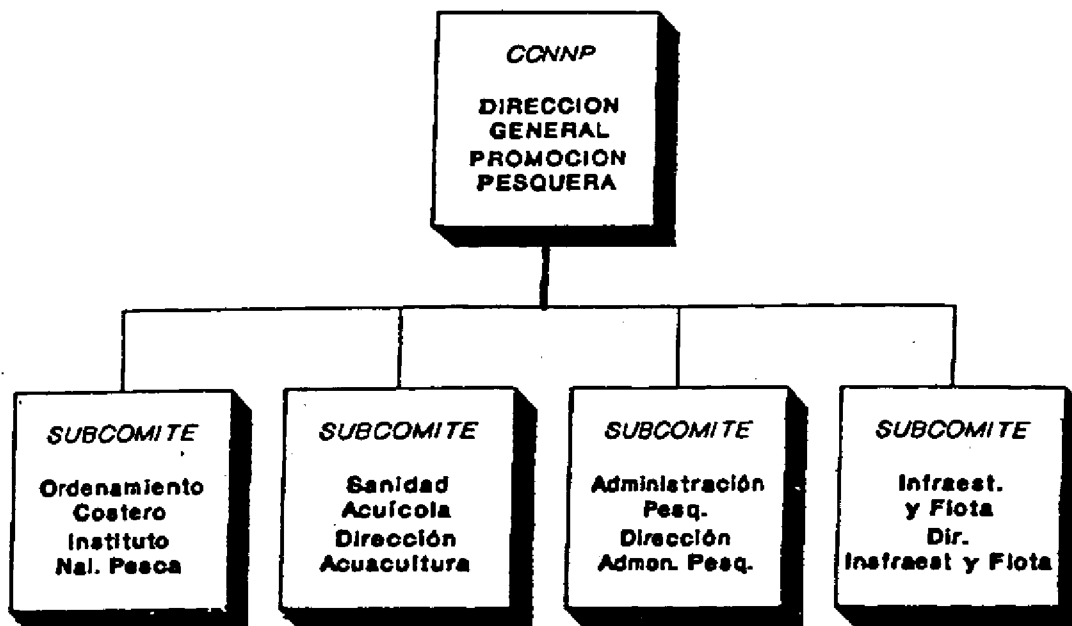
✓ INTEGRACION DE UNA RED NACIONAL DE LABORATORIOS ACREDITADOS POR EL COMITE CENTRAL Y LA ADMINISTRACION DE ALIMENTOS Y MEDICAMENTOS (FDA) DE LOS EE.UU.

- LABORATORIO NACIONAL DE SALUD PUBLICA
- LABOTARORIO DEL INSTITUTO DE INVESTIGACIONES OCEANOLOGICAS DE LA UABC
- LABORATORIOS ESTATALES DE SALUD PUBLICA DE LOS ESTADOS DE SONORA Y GUERRERO

✓ INTEGRACION DE DOCUMENTOS BASICOS (MANUALES)

✓ CERTIFICACION DE AREAS DE PRODUCCION (BAJA CALIFORNIA)

ESTRUCTURA DEL COMITE CONSULTIVO NACIONAL DE NORMALIZACION DE PESCA



SUBCOMITE DE SANIDAD ACUICOLA

OBJETIVOS

- ✓ ESTABLECER LA NORMATIVIDAD A QUE DEBERAN SUJETARSE LOS PROCESOS DE PRODUCCION ACUICOLA Y LA MOVILIZACION DE ORGANISMOS VIVOS.

- ✓ NORMAR Y SUPERVISAR LA OPERACION DE LAS INSTALACIONES Y EQUIPOS DESTINADOS A PROMOVER LA PRODUCCION ACUICOLA.

- ✓ ESTABLECER LAS ESPECIFICACIONES SANITARIAS PARA LA PROTECCION Y PROMOCION DE LA SANIDAD ACUICOLA; MISMÀS QUE SE REFERIRAN A ASPECTOS DE PREVENCION, DIAGNOSTICO Y CONTROL DE ENFERMEDADES.

- ✓ DETERMINAR LAS ESPECIFICACIONES PARA LLEVAR A CABO LOS PROGRAMAS DE VERIFICACION, INSPECCION Y CERTIFICACION PARA EL CUMPLIMIENTO DE LAS NORMAS OFICIALES MEXICANAS EMITIDAS.

ESTRATEGIA

- ✓ PROPUESTA, REVISION, ACTUALIZACION Y EMISION DE LAS NORMAS OFICIALES MEXICANAS.

- ✓ DESARROLLO DE LOS MECANISMOS DE INSPECCION, VERIFICACION Y CERTIFICACION A TRAVES DE:

  - ORGANISMOS DE CERTIFICACION QUE SERAN PERSONAS MORALES PRIVADAS QUE PODRAN ASEGURAR QUE LOS PROCESOS, PRODUCTOS E INSTALACIONES PESQUERAS SE AJUSTEN Y CUMPLAN CON LAS NORMAS OFICIALES MEXICANAS EMITIDAS.

  - LAS UNIDADES DE VERIFICACION SERAN PERSONAS FISICAS O MORALES ACREDITADAS PARA REALIZAR ACTIVIDADES DE INSPECCION PARA CONSTAR OCULARMENTE O A TRAVES DEL MUESTREO Y ANALISIS DE LABORATORIOS ACREDITADOS EL CUMPLIMIENTO DE LAS NORMAS OFICIALES MEXICANAS EMITIDAS.

  - LAS UNIDADES DE APROBACION SERAN LOS LABORATORIOS ACREDITADOS DE LA SECRETARIA DE PESCA, QUIENES SERAN LOS ENCARGADOS DE APROBAR A LAS UNIDADES DE VERIFICACION Y ORGANISMOS DE CERTIFICACION, DILUCIDANDO CUALQUIER TIPO DE CONTROVERSIAS SURGIDAS POR UNA INSPECCION O CERTIFICACION EMITIDA A UNA EMPRESA O UNIDAD DE PRODUCCION.

SISTEMA DE ASEGURAMIENTO DE LA CALIDAD DE LOS PRODUCTOS PESQUEROS

ANTECEDENTES

EN LA PRODUCCION, TRANSFORMACION Y COMERCIALIZACION DE PRODUCTOS PESQUEROS, SE HAN IDENTIFICADO LOS SIGUIENTES PROBLEMAS:

- ✓ PERDIDAS POST-CAPTURA DEBIDO A PRACTICAS INADECUADAS UTILIZADAS DURANTE SU MANEJO, PROCESAMIENTO Y COMERCIALIZACION.

- ✓ PRACTICAS FRAUDULENTAS EN LA COMERCIALIZACION

- ✓ RECURSOS PESQUEROS EXTRAIDOS DE AMBIENTES ACUATICOS CONTAMINADOS

- ✓ CONDICIONES TECNICO-HIGIENICO-SANITARIAS INADECUADAS EN LOS AMBIENTES DE MANIPULACION, TRANSFORMACION, DISTRIBUCION Y VENTA DE PRODUCTOS PESQUEROS.

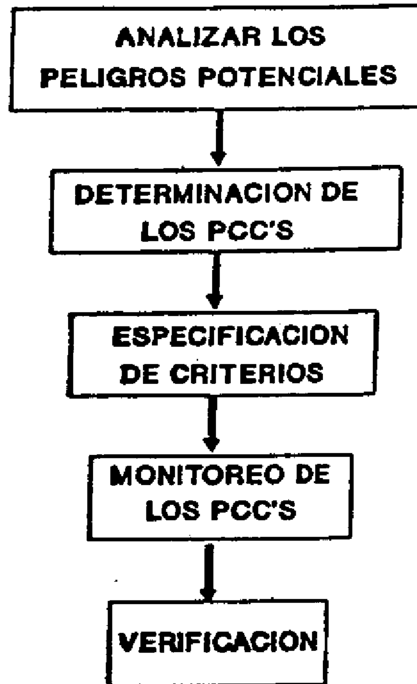
¿ QUE ES EL SISTEMA DE ASEGURAMIENTO DE LA CALIDAD DE PRODUCTOS PESQUEROS ?

ES UN PROGRAMA DE VIGILANCIA DE LA CALIDAD E INOCUIDAD DE LOS PRODUCTOS PESQUEROS, BASADO EN UN ENFOQUE SISTEMATICO DE IDENTIFICACION Y CONTROL DE RIESGOS Y PUNTOS CRITICOS, SOBRE TODOS LOS ESLABONES DE LA CADENA PRODUCCION-CONSUMO.

OBJETIVOS

- ✓ MEJORAR LA IMAGEN DE MEXICO Y LA COMPETITIVIDAD DE SUS PRODUCTOS PESQUEROS EN EL MERCADO INTERNACIONAL.
- ✓ MANTENER UNA CALIDAD UNIFORME DE LOS PRODUCTOS PESQUEROS, SEA QUE SE CONSUMAN EN EL MERCADO NACIONAL O INTERNACIONAL.
- ✓ ESTABLECER LAS NORMAS DE CALIDAD MINIMAS QUE DEBERAN CUBRIR LOS PRODUCTOS PESQUEROS PRODUCIDOS EN EL PAIS Y LOS IMPORTADOS.
- ✓ ESTABLECER UN SISTEMA OFICIAL DE CERTIFICACION DE CALIDAD DE LOS PRODUCTOS PESQUEROS
- ✓ CREAR CONCIENCIA EN EL CONSUMIDOR DE QUE LOS PRODUCTOS PESQUEROS SON DE CALIDAD CONFIABLE
- ✓ PREVENIR Y CONTROLAR LA INTRODUCCION Y DIFUSION DE ENFERMEDADES A LOS ORGANISMOS ACUATICOS.

ETAPAS DEL SISTEMA DE ANALISIS DE RIESGOS Y CONTROL DE LOS PUNTOS CRITICOS



SITUACION ACTUAL DEL SISTEMA DE ASEGURAMIENTO DE LA CALIDAD DE PRODUCTOS DE LA PESCA.
(ACTUAL SITUATION OF THE QUALITY ASSURANCE SYSTEM FOR FISHERIES PRODUCTS)

ING. JOSE GABRIEL SUAREZ TORIELLO
DIRECTOR DE ASEGURAMIENTO DE LA CALIDAD DE LOS PRODUCTOS DE LA PESCA
SECRETARIA DE PESCA
INSTITUTO NACIONAL DE LA PESCA
DR. VALENZUELA No.85, COL.DOCTORES
MEXICO, D.F., 06720, MEXICO

RESUMEN:
SE PRESENTA LA SITUACION ACTUAL DEL SISTEMA MEXICANO DE ASEGURAMIENTO DE LA CALIDAD DE LOS PRODUCTOS DE LA PESCA, QUE LA SECRETARIA DE PESCA A TRAVES DE SU ORGANO DE APOYO TECNICO, EL INSTITUTO NACIONAL DE LA PESCA, ESTA TRATANDO DE IMPLEMENTAR EN EL SECTOR PRODUCTIVO PESQUERO MEXICANO, BUSCANDO SIEMPRE LA MEJORA CONTINUA EN LA CALIDAD TOTAL DE LOS ALIMENTOS PESQUEROS. SE DETALLAN LOS OBJETIVOS, ACCIONES, ESTRATEGIAS Y LOS PLANES DE OFICIALIZACION DEL SISTEMA DE ASEGURAMIENTO DE LA CALIDAD.

LOS TRABAJOS PARA CONFORMAR UN SISTEMA DE ASEGURAMIENTO DE LA CALIDAD EN MEXICO, SE INICIARON EN EL AÑO DE 1989, ANTE LA NECESIDAD DETECTADA DE ESTABLECER UNA ENTIDAD QUE ORIENTARA Y ASESORARA AL SECTOR PRODUCTIVO PESQUERO MEXICANO E INTEGRARA ACCIONES DE LAS DEPENDENCIAS PUBLICAS COMPETENTES QUE HABIAN SIDO INADECUADAMENTE APLICADAS POR DIFERENTES RAZONES.

TODO ELLO CON LA PREMISA DE CANALIZAR ADECUADAMENTE LA EXIGENCIA MUNDIAL DE ASEGURAR LA CALIDAD DE LA PRODUCCION PESQUERA NACIONAL DESTINADA AL COMERCIO INTERNACIONAL, PERO SOBRE TODO ENFOCADO A QUE NUESTRA POBLACION CUENTE CON UNA CALIDAD E INOCUIDAD CONFIABLE EN LOS ALIMENTOS PESQUEROS.

DESPUES DE VARIOS ENSAYOS Y ANALISIS, SE LLEGO EN MAYO DE 1991 AL DISEÑO DEL SISTEMA DE ASEGURAMIENTO DE LA CALIDAD DE LOS PRODUCTOS DE LA PESCA, CON LA VALIOSA AYUDA INTERNACIONAL DE EXPERTOS DE LA FAO Y CON LA PARTICIPACION DE AREAS DE LA SECRETARIA DE PESCA COMO: EL INSTITUTO NACIONAL DE LA PESCA, OCEAN GARDEN PRODUCTS INC. Y LA DIRECCION GENERAL DE PROMOCION PESQUERA.

DICHO DISEÑO CONSIDERO LAS CARACTERISTICAS ESENCIALES DE LOS PRINCIPALES SISTEMAS QUE ESTAN EN VIAS DE CONVERTIRSE DE OBSERVANCIA OBLIGATORIA EN OTROS PAISES, A FIN DE SER HOMOLOGABLE CON CUALQUIER OTRO SISTEMA DE CALIDAD.

LOS PRINCIPALES OBJETIVOS DEL SISTEMA DE ASEGURAMIENTO DE LA CALIDAD DE LOS PRODUCTOS DE LA PESCA SON:

1.- ASEGURAR QUE LOS PRODUCTOS PESQUEROS MEXICANOS CUMPLAN CON LAS NORMAS Y PROCEDIMIENTOS EXIGIDOS POR EL MERCADO.
2.- PROPORCIONAR AL PRODUCTO PESQUERO MEXICANO EL CERTIFICADO QUE GARANTICE SU CALIDAD EN BENEFICIO DE LA POBLACION.
3.- MANTENER LA IMAGEN DEL PAIS Y LA COMPETITIVIDAD DE LOS PRODUCTOS PESQUEROS MEXICANOS EN EL MERCADO INTERNACIONAL.
4.- MEJORAR Y MANTENER UNA CALIDAD UNIFORME DE LOS PRODUCTOS PESQUEROS MEXICANOS.
5.- DAR CONFIANZA AL CONSUMIDOR NACIONAL DE QUE LA CALIDAD E INOCUIDAD DE LOS PRODUCTOS PESQUEROS MEXICANOS, ESTA GARANTIZADA.

EN FUNCION DE ELLO, NUESTRAS ESTRATEGIAS Y ACCIONES ESTAN DEFINIDAS Y ENCAMINADAS BAJO LOS SIGUIENTES PRINCIPIOS; EN FUNCION DE CONTAR CON UNA ARMONIA EQUILIBRADA CON LOS SECTORES:

A) EL SISTEMA DE ASEGURAMIENTO DE CALIDAD PROPUESTO ESTA BASADO EN EL CONCEPTO PARA EL DESARROLLO DE TECNOLOGIAS DE CONTROL DE CALIDAD LLAMADO, ANALISIS DE RIESGOS Y DETERMINACION DE PUNTOS CRITICOS DE CONTROL Y EN EL CONCEPTO DE CALIDAD TOTAL, QUE ES EN EL CASO DE ALIMENTOS PESQUEROS LA CONJUNCION DE SU CALIDAD SANITARIA Y DE SU CALIDAD TECNOLOGICA.

B) EN COORDINACION CON LOS SECTORES PRODUCTIVOS DE CAPTURA, INDUSTRIALIZACION Y COMERCIALIZACION, SE DESARROLLA LA APLICACION DE ESTOS CONCEPTOS PARA CADA RECURSO PESQUERO Y SU APROVECHAMIENTO.

C) EL PRODUCTOR ASUME LA RESPONSABILIDAD DE CONTROLAR LA CALIDAD DE SUS PRODUCTOS. EL INP TIENE A SU CARGO LA ASESORIA, SUPERVISION, AUDITORIA Y VERIFICACION DE LA GESTION DE CONTROL DE LA CALIDAD EJERCIDO POR LOS PRODUCTORES.

D) CADA UNIDAD PRODUCTORA QUE LO POSEA, DEBE PRESENTAR SU PROGRAMA DE CONTROL DE CALIDAD. EL INP LOS REVISA Y REALIZA LAS ADECUACIONES PERTINENTES, O LOS VALIDA SI ESTAN CORRECTOS.

E) EN COORDINACION CON LOS SECTORES PRODUCTIVOS, EL INP ESTABLECE LOS PARAMETROS BASICOS PARA EL DESARROLLO DE LOS PROGRAMAS DE CONTROL DE CALIDAD DE LAS UNIDADES DE PRODUCCION QUE CAREZCAN DE ELLOS, BUSCANDO LA MEJORA CONTINUA EN LA CALIDAD TOTAL.

F) LAS UNIDADES PRODUCTIVAS CUYAS CONDICIONES TECNICO SANITARIAS OBEDEZCAN A LA LEGISLACION VIGENTE Y CUYOS PROGRAMAS DE CONTROL DE CALIDAD SEAN APROBADOS, SON CERTIFICADAS PARA LA PRODUCCION Y COMERCIALIZACION DE SUS PRODUCTOS.

G) EL SISTEMA DE ASEGURAMIENTO DE LA CALIDAD, SE HACE RESPONSABLE DE LA CALIDAD DE LOS PRODUCTOS PESQUEROS EN LOS PUNTOS DE DESCARGA Y MERCADO MAYORISTA, MEDIANTE UN CERTIFICADO DE CALIDAD TOTAL POR EMBARQUE DE ALIMENTO.

ATENTOS AL PROCESO DE MODERNIZACION QUE VIVE NUESTRO PAIS A TRAVES DEL GOBIERNO FEDERAL, Y SIGUIENDO PROCEDIMIENTOS DE DIAGNOSTICO, REHABILITACION Y DE ACTUALIZACION, TAMBIEN HEMOS PROCEDIDO A:

1.- LA ACTUALIZACION DE LOS DIRECTORIOS DE LA PLANTA INDUSTRIAL PESQUERA POR PROCESOS, PESQUERIA, ZONA PRODUCTIVA Y SECTOR (PRIMARIO, SECUNDARIO Y TERCIARIO), CONTANDOSE A LA FECHA CON LAS EDICIONES FINALES CORRESPONDIENTES AL SECTOR PRIMARIO (CAPTURA) Y SECUNDARIO (INDUSTRIALIZACION).

2.- AL DIAGNOSTICO DE LA PLANTA PRODUCTIVA PESQUERA EN RELACION A SUS SISTEMAS DE CONTROL DE CALIDAD, ASI COMO DE SUS REQUERIMIENTOS ESPECIFICOS DE ASESORIA, VERIFICACION Y CERTIFICACION.

3.- A LA REVISION, ADECUACION Y HABILITACION DE LABORATORIOS DE REFERENCIA DEL INP EN SUS INSTALACIONES UBICADAS EN MEXICO,D.F.; TAMPICO, TAMAULIPAS; MAZATLAN, SINALOA Y CIUDAD DEL CARMEN, CAMPECHE.

ESTO ULTIMO SE CONTEMPLO CON EL OBJETO DE UTILIZAR LOS RECURSOS HUMANOS E INFRAESTRUCTURA CON LA QUE ACTUALMENTE DISPONE EL INP Y EN ESTE SENTIDO, SOLO SE HA NECESITADO DE UNA ADECUACION EN SUS LABORATORIOS PARA INTEGRARSE A LOS REQUERIMIENTOS EN MATERIA DE EQUIPO Y PROCESO DE MUESTRAS PARA ANALISIS DE ALIMENTOS PESQUEROS A FIN DE CERTIFICAR SU CALIDAD TOTAL, CON BASE EN LOS LINEAMIENTOS ESTABLECIDOS POR EL SISTEMA NACIONAL DE ACREDITAMIENTO DE LABORATORIOS DE PRUEBA (SINALP).

4.- A LA ORGANIZACION DE CONFERENCIAS, SEMINARIOS Y TALLERES DE TRABAJO PARA EL SECTOR PRODUCTIVO PESQUERO, CON EL OBJETO DE DIFUNDIR EL SISTEMA DE ASEGURAMIENTO DE LA CALIDAD, CONCIENTIZAR, INTERESAR E INVITAR AL SECTOR A SU INGRESO AL SISTEMA A TRAVES DE CONVENIOS DE CONCERTACION, EN LOS QUE EL INP REALIZA APORTACIONES TECNICAS Y EL PRODUCTOR APORTACIONES DE RECURSOS FINANCIEROS.

5.- CON EL OBJETO DE TENER UN SISTEMA LO MAS HOMOGENEO POSIBLE EN RELACION A LO EXISTENTE EN EL MERCADO INTERNACIONAL, SE ESTUDIA EN FORMA PERMANENTE LA SITUACION ENTRE LA NORMATIVIDAD DE MEXICO CON LA DE OTROS PAISES, RALIZANDO EL ACOPIO DE REGULACIONES EXTRANJERAS ACTUALIZADAS

6.- DEBIDO A QUE EXISTE SIMILITUD DE AMBITO DE COMPETENCIA PARA CUBRIR LOS OBJETIVOS DEL SISTEMA, ENTRE ESTE CON OTRAS SECRETARIAS DE LA ADMINISTRACION PUBLICA FEDERAL, EL INP PLANTEARA ANTE ELLAS A MEDIANO Y LARGO PLAZO, LOS MECANISMOS DE COLABORACION PARA QUE ESTE SISTEMA DE CALIDAD EJERZA EN FORMA OFICIAL EL PROCESO DE CERTIFICACION DE LOS ALIMENTOS PESQUEROS Y DE VERIFICACION DE UNIDADES DE PRODUCCION, COADYAVANDO CON ESTO CON LA SECRETARIA DE SALUD Y CON LA DE COMERCIO Y FOMENTO INDUSTRIAL.

EN LA ACTUALIDAD LA SECRETARIA DE SALUD ES LA RESPONSABLE POR LEY DE LA INSPECCION DE UNIDADES DE PRODUCCION Y CERTIFICACION OFICIAL DE CUALQUIER ALIMENTO, PERO UNICAMENTE SE REMITE A ASPECTOS DE CALIDAD SANITARIA.

CON LA NUEVA LEY FEDERAL DE METROLOGIA Y NORMALIZACION DE LA SECRETARIA DE COMERCIO Y FOMENTO INDUSTRIAL, SE ABRIO UN AMPLIO CAMPO EN EL MARCO JURIDICO, PUESTO QUE DICHA LEY CONTEMPLA QUE LAS DEPENDENCIAS PUBLICAS PODRAN APROBAR LABORATORIOS DE PRUEBA, UNIDADES DE VERIFICACION Y ORGANISMOS NACIONALES DE CERTIFICACION.

EN ESTOS MOMENTOS NUESTRO SISTEMA SE ENCUENTRA BAJO UN ESQUEMA VOLUNTARIO NO OFICIAL, PERO CON TENDENCIA A CONVERTIRSE EN UN ORGANO OFICIAL DE VERIFICACION DE UNIDADES PRODUCTIVAS PESQUERAS Y EN UN ORGANO OFICIAL DE CERTIFICACION DE ALIMENTOS PESQUEROS.

EN 1992 TAMBIEN HEMOS INSTRUMENTADO LINEAS ESTRATEGICAS DE ACCION, ENCAMINADAS A ATENDER LAS DEMANDAS QUE EL SECTOR PESQUERO TIENE EN RELACION AL ASEGURAMIENTO DE LA CALIDAD EN SUS PRODUCTOS DE EXPORTACION, ATENTOS AL RETO QUE NOS IMPONE LA APERTURA COMERCIAL DE NUESTRO PAIS EN EL MUNDO.

ADICIONALMENTE Y DURANTE 1992 HEMOS TRABAJADO EN:

1.- DESARROLLAR Y DIFUNDIR LOS DOCUMENTOS TECNICOS DE LAS PESQUERIAS PRIORITARIAS Y DE LAS NO PRIORITARIAS, PARA QUE CONFORMEN LOS PROGRAMAS DE CONTROL TOTAL DE CALIDAD EN EL SECTOR PRODUCTIVO PESQUERO QUE CARECE DE ESTOS, Y PUEDA DE ESTA FORMA OPTIMIZAR Y TENER UNA MEJORA CONTINUA EN EL MANEJO, PROCESAMIENTO, COMERCIALIZACION Y CALIDAD TOTAL DE LOS PRODUCTOS PESQUEROS.

2.- DISEÑAR Y PROMOVER EN EL SECTOR PRODUCTIVO PESQUERO EL MODELO DE GARANTIA DE CALIDAD DE LOS ALIMENTOS PESQUEROS.

3.- DISEÑAR, CONCERTAR Y OPERAR LOS MECANISMOS DE CAPACITACION Y DE EXTENSION PARA LA MATERIALIZACION DE ESTE SISTEMA A TRAVES DE INFORMACION, ASESORIA, SUPERVISION Y VERIFICACION TECNICA AL SECTOR PRODUCTIVO PESQUERO.

PARA 1993 EL SISTEMA PREVE HABER AGRUPADO EN SU ADMINISTRACION A LA MAYORIA DE LOS PRINCIPALES PRODUCTORES, Y TENDRA ENTONCES LAS CARACTERISTICAS Y ELEMENTOS NECESARIOS PARA ALCANZAR LOS OBJETIVOS PLANTEADOS EN EL DOCUMENTO DEL DISEÑO DEL SISTEMA DE ASEGURAMIENTO DE LA CALIDAD DE PRODUCTOS DE LA PESCA, APROBADO EN JUNIO DE 1991.

CON LA OPERACION DE ESTE SISTEMA, SE PRETENDE COADYUVAR EN LA REDUCCION DE PERDIDAS POST-CAPTURAS, TANTO EN VOLUMEN COMO EN EL VALOR DE LA PRODUCCION; ASI COMO EL DE PROMOVER Y DOTAR AL SECTOR PRODUCTIVO PESQUERO DE UNO DE LOS INSTRUMENTOS BASICOS PARA SUS NEGOCIACIONES COMERCIALES, ASEGURANDO Y FOMENTANDO LA MEJORA CONTINUA DE LA CALIDAD TOTAL DE LOS ALIMENTOS PESQUEROS; INCREMENTANDO COLATERALMENTE CON ESTO INGRESOS, CAPTACION DE DIVISAS, EMPLEOS, DEMANDA, PRODUCTIVIDAD, ETC.

PERO SOBRE TODO OTORGANDO LA CONFIANZA A NUESTRA POBLACION, PARA EL CONSUMO DE ESTOS ALIMENTOS BAJO UN ESQUEMA DE CALIDAD TOTAL.

English Addendum
by

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QUALITY ASSURANCE SYSTEM
FOR FISHERY PRODUCTS

I. JUSTIFICATION

AS A RESULT OF THE LASTEST CONTROL SYSTEMS THAT OPERATE IN THE INTERNATIONAL MARKET, IT IS NOT POSSIBLE TO DELAY THE IMPLEMENTATION OF ADEQUATE TECHNICAL STANDARDS TO ENSURE THE QUALITY OF PRODUCTS RELATED TO THE FISHING INDUSTRY. AT THE SAME TIME IS NECESSARY TO LAY DOWN ESSENTIAL RE-QUIEREMENTS RELATED TO THE PREVENTION AND CONTROL OF POLLU-TION AND SPOILAGE DURING FISHING, HANDLING, STORAGE, RANS-PORTATION AND MARKETING OF FISH PRODUCTS.

BECAUSE OF ITS PRIOR EXPERIENCE WITH REGARD TO PROMOTING THE PRODUCTION AND MARKETING OF FISH PRODUCTS, THE MINISTY OF FISHERIES PROPOSES OVERSEENG THE QUALITY ASURANCE SYSTEM OF SUCH PRODUCTS.

II. OBJETIVES

A) ENSURE THAT THE FISH PRODUCTS ARE OF SUFFICIENT QUALITY SO THAT THEY MAINTAIN THEIR NUTRITIONAL VALUE.

B) ARCHIVE AND MAINTAIN A UNIFORM QUALITY FOR FISH PRODUCTS.

C) ENSURE THAT ONLY ESSENTIAL FISHING IS CARRIED OUT TO AVOID POSTCAPTURE WASTAGE.

D) MAKE SURE THAT THE FISH PRODUCTS COMPLY WITH ALL HEALTH AND QUALITY STANDARDS.

E) AS FAR AS IS, ENSURE THAT THE CONSUMPTION OF FISH PRODUCTS DOES NOT PRESENT A PUBLIC HEALTH HAZARD.

III. STRATEGY

A) THE QUALITY ASSURANCE SYSTEM WILL BE HEADED BY A DIRECTOR WHO WILL HAVE THE AUTHORITY TO DECIDE ON TECHNICAL AND OTHER RELATED MATTERS.
B) THIS SYSTEM WILL FUNCTION ON THE BASIS OF RISK ANALYSIS AND DECIDING THE CONTROL POINTS RELATING TO QUALITY CONTROL.
C) IN CONJUNCTION WITH THE PRODUCTION SECTORS, THIS QUALITY ASURANCE SYSTEM WILL BE GIVEN TO EACH FISHERY FOR IMPLEMENTATION.
D) TOGETHER WITH THOSE IN CHARGE OF PRODUCTION. SEPESCA WILL ESTABLISH BASIC GUIDELINES IN ORDER TO SET UP THESE QUALITY CONTROL PROCEDURES.
E) THE PRODUCER WILL ASSUME RESPONSIBILITY FOR THE QUALITY CONTROL OF HIS PRODUCTS.
F) SEPESCA WILL BE RESPONSIBLE FOR INSPECTING, VERIFYNG, SUPERVISING AND MONITORING THE QUALITY OF FISH PRODUCTS THAT WILL ALREADY HAVE BEEN CHECKED BY PRODUCER HIMSELF.

G) EACH PRODUCER WILL BE OBLIGED TO PRESENT HIS OWN QUALITY CONTROL PROGRAM.
H) SEPESCA WILL SUBSEQUENTLY ASSESS EACH PROGRAM AND MAKE ANY NECESSARY CHANGES.
I) EACH PRODUCER WHOSE TECHNICAL/SANITARY PROCEDURES CONFORM TO CURRENT LEGAL REQUIREMENTS AND WHOSE QUALITY CONTROL PROGRAM COMPLIES WITH THE ESTABLISHED NORMS.- WILL BE OFFICIALY CERTIFIED TO PRODUCE AND MARKET ITS PRODUCTS.
J) THIS QUALITY ASURANCE SYSTEM WILL PROVIDE CONTINUITY TO THE GUIDELINES SET OUT BY THE NATIONAL COUNSELLING COMMITTEE FOR ESTABLISHING NORMS FOR FISH PRODUCTS. (COMITE CONSULTIVO NACIONAL DE NORMALIZACION PARA LOS PRODUCTOS DE LA PESCA).
K) TAKING INTO THE MATERIAL ALREADY AVAILABLE, OPERATIONAL MANUALS WILL BE DEVELOPED AND PRODUCED TOGETHER WITH THE PRODUCERS. THESE MANUALS WILL STATE THE SPECIFICATION TO FOLLOW CONCERNING THE CRITICAL CONTROL POINTS FOR THE CONTROL OF RAW MATERIALS, PROCESSING, HYGIENE, FINISHED PRODUCTS, TRANSPORTATION, WHAREHOUSING, MARKETING, ONBOARD HANDLING AND MARINE ENVIRONMENT.

IV. MECHANISM

A) SELECTION AND TRAINING OF ALL PERSONNEL.
B) DEFINING THE PHYSICAL LOCATION OF PERSONNEL AND MATERIAL NEEDED FOR THE QUALITY CONTROL SYSTEM, BOTH CENTRAL AND REGIONAL.
C) ESTABLISHING WRITTEN AGREEMENTS AND COORDINATION GUIDELINES TO LIMIT THE FUNCTIONS, ACTIVITIES AND RESPONSABILITIES OF THE VARIOUS REGIONAL OFFICES.
D) ASSES THE TECHNICAL/SANITARY STANDARDS OF EACH PRODUCER IN RELATION TO LEGAL REQUIREMENTS.
E) DRAW UP, REVISE, ADAPT AND IMPLEMENT THE QUALITY ASSURANCE STANDARDS FOR FISH PRODUCTS.
F) DRAW UP AND ADAPT THE QUALITY ASSURANCE OPERATIONAL MANUALS FOR FISH PRODUCTS.
G) SET UP OPERATIONAL MANUALS IN CONJUNCTION WITH THE PRODUCERS, TAKING INTO ACCOUNT MATERIAL ALREADY IN EXISTENC THESE MANUALS WILL CONTAIN SPECIFICATIONS FOR THE CONTF OF CRITICAL POINTS RELATING TO RAW MATERIALS, PROCESSINC HYGIENE OF PERSONNEL, FINISHED PRODUCTS, TRANSPORTATIOI WAREHOUSING, MARKETING, ONBOARD HANDLING AND MARINE ENVIRONMENT.

H) PREPARE, EVALUE AND APPROVE THE QUALITY CONTROL PROGRAMS OF EACH PRODUCER.
   1.- SET UP A WORK GROUP IN CONJUNCTION WITH THE PRODUCERS IN ORDER TO PREPARE THE MODEL ON WHICH THE QUALITY CONTROL PROGRAMS WILL BE BASED.
   2.- ADVISE AND EDUCATE THE PRODUCERS AS TO THE ADVANTAGES OF USING THE PROPOSED SYSTEM.
   3.- TRAIN THE PERSONNEL TO PREPATRE AND REVISE THE QUALITY CONTROL PROGRAMS.
   4.- ESTABLISH A TIME LIMIT FOR SUBMITTING THE QUALITY CONTROL PROGRAMS.
   5.- ASSESS QUALITY CONTROL PROGRAMS.
   6.- DRAW UP'A LIST OF PRODUCERS IN ORDER TO ESTABLISH A PROGRAM OF PERIODIC INSPECTIONS AND PRODUCT SAMPLING.

I) IN ACCORDANCE WITH ALREADY EXISTING GUIDELINES,- NORMATIVE ADVISORES AND OUTSIDE SERVISES TO ASSESS THE QUALITY LEVELS (LABORATORIES, TECHNICAL CONSULTANTS, ETC).

J) SET UP PROCEDURES TO CERTIFY THE QUALITY OF FISH PRODUCTS.

K) ESTABLISH DIFFERENT PRIORITY LEVELS TO SUPERVICE THE SYSTEM.

L) ASSESS THE COSTS INCURRED IN PROVIDING THE SERVICES NECESSARY FOR THE SYSTEM AND SET UP A MECHANISM FOR RECUPERATING SUCH COSTS.

V. INFRASTRUCTURE

THE SYSTEM WILL HAVE A CENTRAL HEADQUARTERS AS WELL AS FOUR REGIONAL OFFICES IN ADITION TO A CENTRAL REFERENCE LABORATORY AND LABORATORIES IN EACH ONE OF THE REGIONAL OFFICES.

THE AUTHORIZED EXTERNAL SERVICES (LABORATORIES, TECHNICAL CONSULTANTS, ETC) WILL FORM PART OF THE SYSTEM.

TACKING INTO ACCOUNT THE GEOGRAPHICAL DISTRIBUTION OF THE FISHING AREAS, THE FOUR REGIONAL OFFICES WILL OPERATE AS FOLLOWS:

NORTH PACIFIC	MID-SOUTH PACIFIC	GULF AND CARIBBEAN	INNER STATES
BAJA CALIFORNIA NORTE.	NAYARIT.	TAMAULIPAS.	ALL STATES WITHOUT A COASTLINE
BAJA CALIFORNIA SUR.	JALISCO.	VERACRUZ.	
SONORA.	COLIMA.	TABASCO.	
SINALOA.	MICHOACAN.	CAMPECHE.	
	GUERRERO.	YUCATAN.	
	OAXACA.	QUINTANA ROO.	
	CHIAPAS.		

VI. FUNCTIONS

A) THE CENTRAL HEADQUARTERS WILL BE RESPONSIBLE FOR THE DIRECTION, COORDINATION AND SUPERVISION OF THE SYSTEM'S FUNCTIONS, AS WELL AS COORDINATION OF OTHER NATIONAL AND INTERNATIONAL, PUBLIC AND PRIVATE AGENCIES

B) CENTRAL HEADQUARTERS AND EACH REGIONAL OFFICE WILL BE RESPONSIBLE FOR RECORDING, EVALUATING AND CONTROLING THE PRODUCERS INVOLVED.

C) CENTRAL HEADQUARTERS AND EACH REGIONAL OFFICE WILL BE RESPONSIBLE FOR EVALUATING THE QUALITY CONTROL PROGRAMS OF THE PRODUCERS.

D) REGIONAL OFFICES WILL BE RESPONSIBLE FOR CERTIFYNG THE QUALITY OF FISH PRODUCTS.

E) CENTRAL HEADQUARTERS WILL BE RESPONSIBLE FOR APROVING AND REGISTERING THE CORRESPONDING PRODUCTS AND LABELS.

F) CENTRAL HEADQUARTERS AND EACH REGIONAL OFFICE WILL BE RESPONSIBLE FOR PREPARING, REVISING, ADAPTING AND IMPLEMENTING THE NORMS AS WELL OF THE OPERATIONAL AND QUALIT CONTROL MANUALS OF THE PRODUCERS.

G) CENTRAL HEADQUARTERS AND EACH REGIONAL OFFICE WILL BE RESPONSIBLE FOR TRAINING PERSONNEL.

H) THE CENTRAL REFERENCE LABORATORY AND EACH REGIONAL LABORATORY WILL BE RESPONSIBLE FOR IT'S OPERATIONS.

I) EACH UNIT WITHIN THE SYSTEM WILL BE RESPONSIBLE FOR ANY SANITARY EMERGENCY.

J) CENTRAL HEADQUARTERS WILL BE RESPONSIBLE FOR APPOINTING QUALITY CONTROL CONSULTANTS AND OUTSIDE SERVICES.

VII. SOURSES OF FINANCING

FINANCING WILL BE PROVIDED BY THE

FOLLOWING SOURSES:

A) PUBLIC SECTOR

B) PRODUCERS

- WILL BE CHARGED FOR OUTSIDE SEVISES (INSPECTION,- ADVISORS, TRAINING, ANALYSIS, SAMPLING AND CERTIFICATION).
- CONTRIBUTION BY EACH RELATED INDUSTRY
- CONTRIBUTION BY COOPERATIVES THROUGH THE NATIONAL CHAMBER OF THE FISHING INDUSTRY (CAMARA NACIONAL DE LA INDUSTRIA PESQUERA).

C) INITIAL INTERNATIONAL CONTRIBUTION.

VII. LEGAL FRAMEWORK

IN ACCORDANCE WITH MEXICAN FISHING LAWS, THIS MINISTRY IS AUTHORIZED TO PROMOTE THE FISHING INDUSTRY AND THE CONSUMPTION OF FISH PRODUCTS AS WELL AS ESTABLISH TECHNICAL SANITARY STANDARDS FOR LIVE AQUATIC SPECIES.
THE MINISTRY ALSO HAS THE AUTHORITHY TO SET UP QUALITY CONTROL STANDARDS FOR FISH PRODUCTS AND BY-PRODUCTS.

BY MEANS OF THE ORGANIC LAW LAID DOWN BY THE FEDERAL PUBLIC ADMINISTRATION, THE MINISTRY OF COMMERCE & INDUSTRIAL DEVELOPMENT (SECOFI) HAS THE AUTHORITY TO SET UP AND SUPERVISE QUALITY CONTROL STANDARDS.

SECOFI IS RESPONSIBLE FOR ENFORCING FEDERAL LAW REGARDING METROLOGY AND STANDARIZATION FOR THE PURPOSE OF PROMOTING THE DEVELOPMENT AND ENFORCEMENT OF NORMS LAID DOWN BY THE MEXICAN LAW, AND IMPROVING THE QUALITY OF NATIONAL PRODUCTS AND SERVISES.

IN ORDER TO ATTAIN THE OBJETIVES TO DEVELOP THE FISHING INDUSTRY AND PROMOTE DOMESTIC AND FOREING CONSUMPTION OF FISH PRODUCTS, AND RESPECTING THE AUTONOMY OF EACH MINISTRY INTERMINISTERIAL COORDINATION AGREEMENTS WILL BE SIGNED. THESE WILL GIVE SEPESCA THE AUTHORITY TO OVERSEE THE OPERATION OF THE "QUALITY CONTROL SYSTEM FOR FISH PRODUCTS", MAKING USE OF THE LEGAL AND INSTITUTIONAL MECHANISMS OF THE NATIONAL STANDARDIZATION COMMISSION, SO THAT IN CONJUNTION WITH SECOFI AND S.S., COORDINATION AGREEMENTS WILL BE SIGNED TO SET UP THE TERMS AND METHODS TO IMPLEMENT THE SYSTEM. A A RESULT, SEPESCA HAS THE AUTHORITY TO INTERVENE IN ACTIVITIES AFTER FISHING HAS BEEN CARRIED OUT, SUCH AS THE PROCESSING AND MARKETING OF FISHING PRODUCTS.

SEPESCA HAS THE LEGAL AUTHORITY TO DEVELOP AND IMPLEMENT THIS SYSTEM. IN ORDER TO ACHIEVE ITS OBJETIVES, IT WILL COORDINATE WITH SECOFI THROUGH AN INTERMINISTERIAL AGREEMENT, SO THAT SECOFI CAN ESTABLISH QUALITY STANDARDS FOR FISH PRODUCTS, WHICH HAVE YET TO BE DEFINED. THIS WILL REQUIRE OBSERVANCE OF THE GENERAL LAW OF HEALTH.

U.S. FDA'S SEAFOOD INITIATIVES - A REGULATORY UPDATE

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It is a pleasure to be here in Merida, Mexico and to share with you the U.S. Seafood Initiatives. Seafood has become much more important to North America, in terms of its contribution to the diet as well as its contribution to the overall economic base. In the United States alone, we estimate that consumers spend approximately $27 billion dollars (1990 figure) on seafood. 60% of that is imported. This is even more important considering the impact that the Free Trade Agreement will have on the economies of Mexico, Canada and the U.S. As consumption has increased, so has concern about the safety of the seafood supply. In response to this concern and with the help of a budget increase from the Congress, FDA created its Office of Seafood in February 1991. The Office is responsible for coordinating and implementing all of the agency's activities in seafood inspection, research, enforcement, education and training. Today, I want to tell you of the exciting and difficult tasks the Food and Drug Administration is undertaking to assure seafood safety, wholesomeness and truthfulness in labeling.

PHOSPHATES IN SCALLOPS

In August, the agency announced a new interim labeling policy for scallops treated with Sodium Tripolyphosphate. Let me emphasize that this was in response to requests from the industry for us to establish a common or usual name for scallops with added moisture, and also in follow-up to a host of letters the agency sent warning against overuse of phosphates and the illegal inclusion of water in purchase price weight of scallops.

Available data indicate that sea scallops consist of about 75 - 79% water. Once harvested, they can lose a moisture rather quickly. STP, or Sodium Tripolyphosphate, is considered to be Generally Recognized As Safe (GRAS), and is used legally, to prevent moisture loss, or "drip loss" in scallops. Unfortunately, prolonged soaking results in scallops taking up excessive water, adding to the overall weight of the product. This water weight is added to the price per pound of the product and constitutes economic fraud. In extreme cases, we have found scallops that had been soaked for so long and at such high concentrations that the product was badly decomposed.

We indicated over a year ago our concerns regarding the misuse of phosphates, and the illegal inclusion of added water in scallops. Follow-up activities included meetings with the concerned industry, inspections, warning letters and appropriate follow-up regulatory efforts.

So, in response to industry requests, we have implemented a six month interim labeling policy. Scallops that have been treated with STP and have picked up

water, must be labeled with the identity statement of "X % Water Added Scallop Product". This labeling must be applied to any scallop product with 80 percent water or more. In addition, the statement "Processed with Sodium Tripolyphosphate" or any other polyphosphate that is used, must appear on the label. The ingredients statement, too, must indicate that water, STP or other phosphates have been added.

In the meantime, the industry and academic scientists are developing data to determine the effects of various STP concentrations and soak times, and whether STP soaking has functional benefits beyond the prevention of drip loss, as some in the industry claim. Data are also being developed to determine the effects of the various treatments on key nutrients. Additionally, scallops consisting of 79% water or less are considered to be scallops and should be labeled as such. Anything consisting of 79 - 84% must be labeled as a "scallop product", and anything over 84% cannot be marketed.

We have instructed our field offices to actively pursue the examination, sampling and analysis of scallops, both imported and domestic for compliance with the interim policy. We are taking action down to the retail level against product found not to be in compliance with the interim policy and will continue to do so until a final policy is established.

COLOR ADDITIVES

An issue that has been a bit of a sticky wicket has to do with the use of color additives in farm-raised fish and the industry's desire to call some of these fish a name other than the FDA acceptable market name.

Canthaxanthin use is approved for coloring food and for coloring chicken feed under 21 CFR 73.75(c)(1) and 21 CFR 73.75(c)(2). At the time that this provision was passed, it was not the agency's intent to include the use of this color additive in fish feed, although we don't explicitly prohibit it. This ambiguity has led to a lot of confusion in the industry AND in the agency. We are drafting a Federal Register notice which we hope will clarify our position. Pending publication of the FR notice, the Agency is not likely to take regulatory action as long as the product is properly labeled as having color added. We continue to encourage the industry to submit a petition for the use of canthaxanthin in fish feed.

In addition, Hoffman LaRoche submitted sometime ago a petition for the use of synthetic astaxanthin in fish feed. Neither natural, nor synthetic astaxanthin are allowed for use in animal or human food at this time. We are still reviewing new data submitted by them a few weeks ago.

INSPECTIONS

As a follow-up to the limited GMP survey we did last year of the domestic seafood processors industry, we planned and carried out more in depth inspections of producers of high risk products. These included cooked-ready-to-eat products; products in modified atmosphere packaging; and scombroid-type species, including tuna, mackerel, mahi mahi and bluefish; and certain specialty products such as stuffed and breaded items. We have begun compiling the extensive inspectional observations collected at each facility.

The information gleaned from these surveys will help us in targeting future activities based upon a better understanding of the latest industry practices. Meanwhile we are upgrading our inspection frequencies by inspecting the higher risk firms at least annually and all others every other year.

A longer term goal, of course, is to incorporate HACCP principles into all our seafood inspections, something that is near and dear to the heart of Commissioner Kessler. HACCP provides processors with an important quality control tool to prevent problems before they begin. FDA benefits in that our inspectors can audit the pertinent processors' records made during industry's continuous monitoring of critical control points, and therefore have more than just a snapshot of the operation. The ultimate beneficiary will be the consumer.

In July 1992, the National Oceanic and Atmospheric Administration (NOAA) announced in the Federal Register the availability of a new HACCP-based seafood inspection service. This is a major revision of NOAA's traditional voluntary inspection program and a modification of their existing fee-for-service program. It is also in addition to the FDA/NOAA Voluntary Seafood Program that we are developing cooperatively under the Memorandum of Understanding. Once the joint program is available, we intend to build on NOAA's experience.

FDA/NOAA VOLUNTARY SEAFOOD PROGRAM

The FDA/NOAA Voluntary Seafood Program has been a long time in coming. We have completed the pilots for domestic, foreign processor and retail. We are preparing one for food service and are putting the last touches on the Federal Register announcement and hope to have that out soon. The Agency plans to implement the Joint program January 1, 1993. That is, it will be available and offered to U.S. processors.

BLUE CRAB

As some of you are aware, FDA has a Compliance Policy Guide which states that product labeled as "crabmeat", with no qualification, must be derived from the blue crab, Callinectes sapidus. At a recent meeting with the blue crab industry, we received a formal complaint about imported product from other species which is being substituted for and labeled as crabmeat, with no regard for FDA's policy and requirements, or even that of the U. S. Customs Service's country of origin requirement. We have this under investigation and are working with the industry to solve the problem. In fact, the State of Maryland has recently made a large seizure of imported crabmeat packed as Maryland crabmeat.

DUNGENESS CRAB

In October, the Agency issued two notices to State and Local regulatory agencies through our National Regional-State Telecommunications Exchange System (NRSTEN). Letters are being sent to the affected industry as well. The notices explained our regulatory position on the incidence of domoic acid which causes Amnesic Shellfish poisoning and saxitoxins which cause Paralytic Shellfish Poisoning (PSP) in the viscera of cooked Dungeness crab. FDA is taking regulatory action against Dungeness crab that is found to have 20 ppm or more of domoic acid in the viscera, as well as Dungeness crab that is found to have 80 μg per 100 grams or more of saxitoxin in the viscera.

This action is in response to several events and new information available to the agency with respect to PSP and ASP. Canadian authorities have recently closed harvesting areas near Quatsino Sound of Vancouver Island. Alaska too has issued an advisory to consumers and to the industry there, recommending evisceration of affected crab and tagging or warning labels. We support these efforts, as well as encouraging the affected states to put in place monitoring and closure systems modeled after the very successful PSP monitoring system for molluscan shellfish.

PARASITES

In June, we received an interim report on Parasites in Fish from the National Fisheries Institute's Industry Task Force . This report was somewhat limited, and we hope the final report will be more conclusive. In the meantime, we have issued an assignment to our Field offices to examine, sample and analyze finfish for parasites. This is an on-going project and will enable us to evaluate industry practices for the control of parasites in fish products.

The information gleaned from the task force, our own inspectional and analytical efforts, current international standards, as well as a search the literature, will all be used to develop Good Manufacturing Practice (GMP) parasite tolerance levels on a species group basis. The first groups to be addressed will be the gadids and the flounders. Then less utilized species will be considered.

IMPORTS

According to a profile of the industry recently completed by the agency's Office of Planning and Evaluation, we estimate that imports account for 5.6 billion pounds of product or 43% of the edible seafood in the United States. In the past, the agency has been too forgiving about unsafe or poor quality product entering the country. Importers must do a better job - they must be more responsible to make sure the product they send to the U.S. meets our requirements.

We have established a new import strategy. It includes closer cooperation with state and local agencies in order to identify imports that reach the retail market; the initiation of civil and criminal judicial actions against importers who flagrantly violate FDA regulations; and short term surveys of specific product categories; and education.

In addition, we are pursuing a new strategy with respect to Memoranda of Understanding (MOUs) with foreign countries. Instead of pursuing agreements with countries that continually have problems, we intend to look for agreements with countries that historically have done a good job. We call these the "Good Guy MOUs". This new philosophy will facilitate the entry of product from these "good" countries, and will permit us to concentrate our resources on the bad guys. During this fiscal year, we intend to pursue MOUs with Iceland, Canada, Australia, New Zealand, and Norway.

A relatively new issue has to do with our policy for handling imported perishable fish and seafood. Under our current regulations (21 CFR 1.90), when a sample of an imported product is taken, the owner of the product must hold that product until notified of the results of the laboratory examination. Unfortunately, it can take up to 18 days to receive the laboratory reports on import samples. This is a real problem when the product is fresh, for obvious reasons. The only present exception to the rule is when the product is being tested for pesticide residues, in which case, the analysis can be completed in 24 hours. There is a vast difference between 18 days and 24 hours! When this policy was originally developed, it was primarily designed to apply only to fruits and vegetables. Now, however, we are receiving complaints from the industry about the inexorable length of time it takes to receive the results, and what to do with perishable product in the meantime. We are exploring a number of options, including adjusting the policy to reflect all analyses on seafood products; establishing parameters for expediting seafood analyses; and in the longer term, identifying seafood ports of entry near laboratories that could specialize in analyzing seafood products.

CHEMICAL CONTAMINANTS

A major concern on the part of the American public has to do with chemical contaminants in seafood. Certainly, FDA is always being asked to make a judgment on the public health significance of contaminants in both freshwater and marine species. We've taken a number of steps in an effort to provide such guidance and to gather new information.

We have completed development of guidance documents on five contaminants found in molluscan shellfish - cadmium, lead, nickel, arsenic and chromium. These are designed to provide relevant scientific information to state and local regulatory personnel. They can then evaluate the public health significance of local and regional contamination, and issue appropriate public advisories or initiate closures of harvesting waters. These documents are under review by a subcommittee of the Association of Food and Drug Officials (AFDO) on fish contaminants. I hope they will be available soon. We intend to develop other documents on other contaminants in time.

We are also hosting a two-day conference on chemical contaminants in seafood, in January, in Washington, D.C. The purpose of the conference is to explore the known and unknowns about chemical contaminants, and to assist FDA in determining the kind of monitoring and research that are still needed. I hope some of you will plan to attend this meeting and assist us in determining where we need to go from here.

NUTRITION LABELING

On November 8, the initial requirements issued under the Nutrition Labeling and Education Act (NLEA) go into effect. At this point in time, it does not look as if this deadline will be extended. We have received comments from the seafood industry and have them under consideration. An issue related to this, and one with which we will all have to contend is that of labeling implications of aquaculture products vs. wild harvested due to different and often higher fat composition.

LISTERIA

Listeria in seafood is a difficult problem. Currently our policy is that if we find it, we take action. The Canadians have a policy that seems to be working in which the firms must adhere to strict government GMPs, which minimizes the occurrence of listeria in ready-to-eat products. On this basis, it is not necessary for them to routinely monitor listeria in end product.

CHOLERA

In the last year, we have had to deal with a number of unanticipated problems, including the cholera epidemics in Latin America. The first cases were reported in January 1991, and by the end of the year, 12 countries were reporting illnesses and deaths. In the United States, there have been over 90 cases of cholera reported this year. The majority of these were travel related. To illustrate, one incident involved Seventy six airline passengers who became ill. One subsequently died. The flight originated in South America and picked up food from a caterer in another South American country where cholera is epidemic, before continuing to Los Angeles. Although the Los Angeles health department could not determine with certainty what made the passengers ill, the shrimp salad became the main suspect.

FDA has been working closely with the Centers for Disease Control, the Pan American Health Organization and other international health agencies to stop

the spread of this disease. Our field inspectors are monitoring seafood and produce being imported into the U.S. from affected countries. None of the products tested has contained the epidemic strain. However, this monitoring effort did allow us to detect Vibrio cholerae in imitation crabmeat imported from Korea.

In addition, our laboratory at Dauphin Island, AL found shellfish and fish samples positive for Vibrio cholerae in Mobile Bay. This resulted in the testing of samples of ballast, bilge and holding tank water from ships arriving in the Bay from Central and South American ports. A total of 108 ships were sampled and 181 samples collected. Of these, seven samples (3 ballast, 2 bilge, 1 firemain, and 1 holding tank) from three ships were found positive for toxigenic V. cholerae 01, El Tor, Inaba and genetically indistinguishable from the epidemic strain from the affected countries. Another ballast water sample from a ship in New Orleans was also found positive. The last ports of call for the four positive ships were Brazil, Colombia and Chile.

We are continuing our investigation and monitoring of products, as well as our cooperation with other consumer protection groups around the world.

DECOMPOSITION

We have been putting a lot of effort into the area of decomposition, trying to improve the consistent detection of decomposition that occurs by looking at both microbiological and chemical indicators. The microbiological indicators have not worked so well because they can change depending on ambient conditions. The chemical indicators, on the other hand, have proven to work well. There are variations, though, and we are finding that the relative levels of chemical indicators and the rates of decomposition are highly variable and related to the different categories of seafood.

We are putting together a plan and schedule for this area to include the establishment of a 5 ppm defect action level for histamine in all scombridae fish; a 1 ppm cadaverine defect action level for mahi mahi, tuna and other fishery products; and the implementation of a PASS/FAIL system for assessing statistically taken samples. Those are just some of the highlights.

ECONOMIC FRAUD

In a speech to the National Fisheries Institute in 1991, Dr. David Kessler, Commissioner of Food and Drug, stated, "There is no place in the seafood industry for those who substitute a less expensive or less desirable specie of fish for one that consumers value more. We will seek out those who perpetrate fraud - and we will bring them to justice". He received a standing ovation from the industry. This enthusiastic response was not in vain. Over the last year we have taken a vigorous stand against economic fraud. Our budget for this has doubled from 2% to 4%, not insignificant, considering our accomplishments.

From our office alone, we sent over 1,000 letters warning the industry against overglazing. Our Field offices added to this by sending their own warning letters on overglazing and on other regional issues, such as soaking of scallops in phosphate solutions.

We have taken a number of actions against species substitution, including rockfish for red snapper; Pollock for cod; fresh water fish for salt water; and Oreo dory for Orange roughy. And we intend to continue to vigorously pursue action against this and other means of economic fraud.

IRRADIATION OF SEAFOOD

The possible irradiation of seafood to reduce pathogenic or decomposition organisms is, indeed, a primary seed of change. This is a safe, scientifically sound technology that is cleared for use in about 35 countries. If properly used, it will give the consumer a higher quality product, as well as a safer product in terms of harmful microorganisms. An editorial in the September/October 1992 issue of Public Health Reports says, "Food irradiation can be compared with pasteurization in its promise for the public health. Not only does the technology extend the shelf life of produce by inhibiting ripening or sprouting, it kills or renders noninfective many harmful food-borne organisms." The editorial goes on to cite the incidence of both Vibrio and cholera in seafood, leaving the idea that irradiation would go a long way in solving these problems. In addition, the use of irradiation will give industry greater flexibility in terms of available species and distribution and will help balance international supply and demand.

Two petitions are currently under review in the Agency to permit the application of this technology to fishery products. It is interesting to note that we are beginning to receive inquiries about the technology - from consumers, that is. It is clear there will need to be greater consumer education to achieve acceptance and understanding - the poultry industry isn't exactly being overwhelmed with requests to provide the marketplace with irradiated product!
And industry and government must work together to establish appropriate safeguards to prevent misuse of the technology. In the meantime, if this seed of change is to go anywhere, the industry will have to utilize the FDA petition process.

LEGISLATION

Legislatively, it has been pretty quiet this year, compared to the last two years. We did have one Senate hearing in June on the Hollings bill. This was designed to address virtually every aspect of the program and then divide it among three Federal agencies - the Department of Agriculture, the Department of Commerce and the Food and Drug Administration.

This division of labor did reflect an effort at compromise, but the Administration urged the Senate to resist the temptation in this case on the grounds that such an arrangement would likely entail many hidden costs, threaten program consistency and be difficult to manage. We understand that the bill may be reported out of the Senate Commerce Committee before the end of the session this fall. But the odds are against passage of any seafood legislation in this Congress.

Frankly, legislation is not going to solve the problems or make the critics go away. Instead, it is going to take a concerted and dedicated effort on the part of industry and government to assure the safety of seafood and instill consumer confidence.

FDA SEAFOOD HOTLINE

Last, but certainly not least is the initiation of the FDA Seafood Hotline on October 1, 1992. This too has been long in coming, but is an important part of our comprehensive educational program designed to enlighten the public about the safety of the seafood supply. The Hotline number is 1-800-FDA-4010, or in the Washington, DC area, 205-4314.

The Hotline is available 24 hours a day through a computerized information retrieval system that permits callers with touchtone phones to request FDA seafood publications, listen to pre-recorded seafood safety messages and access other information. Information can even be FLASHFAXED using this system. In addition, between 10 am and 2 pm, Eastern Time, Monday through Friday, FDA Consumer Affairs Specialists are available to answer questions

directly. Since the Hotline began officially, we are averaging over 25 calls per day. We will be receiving rather extensive reports that will include information on the numbers of questions asked, the topics and publications requested. This will help us immensely in targeting future consumer information activities and subjects.

To wrap things up, we were recently accused of following a policy of denial regarding problems with seafood safety, wholesomeness and labeling. Given what is on our plate and all the things we've been doing, we find that very suggestion to be repulsive and badly off the mark. Rather, we are convinced, we have looked our responsibility squarely in the face, shouldered it and are making intelligent and competent decisions on how to deal with the problems of seafood safety. We can't do this alone and appreciate the support and cooperation of the industry. But industry also has a responsibility - to take the action to put things right - and earn the greater confidence of consumers.

I wish to thank Mrs. Cynthia C. Leggett of the Office of Seafood for her valuable assistance in preparing this presentation.

PERSISTENCE OF *VIBRIO CHOLERAE* O1 IN ENVIRONMENTAL WATERS AND SHELLFISH

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Vibrio cholerae is the etiological agent of cholera. Strains implicated in cholera epidemics are toxigenic (i.e. produce cholera toxin) and are usually serotype O group 1 (O1). *V. cholerae* O1 strains can also be typed as serotype Ogawa or Inaba, and as classical or El Tor biotype. Symptoms of cholera include watery diarrhea, vomiting and electrolyte imbalance. Cholera is commonly transmitted by contaminated food and water, and through infected travelers. Its marked ability to survive in seawater and freshwater constitutes a continued health hazard when persons obtained food and water from aquatic environments.

In January of 1991, the first epidemic of cholera in over one hundred years was reported in Peru (1). Since then it has spread rapidly to nearly all South and Central American countries through contaminated food, water, and diseased individuals. In Peru, high levels and frequent isolations of *V. cholerae* O1 were observed in seawater, river water, sewage, plankton, finfish, and shellfish in counts ranging from 100 to 100,000 cells per gram/ml (13). In July of 1991, this virulent strain contaminated shellfish-growing waters near Mobile Bay, Alabama, and persisted in oysters for over one year, thus intensifying the need to determine the effect of post-harvest techniques, such as controlled purification, which might reduce *V. cholerae* O1 numbers in live molluscan shellfish.

Controlled purification (i.e. depuration) is an important critical control point in shellfish processing which can increase the safety and value of live shellfish products. Controlled purification is intended to reduce the number of pathogenic organisms in shellfish harvested from moderately polluted (restricted)

waters to such levels that shellfish will be acceptable for human consumption without further processing (14).

Research demonstrates that currently approved conditions of controlled purification are usually effective in reducing fecal coliforms and *Salmonella* (7,9), but are not always sufficient for elimination of viruses (8) and *Vibrio* spp. (7,10). Tamplin and Capers (10) report that in controlled purification systems, *V. vulnificus* can increase in oyster meats and be released into surrounding seawater at numbers of over 100,000 *V. vulnificus* cells/oyster/h at 23°C, a temperature which is approved by the US Food and Drug Administration (14). Persistence of vibrios during controlled purification indicates their ability to attach to oyster tissues, and sometimes replicate at rates exceeding that of elimination (10). However, when oysters are similarly processed in 15°C seawater, multiplication of *V. vulnificus* is markedly inhibited (10). Other research of *V. parahaemolyticus*, *V. harveyi*, and non-O1 *V. cholerae* shows that controlled purification is similarly inadequate (3,6,7). Reports of the effect of controlled purification on *V. cholerae* O1 are lacking.

Agglutinins, or lectins, present in oyster tissues may also influence retention of *V. cholerae* O1 in oysters. Tamplin and Fisher (11) reported a lectin in cell-free oyster hemolymph which showed high specificity for *V. cholerae* O1. This lectin has been demonstrated in *Crassostrea virginica* from various environments along the Gulf and Atlantic Coasts. However, its effect on survival of *V. cholerae* O1 in oysters has not been reported.

For these reasons, it is necessary to investigate the effect of post-harvest techniques which may reduce levels of *V. cholerae* O1 in molluscan shellfish, particularly oysters from the Gulf of Mexico where high water temperature increases the threat and numbers of pathogenic vibrios in oysters. The following studies determined the effect of controlled purification methods, and *V. cholerae* O1-specific oyster lectins, on accumulation and retention of *V. cholerae* O1 in oysters.

MATERIALS AND METHODS

Bacterial maintenance and culture

One day prior to experimentation, three *V. cholerae* O1 strains isolated from Peru waters, *E. coli* (strain 52), and *S. tallahassee* were plated on tryptic soy agar containing 1% NaCl (TSA-1%), and cultured overnight at 37°C. Colonies were transferred into 100 ml tryptic soy broth and incubated at 37°C until logarithmic growth was observed. The culture was centrifuged for 15 min at 3000 x *g*, the supernatant removed, and the cell pellet suspended in 50 ml of phosphate buffered saline (PBS). Cells were washed three times with PBS and the final pellet adjusted to approximately 10^{10} colony forming units (CFU) per ml.

Preparation of oysters

Oysters were collected from approved waters near Cedar Key, Florida and scrubbed to remove loose dirt and other marine debris prior to experimentation.

For bacterial analyses, oyster shells were rinsed with deionized water and meats of 10 specimens shucked into sterile blender jars. Samples were diluted 1:1 (weight:weight) with PBS and homogenized for 90 sec on high speed.

Isolation and enumeration of bacteria

Vibrio cholerae O1 and *V. vulnificus* were enumerated by the three-tube MPN enrichment method, using APW (2,12). After six to eight h incubation at 37°C, all turbid tubes were streaked onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar for isolation of *V. cholerae* O1. APW was incubated at 37°C for a total of 12 to 16 h, and streaked to modified colistin-polymyxin-cellobiose (mCPC) agar for isolation of *V. vulnificus*. TCBS and mCPC plates were incubated at 37°C for 18 to 24 h. Colonies were identified as *V. cholerae* O1 by an immunoassay described by Tamplin et. al. (12).

Escherichia coli was enumerated by the five-tube MPN method. All turbid enrichment tubes were transferred to 10 ml EC broth containing 50 mg/L 4-methylumbelliferyl-beta-D-glucuronide (MUG) and inverted Durham tubes, and incubated at 44.5°C for 22 to 26 h for confirmation of *E. coli*.

Salmonella tallahassee was enumerated following methods established by the U.S. Food and Drug Administration's Bacteriological Analytical Manual, 7th edition. Colonies were confirmed by biochemical tests using an API 20E kit.

Controlled purification system

To simulate a commercial closed-type depuration system as outlined in the Food and Drug Administration's National Shellfish Sanitation Program Manual of Operation (14), a 30 gallon aquarium was filled with 41 L of artificial seawater (Instant Ocean), and recirculated by pump through a 5 μm cartridge filter and 30 watt UV light at a rate of 1,928 liters/h. Controlled purification experiments were conducted at room temperature (25 to 26°C), and at 15°C and 19°C.

Bactericidal effect of ultraviolet light

To determine the disinfecting efficiency of UV light in the controlled purification system, *V. cholerae* O1, *E. coli*, and *S. tallahassee* were separately added to 41 L of 25 part-per-thousand (ppt) seawater at 25°C. Each bacterial species was prepared for inoculation as described above and added to the tank at a final concentration of ~ 10^7 *V. cholerae* O1/ml and ~ 10^5 *E. coli* and *S. tallahassee*/ml. Samples were removed at selected time intervals over seven h. Bacteria were enumerated as previously described.

Uptake of *V. cholerae* O1 by oysters

Approximately 100 oysters were placed in a tank containing 41 L of recirculated 25 ppt seawater (25°C). *Vibrio cholerae* O1 were added to tank water to obtain levels of *V. cholerae* O1/ml similar those observed in seawater in Peru (~ 10^4/ml) (13). Ten oysters were randomly selected and removed from the tank at selected time intervals over seven h.

Effect of temperature and comparison of uptake of *V. cholerae* O1, *S. tallahassee*, and *E. coli* by oysters

Vibrio cholerae O1, *E. coli*, and *S. tallahassee* were added to 41 L of seawater (25 ppt, 25°C) at a concentration of ~ 10^4/ml. At selected time intervals, 10 oysters were removed and bacteria enumerated. In a second experiment, seawater was maintained at 19°C and inoculated with ~ 10^4 CFU/ml

of each species.

Fate of *V. cholerae* O1 under conditions of controlled purification

To measure retention of *V. cholerae* O1 in oysters, shellfish were collected and placed in 41 L 25 ppt seawater at 25°C. The tank was inoculated with ~ 10^4 *V. cholerae* O1/ml, and the oysters exposed to *V. cholerae* O1 for four h. Ten oysters were removed and enumerated for *V. cholerae* O1 before experimentation, after four h of uptake, and at selected time intervals of controlled purification over 48 h.

Effect of temperature and comparison of the fate of *V. cholerae* O1, *E. coli*, *S. tallahassee*, and *V. vulnificus* under conditions of controlled purification

Experiments were conducted to compare retention of *V. cholerae* O1, *E. coli*, and *S. tallahassee* under conditions of controlled purification with seawater held at 15, 19, and 25°C. In the first experiment, prior to depuration conditions, seawater (19°C) was inoculated with ~ 10^2 *V. cholerae* O1, *E. coli*, and *S. tallahassee*/ml, and oysters exposed for four h. After activation of UV lights, 10 oysters were sampled at selected time intervals over 48 h and *V. cholerae* O1, *E. coli*, and *S. tallahassee* enumerated in meats. Endogenous levels of *V. vulnificus* were also measured. In a separate experiment, oysters were exposed to bacteria at 25°C for four h. Oysters were then divided into equal numbers and placed in controlled purification tanks at either 15°C or 25°C. A sample of 10 oysters was removed from each tank and enumerated for the three bacterial species at selected time intervals over 48 h.

Effect of hemolymph lectin level on uptake of *V. cholerae* O1 by oysters

Oysters collected in July, 1992 were separated into groups of low and high lectin titer as determined by the bacterial agglutination assay described by Tamplin and Fisher (11). After lectin titers were determined, oysters were separated into low titer (approximately 2^0) and high titer (approximately $>2^{64}$) groups. Next, oysters were placed into a tank of 25 ppt seawater at 25°C for 30 minutes to acclimatize animals. Low and high titer oysters were then placed into two separate ten gallon tanks containing recirculated seawater (25 ppt, 25°C). Tanks were inoculated with ~ 10^2 *V. cholerae* O1/ml. At selected time intervals, five animals were removed from each tank and numerated for *V. cholerae* O1.

Effect of hemolymph lectin on retention of *V. cholerae* O1 by oysters

Oysters collected in January, 1993 were separated into groups of low and high lectin titer as determined by the bacterial agglutination assay described above. After four h of exposure in the same 30 gallon tank (25 ppt, 25°C), containing ~ 10^4 *V. cholerae* O1/ml seawater, the two groups were placed separately in two 10 gallon tanks of recirculated seawater (15°C) which was disinfected with UV light. Five animals from each tank were removed and enumerated for *V. cholerae* O1 before exposure to bacteria, after four h of exposure to *V. cholerae* O1, and at selected time intervals of controlled purification.

Statistical methods

Significant differences in mean values were determined using the two-tailed Students's *t* distribution test ($p \leq 0.05$; degrees of freedom = 4).

RESULTS

Bactericidal effect of ultraviolet light on *V. cholerae* O1, *E. coli*, and *S. tallahassee*

The bactericidal efficiency of UV light was tested by inoculating 25°C seawater with ~ 10^7 *V. cholerae* O1/ml, and ~ 10^5 *E. coli* and *S. tallahassee*/ml. *Vibrio cholerae* O1 was not detected at four h of treatment (not shown). *Escherichia coli* and *S. tallahassee* were not detected at two and three h respectively.

Uptake of *V. cholerae* O1, *E. coli*, and *S. tallahassee* by *Crassostrea virginica*

To determine uptake of *V. cholerae* O1 by oysters, approximately 100 specimens were exposed to 25°C seawater containing ~ 10^4 *V. cholerae* O1, *E. coli*, and *S. tallahassee*/ml. Oysters contained no detectable *V. cholerae* O1 or *S. tallahassee*, and 1.4 *E. coli*/g prior to exposure. After four h exposure, *E. coli* and *S. tallahassee* reached ~ 10^4/g oyster tissue and remained stable through 12 h (Figure 1). At four h, oysters contained greater than 10^5 *V. cholerae* O1/g. More *V. cholerae* O1 was accumulated than *E. coli* and *S. tallahassee* throughout the 12 h exposure. To determine the effect of reduced temperature on uptake of each species, oysters were exposed to ~ 10^4/ml of each of the bacteria strains in seawater maintained at 19°C. In contrast to the previous experiment, after 12 h exposure, oysters contained ~ 10^3 *V. cholerae* O1/g, and less than 10 *E. coli* and *S. tallahassee*/g (not shown).

Fate of *V. cholerae* O1, *E. coli*, *S. tallahassee* under conditions of controlled purification

To compare retention of *V. cholerae* O1 *E. coli* and *S. tallahassee*, oysters containing non-detectable levels of *V. cholerae* O1 and *S. tallahassee*, and 0.2 *E. coli*/g prior to exposure, were placed in 19°C seawater containing ~ 10^2 *V. cholerae*, *E. coli*, and *S. tallahassee*/ml for four h. Following exposure, *V. cholerae* O1, *E. coli*, and *S. tallahassee* levels in oysters were 4.3 x 10^2, 2.3 x 10^2, and 2.3 x 10^2/g, respectively (Figure 2). The level of naturally-occurring *V. vulnificus* following the four h exposure was 23/g. After 48 hours of controlled purification, oysters retained 4.3 *V. cholerae* O1/g and 1.5 x 10^2 *V. vulnificus*/g. *Escherichia coli* and *S. tallahassee* were not detected after 48 h of controlled purification (Figure 2).

In a separate experiment, oysters were exposed to the three bacterial species at 25°C, and then separated into two tanks with seawater maintained at 15°C or 25°C. Prior to experimentation, oysters contained no detectable *V. cholerae* O1 or *S. tallahassee*, and 0.56 *E. coli*/g. After four h exposure to seawater containing ~ 10^5 *V. cholerae* O1, *E. coli*, and *S. tallahassee*/ml, oysters contained ~ 10^2 *V. cholerae* O1/g, ~ 10^3 *E. coli*/g, and ~ 10^2 *S. tallahassee*/g (Figure 3). After 48 h of controlled purification, oysters at 15°C and 26°C retained greater levels of *V. cholerae* O1 than *E. coli* or *S. tallahassee*. Specifically, at 15°C, *V. cholerae* O1 was 30 times greater than *S. tallahassee* and 540 times greater than *E. coli*. At 26°C, *V. cholerae* O1 reached levels 100 times greater than S. tallahassee and 10 times greater than *E. coli*. Greater retention

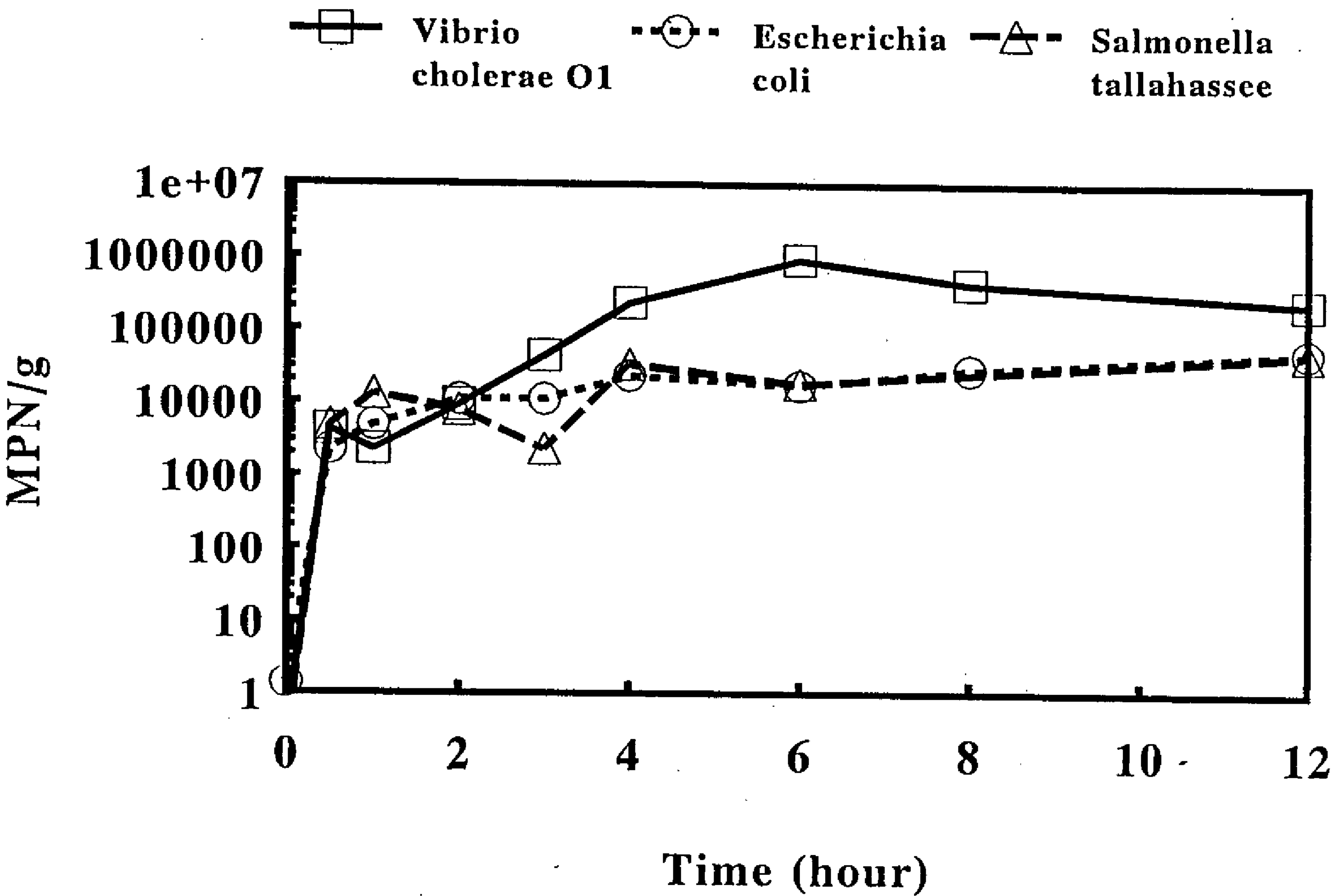

Figure 1. Uptake of *V. cholerae* O1, *E. coli*, and *S. tallahassee* by oysters at 25°C.

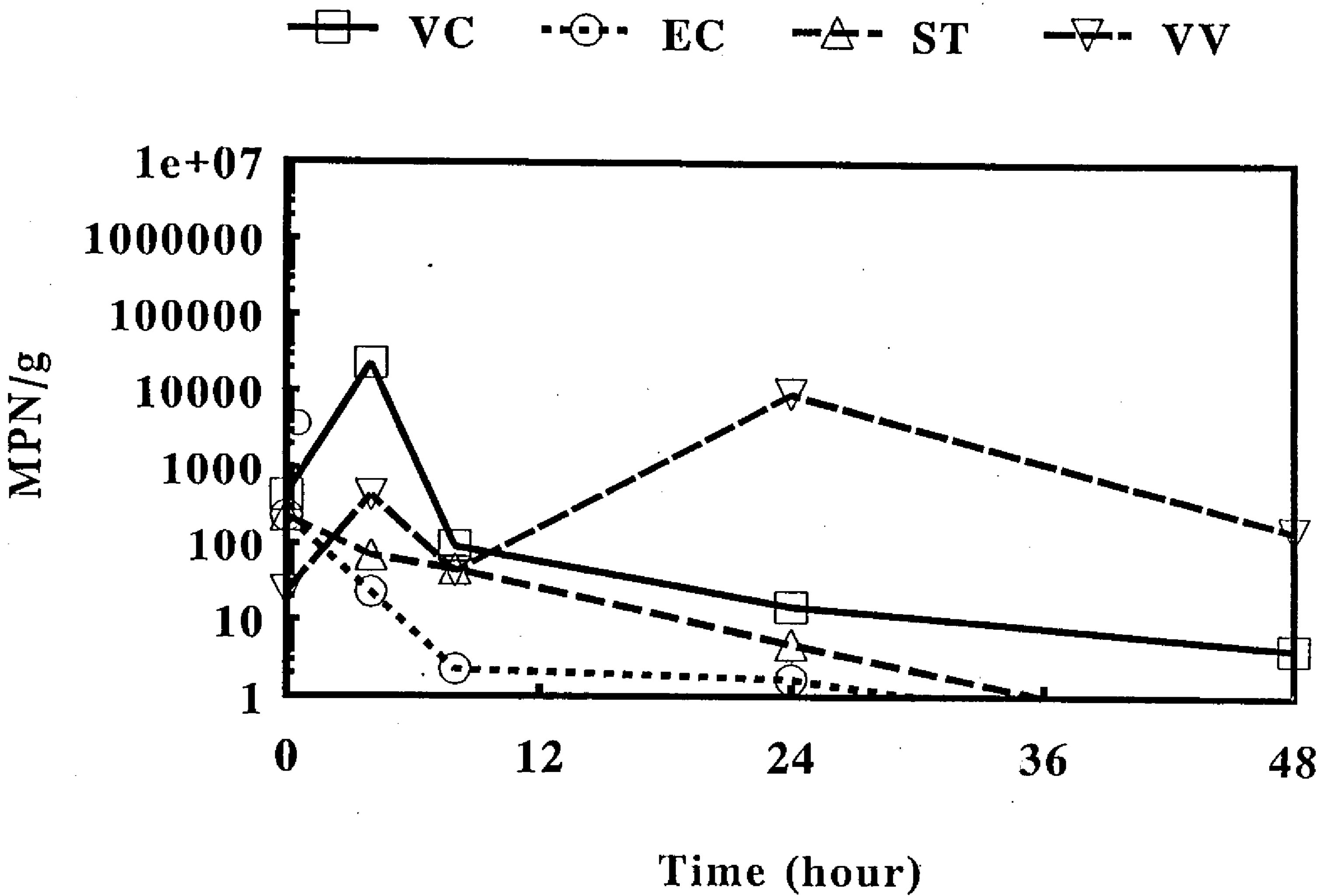

Figure 2. Retention of *V. cholerae* O1 (VC), *E. coli* (EC), *S. tallahassee* (ST), and *V. vulnificus* (VV) at 19°C.

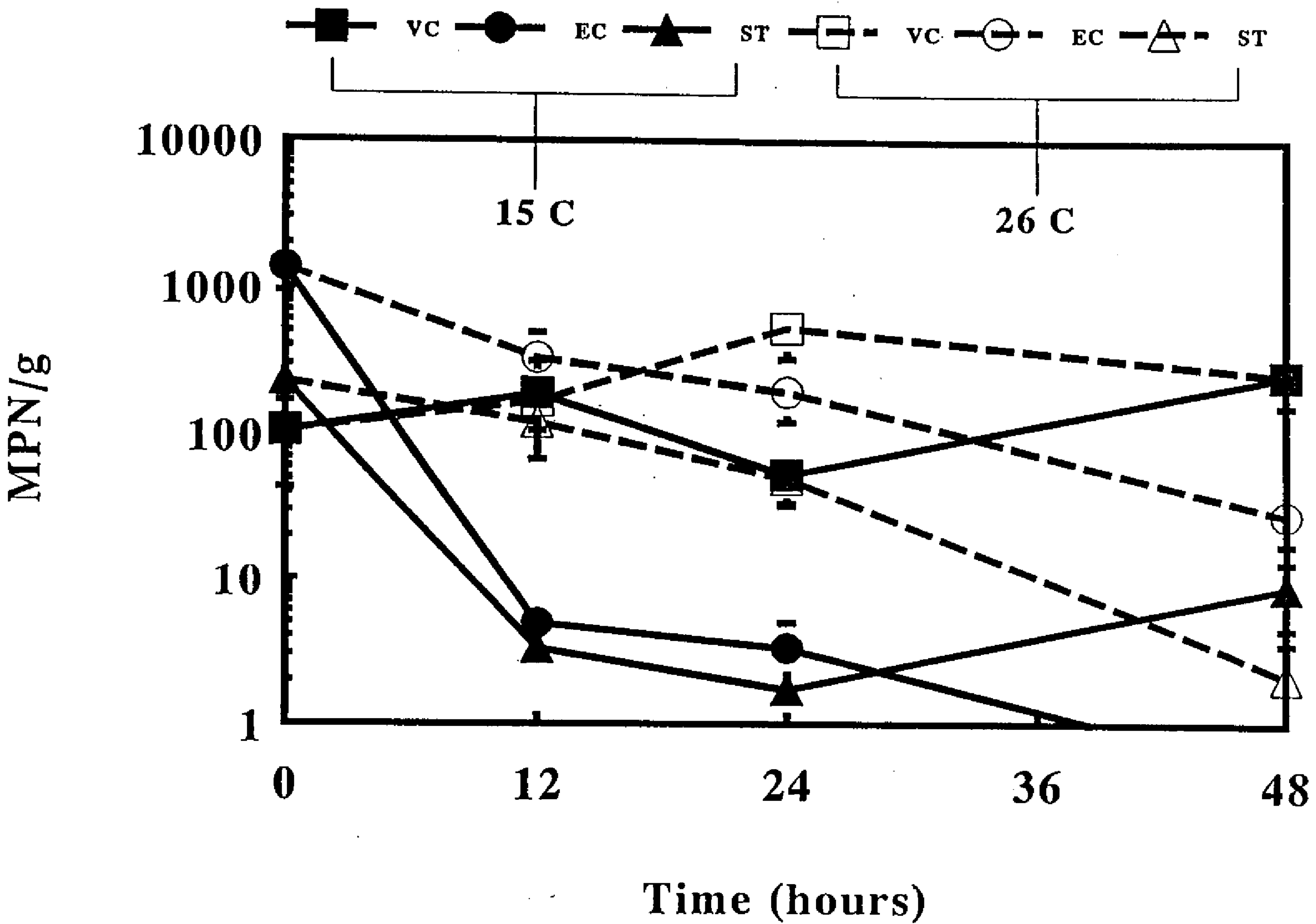

Figure 3. Retention of *V. cholerae* O1 (VC), *E. coli* (EC), and *S. tallahassee* (ST) at 15°C and 26°C.

of *E. coli* occurred at 25°C, versus 15°C, through 48 h. Greater numbers of *S. tallahassee* were retained at 25°C , versus 15°C, at 24 h, but at 48 h, higher levels were observed at 15°C (Figure 3).

<u>Effect of hemolymph lectin titer on uptake and retention of *V. cholerae* O1</u>

There was no significant difference in uptake of *V. cholerae* O1 between low and high lectin titer oysters at all time points (not shown). Low titer oysters contained 1.1 x 10^4 *V. cholerae* O1/g and the high titer group contained 3.6 x 10^4/g after eight h exposure.

To measure retention of *V. cholerae* relative to low and high lectin titer, oysters were exposed to ~ 10^4 *V. cholerae* O1/ml for four h at 25°C. The oysters were then subjected to conditions of controlled purification at 15°C and tested for retention of *V. cholerae* O1 over 48 h. There was no difference in *V. cholerae* O1 levels between high and low lectin titer groups over 48 h (not shown). Both the low and high titer oysters had no detectable (<0.3/g) *V. cholerae* O1 after 48 h of treatment.

DISCUSSION

Although contaminated seafoods have been implicated in the transmission and persistence of *V. cholerae* O1, a review of the literature yields no reports of controlled purification methods for reducing this pathogen in raw molluscan shellfish. The present research investigated the effectiveness of controlled purification in reducing *V. cholerae* O1 in oysters.

Results showed that oysters exposed to seawater containing *V. cholerae* O1, at levels similar to the infected waters in Peru, accumulated *V. cholerae* O1 in as few as four h of exposure, and, levels continued to rise through 12 h. This may have resulted from multiplication of *V. cholerae* O1 in oyster tissues, as has been suggested for *V. vulnificus* (10). When compared to *E. coli* and *S. tallahassee* in both 19°C and 25°C seawater, *V. cholerae* O1 was accumulated in greater numbers over 12 h. However fewer bacteria were accumulated at 19°C versus 25°C, indicating that reduced temperature may affect; 1) oyster physiology, (i.e. pumping) allowing less accumulation of bacteria, 2) surface affinity between bacteria and oyster tissue, and 3) survival of bacteria in oyster tissues.

Initial investigation of the retention of *V. cholerae* O1 under controlled purification with seawater at 25°C indicated persistence of *V. cholerae* O1 in oyster tissues. The level of *V. cholerae* O1 in oysters after 48 h of controlled purification was slightly higher than levels after four h of exposure. Again, this observation may result from multiplication of *V. cholerae* O1 in oyster tissues with process seawater maintained at 25°C. However, when comparing retention of *V. cholerae* O1, *V. vulnificus*, *E. coli*, and *S. tallahassee* during controlled purification with seawater at 19°C, *V. cholerae* O1 was reduced 100-fold after 48 h, indicating that decreased temperature may prevent multiplication of *V. cholerae* O1 in tissues and in the controlled purification system, allowing UV light to

reduce the numbers of *V. cholerae* O1 in seawater, which in turn allows less accumulation in oysters. *E. coli* and *S. tallahassee* were reduced to non-detectable levels by 36 h.

Natural *V. vulnificus* populations increased one log after 48 h of controlled purification, agreeing with Tamplin and Capers (10) that controlled purification is not effective in reducing *V. vulnificus*, and that *V. vulnificus* levels will increase in seawater above 15°C.

The contrasting data on retention of *V. cholerae* O1 at 19°C and 25°C led to an experiment to compare retention of *V. cholerae* O1, *E. coli*, and *S. tallahassee* in oysters subjected to controlled purification at 25°C and 15°C, since the latter temperature has been recommended for controlling replication of *V. vulnificus* and is used by commercial controlled purification facilities. Oysters were exposed to bacteria at 25°C and then placed in controlled purification tanks containing seawater at 15°C and 25°C. In this experiment, greater retention of *V. cholerae* O1 versus *E. coli* and *S. tallahassee* occurred at 15°C and 25°C. At 24 h, slightly more *V. cholerae* O1 was retained in oysters subjected to 25°C controlled purification, however, levels of *V. cholerae* O1 in oysters at 48 h were identical at both temperatures. Perhaps harvesting these particular oysters from temperate waters (20°C), subjecting them to increased temperature (25°C) during exposure, and maintaining them in seawater at 15°C during controlled purification, stressed the physiological state of the animals resulting in higher than expected *V. cholerae* O1 at 48 h. Nevertheless, *V. cholerae* O1 was retained 10 to 540 times greater than *E. coli* or *S. tallahassee* at either temperature.

There was a marked contrast in titers of oyster hemolymph *V. cholerae*-O1 agglutinin between warm and cold water collections. Fisher (4,5) has reported that *V. cholerae* O1 agglutinin levels are lower in summer and higher in winter. The low bacterial agglutination titer during the warm water collections (July, 1992) may have resulted from the reproductive state (spawning) of oysters which stresses their physiology. In the July harvest, many oysters contained no detectable agglutinin (< 2, $n = 33$), while no oysters sampled contained lectin titer of > 128. In contrast, the majority of oysters collected in January had high lectin titer (> 128, $n = 44$) and few had no detectable titer (< 2, $n = 2$).

There was no significant difference in accumulation of *V. cholerae* O1 between the low and high titer lectin groups. The comparison of retention of *V. cholerae* O1 under conditions of controlled purification also indicated no significant difference between low and high titer groups.

There are several explanations for these results. First, the bacterial agglutination assay as described by Tamplin and Fisher (11) determines lectin titer of oyster hemolymph collected from the sinus of the adductor muscle and may not reflect the amount of lectin present in oyster tissue, which represent the largest surface areas exposed to *V. cholerae* O1. Second, the bacterial agglutination assay uses a high concentration of *V. cholerae* O1/ml (i.e. $\sim 10^{10}$), and results may not coincide with low *V. cholerae* O1 levels used in these experiments (i.e. $\leq 10^5$), which may reduce the interaction between bacteria and lectin. A third possibility recognizes that removing hemolymph from oysters, and storing them at 10°C overnight, may not be sufficient to allow the oyster to replenish

hemolymph causing differences in lectin titer, oyster physiology, and controlled purification profiles.

This research provides information on the potential of a post-harvest process for eliminating *V. cholerae* O1 from raw molluscan shellfish. Importantly, current US FDA regulations recommending depuration and wet storage temperatures of 10°C to 25°C must be reevaluated to determine optimal temperatures for eliminating fecal coliforms and *Salmonella*, with consideration for the effect on *V. vulnificus* and *V. cholerae* O1, since these results show that elevated seawater temperature may produce a more hazardous product.

In addition, these studies do not support the hypothesis that oysters containing high concentrations of hemolymph agglutinin retain greater amounts of *V. cholerae* O1. Further studies are needed to determine the amount of lectin in specific oyster tissues, and develop better methods for optimizing oyster physiology during experimentation.

The recent introduction of a new virulent *V. cholerae* O1 strain into the Gulf of Mexico signals a new threat to shellfish industries and public health. The potential persistence of this pathogen in estuaries poses new challenges to develop effective post-harvest processes which can maintain a commercially-viable raw shellfish product.

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BACTERIA OF SIGNIFICANCE IN THE INTERNATIONAL TRADE OF SHRIMP

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Shrimp is an important and expensive commodity in international trade. Japan, Europe, and the United States consume 2,145,000,000 pounds per year of which 80% is imported. Mexico, Ecuador, China, Thailand, Indonesia, India, Vietnam, Taiwan, and the Philippines are the largest exporting countries (20). The Food and Drug Administration (FDA) has identified seafood as the largest of all food import problems (1). Reasons for detention and/or rejection have included filth, decomposition, pathogens and illegal or undeclared additives. At ports of entry, there may be little information available as to the processing history of the shrimp. In response, the FDA has used the presence of *Salmonella* as an indicator of poor sanitation. In domestic, or pond raised shrimp, however, there is evidence that this may not be an appropriate indicator of proper sanitation and hygiene and to apply the same standards as those assigned to imported shrimp may be inappropriate.

RESULTS

Spoilage and decomposition

The loss of freshness in shrimp due to the multiplication of bacteria can take two different paths. One occurs at refrigeration temperatures from the metabolism of psychrotrophic bacteria and is commonly referred to as spoilage. The other is the

result of the metabolism of mesophilic bacteria at ambient temperature and is more aptly referred to as decomposition. Both can be minimized through good sanitation and proper time/temperature control.

Spoilage generally is caused by gram-negative psychrotrophs in the *Pseudomonas/Moraxella/Acinetobacter* groups. These organisms are usually not part of the normal flora of wild-caught or pond raised shrimp. Instead, they are introduced onto the shrimp during various handling steps and become the predominate flora under refrigerated storage. A primary by-product of spoilage is ammonia. As spoilage bacteria deaminate amino acids from the free amino acid pool or shrimp tissue protein, there is a concomitant rise in the pH of the shrimp. Generally, fresh shrimp will have a pH of 7.25 - 7.50, marginal quality shrimp between 7.50 - 7.75, and spoiled shrimp above 7.75 (6). Monetary incentives paid for shrimp landed at the dock with pH values below 7.50 have proven to be effective in encouraging good handling practices.

Decomposition is the result of the action of organisms like *Klebsiella, Proteus, Enterobacter, Citrobacter* and other mesophilic *Enterobacteriaceae*. The primary products of decomposition are cadaverine, putracine, skatol and, specifically indole, which is produced from the decarboxylation of the amino acid tryptophane. The FDA has established three classes of decomposition for shrimp. Class I includes shrimp with fresh aroma and no evidence of off-odors. Class II shrimp possess a slight odor of decomposition. Class III includes shrimp that are obviously decomposed.

A production lot of shrimp is rejected if more than 20% of the product is Class II or more than 5% is Class III. It can also be rejected if it contains both Class II and Class III shrimp. Here Class III shrimp are given four times the weight of those in Class II and the lot is rejected if it exceeds 20%. For example, if within a production lot of shrimp 10% are categorized as Class II, and 5% are Class III (4 x 5% = 20%) the lot of shrimp exceeds Class II guidelines by 10% and is rejected (10% + 20% = 30%). Since these evaluations are subjective, quantitative analysis for indole confirms the organoleptic evidence of decomposition, as indole is an absolute indicator of high temperature abuse. The indole level in Class I determinations is less than 25 μg/100g, in Class II is equal to or greater than 25 μg/100g, and in Class III is equal to or exceeds 50 μg/100g (8). Shrimp with indole levels of 25 mg/100 g and higher are considered decomposed.

Pathogenic bacteria

Primary pathogens of concern in the international trade of shrimp are *Salmonella, Listeria,* and *Vibrio cholera.* The following sections will give some historical perspective and update current literature available on each pathogenic group.

Salmonella

Salmonellosis has been increasing for the past 40 years and plays a role in at least 1,000 deaths a year in the United States. Costs of up to a billion dollars a year for patient care are estimated (26).

In the late 1960s, a large volume of shrimp imported from India, Bangladesh, and Pakistan was contaminated with *Salmonella.* Although there were no reported outbreaks from these shrimp and most were raw product destined for cooking, the presence of salmonellae was considered an excellent indication of poor sanitation and handling practices.

The FDA now applies a Category III sampling plan for raw imported shrimp. This classification normally applies to foods that are subjected to processes lethal to *Salmonella* between sampling and consumption. A total of 15 sample units (100 g each) of product are collected and 25g portions of each are analyzed either individually or as composites for the presence of *Salmonella.*

Properly handled sea-caught shrimp should be free of enteric pathogens, specifically salmonellae. However, the product may become contaminated during subsequent handling from contaminated water. Thus, the presence of *Salmonella* in sea-caught shrimp has been regarded as an indication of poor hygienic practices. The development of technologies to allow for the reproduction of shrimp in captivity and the availability of low-cost coastal land have created an explosion in the world-wide production of pond-raised shrimp.

In one study conducted in the Philippines (23), researchers concluded that brackish water ponds and shrimp taken from them were inherently contaminated with enteric pathogens. *Salmonella* spp. were present in 16% of prawn samples and 22% of mud samples analyzed. In a separate study from the Philippines (13), researchers reported *Salmonella* spp. in zero to 23% of mud samples from ponds, but none from freshly harvested shrimp or from the processing environment. These researchers concluded that salmonellae were not part of the natural flora of the shrimp. Instead, they were the result of contamination from animal manure or nearby human settlements with inadequate sewage disposal. In a report from

Indonesia, salmonellae were isolated frequently from sediment samples taken from ponds, but not from the shrimp itself (21).

One would expect *Salmonella* in the water and shrimp from ponds fertilized with raw poultry feces or in close proximity to small villages and livestock producing areas. It is doubtful that salmonellae could ever be totally eliminated from the sediment, water or shrimp produced in such ponds. Indigenous wildlife such as snakes, frogs, turtles, and birds will ensure some low-level incidence of *Salmonella* in ponds world-wide.

However, there is clear evidence that the incidence of salmonellae in pond raised shrimp can be reduced through good management practices. Salmonellae were not isolated from prawn or water samples collected from farms in Sri Lanka (9). This particular study stressed good pond management and harvesting practices in producing uncontaminated product. It follows that good pond management can be effective in reducing the incidence of *Salmonella* in pond-raised shrimp.

Every effort should be made to reduce the incidence of *Salmonella* in pond raised shrimp, but the use of *Salmonella* as an index of sanitation in pond shrimp may not be appropriate. Current sampling and testing programs for salmonellae could be a devastating setback to the production of pond raised shrimp. Other raw foods of animal origin, such as poultry and pork are inherently contaminated with salmonellae and it is accepted that it is not economically feasible to set a standard of *Salmonella* negative for these products. The same consideration should be given to pond raised shrimp.

Listeria

Published literature contains information on *Listeria* isolations from soil, animals, birds, sewage, silage, stream water, mud, trout and crustaceans. Public health concerns have expanded from dairy products to raw vegetables, meat and seafood products. In a recent article (7), the author states that seafood products have received less study than other food and have been epidemiologically implicated in two listeriosis outbreaks. In addition, many products in North America, including cooked shrimp, have been recalled from market (25).

Recent surveys report the presence of *Listeria monocytogenes* in various shrimp products throughout the world. *Listeria monocytogenes* was isolated from nine percent of ceviche (lime juice marinated, raw product) samples in Peru (10). The results indicated that *Listeria monocytogenes* could survive short exposure

times (same day of manufacture) in the pH range of 3.8 to 4.8 as a result of the buffering capacity of shrimp. Seventy-five percent of the samples were positive for *Listeria innocua*.

Icelandic researchers found *Listeria monocytogenes* in nine percent of cooked shrimp samples (12). Interestingly, the incidence level in shrimp salads was 23%. They indicated that cooking at 60 to 80°C for a short time might not be adequate for the destruction of the organism. In a survey of foods from Taiwan, 10.5% of the seafood analyzed was positive for *L. monocytogenes* (29). Eighteen percent of the brine pickled shrimp with a pH of 6.0 in Norway were found to be positive for *L. monocytogenes* (24).

Of 57 retail raw and cooked seafood samples examined in U.S. markets, 35 (61%) were positive for *Listeria* spp. and 15 (26%) were positive for *L. monocytogenes*. Crabmeat, shrimp, lobster tail, langostinos, scallops, and surimi were included in the positive samples. Of 74 shrimp samples collected from the Gulf of Mexico, eight (11%) were positive for *L. monocytogenes* (16). A higher incidence of *Listeria* was observed when temperatures of the harvest water were 20°C or above and there was no correlation to salinity or fecal coliform levels.

The close association of *Listeria* with soil and water explains the incidence of *Listeria* in raw shrimp, especially those from brackish or fresh water. Although the incidence of *Listeria* in shrimp may seem high, the levels may be low. In a comparison of enrichment procedures for the detection of *Listeria* spp. in naturally contaminated seafoods, it was noted that composite sample portions were not identical because of low levels of the microorganism (18).

Unprocessed raw shrimp may represent a potential source of contamination to processing equipment and to other processed seafood products. Therefore, the data obtained from environmental swab samples collected from processing plants should be interpreted carefully. Positive environmental samples may be attributed to the normal flora of the pond or farm where the shrimp was raised rather than unsanitary practices in the plant. As discussed previously for *Salmonella*, application of GMPs and the identification of critical control points will allow shrimp processors to control but not completely eliminate *Listeria*.

Information on the effectiveness of sanitizers on *Listeria* should be evaluated carefully. Many studies are performed with suspended cells. Higher concentrations of sanitizers may be required to inactivate *Listeria* attached to surfaces.
Mafu et al. (14) indicated that *Listeria* attached to surfaces were more resistant to sanitizers than those suspended in a media or buffer. They reported sodium

hypochlorite to be more effective at the recommended manufacturers concentration (200 ppm for nonporous and 800 ppm for porous surfaces) than quaternary ammonium compounds (200 ppm). The researchers also reported that concentrations of two to three times greater are required when applications were at 4°C versus 20°C. These concentrations, however, do not seem to be appropriate for commercial applications. Another report (2) indicated *Listeria* biofilms attached to chitin are more resistant to chlorine and iodine than to quaternary ammonium compounds. This was true even when higher than normal concentrations and longer contact times were analyzed. Quaternary ammonium compound used at double strength for five times the recommended exposure period was the most effective concentration examined in this study.

It is well documented that *Listeria* can grow under conditions found in a variety of refrigerated foods including brine pickled shrimp (24). Harrison et al. (11) examined the survival and growth of *L. monocytogenes* in seafood, specifically shrimp. Shrimp inoculated with *L. monocytogenes* (Scott A) were stored with and without vacuum packaging on ice (approximately 1°C) and in frozen storage. There was no increase in *L. monocytogenes* after 21 days storage on ice for either packaging variable. Populations decreased slightly after three months of frozen storage. Thus, growth of *L. monocytogenes* may occur in shrimp under normal refrigerated storage (>/= 40°F) but is limited by storage on ice, with some decrease observed during frozen storage.

Vibrio cholera

There are two groups of serotypes in the species *Vibrio cholera*. They are commonly referred to as the O1 and the non-O1. The O1 group contains two biotypes, Classical and El Tor. This group, which agglutinates the O Group 1 antiserum, is responsible for the world epidemics of cholera. The non-O1 group is common in marine environments and is referred to as NAG, nonagglutinable to the O Group 1 antiserum. It tends to be endemic rather than epidemic.

The disease produced by the pandemic O1 cholera causes a devastating loss of body fluids from diarrhea and large numbers of deaths where medical treatment is inadequate or not available. Interestingly, the ancient Chinese used tea as an antidote. Black tea contains catechins and theaflavins which have been shown to be bactericidal (27). Recent research from Japan showed tea extract inhibited the hemolysin activity of *V. cholera* O1 El Tor, leading the authors to suggest tea as a possible preventive and therapeutic agent against cholera in developing countries.

The non-O1 endemic cases are more similar to "traveler's diarrhea" and may be associated with the consumption of improperly handled seafood, polluted water or general lack of sanitation in undeveloped countries.

Current concerns about cholera in the U.S. were intensified with the large outbreak in Peru. On February 15, 1991, the FDA issued an import alert for 100% sampling of seafood and water-processed produce from Peru for *V. cholera*. By August of 1991, the spread of cholera had been reported from Peru, Ecuador, Colombia, Chile, Mexico, Brazil and the United States (Table 1).

Table 1. Cholera cases reported to Pan American Health Organization – western hemisphere, as of August 7, 1991.

COUNTRY	NO. CASES [a]	NO. HOSPITALIZED	NO. DEATHS	DATE OF REPORT
Peru	238,261	92,022	2,387	Aug. 1
Ecuador	31,881	24,361	505	July 13
Colombia	4,279	3,166	76	July 30
Mexico	257	69	2	July 27
Chile	41	NR [b]	2	July 22
Brazil	31	19	0	July 27
United States	14	7	0	July 30
Guatemala	3	NR	0	July 24
Canada	1 [c]	NR	0	July 19
Total	274,768	119,644	2,972	

(a) Probable and confirmed cases.
(b) Not reported.
(c) Associated with travel to non-Western Hemisphere countries with cholera.

In August the increasing number of cases in Mexico intensified the FDA's sampling to include water and ice, vegetables, fruits and seafood imports from Mexico for *V. cholera*.

Four of the confirmed U.S. cases occurred in New York and were traced to crab purchased in Ecuador (5). All individuals had diarrhea and showed high blood titers for antibodies to vibrio antigens. Although only one individual had visited Ecuador, the remaining three had consumed a salad containing crab meat from Guayaquil, Ecuador. Crabs purchased by this person at a local pier, were boiled, shelled and then the meat and claws were stored in a plastic bag in a freezer.

The crab meat was returned in the traveler's suitcase and placed in his home freezer (still frosted). The next day, it was thawed in a double boiler; then two hours later, without further cooking it was served in a cold crab salad and as cold crab in

the shell. Over a six hour period, three other individuals consumed the crab and subsequently became ill. Crab from Ecuador was the most probable source of the infection since *V. cholera* O1 type El Tor can survive in crabs boiled up to eight minutes. In this case, the vibrios may have survived the boiling, contaminated the meat during the shelling and then multiplied during storage at ambient temperature.

An isolation of O1 cholera from Mobile Bay prompted its closure to oyster harvesting in July 1991. Some speculate the organism may have gained access to the bay water from a passing foreign freighter. Researchers in Japan were unable to detect the toxic gene in 225 isolates from natural waters, but found it in 26.6% of isolates from imported seafoods (15). Their results suggested that toxin-positive *V.cholera* O1 had been imported into Japan through seafoods and/or travelers. Aquatic birds are known carriers of *Vibrio cholera* and incidence levels of 17% have been reported in aquatic birds from non-coastal areas in Utah and Colorado (19).

Pond-raised shrimp may again present a unique public health question with respect to *V. cholera*. Researchers in India (17) surveyed 131 samples including five different species of shrimp cultured in paddy fields for vibrios. *V. cholera* isolates were recovered from 81.7% of the samples but none agglutinated the O Group antisera. The authors reported it would be virtually impossible to eliminate vibrios from these shrimp since *V. cholera* and *V. parahaemolyticus* are autochthonous to brackish waters.

Processing plant sanitation, proper cooking, freezing, avoidance of cross contamination and proper handling should eliminate any risk from *V. cholera*. Cooked, ready-to-eat shrimp products should be free of *V. cholera*, however, preparation of ready to eat crab products, may require more extensive cooking to remove the risk of *V. cholera*.

DISCUSSION

The Seafood Working Group of the National Advisory Committee on Microbiological Criteria for Foods recommended four microbiological criteria for the verification of Hazard Analysis Critical Control Point (HACCP) programs in cooked, ready-to-eat shrimp.[4] These criteria are recommended on the following basis: (i) shrimp is in international trade; (ii) the history of the product is unknown; (iii) all criteria were associated with safety issues; (iv) reasonable and reliable methodologies are available for testing; (v) the criteria can be applied at any point in the

distribution system; and (vi) cooked, ready-to-eat shrimp can be a potential public health problem when abused in the production-distribution-retail-consumer chain.

The criteria focus on two important factors that create the conditions for pathogens to be present: underprocessing and post-processing contamination. The criteria are presented in both 2-class and 3-class plans. For the 2-class plan "n" is the number of sample units tested and "c" the maximum allowable number of defective units. For the 3-class plan "n" is the number of sample units, "c" is the number of marginally acceptable units, "m" the level of bacteria at which a sample is considered marginal, and a level at "M" causes rejection. For *Salmonella* and *Listeria*, the proposed standards use 2-class plans; none of the samples can be positive for the lot to be accepted.

Thermal tolerant coliforms are those that grow at higher incubation temperatures (43°C) and are not known to be psychrotrophic (do not multiply in a refrigerated product). They are considered to be good indicators of process integrity and temperature abuse in storage. It was not the intent of the Committee to have thermal tolerant coliforms be used as a single criteria for the rejection of a product. Whereas the other three criteria are proposed standards, the thermal tolerant coliforms are proposed as guidelines (Table 2).

Table 2. Criteria for verification of HACCP in cooked, ready-to-eat shrimp.

ORGANISM	n	c	m	M
Salmonella	30	0	0	0
L. monocytogenes	5	0	0	0
S. aureus	5	2	50/g	500/g
Thermal tolerant coliforms	5	2	100/g	1000/g

Source: The Seafood Working Group of the National Advisory Committee on Microbiological Criteria for Foods.

As an example, a cooked ready-to-eat shrimp product is evaluated by the preceding criteria for *S. aureus*. Of the five samples, two could have levels between 50 and 500 per gram and the lot would be accepted. The lot would be rejected if more than two samples were between 50 and 500 per gram, or any single sample

exceeded 500 per gram. This approach to sampling was recommended by the International Commission on the Microbiological Specifications for Foods.

Product from processing operations operating under good manufacturing practices coupled with a good HACCP program should have little difficulty meeting these criteria at any point in the distribution chain.

CONCLUSION AND REFERENCES

Demand for shrimp and other seafoods remains high and is expected to increase. Thus, world shrimp production will continue to expand and the international trade of shrimp continue to grow. However, as stated in the introduction, the FDA has identified imported seafood as a primary concern and recent television series and consumer group reports clearly indicate a concern about the safety of shrimp and other seafood (3).

Most of the references used in this presentation are very recent (1990-1991) indicating a high level of interest in this very valuable and palatable international trading commodity. Continued efforts by researchers, harvesters, cultivators, processors, and regulators will ensure a consistent supply of high quality and wholesome shrimp to the world's consumers.

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SCOMBROID POISONING FROM SEAFOOD

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Seafood products that are initially wholesome when they are brought from the ocean can deteriorate if they are not handled properly. Spoilage due to bacterial contamination and poor handling is often apparent to processor or consumer, resulting in rejection and no risk to health. Some forms of decomposition, however, result in changes that are not readily detected especially by consumers, and can lead to adverse health effects. Scombroid poisoning, which results from the ingestion of fish which have decomposed under conditions that permit the formation of toxic levels of histamine and other compounds such as cadaverine, represents such a change and is a worldwide problem that can arise from scombroid fish such as tuna and from non-scombroid fish such as bluefish.

It has been estimated that scombroid poisoning on a worldwide basis accounts for the greatest morbidity of any type of fish poisoning. Documentation of episodes of poisoning have been sporadic and disorganized due to a lack of definition for the poisoning and insufficient knowledge about it in the medical community. Its symptoms can be confused with those of other types of seafood toxins. The poisoning affects people of all economic levels and ages, and is encountered in every eating environment; at home, in restaurants, schools, hospital feeding programs and work cafeterias. Incidents of poisonings in the U.S. are cataloged by CDC using both clinical and laboratory findings. The collected data indicate that most poisonings occur in restaurants and no seasonal effect occurs. Changes in the number of reported poisonings appear to be due to shifts in the types of imported seafood. In 1980, an increase in incidents were due to imported mahimahi. Detentions of this product rose from 250,000 lb in 1979 to 3 million lb during the following 3 years. Over 100 cases of poisonings were reported. A review of poisonings has been published by Taylor (1).

Since scombroid poisoning is a chemical intoxication, the incubation period is short, usually ranging from an immediate reaction to several hours after ingestion. The duration of the illness is usually a few hours but in some cases, symptoms lasting several days have been reported (2). A range of symptoms can occur during incidents of

histamine poisoning. These vary from rashes and localized inflammation to gastrointestinal effects including nausea, vomiting and diarrhea. A burning sensation of certain body tissues such as the lips has also been reported. Cardiac involvement is also experienced, ranging from headaches to hypotension. Because of the range of effects reported, the potential role of other decomposition metabolites in the mechanism of poisonings has received much attention but without conclusive results.

Histamine and other suspected agents.

Historically, scombroid poisoning has been associated with the presence of high levels of histamine. The properties of this compound regarding solubility in water, stability to heat, amine functionality and reaction to the guinea pig ileum bioassay are all consistent with the toxic compound. Although the amine when given in pure dosage form is normally detoxified in the intestinal tract, it becomes physiologically active when ingested with food. In research at FDA (HENRY, S., T. Sobotka, W. Staruszkiewicz, V. Olivito and T. Farber. 1980. Investigations on scombroid toxicity in the beagle dog. Meeting of Society of Toxicology, Washington, D.C.) it was shown that addition of histamine to extracts of good tuna caused severe emetic and cardiovascular effects in beagles.

Questions have been raised about the possibility of other toxic agents and potentiators of toxicity. Kawabata (3) reported finding a compound he called "saurine" that was more active than histamine. His finding could not be confirmed in other laboratories and it has been suggested that "saurine" was actually a salt of histamine (4). Bjeldanes (5) reported that the addition of cadaverine to toxic extracts increased the mortality of guinea pigs but putrescine did not have a significant effect. This finding was in contrast to that of Parrot (6) who found putrescine to be a potentiator of the histamine reaction when using mice as a test animal. Terada (7) studied extracts of decomposed mackerel, crab, octopus and sea bream for synergic actions of formed compounds using the guinea pig ileum test. His data suggested that cadaverine and agmatine were potentiators of the histamine reaction. The addition of these compounds to histamine resulted in 25 mg% levels exhibiting toxic reactions. Putrescine was inactive.

Other compounds such as the vasoactive amines tyramine, tryptamine, dopamine, and serotonin have not been demonstrated to be of significance in scombroid poisoning. The possibility that bacterial endotoxins might act in conjunction with histamine and cause a hypersensitivity has not been proven. Geiger (8) suggested that the use of alcohol or specially seasoned foods prepared from spoiled fish might

alter intestinal conditions and facilitate the absorption of histamine. Ferencik (9) postulated three types of toxins; histamine, other active amines, and N substituted compounds with a cholinotropic effect.

The common requirement for a toxic reaction is that the fish has decomposed with the release of histamine and other compounds. While the significance of each combination of metabolites is of interest, there is no evidence for problems associated with fresh acceptable quality seafood.

Decomposition in seafood.

The conditions which lead to the formation of histamine and its suspected synergists form a subset of the many forms of decomposition which occur in seafood. The principal causes are high temperature conditions in the presence of bacteria which have the capability to decarboxylate histidine found in the muscle of the spoiling fish. A characteristic of such spoilage conditions is that obvious odors of decomposition are not formed rapidly and a consumer has no chance of identifying a problem before eating the fish. High levels of the amines can also form at lower temperatures but usually with the formation of typical odors of decomposition. If spoilage begins at a high temperature and the fish are cooled insufficiently or undergo later temperature abuse in a plant, at an airport etc., histamine can again increase significantly.

Bacteria are always present in the gills, the intestinal contents, and on the slime of the fish. The initial flora is quite heterogenous, and consists of many different types. It will depend upon the species of fish, the geographical location, the season of the year, and the further impacts of organisms in the waters of capture and on the fishing vessel. The flesh of living fish which is free of bacteria, rapidly become susceptible to invasion along a number of routes if handling conditions permit the growth of bacteria. The mixture of bacteria are in competition and their composition changes with holding conditions. Psychrotrophs which grow at lower temperatures will predominate under refrigeration conditions while mesophiles will be more important at higher temperatures. When a seafood is subjected to combinations of temperatures, a different set of end products and odors will be produced. Other factors such as salinity, pH, degree of exposure to oxygen and various combinations, will favor the growth of a segment of the initial population that is best adapted to the particular blend of conditions. One is faced with many varieties of spoilage, as well as products of spoilage, that may have different odors, or even no offensive odor at all. The danger from poisoning varies as well in an uncertain way. Waters that may be as warm as 30°C with the temperature of some fish perhaps 8°C

or so degrees higher, is about right for the mesophilic bacteria to grow at a high rate.

Levels of Histamine Associated with Poisoning.

Too often, only related samples of similar nature from a lot of fish which has caused scombroid poisoning have been available for analysis. In such samples histamine levels in excess of 300 mg% have been found. Lower levels are frequently found as well. Wurziger (10) reported that mackerel containing 3 to 30 mg% resulted in moderate illnesses and in a second occurrence levels were 10-20 mg%. He concluded that fish containing more than 8 mg% should not be considered safe to eat. In a review by Ienestia (11), it was concluded that the ingestion of 8 to 40 mg histamine could cause adverse reactions, moderately severe reactions from 70 to 100 mg, and severe reactions beyond that level. Simidu (12) reported that 60 mg% represented a toxic level based on his investigations of several poisonings. Other reported toxic levels have been 30 mg% (tuna), 50 mg% (herring), and 60 mg% (mackerel). It appears that high levels of histamine can frequently be encountered in decomposed fish and cause very severe reactions, but in general, values of 20 mg% or more should be considered to indicate a hazardous condition.

Formation of Histamine in Fish.

The formation of histamine in tuna and in mahimahi as a function of time and temperature has been studied in two research projects at the University of Hawaii (NOAA Contract 03-6-208-35369 and FDA Contract 223-80-2295 XVIII). The results from these two studies illustrate not only the production of histamine but also its anatomical distribution and the formation and distribution of other compounds such as cadaverine as well. In a study on tuna, the fresh fish contained essentially no free histamine; a maximum of 0.1 mg% was present in any fish section and was frequently lower (13). The formation of histamine during spoilage was studied over a temperature range of 60 to 120^{0} F for periods of time representing those experienced in commercial practice. Rapid production of histamine was found at temperatures of 70^{0} F and higher with an optimum temperature of 100^{0} F. Each fish was divided into five transverse sections for chemical analyses. The characteristic pattern found in the study was a maximum concentration of histamine in the anterior end with decreasing amounts proceeding towards the posterior end. The belly flaps were exceptions to this gradient and usually had histamine levels that were as high as the anterior end. For the first six hours of spoilage, histamine formation was minimal but was 100 times higher by 12 hours of decomposition. During this period of decomposition, the histamine levels in the remaining

sections of fish averaged 5.1 mg%. After 12 hours, histamine increased significantly in all sections of the decomposing tuna. Since this research was conducted, the analyses of many commercial samples of decomposed fish have shown that, on occasion, the maximum concentration of histamine appears in the posterior end of a fillet. Furthermore, the gradient across the loin differs between small and large fish and varies with the time of spoilage. When spoilage occurs at lower temperatures, thus at a slower pace, deterioration is evident throughout the loins. If the fish are damaged (broken flesh, intrusions into the muscle, etc.) the spoilage pattern is also disrupted and fish tend to decompose much more rapidly. A further practical concern in commercial practice is the difficulty of removing body heat from large tuna after capture. The temperature of the tuna can be 8^0 F above ambient and if many fish are taken at one time it is necessary to place them in prechilled brine water which is circulated to drop the temperature below a point where spoilage may occur. The effect of these variables on the canned product at the consumer's level is illustrated by the histamine data shown in Table 1 for three different samples of canned tuna. Some cans contain toxic levels of histamine while others have low levels of the amine.

Table 1. Histamine (mg%) in Individual Cans of Tuna.

CAN #	SAMPLE I	SAMPLE II	SAMPLE III
1	13.8	0.6	1.7
2	15.1	1.0	42.9
3	15.5	96.0	3.0
4	12.0	3.0	23.0
5	7.0	1.0	3.0
6	20.3	2.0	11.0

Research on mahimahi resulted in similar findings. Formation of histamine was a function of the spoilage temperature and the compound was distributed in a gradient across the fillets. In research being concluded at FDA, the formation and distribution of cadaverine and putrescine with decomposition was also determined and correlated with the presence of histamine and odors of decomposition. Putrescine was not generally found at high levels except in an advanced decomposed state. Cadaverine was always found in decomposed fish, even when spoilage temperatures were below 70^0 F. In the study on mahimahi,

decomposition was studied from iced conditions to 90° F. The results show that cadaverine forms more rapidly than histamine, over a wider temperature range, and is increased over a wider area of the loins than is histamine. It was found that many of the spoilage bacteria were poor histidine decarboxlyate formers but frequently produced large amounts of lysine decarboxylate which led to the formation of cadaverine.

Variations in temperature during decomposition produce divergent results. If fish are spoiled after freezing, high levels of histamine are not commonly found (Table 2) and greater reliance must be placed on the diamines for detecting decomposition. If spoilage begins at a high temperature (> 70° F) and the fish are cooled but not frozen, then subjected to further temperature abuse, histamine is likely to continue to form but at an unpredictable rate. The critical period is immediately after capture; fish must be chilled as quickly as possible to avoid the formation of histamine.

Table 2. Effect of Decomposition Temperature on Formation of Amines.

SPOILAGE TEMP, °F	TIME OF SPOILAGE	HISTAMINE (mg%)	CADAVERINE (ppm)	PUTRESCINE (ppm)
32	14 Days	6.1	24.0	11.9
50	5 Days	5.9	241.8	28.3
70	40 Hours	320.5	130.4	22.1
90	15 Hours	108.5	223.1	41.1

Analytical Methods for the Detection of Scombrotoxic Fish.

Because scombrotoxic fish are produced as a result of decomposition, methods of detection can focus on the physiological activity of the fish, the direct determination of histamine, or the detection of decomposition regardless of the possibility of a toxic threat. The detection of decomposition can be subdivided into sensory techniques, physical changes, and the chemical determination of decomposition metabolites, which includes the detection of histamine. A determination of the histamine-like activity of fish as a measure of its safety is unwieldy, lacking in specificity and not amenable to the commercial environment. In addition, such a test would require that a product already be in an unsafe condition in order for a test to function effectively. Preferably, a test should be sufficiently quantitative to detect problems prior to a hazardous condition and be implemented by any well trained analyst on production samples.

Sensory analyses have been an accepted part of both commercial and regulatory programs for rejecting unacceptable fish. In addition to the usual difficulties with sensory analyses, high temperature spoilage frequently does not produce the typical decomposition odors associated with unacceptable products. Many analysts are trained on spoilage packs prepared under iced conditions which produce a different set of odors. Training analysts on packs spoiled at temperatures above 70° F can improve their performance. However, a significant number of scombrotoxic fish are still passed undetected. Physical changes such as honeycombed tissue are useful but do not appear until the fish are cooked which limits such an approach.

Chemical testing overcomes the problems associated with the foregoing approaches. Methods for the determination of histamine fall into five categories: the AOAC fluorometric method which is used for regulatory analyses in the U.S.; thin layer chromatography; liquid chromatography; flow injection procedures; and enzymatic procedures using an oxygen electrode as a detection system. Each of these procedures have advantages and limitations for various applications.

The AOAC fluorometric method (14) has been used for 15 years at FDA and is the basis for the establishment of defect action levels for histamine in fish. The only modification to the original procedure is the use of 75% methanol for extractions in place of 100% methanol. While this change does not materially affect histamine assays in canned tuna, it provides more consistent analytical results on unprocessed fish. More importantly, it improves the recovery of the diamines, especially putrescine, from unprocessed fish. The method requires the sample to be blended with 75% methanol for two minutes, and after heating and taking to volume, an aliquot of the extract is passed through a short column of an anion exchange resin to remove amino acids. The effluent is derivatized with orthophthalaldehyde (OPA) for 4 minutes and the fluorescence measured after adjusting the pH to < 2. Neither sensitivity or specificity are limitations. While the method can be automated, it is usually used in a batch format.

Several procedures for thin-layer chromatography have been developed (15, 16). Although these procedures are relatively inexpensive and multiple samples can be screened on a single plate, these methods are generally limited by their sensitivity. They are usually used as screening procedures.

Liquid chromatography offers the opportunity to assay all of the amines of interest in a single run: histamine, cadaverine, and putrescine and be automated (17). Two primary approaches are precolumn derivatization with dansyl chloride and postcolumn reaction with OPA.

Despite the potential advantages of HPLC, this laboratory has collaborated on many proposed procedures with only marginal success. In order to use precolumn dansylation, the fish extract requires removal of amino acids to permit routine quantitation without interfering peaks. Liquid/liquid extractions are, at best, semiquantitative since histamine is very water soluble. In addition to limitations in the chemistry, liquid chromatographic pumps are a frequent source of variations in assays, particularly if buffers are part of a mobile phase as is the case for separation systems using postcolumn OPA reactions. With an improved procedure, HPLC may yet prove useful in confirming the presence of scombrotoxic fish.

Flow injection analysis for histamine offers both advantages and limitations of several of the above systems (18). An extract of fish is prepared which is not treated prior to application of flow analysis. OPA derivatives are formed by constantly pumping solutions of the appropriate reagents into a moving stream containing the fish extract. Thus all amines and amino acids can react to form fluorescent derivatives. By careful selection of reagent concentrations and accurate control of flow rates, fluorescence of the histamine derivative is maximized relative to other reactive amines and amino acids. The disadvantage is the need to accurately control flows of 4 pumps in order to maintain specificity for the histamine derivative.

A novel approach which does not require any pumps or chromatographs is the procedure of Ohashi (OHASHI, M. 1993. Personnel communication. Ochanomizu University, Tokyo, Japan.). In his procedure, an aliquot of a fish extract is buffered and diamine oxidase added. Using an oxygen electrode, the decrease in oxygen content of the solution due to its reaction with histamine in the presence of the enzyme is used to quantify the amine. The procedure is reported to have sufficient sensitivity and is very rapid. Evaluations are underway in several laboratories to assess the practical utility of the enzyme/oxygen electrode system.

A GLC procedure for the determination of cadaverine and putrescine was published in 1981 (19) and has found extensive applications in the analyses of seafood. Modifications have been made to the GLC method to reduce the number of steps and shorten the time it takes to analyze a sample. The original method involved four steps: extraction of the sample, making a fluorinated derivative, purification of the reaction mixture by column chromatography, and detection of the diamines by GLC. Modifications have been made in the extraction procedure (using 75% methanol in water instead of 100% methanol), by elimination of the evaporation step after the PFP reaction, and by replacing the column chromatography with solid phase extraction (SPE).

The same extract (75% methanol in water) has been used to determine histamine in seafood using the AOAC fluorometric method. The revised method is available as a Laboratory Information Bulletin from FDA.

All of the current chemical procedures require that fish be extracted prior to analysis. This time consuming step can be accommodated in a laboratory where confirmation of a problem is needed. However, especially for shipments of unfrozen fish, a rapid on-site test is still needed.

Applications of Chemical Data.

Decisions based on chemical data determine whether a shipment of product is acceptable or rejected as decomposed, and possibly scombrotoxic. At present, a level of histamine at or above 50 mg% is considered evidence of a hazardous product. It is preferable to reject decomposed fish before they are permitted to reach a hazardous condition. Actions to reject fish below histamine levels of 50 mg% are based on evidence of decomposition. A histamine level of 5 mg% (50 ppm) is considered evidence of decomposition for regulatory actions. For quality control purposes, a histamine level of 20 ppm or more indicates some spoilage is probably present and corrective actions should be taken. This value is based on the research findings and on practical experience and on product surveys. As indicated above, research has shown that freshly harvested fish do not contain significant amounts of histamine. A survey of commercial canned tuna in 1981, found that the average level of histamine in acceptable quality product was 0.6 mg% (6 ppm). Recent examinations of frozen, acceptable quality mahimahi, albacore, yellowfin, skipjack and bonito average 0.2 mg% (2 ppm). In addition, research has demonstrated that a fish which contains a level of 5 mg% in one part of a fish may also contain 50 mg% in a different part of the loin.

Cadaverine has been shown to be formed together with histamine but at a faster rate which can be used to provide a rejection point prior to histamine reaching scombrotoxic levels. This compound also is a more general chemical indicator of decomposition. Levels of cadaverine in acceptable quality fish are in the 0 ppm to approximately 0.2 ppm range. For tuna, rejection of product for sensory evidence of decomposition correlates with cadaverine levels of approximately 0.6 to 1.0 ppm. Samples which contain in excess of these levels contain decomposed tissue. Although the procedure is being applied to other fishery products, the data is not sufficient at this time to estimate reject levels.

Prevention of Poisoning.

The most important guideline for avoiding the production of scombrotoxic fish is to rapidly cool the fish upon capture. Maintaining a sanitary environment aboard the fishing vessel and in processing plants and avoiding damage to the fish muscle assist in maintaining a low bacterial population and decreases chances for decomposition to occur. It has been shown (Table 3) in FDA sponsored research that rapid cooling to a frozen condition can have a dramatic effect on histamine levels during later spoilage.

Table 3. Effect of Frozen Storage on Histamine Formation, mg%.

FROZEN STORAGE (weeks)	SPOILAGE AT 90° F		SPOILAGE AT 70° F	
	12 Hours	24 Hours	24 Hours	48 Hours
0	2.0	292.0	1.0	154.0
24	0.1	85.0	0.1	0.2
40	0.1	1.0	0.1	0.2

Unfortunately, a low product temperature when fish are received is no assurance of safety since temperature abuse may have occurred prior to icing and freezing for shipment. Storage of fish such as mahimahi for 5-7 days at 50° F can result in the formation of scombrotoxic levels of histamine. If fish are subjected to 6-12 hours of holding at tropical temperatures of 85-90° F and slowly chilled to 50° F, it can be expected that levels of histamine will exceed 50 mg% within 24 hours and may continue to form slowly even under poorly iced conditions or when only a few gel packs are used for cooling. Careful quality control measures are required for the examination of seafood including evaluation of odor, internal condition, muscle condition and other physical attributes. Good quality fish should contain less than 20 ppm histamine and less than 0.6 ppm cadaverine when determined with appropriate methods.

SUMMARY

The potential for scombroid poisoning upon the ingestion of decomposed fish such as tuna and mahimahi has been known for decades. While commercial canned tuna seldom causes illnesses, other

fishery products are frequently responsible for reactions in consumers. The toxic reactions appear to be due to the formation of histamine during decomposition as well as the production of potential synergists such as cadaverine. Because this type of decomposition does not result in large amounts of spoilage odors and the amines are distributed in a heterogenous manner within and between fish in a lot, effective quality control measures require adequate sampling and analysis by highly qualified organoleptic analysts supported by chemical tests.

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USE OF SULFITES AND PHOSPHATES WITH SHRIMP

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The use of sulfites and phosphates to enhance and prolong the shelflife of seafoods is best exemplified by commercial practices with penaeid shrimp. These food processing aids were first introduced to the shrimp industry during the 1950's and 1960's, respectively. The initial methods for application were borrowed from the potato and meat industries. Today these compounds remain as vital ingredients in various segments of the shrimp industry, but current regulatory and buyer concerns for various product attributes are calling for further scrutiny and controlled use.

SULFITES

The sulfite agents most commonly used to treated shrimp are sodium bisulfite ($NaHSO_3$) and sodium metabisulfite ($Na_2S_2O_5$). These compounds are sold dry in 50 pound (22.7 kg) bags or plastic tubs distributed through regional fishermen supply firms. These powders are blended with tap water or clean seawater to prepare dip concentrations of approximately 1.25% (weight/weight). Fresh harvested whole or headless shell-on shrimp from trawls, traps or culture ponds are first washed then placed in the sulfite dips for approximately 1 minute. The dip concentration and soak time (1.25% for 1 min.) corresponds to an average residual sulfite level (measured as liberated SO_2) of 100ppm (100 parts SO_2 per one million parts of edible shrimp). This residual level has been demonstrated to effectively prevent the formation of shrimp "black-spot" or melanosis. Melanosis is the development of black pigments that can adversely discolor the shrimp shell and meat.

Melanosis is a natural enzyme reaction that occurs after the death in many crustaceans. The polyphenoloxidase enzymes associated with the shell rehardening process in live crustaceans can produce black pigments (melanins) about the shell-meat surface after the shrimp die. The rate of onset and amount of melanin formed can vary considerably by shrimp species, stage of shedding, storage temperatures and handling conditions. Exposure to elevated temperatures, oxygen in the atmosphere (air), and sunlight can accelerate the development of melanin. This natural reaction is not caused by bacteria, but it can be associated with handling and storage conditions that promote the growth of spoilage type bacteria. Proper and immediate washing and icing can reduce the development of melanin, but not completely stop it. Likewise, freezing can stop melanosis, but the discoloration process or enzyme activity will begin as the product is thawed. In problematic species, thawed product can develop melanin more so than for the same fresh product stored properly in ice.

Sulfites block the enzyme activity and can bleach some of the developed melanin. In the United States the sulfite compounds are approved as 'GRAS' substances ("generally recognized as safe") based on their previous use and prior sanction by the U.S. Food and Drug Administration (FDA) in 1956. More recent FDA regulations have specified the legal residual

sulfite level allowed on the edible portion of shrimp is 100ppm (SO_2). A 10ppm residual level is the detectable amount that determines the requirements to label treated product. Recommended package labeling on treated product should state "- ingredients: shrimp, sulfites used as a preservative". Product with residuals greater than 100ppm are considered adulterated and should not be sold. The international Codex Alimentirus recommendations for residual limits on shrimp are also 100ppm on raw products and 30ppm on cooked shrimp.

Residual sulfite limitations are necessary to prevent possible adverse health consequences associated with exposure to sulphur dioxide (SO_2). This gas has been the cause of severe respiratory and anaphlax (msp.) reactions to foods with elevated residues. This problem is rare and has been more commonly associated with asthmatic consumers hypersensitive to SO_2 exposure. The more problematic foods have been carbohydrate based such as certain potato products, salads and fruits. There is only minor concern for health consequences due to consumption of sulfited shrimp, a protein based food that can bind the SO_2 residual. Likewise, the legal residual limits provide an additional safety guideline. A rare, but more serious concern can involve fishermen and other users of the sulfite compounds. If the dry powders become wet during storage or if the dip concentrations are improperly made too strong, these situations can release toxic levels of SO_2 gas. If this occurs in enclosed or poorly vented areas the workers could inhale a lethal dose.

The official analytical test for sulfite residual on foods is an involved heat distillation procedure known as the Monier Williams test. A more simplified and rapid field method is the use of sulfite test strips. These latter, unofficial methods cannot be used for regulatory purposes because they are not sensitive or accurate relative to exact residual concentrations, but they do provide a convenient test to check for previous treatments or potential product abuse.

Recently a treatment alternative to sulfites was developed using 4-hexylresorcinol. This unique compound originally sold as 'Everfresh' is currently distributed by Pfizer Inc., through various regional dealers. A similar dip type application is required and the resulting residual is approximately 1.0ppm. Residual concentration and treatment costs are controlled by prepackaged portions sized for typical 50 to 60 lb (22.7 to 27.2 kg) shrimp baskets used on shrimp trawlers. Treatments should be applied immediately after harvest and/or in conjunction with thawing procedures for untreated shrimp to prevent the onset of melanosis. The 4-hexylrecorcinal treatment does not bleach any preformed melanin as sulfites do. Likewise, 4-hexylresorcinol results vary per shrimp species and harvest conditions. Initial trial tests should be conducted to confirm the most effective treatment. Less than 30 seconds soak time is usually successful for certain shrimp species. Most importantly the 4-hexyrecorcinol does not pose hazardous health concerns for consumers.

Future use of any anti-melanosis treatments will continue in the seafood industry. Use of sulfites alone could be challenged, particularly for imported seafoods due to potential consumer health consequences. Further work will focus on developing blends which combine the cosmetic benefits of anti-melanosis compounds with additional ingredients to reduce microbial contaminates and prolong product shelflife.

PHOSPHATES

Use of phosphates with shrimp was initially based on commercial practice with hams, poultry and other meats. The primary benefit was to retain moisture loss that was otherwise lost during subsequent processing, chilling, freezing, frozen storage, thawing, refrigeration and cooking. The initial compound of choice was a brand of sodium tripolyphosphate ($Na_3P_2O_{10}$). Overtime additional phosphates and phosphate blends were introduced for variable effects and applications (Table 1). The blends combine various phosphates and other approved food ingredients which may influence the pH (acidity) and/or antimicrobial attributes of the mix.

Some of the larger corporate suppliers of these phosphates are listed in Table 1. These substances were all reaffirmed as 'GRAS' ingredients by an FDA review published in the December issue of the 1979 federal register.

The methods for applying phosphates requires product exposure to prepared solutions. The exposure can be by spray, dips, soaks, soaks with or without mechanical tumbling (with or without vacuum), and simply direct packaging with product just prior to freezing. Depending on the product form and method of application, the concentration of the phosphate solutions can vary from 1.0 to 10.0%. A 2.0 to 5.0% concentration is more common, and for shrimp the treatment may contain a small portion (0.25 to 1.0%) sodium chloride to assist with product penetration (muscle protein interaction via surface solubility). Product form can be raw muscle or shell-on shrimp destine for direct freezing or freezing after cooking or breading.

All treated fishery products sold in the United States must be labelled to designate the use of an approved phosphate ingredient. There is no formally approved level for phosphates in previously treated fishery products. A previous, nonapproved FDA proposal designated a residual limit of "0.5% for fishery products...as served" as a maximum level as results from 'good manufacturing practices" (GMP's). It is still unclear what this 0.5% level means. Likewise, analytical methods to distinguish added phosphates from the natural background phosphates in the shrimp have been complicated by the tendency of the phosphate compounds to gradually and continuously change after they are added to the shrimp. This situation has forced recent regulatory concerns to focus analytical limits on the total moisture content allowed in treated products. An example is the recent temporary FDA guideline established for Atlantic sea scallops (Table 2). This simplified regulatory approach does place attention on the principle component of concern - water. Water retention versus addition is in question. Excessive additions could be designated as adulteration for concern as an economic fraud. Analytical measures for moisture relative to adulteration remains complicated for a lack of understanding of the natural and variable moisture content in untreated products. This situation dominated the current regulatory and commercial debates for proper use of phosphates with seafoods. Additional confusion is anticipated for breaded shrimp which is controlled by a previous established federal "standard of identity" which implies no phosphates can be used with these products. This implication and federal standard will be challenged beginning in 1993.

The debate for proper use of phosphates centers on concern for adding "excessive" water to the original product. Any excess could be considered an adulterant that results in economic fraud for the consumers. In contrast, consumer perception studies for the phosphated shrimp indicated a consumer preference for treated products (Applewhite et al, 1993 this proceedings). This implies consumer benefits from moisture additions and retention. Definitions for excessive additions and consumer benefits remain unresolved.

Future use of phosphates with fishery products in the United States will require regulatory clarification for treated shrimp. Decisions will depend on understanding the relationships between phosphate residuals and moisture consequences versus product quality attributes as noted by sensory character, product shelflife, nutrient content and consumer acceptance.

ACKNOWLEDGEMENT

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Table 1. Some GRAS phosphates agents used to influence moisture in muscle foods

Monosodium phosphate	Sodium acid pyrophosphate
Sodium hexametaphosphate	Dipotassium phosphate
Sodium metaphospate, insoluble	Dipotassium phosphate
Sodium tripolyosphate	Potassium tripolyphosphate
Sodium pyrophospate	Potassium tripolyphosphate

Major suppliers: BK Ladenburg, Budenheim, Monsanto and Rhône-Poulenc.

Table 2. FDA's interim or temporary policy to regulate use of phosphates used to maintain moisture content in scallops. Policy issued in August 1992.

Moisture Content in scallop meat	Product Designation
less than 80%	Considered a 'scallop' and can be labelled as such
greater than 80% up to 84%	Considered a 'scallop product and must be labelled as such'*
greater than 84%	Illegal, adulterated scallop subject to seizure

* The 84% moisture content was considered equivalent to "25% water added scallop product". This is the recommended label statement.

Clostridium botulinum Type E Outgrowth and Toxin Production in Vacuum-Skin Packaged Shrimp

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Due to the increasing consumer demand for high quality, convenient seafood products, more interest has been directed towards new packaging methods that will extend the shelf-life of seafood products, as well as maintain quality under refrigerated temperatures (4). Vacuum packaging may be the means of meeting this consumer demand. However, the use of this packaging method may be limited in commercial application in fresh seafood storage because of the possible increased risk from the growth and toxin production by *Clostridium botulinum* (10). This concern is due to the fact that: (a) *C. botulinum* type E spores which are widely distributed in marine environments, are capable of growth at temperatures as low as 3.3°C to 4°C; (b) the reduced oxygen environment in vacuum-packaged products may eliminate bacterial competition allowing *C. botulinum* growth at slightly abused storage temperatures; and (c) the possibility that noticeable spoilage of seafood products may not precede toxin production (10).

Studies indicate that maintaining a temperature below 3°C during distribution and storage may prevent *C. botulinum* growth and toxin production in vacuum-packaged seafood products, but this can not always be guaranteed with commercial practices that exist presently (12). The objective of the present study was to investigate the potential for *Clostridium botulinum* outgrowth and toxin production in vacuum-skin packaged shrimp at two temperatures: 4°C and 10°C. From the data obtained, a decision can be made on whether the risk of toxin production results from the packaging type used or the storage temperature used.

MATERIALS AND METHODS

Spore preparation

Four strains of *C. botulinum* type E were maintained at room temperature in reinforced clostridial broth (Oxoid Ltd., Basingstoke, Hants., England). Prior to inoculation of the shrimp, spores of each strain were prepared and enumerated by the method of Lindroth and Genigeorgis (14).

Experimental design

White and brown shrimp (*Penaeus* spp.) were harvested from the Georgia coast near the University of Georgia Marine Experiment Station in Brunswick, GA. Shrimp were deheaded, held at 0-3°C, and transported to the laboratory for use the following day. Shrimp were inoculated with a mixed pool of four strains of *C. botulinum* type E spores (Beluga, Minnesota, G21-5, 070) obtained from the Food and Drug Administration, Washington, D.C. Shrimp were inoculated by dipping them into a Butterfield's phosphate buffer solution containing $\log_{10}$ 6-7 *C. botulinum* spores/ml (17). After a 30 minute exposure, the shrimp were placed on a sterile screen and the excess liquid was allowed to drain over a 30 minute period. The dipping procedure allowed for a target spore load on the shrimp of $\log_{10}$ 3-4 spores/g. Controls samples were dipped the same way except the solution did not contain *C. botulinum*. Oxygen barrier film (Trigon Intact skin packaging film), designed for the RM331 Mark III Mini Intact Machine (Trigon National Corp., Redmond, WA) was used to vacuum-skin package the shrimp. Packages were prepared film-to-film with a sealing temperature of 120°C for 20 seconds. Packaged shrimp were stored at either 4°C for 21 days or 10°C for 15 days. For the 4°C storage, samples were analyzed at 3 day intervals. Samples held at 10° C were analyzed on 0, 1, 2, 3, 6, 9, 12, and 15 days. For each storage temperature, three replications of both inoculated and control samples were performed.

Most Probable Number procedure for C. botulinum

The five-tube MPN method using TPYGT enrichment broth developed by Lilly et al. (15) was used. Trypsin was added to the broth to inactivate the bacteriocins produced by nontoxigenic organisms and aide in isolating *C. botulinum* type E from mixed cultures (15). Shrimp samples ranging from 10-15 g were homogenized by a stomacher (Stomacher Lab Blender 400, Tekmar Co., model #S10-400) and serial dilutions were made with Butterfield's phosphate buffer (17). Dilutions were transferred to the TPYGT broth tubes, and the tubes were incubated anaerobically at 30°C for 48 hours. Calculations to determine MPN counts were made using tables found in the *Compendium of Methods for the Microbiological Examination of Foods* (16).

Psychrotrophic enumeration

Psychrotrophic populations in shrimp samples were determined on the initial packaging day and at intervals stated previously. The serial dilutions made for the MPN enumeration were plated onto Plate Count Agar (Difco Laboratories, Detroit, Michigan, USA) plates which were incubated aerobically at 4°C for 10 days prior to counting the colony forming units which developed.

Toxin analysis

The procedure to assay botulinum toxin followed the Centers for Disease Control protocol with a slight modification (7). The overnight suspension prepared with homogenized shrimp and equal parts gelatin phosphate buffer was centrifuged at 3000 rpm for 30 min. The resulting supernatant was retained and analyzed as outlined in the CDC protocol using male Swiss ICR mice.

Spoilage endpoint

Stored shrimp was considered spoiled when the psychrotrophic populations exceeded approximately $\log_{10}$5-6 CFU/g and there was obvious presence of noticeable off-odors. Since the product was potentially contaminated with *C. botulinum* toxin, spoilage detection by organoleptic means other than aroma was not possible for safety reasons.

RESULTS AND DISCUSSION

Shrimp inoculated with *C. botulinum* and held at 4°C showed a 3.5 log increase in psychrotrophic populations and a 4.3 log increase in anaerobic populations (Fig. I and Fig. II). The products appeared spoiled between 6-9 days of storage based on psychrotrophic populations and off-odor presence. No botulinum toxin was detected in any of the packages during the 21 days of storage (Table I). Shrimp inoculated with *C. botulinum* and held at 10°C showed a 3.6 log increase in psychrotrophic populations and a 4.9 log increase in anaerobic populations, but spoilage occurred at a more rapid rate (Fig. III and Fig. IV). Toxin was produced on shrimp inoculated with *C. botulinum* and stored at 10°C by day 6 (Table II). Inoculated samples were unacceptable for consumption between the 3 to 6 day range based on psychrotrophic populations and off-odor characteristics. These findings were supported by several investigators who have found that "generally" spoilage was apparent before *C. botulinum* toxigenesis in vacuum-packaged raw seafood products held below 10°C (3, 9, 11). However, these findings contrast many other investigators who have revealed that toxin production by *C. botulinum* may precede organoleptic spoilage in fish samples that have been vacuum-packaged (2, 8, 9, 10, 11, 13, 14). Based on these contrasting results, it can be concluded that spoilage should not be used as the sole indicator which determines toxigenesis in vacuum-packaged seafood products (2).

Obviously, more attention should be directed towards maintaining appropriate time-temperature storage conditions throughout distribution in evaluating *C. botulinum* toxigenesis. This inoculated pack study indicated that temperature appeared to have an impact on the amount of spoilage and toxin production in vacuum-packaged shrimp. This study confirmed results obtained by several investigators that abusive time-temperature storage conditions of seafood products more significantly affected toxigenesis than the type packaging material used (1, 2, 5, 6).

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Table I: Mouse bioassay of vacuum-skin packaged shrimp samples inoculated with *Clostridium botulinum* type E spores and uninoculated controls stored at 4°C.

DAY	CONTROL	INOCULATED
0	0[a]/3[b]	0[a]/3[b]
3	0 /3	0 /3
6	0 /3	0 /3
9	0 /3	0 /3
12	0 /3	0 /3
15	0 /3	0 /3
18	0 /3	0 /3
21	0 /3	0 /3

[a]: the number of positive toxic shrimp samples.
[b]: the total number of shrimp samples tested for toxin.

Table II: Mouse bioassay of vacuum-skin packaged shrimp samples inoculated with *Clostridium botulinum* type E spores and uninoculated controls stored at 10°C.

DAY	CONTROL	INOCULATED
0	0[a]/3[b]	0[a]/3[b]
1	0 /3	0 /3
2	0 /3	0 /3
3	0 /3	0 /3
6	0 /3	2 /3
9	0 /3	3 /3
14	0 /3	3 /3

[a]: the number of positive toxic shrimp samples.
[b]: the total number of shrimp samples tested for toxin.

NUCLEOTIDE DEGRADATION PRODUCTS AS A QUALITY INDEX OF AQUATIC FOODS PACKAGED IN MODIFIED ATMOSPHERES

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INTRODUCTION

Seafood consumption in the United States has been steadily increasing since the mid-1950s (USDC, 1992). The fact that fresh seafood is susceptible to rapid deterioration created a need for extending the shelf-life. Modified atmosphere (MA) packaging is a recently developed technique which can be used for extending the shelf-life of seafood.

Modified Atmosphere Packaging uses gas mixture to replace the air in the package. Three gases are commonly used in this packaging. They are CO_2 N_2 and O_2 CO_2 inhibits aerobic bacterial, yeast and mold activity in foods. It prolongs the lag phase and generation time of microorganisms. N_2 however, is a chemically inert gas used primarily as an inert filler to retain the shape of a package. O_2 is used along with CO_2 and N_2 in some packaging to retard the growth of anaerobic bacteria. The advantages of MA Packaging include: to extend the shell-life and improve quality (such as appearance, odor and freshness), to allow the product to be transported longer distance for distribution and marketing, and to reduce economic losses as a result of delayed spoilage. However, there are some disadvantages. The MA packaging may provide conditions for growth and toxin production by anaerobic pathogens. It requires special packaging materials and gases. It may cause the collapse of the package if the atmosphere is too rich in CO_2

There are many methods used to assess the fish quality. However, each has its particular advantages and limitation. So far, the sensory evaluation is the most common and best method. It uses the human senses of sight, smell and touch. This approach gives an immediate appraisal of quality. However, there are some difficulties, because it requires the trained and experienced persons to do the job. The method is also not very objective. Several physical methods have been

developed to monitor the physical changes that take place in fish as they age. These methods include the measurement of the refractive index, textural changes, and electrical conductivity. However, these tests have not shown a good correlation with freshness in fish. The Torrymeter is a compact and portable instrument which can be used in any places such as the fish processing plant and market. The principle of operation is the measurement on the alteration of dielectric properties of fish skin during spoilage.

Microbiological methods on other hand, would provide an index of freshness, but not of quality changes. However, there are some objections to this approach. First, not all species of bacteria organisms are proportional to the total bacteria population changes. Second, most bacteriological tests require two to three days to complete. Third, as various new methods of preserving fish are tested, the spoilage would no longer be basically microbiological.

Many chemical methods have been developed to measure the extent of the deterioration of fish in the chilled state. They include Trimethylamine (TMA), Total Volatile Bases (TVB) and Ammonia. However, chemical method can be used for the spoilage index and not the freshness index.

During the seafood spoilage, the first changes result from autolytic reactions controlled by native enzymes in the tissue. These enzymes retain much of their activity after the animal dies. Autolysis is especially important during the first seven to ten days of commercial storage in melting ice. It is obvious that seafood quality depends on a complex set of biochemical changes. These changes can be use as freshness test. Adenosine nucleotide degradation is one of the most important reactions. It correlates well with the flavor change and the loss of freshness in a wide range of species (Jones and Murray 1962, 1964; Spinelli et al. 1064).

The sequence of nucleotide degradation follows a well-defined process. The predominant nucleotide in resting muscle is ATP. This undergoes enzymatic dephosphorylation to form ADP and AMP. Deaminoation of AMP produces IMP. These steps are fast and give a rapid accumulation of IMP. However, inosine and hypoxanthine are accumulated with the increase of storage time. The concentration of these two compounds provide a basis for valid and useful indices of fish quality. The K value has received much attention. It is defined as the ratio of inosine plus hypoxanthine to the total adenosine triphosphate (ATP) and related compounds (ADP, AMP, IMP, HxR, and Hx) (Saito et al. 1959). Many studies has also confirmed the relationship between fish freshness and K-value (Ehira et al. 1970; Ehira and Uchiyama 1974).

There are several methods used in the analysis of nucleotides. Hypoxanthine can be measured by either a colormetric test or by using an enzyme sensor. But both methods generally require a long time for the determination. Two other methods commonly used to measure IMP, inosine and hypoxanthine are the color reaction method and polarography method. However they are too expensive.

Nucleotides are also measured by using capillary electrophoresis and HPLC. Both of them can be done within 15 to 20 minutes. In this study, a reversed-phase HPLC method is used. HPLC has been applied to the determination of nucleotides, nucleosides and bases in tissue extracts only recently. Traditionally, nucleotides have been determined by ion-exchange HPLC. This technique only partially separated the nucleotide or required lengthy analysis time. Results obtained by reversed-phase HPLC showed some advantages over the ion-exchange mode because of its reproducibility, versatility and ease of operation.

The objectives of this project were to determine the shelf-life of shrimp in MA packaging, to use reversed-phase HPLC method to analyze the changes of adenosine nucleotides and the breakdown products during the MA storage, to assess the potential uses as a freshness index for the MA packaged shrimp, to statistically analyze the correlation between the changes of adenine nucleotides, the breakdown products and the changes of chemical and microbiological qualities.

MATERIALS AND METHODS

Sample Preparation

Fresh shrimp were caught off the Georgia coast in Brunswick. After harvest, the shrimp were immediately washed, headed, stored in ice-chest and transported to the department of Food Science.

On arrival at the Laboratory, the shrimp were immediately packaged in oxygen barrier-bags. Eight shrimp were placed into each B-bag. The bag was first vacuum-sealed and then directly injected with one of the six atmospheres where the bag was inflated and sealed.

Six atmospheres including (1) 100% air (served as control), (2) 90% CO_2(balanced with 10% air), (3) 90% CO_2(balanced with N_2), (4) 80% CO_2(balanced with N_2), (5) 70% CO_2(balanced with N_2), (6) 60% CO_2 (balanced with N_2) were used. Packaged shrimp samples were stored in 4°C walk-in cooler, and removed at 3, 7, 11, 15 and 18 days for objective quality assessment.

Quality Analyses

At each sample time, three bags of each six different atmosphere packaged shrimp were used. Plates for psychrotrophic bacteria count were incubated at 7°C for 14 days. Total anaerobic bacteria were incubated in an anaerobic jar at 30°C for 4 days.

Ammonia level and pH value were also measured. An ammonia ion selective electrode was used to determine ammonia level. Nucleotide and the catabolites were analyzed by reversed-phase HPLC. For extraction, 2g of well mixed shrimp homogenized with 50ml cold PCA at high speed for 2min. Sample slurry was then filtrated with No.5 Whatman filter paper. Then the filtrate was adjusted pH to 6.7 with 10N KOH and centrifuged at 3000 RPM for 10 min. The supernatant was filtered through a 0.45μm membrane. The whole extraction procedure was under the cool condition.

In our study, an ISCO HPLC system with a UV-Vis detector was used for all nucleotides analysis at 254nm with 0.05 sensitivity. Separation was achieved on a reversed-phase column at room temperature. The mobile phase consisted of buffer A and Buffer B. Buffer A is Potassium Phosphate with pH 6.0. buffer B is buffer A and 30% of methanol. The flow rate was 1.5ml/min. The chromatographic conduction was started with 2.5 min at 100% buffer A, then 80% of buffer A, 60% of buffer A, and 100% of buffer B hold 5 min. The gradient was then rapidly returned to 100% buffer A and initial conduction for 7 min.

Peaks obtained from shrimp extracts were identified by co-chromatography with standard solutions. The recoveries of each adenosine nucleotides nd catabolite were determined by spiking aliquot of shrimp/PAC homogenate with varying amounts of each these compounds. All the analyses were carried out in duplicate.

RESULTS AND DISCUSSION

The recoveries of nucleotide degradation compounds were in the range from 79.4 to 117.6% with a mean recovery of each compound varying from 82.7 to 110.4%. Standard curve shows that Peak areas increased linearly with increasing concentrations of standard compounds.

The psychrotrophs population on shrimp in the air and five different mixed gases packs were presented in Figure 1. On the day of catch, psychrotrophs population in shrimp had low counts of 2.4 log CFU/g. By day 11, the psychrotrophs counts reached 6.6 log CFU/g, which indicated the onset of spoilage. In contrast to the air packed samples, shrimp in modified atmosphere pack had much lower psychrotrophs growth throughout the entire 18 days of storage. Analysis of variance verified that psychrotrophs of air packed samples had a significant

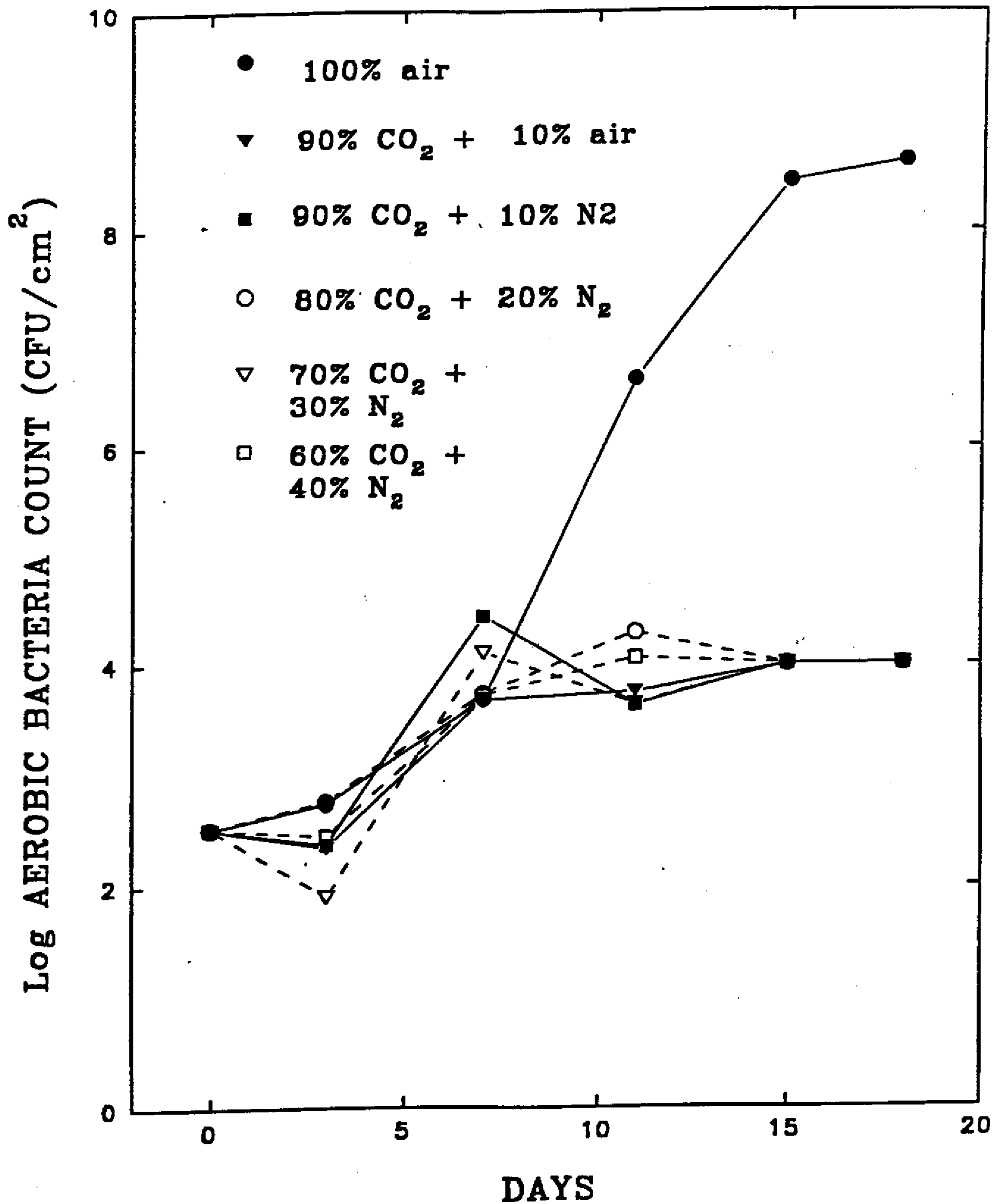

Figure 1. Aerobic bacterial population of shrimp held in different modified atmospheres during refrigerated storage

increase after the 11th day. However, no significant difference among these five different MA packs was found. The results supported that the bacteriostatic effect of CO_2 as reported in brown shrimp (Lannelongue et al. 1982).

The change in population of anaerobic bacteria was similar to that of psychrotrophs (Figure 2), but it was about 0.5 log lower than that of psychrotrophs in air packed samples. Results of analysis of variance showed the same results as the psychrotrophic count. In this study, CO_2 concentration on the bacteriostatic effect was not shown as reported by previous researchers that the bacteriostatic effect increase with the concentration.

The content of ammonia (mg/g) in air pack and MA pack shrimp is shown on Figure 3. Ammonia production is well recognized as a product due to the bacteria breakdown of proteins and peptides in fish muscle during extend periods of storage. In this study, the initial ammonia content in shrimp was 0.12 mg/g. Ammonia content of shrimp held in all packaging conditions increased during storage period. By day 7, ammonia content among the air pack and MA package samples had no significant difference. However, on day 11, a significant difference was found between the air pack and MA pack samples, but no significant difference existed among the MA packed shrimps with the exception of the at 18th day. The change of ammonia content seemed to follow the pattern of bacteria growth.

pH determinations of shrimp packed under air and modified atmosphere are shown in Figure 4. The pH of very fresh shrimp initially at approximately 6.84 and then increased up to the range of 7.97-8.05. The pH in air packed shrimp was lower than shrimps held in MA. The difference became narrow by the 18th day of storage. Analysis of variance showed that pH in air pack samples at day 11 and 15 significantly were lower than that in MA packs. The result was opposite to the previous report in which the pH decreased as the CO_2 was dissolved in the drip or absorbed by fish tissue during the modified atmosphere storage. This pH decrease has been found to be proportional to the CO_2 content in the packaging atmosphere.

In the very fresh shrimp ATP and ADP had 4.0 and 3.1 µmol/, respectively. ATP and ADP fell rapidly to very low levels after 3 days. The AMP decreased from 4.09 µmol/g to the range of 2.0 to 2.49 µmol/g on the 3rd day for all packs. By the 15th day, the concentration of AMP decreased to only 0.09 to 0.24 µmol/g (Figure 5). Analysis of variance showed that at day 7 and 11, air packed samples had a significantly higher AMP level than MA pack (except 10% air).

The rapid breakdown of ATP via ADP and AMP caused the accumulation of IMP in the early stage of storage. As shown in Figure 6, the initial IMP of 4.0 µmol/g increased rapidly to a maximum of 12.3 µmol/g in MA pack with 60% CO_2 and 12.1 µmol/g in 70% CO_2. The AMP increased to a range of 9.6 to 12.3 µmol/g after 3 days, then gradually decreased to a range of 2.90 to 4.41 µmol/g on 18th day. Analysis of variance shown that by the 7th day, the IMP level in the pack of 80% CO_3 20% N_2 was significantly ($p<0.05$) lower than those of others. On the 15th day, the concentration of the IMP in the air packed sample became the lowest among packs and no significant difference ($p<0.05$) existed on 18th day.

The degradation of IMP to HxR and Hx were very slow. The amount of HxR at the 0 day was low and continued to increase with the storage time. The same pattern existed among the packs (Figure 7). At the day 15, HxR in the air packed sample was 4.83 µmol/g and that in MA packed samples were in the range of 4.52 to 6.32. Significant increments of HxR were found in most periods of sampling. Statistic analysis also showed that a significant difference of HxR existed between air and MA packs in day 3,7,11 and 18.

Similar to HxR, the Hx detected at 0 day was low and continued to increase with the storage (Figure 8). By the end of storage, Hx in air was 6.9 umol/g, and that in MA packs were in the range of 3.9 to 4.5 except in 60% CO2. Analysis of variance showed a significant difference between air and MA packs after 11 days except in the 60% CO_2 packs at day 18. This result disagreed with reports of a very erratic pattern of Hx accumulation in the rockfish and silver salmon. In this study, the results suggested that shrimp in air pack accumulated more Hx than HxR. Shrimp in MA packs accumulated more HxR than Hx.

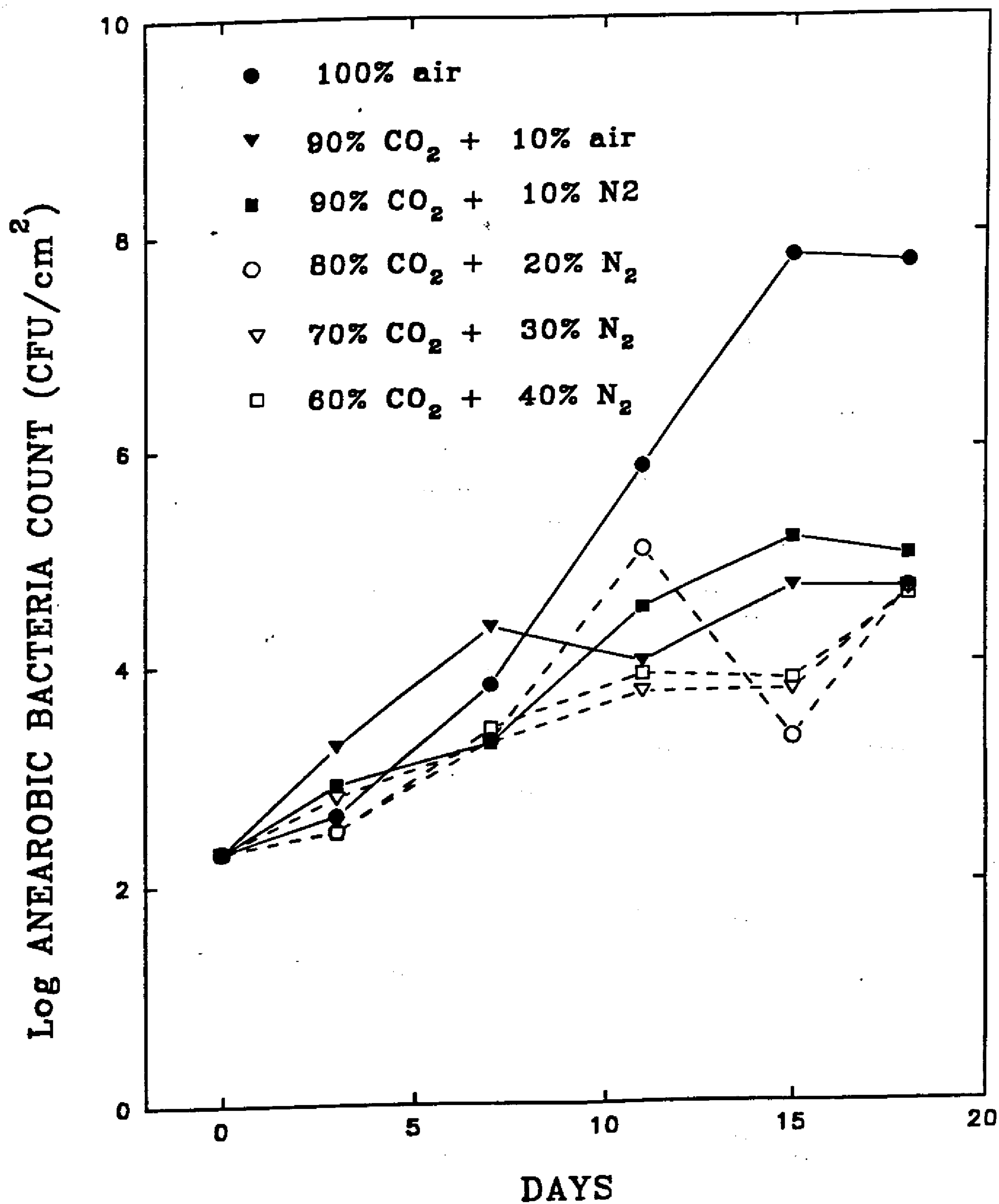

Figure 2. Anaerobic bacterial population of shrimp held in different modified atmospheres during refrigerated storage

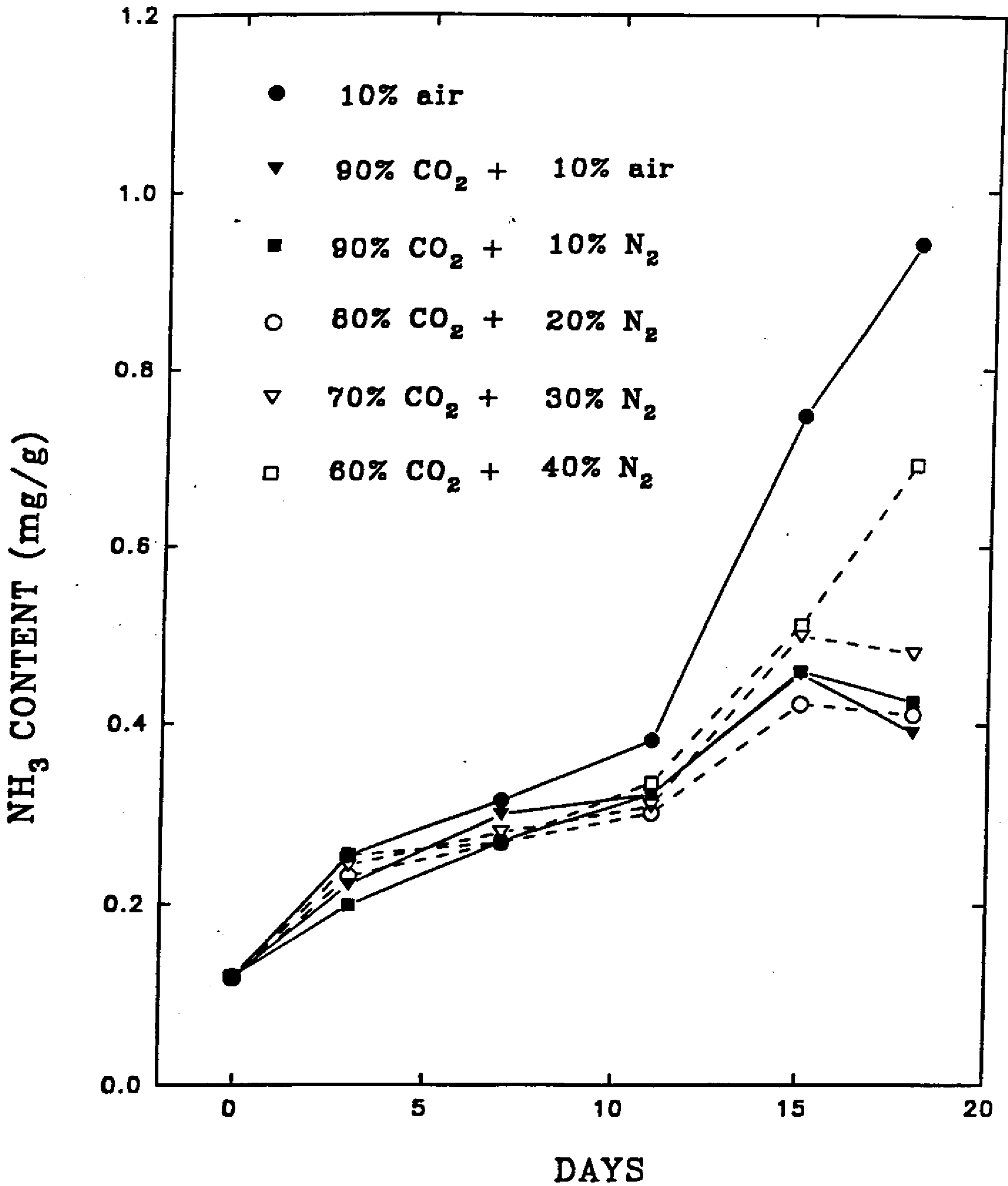

Figure 3. Ammonia content of shrimp held in different modified atmospheres during refrigerated storage

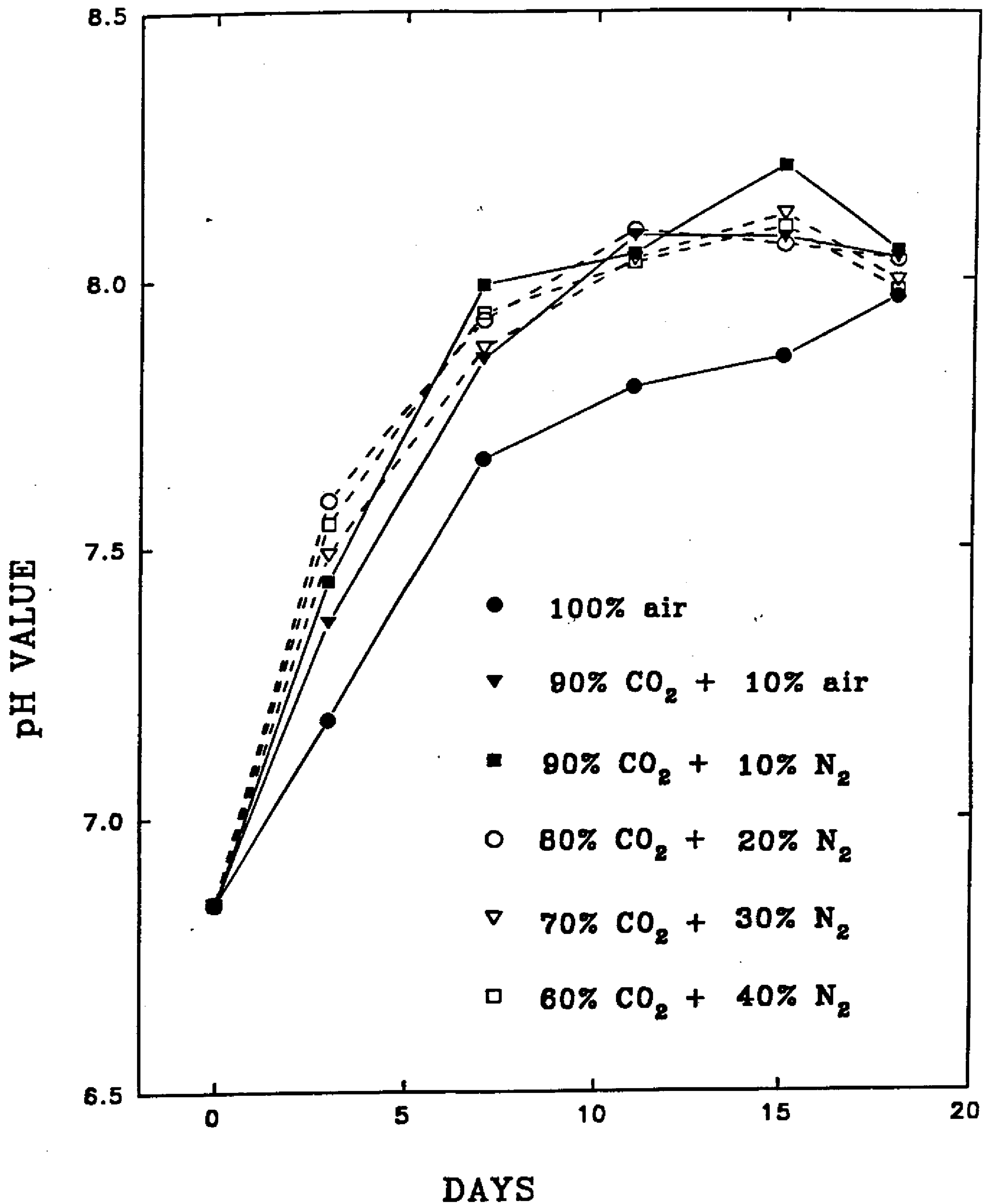

Figure 4. pH value of shrimp held in different modified atmospheres during refrigerated storage

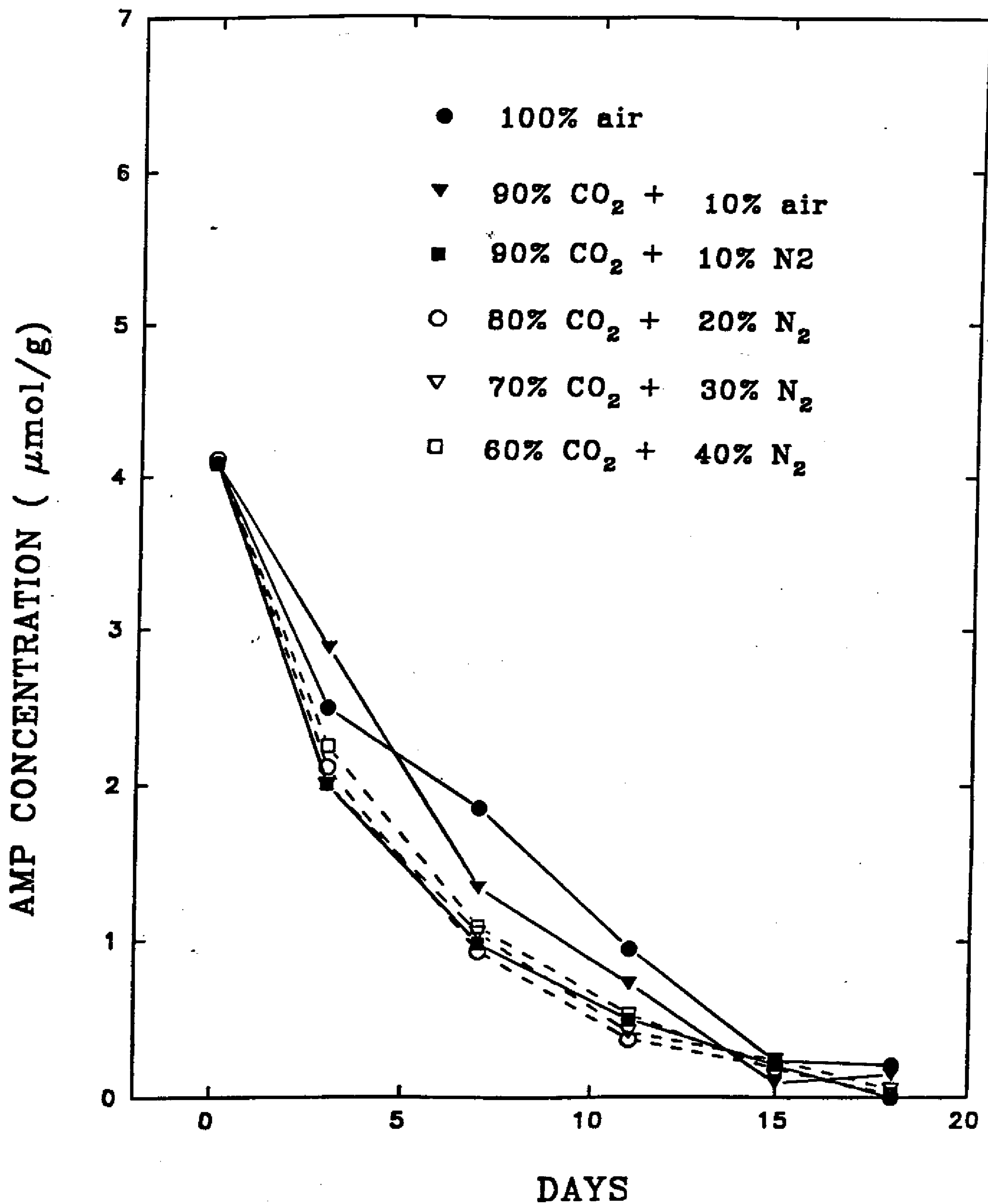

Figure 5. AMP concentration of shrimp held in different modified atmospheres during refrigerated storage

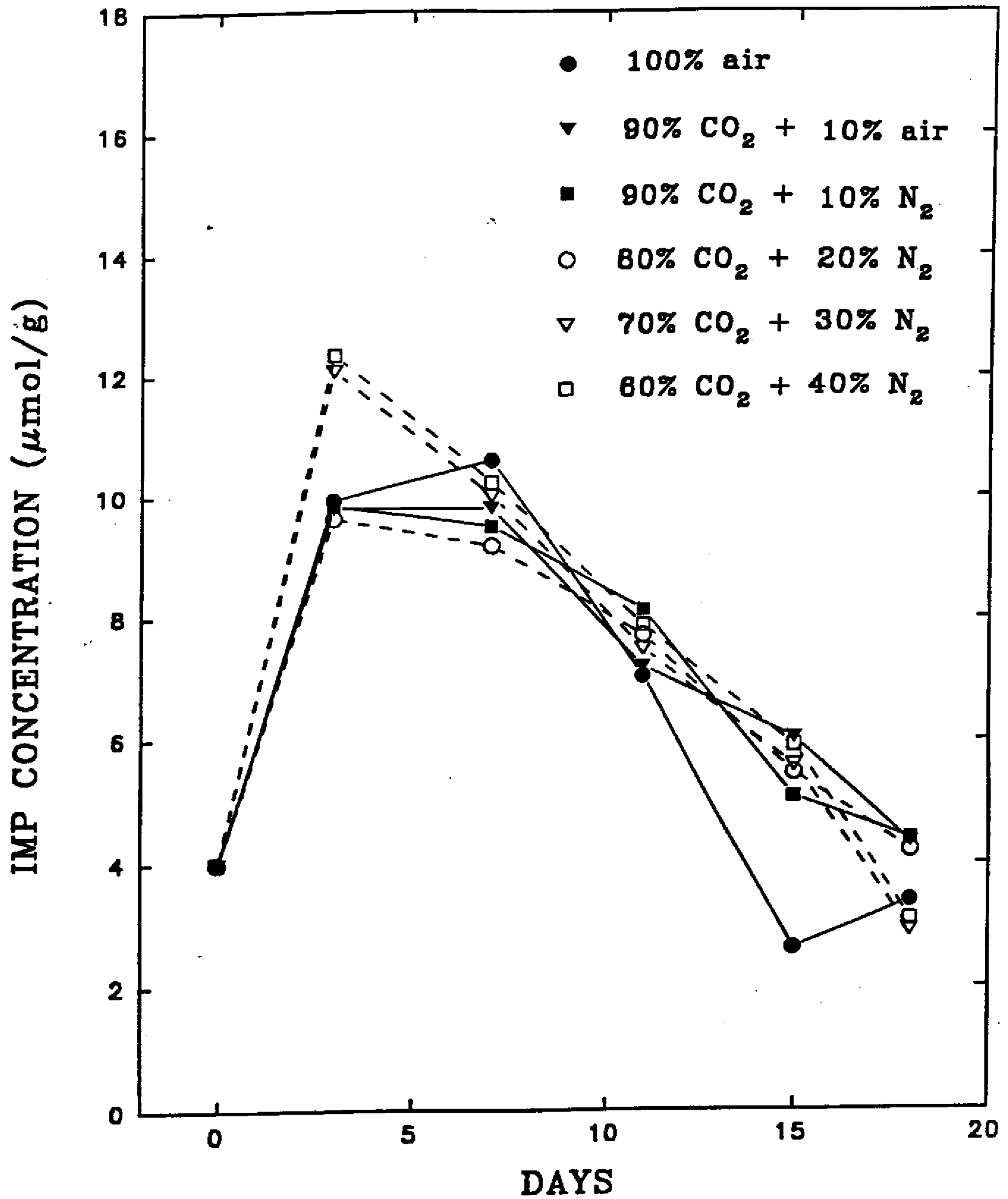

Figure 6. IMP concentration of shrimp held in different modified atmospheres during refrigerated storage

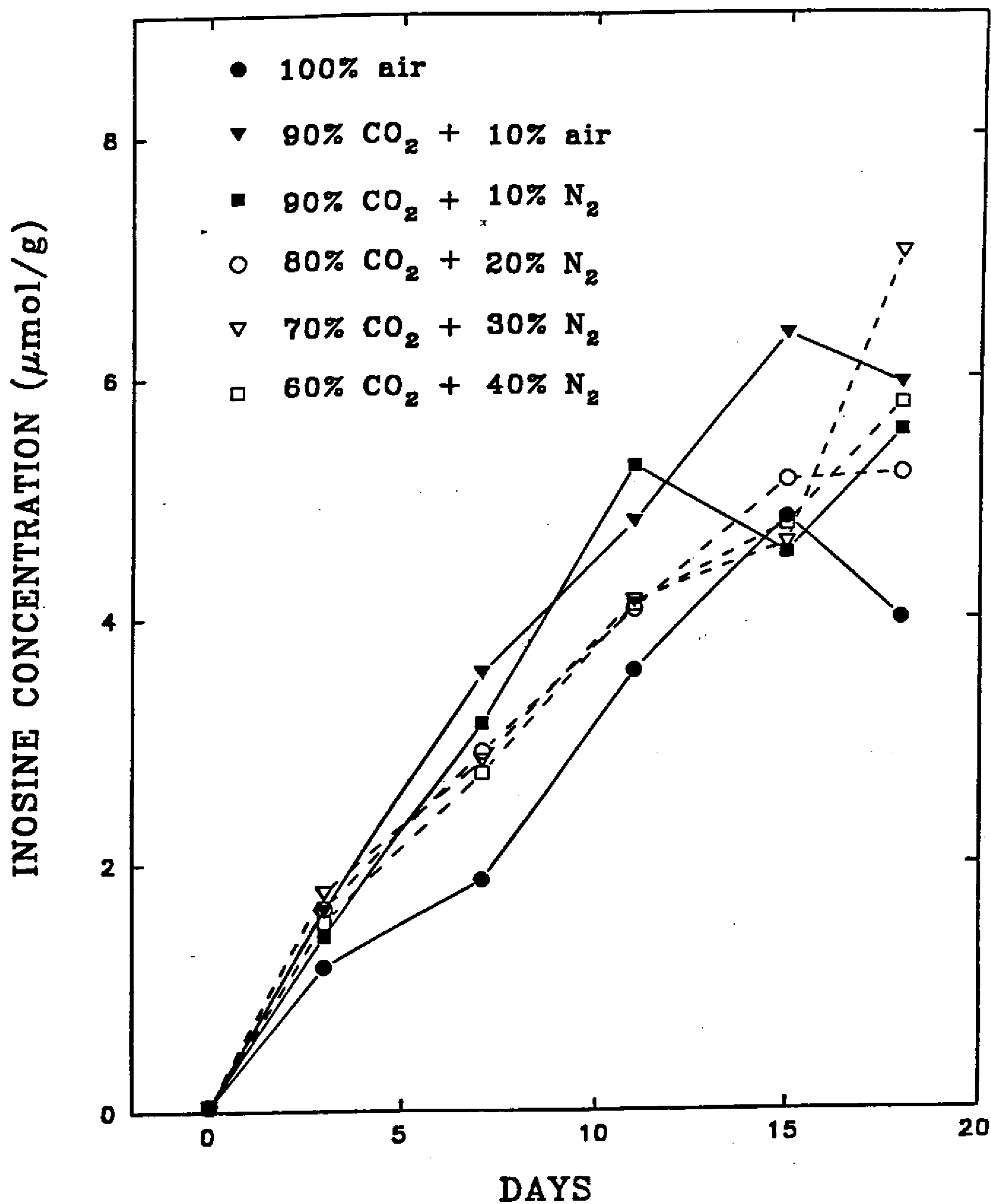

Figure 7. Inosine concentration of shrimp held in different modified atmospheres during refrigerated storage

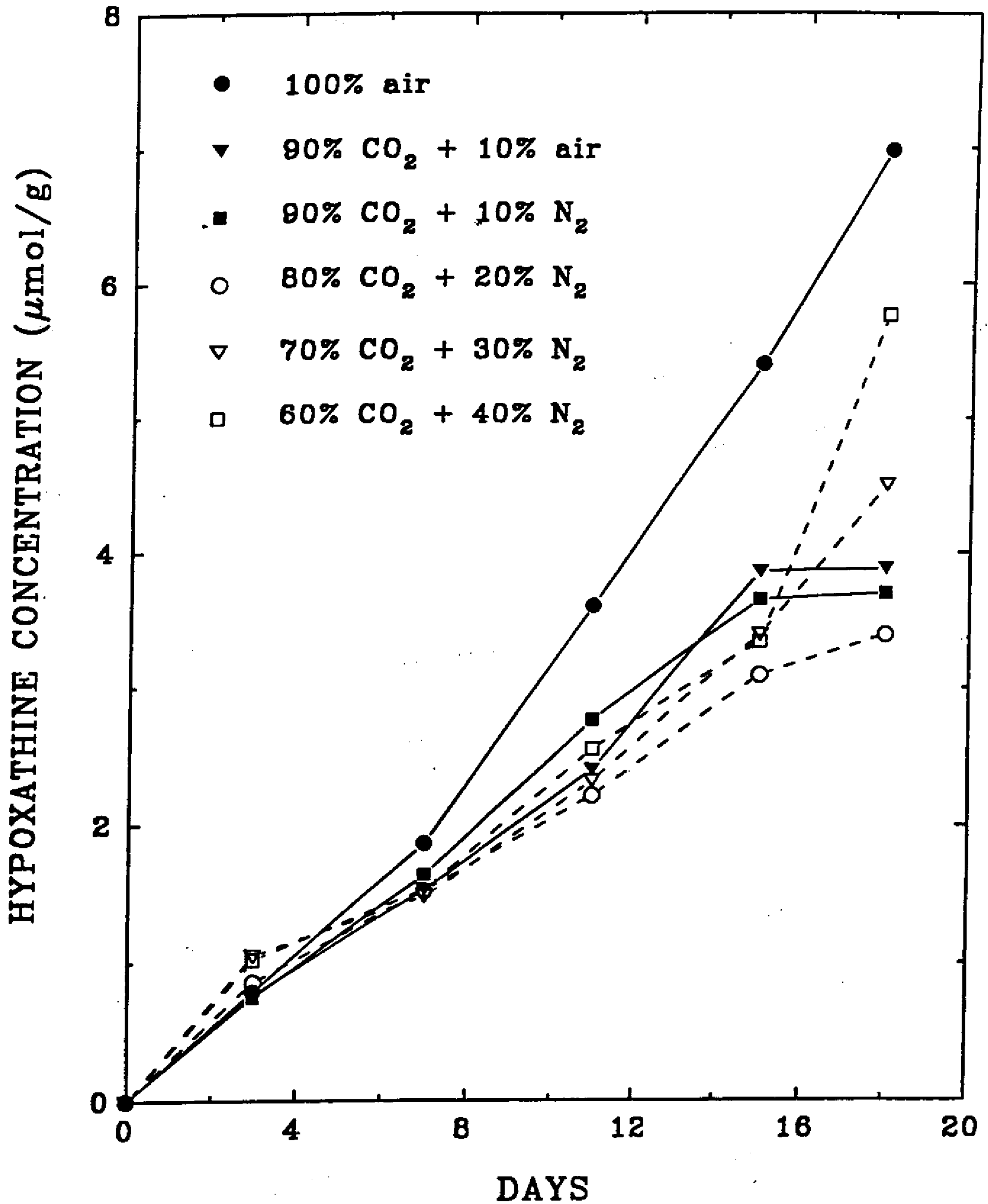

Figure 8. Hypoxanthine concentration of shrimp held in different modified atmospheres during refrigerated storage

Figure 9 showed the change in the K value during the storage period. The initial K value of 0.26% linearly increased with the increments of storage period, and finally reached the 73.2% in air pack, and 67.17% in MA of 90% CO_2 10% air. Analysis of variance ($p<0.05$) showed that K values among all packs had significant increases during the period of storage. However, no significant difference between packs were found except on 15th day which the K value of shrimp in air pack was significant higher ($p<0.05$) than that of MA packs. Attempts were made in this study to establish a relationship between chemical quality, microbiological quality, catabolites of adenine nucleotide and K value. Results showed that significant correlations existed between the K value and the other indices.

In summary, the reversed-phase HPLC method has the advantages of simplicity and is rapid in the measurement of the adenine nucleotides and the breakdown products. In MA packages, the shelf-life of shrimp can be extended up to 18 days without spoilage. Modified atmospheres affected the breakdown rate from IMP to HxR and Hx and caused less Hx accumulation. Statistical analysis indicated that HxR, Hx and K value had a significant correlation ($p<0.001$) with chemical and microbiological changes. K value did not predict the difference between the air packaged samples and MA packaged samples. However, HxR and Hx can be considered as a potential index of freshness.

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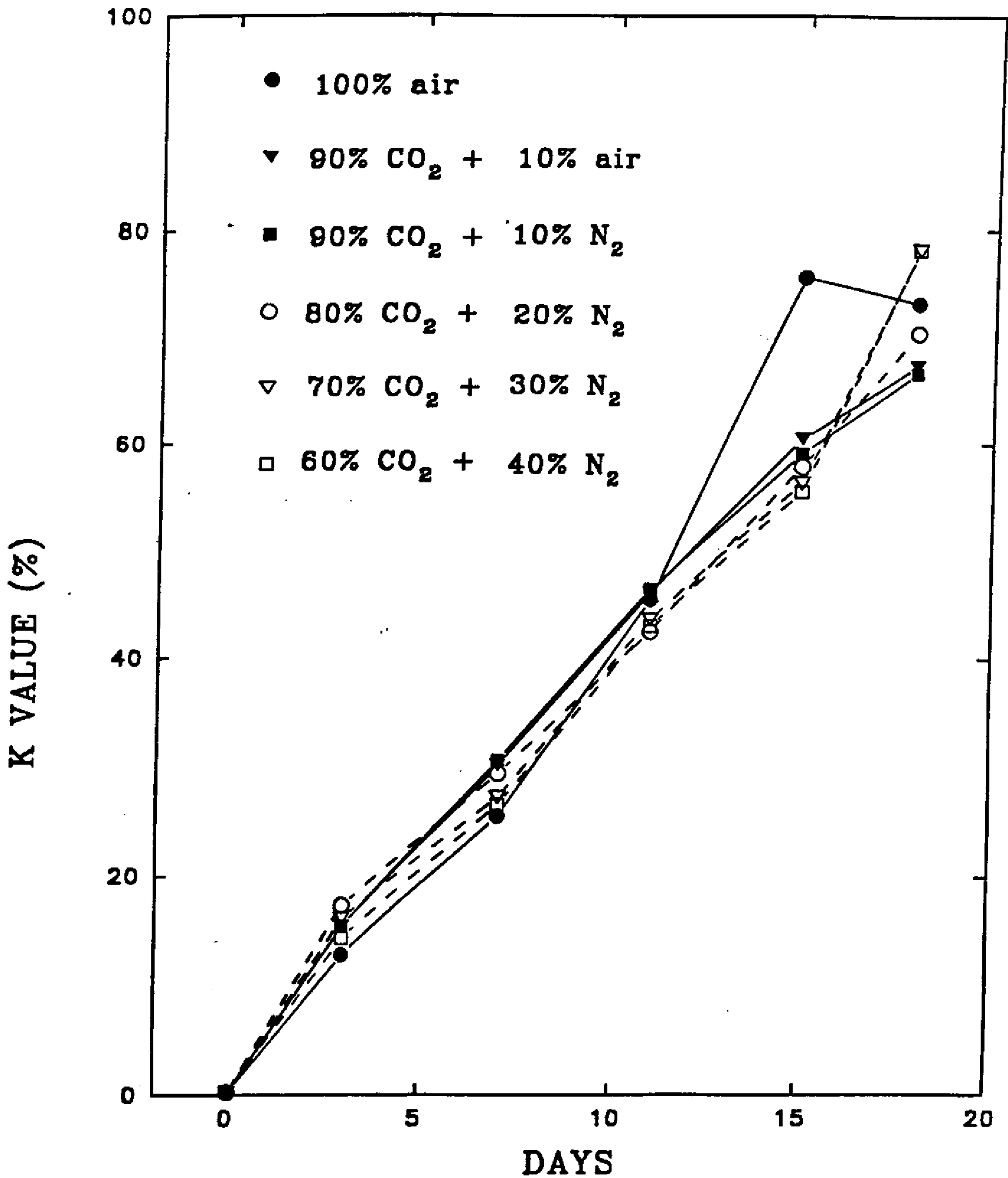

Figure 9. K value of shrimp held in different modified atmospheres during refrigerated storage

INITIAL STUDIES TO MEASURE CONSUMER PERCEPTION OF WATER ADDED TO SHRIMP TREATED WITH PHOSPHATES

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Consumer acceptance studies are necessary to better define the intended use of phosphates in foods, specifically seafoods. Similar studies have been published for some red meats and poultry, but there are no reported studies for seafood. There is also no distinct declared intent for the use of phosphates in treating seafoods. The primary use of phosphates with muscle protein has been to reduce dehydration and "drip loss" during processing, frozen storage and thawing. The objective of this study was to record consumer ratings for various organoleptic attributes perceived during the evaluation of cooked shrimp which had been previously phosphated to provide varying amounts of added water.

METHODS

Whole, untreated pink shrimp (Penaeus duorarum) from Key West, FL were headed, peeled and deveined without excessive water contact or any soaking. These processed shrimp varied in size from 30 to 40 individuals per pound. The shrimp were individually quick frozen, bagged and stored at -20°C. Before phosphating and freezing, the shrimp were thawed overnight in refrigeration. Phosphate treatments were selected based on preliminary trials, to impart "targeted" moisture uptakes based on increased raw weights of 0,5,10,15 and 20 percent. These changes in weight correspond to moisture gains between 1 and 5% based on the initial moisture content in the shrimp. The respective treatments to achieve these moisture additions were no phosphates (controls), 2% sodium tripolyphosphate (STP) for a 30 minute static soak, 1.5% STP for 15 minute tumble-soak, 4% STP for 15 minutes tumble-soak, and 6% STP for 30 minutes tumble-soak. All phosphate solutions contained 1% sodium chloride and the ratio of shrimp to solution (room temperature) was 1 to 2 (w/w). After treatments, each batch was thoroughly drained, weighed, bagged then frozen (-20°C, walk-in).

A series of taste panels were performed at the University of Florida with "experienced" panelist. These panelist were faculty, staff, administrators or students in the Food Science and Human Nutrition Department that were aware that the shrimp were different but were unaware of the treatments. Triangle tests were performed with each treatment verses the control (untreated shrimp). The 20 to 24 panelist evaluated the shrimp in duplicate. One plate contained 2 treatment samples and 1 control and the other contained 1 treatment sample and 2 controls. The objective of the triangle test was to determine if differences could be noted between the various phosphate treatments and the nonphosphated shrimp.

For the consumer evaluations, the thawed shrimp samples were reweighed and cooked by a standard, immersed boiling procedure. Exposure time in boiling water was predetermined to be less than 1 minute to allow a minimum internal cook temperature of 160°F. The cooked shrimp were drained, cooled in refrigeration and served to the panelist. Consumer panelists (n=125) were prerecruited, based on their frequency of shrimp consumption, to participate in a central location test in Greenville, SC. The consumers were prescreened for age, sex, and level of income. Each panelist was paid $25.00 to assure their concentrated effort. Cooked shrimp from the various treatments were randomly presented on color coded plates. Each coded sample was presented and evaluated before presentation of the next sample. Ratings were based on a 1 to 7 scale for a series of questions addressing appearance, aroma, flavor and texture characteristics, plus quality and value perceptions. The ratings were analyzed for variance, and mean differences were measured for significance based on the Walker-Duncan k-ratio test.

RESULTS AND DISCUSSIONS

The boiling procedure resulted in an average product weight (moisture) loss for the respective treatments as -30.8, -33.9, -28.7, -22.5 and -9.3. In the series of triangle tests, an average of 58% of the "experienced" panelist could detect differences in the "5% uptake" sample and untreated sample 100% of the time. The following averages show that as the percent moisture uptake of the treated sample increased so did the averages: 73% detected differences between the "10% uptake" samples and the control; 85% detected differences between the "15% uptake" samples; and 100% detected differences between the "20% uptake" samples. thus the human palate could detect differences in sensory attributes in phosphated shrimp.

Overall the consumer panelist rated preference for all phosphated shrimp significantly higher (x=4.7 to 5.2) than for the untreated control (x=3.6), thus indicating a stronger acceptance of the treated product. This acceptance was consistent in ratings for general appearance, flavor, and overall quality. There was no significant differences in ratings for aroma of aftertaste, and ratings for saltiness (1.6 to 2.7) averaged below the "preferred" salt taste level.

This one time study with one shrimp species and one product for cooked in boiling water indicates phosphate treatments for shrimp can impart some quality benefits as perceived by typical consumers. These results should be supplemented with similar studies with additional variables and seafoods.

CREDITS AND SUPPORT

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The excellent sensory analysis is recognized as provided by Sharon Hargett, Sensory Evaluation Consultant, Greer, SC in liaison with Market Insight, Greenville, SC

OHMIC THAWING OF SHRIMP BLOCKS

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INTRODUCTION

The current practice of thawing shrimp blocks consists of placing the blocks in large water tanks in which warm water is continuously fed, and by agitation of the water in the thaw tanks. The daily use of water in thaw tanks in a large shrimp processing plant in Florida is about 155,000 gallons. The current practice is costly to the company and to the community where fresh water may not be abundant. It generates large amounts of waste water costing the company large sewer bills and stressing the environment, and affects the quality of the shrimp by exposing it to potential cross-contamination. Since the outer layers of the shrimp block thaw first and have the same temperature as the thaw water (about 20-25°C) there is also the possibility of microbial growth. The typical 2.4 kg shrimp block takes about 2 hrs to thaw in these conditions. The only apparent advantage of water thawing is the water uptake of the shrimp, and its increase in weight. However, the waste water generated contains soluble proteins and other nutrients. There is also waste of energy since the typical conversion efficiency of electricity to heat by way of steam is about 40%. Ohmic heating can be used in thawing frozen food blocks in general, and shrimp blocks in particular, therefore eliminating the concerns mentioned above.

Ohmic thawing

The principle of ohmic heating is to use the internal electrical resistance of the food to convert the electrical energy directly to heat. This method differs from the conventional "surface" heating

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of the food. In conventional heating, heat is supplied to the surface of the food by steam, hot water, etc. This step is quick and easy. However, heat must then conduct into the food, and this is the slow step in the process. In ohmic heating, as in microwave heating, heat is generated throughout the volume of the food. Therefore, the process is quick, and the temperature gradients are modest in "normal" operations. Since all the electricity is converted to heat, this is also a very efficient system.

Most foods contain enough free water and dissolved ionic salts to conduct electricity. There is evidence that ohmic heating can be very successful. This process received a great deal of interest in recent years. In 1991, a new research center devoted to ohmic heating was announced (1). A line of ohmic heaters were introduced in the area of food sterilization. The bulk of the research is presently focused on the sterilization of particulate food materials in a liquid (9). In pilot scale operations, liquid foods with flow rates of 400 kg/h were sterilized by a 45 KW transformer. The time required to sterilize liquid foods was as short as 90 sec. However, ohmic heating cannot be applied to fats, oils, alcohol or bony tissue. Since electrical conductivity increases with temperature, there will be a need to modulate the energy input to the system (10). There is physical data being accumulated for different foods (5, 6, 7, 8). In food sterilization, the cost of using ohmic heating is very competitive with that of retorting with steam, as evidenced by the intense research and commercial development efforts in this area. Physical principles and equations describing heating rates depending on current, geometry etc. have been developed (4, 3)

Electrical energy can be used to thaw frozen food materials. The process is more complicated than in the case of sterilization, since phase change must be accommodated. Also, electrical and physical properties of foods are different in the thawed and frozen states. This implies potential local overheating problems. However, these can be eliminated or minimized by knowing the properties of the food, and by appropriate control of the operation.

Ohmic thawing was anticipated to eliminate the use of the thaw water and the resulting waste water management and handling problems. It was also expected that by using direct conversion of electricity into heat, substantial energy savings would be realized, and by accelerating the thawing process economic benefits would be possible. We also anticipated that the quality of the shrimp would be comparable if not better than the water thawing process.

Our objectives for this pilot study were to determine the technical feasibility of thawing shrimp blocks by ohmic heating. The effect of salt content on the thawing times was investigated. Commercial shrimp blocks were then ohmically thawed and the electrical energy use was calculated and compared with theoretical heat loads in different experimental conditions.

MATERIALS AND METHODS

Effect of salt content on shrimp thawing

A factor strongly affecting the electrical conversion to heat is the conductivity of the material, and for foods the salt content. Higher salt contents result in more electricity passing through the material, and therefore higher heat generation. No published data on the conductivity of shrimp, or other seafood were found. Recent data on lean beef and chicken are available (14).

A home-built apparatus was used. Two circular stainless steel electrodes of 6" diameter were attached to the regular electrical supply (220 volts AC, 60 Hz). The outlet was connected to a Variac (Staco Energy, Dayton, OH) to allow for control of the voltage. An induction type AC ammeter (Amprobe, Pyramid Instruments, Lynbrook, NY) was placed around the wire to measure current flow.

Untreated pink shrimp which had been kept frozen for 6 months at -30°C were thawed. Samples of approximately 65 g shrimp were placed in single layers at the bottom of plastic containers (3.5" diameter) with just enough NaCl solution (0%, 0.5%, or 5%) to cover the shrimp. Approximately 93 g of NaCl solution was needed. The containers were covered with Saran wrap held in place with a rubber band and placed into the cold room at 4°C. Another set of samples were prepared as above using approximately 186 g of shrimp placed in 3 layers. Approximately 200 g of NaCl solution was needed to cover the shrimp. The next day 2 mL of the salt solution covering shrimp samples were pipetted off and transferred to test tubes. This solution was tested for %Na using a flame photometer (PFP-7, Buck Scientific, East Norwalk, CT). The shrimp and solutions were placed in a -20°C cold room for freezing.

Two similar samples were removed from the freezer and weighed. The control was rinsed with tap water and put between two stainless steel circular 6" diameter plates. The sample was rinsed with tap water to moisten the block surface and placed between the 2 stainless steel 6" electrode plates of the ohmic thawing system. Electricity was turned on. The voltage was set at 100% (120V) until the amperage reached 1 amp. Voltage was then continuously adjusted to maintain the amperage at 1 amp. Time, volt% and amps were recorded every 30 seconds. When the amperage dropped suddenly the system was turned off and the sample rearranged so that shrimp was contacting the electrodes instead of ice. Thawed shrimp and slushy ice were removed at this time. The system was turned back on until the shrimp were thawed or nearly thawed. Some ice pieces were still present. Similar experiments were performed on all samples with different salt contents.

Thawing of commercial shrimp blocks

Five lbs rectangular (approximately 10x12x3") shrimp blocks (headless, uncooked, butterflied, 50 to 60 count, shell off) were supplied by Singleton Seafood, Tampa, FL. They were transported to

the lab in a frozen state, and placed in a -28°C freezer after being wrapped individually in plastic bags.

A larger ohmic heating unit was built (Figure 1), consisting of an alternating current variable transformer (6020CT-25, Staco Energy, Dayton, OH) with an input of 240V, output 480 volts, a sample box made of Lexan (22x13x12"), and various circuit components. Inside this box, there was a platform to hold the sample (13x10x3), raised from the bottom to separate thaw drip from the samples. The material was placed between the electrodes (rectangular shape, 12x9.5x1/16" stainless steel), the voltage was applied, and the current flow and voltage were recorded at regular intervals. From time to time, the current was turned off and the block was checked for hot spots. Thawed shrimp was manually removed from the rest of the block at those times. Experiments at different conditions were conducted where the voltage and amperage levels were changed.

Qualitative experiments were also conducted in thawing frozen raw yellowfin tuna steaks. The purpose was to see if frozen fish could be thawed by ohmic means.

RESULTS

Effect of Salt Content on Shrimp Thawing

Table 1 summarizes the results obtained in these experiments. It was observed that by leaving the shrimp in water for one day changed the Sodium content of the water. For samples with no salt added, there was detectable Sodium in the water at the end of 1 day of storage, e.g. from 0 to 0.05% Sodium. For samples with added NaCl, the Sodium level changed with storage. For 5% NaCl added, there was some absorption of salt into the shrimp, with a decrease of the Sodium level from an initial of 1.97% to a final value of 1.32%. For 0.5% initial level of NaCl (or 0.197% Sodium), the final level was e.g. 0.15% Sodium. This means that there can be an increase in the normal Sodium level of shrimp by storage in NaCl solutions, and this may positively affect its behavior in an ohmic heating environment.

In all experiments, there was some loss of water compared to the initial weight of the frozen sample. This can be explained by the fact that the samples were wetted at the beginning of the experiment, and some ice may have melted as a result. Therefore, calculations presented in Table 1 were based on the final weights.

The calculation method for the estimation of the total electrical energy input by ohmic thawing was as follows :

1. Experimental heat generated : By monitoring the voltage V, and the amperage A with time during the experiment, we can calculate the heat generated by electricity during a time interval as :

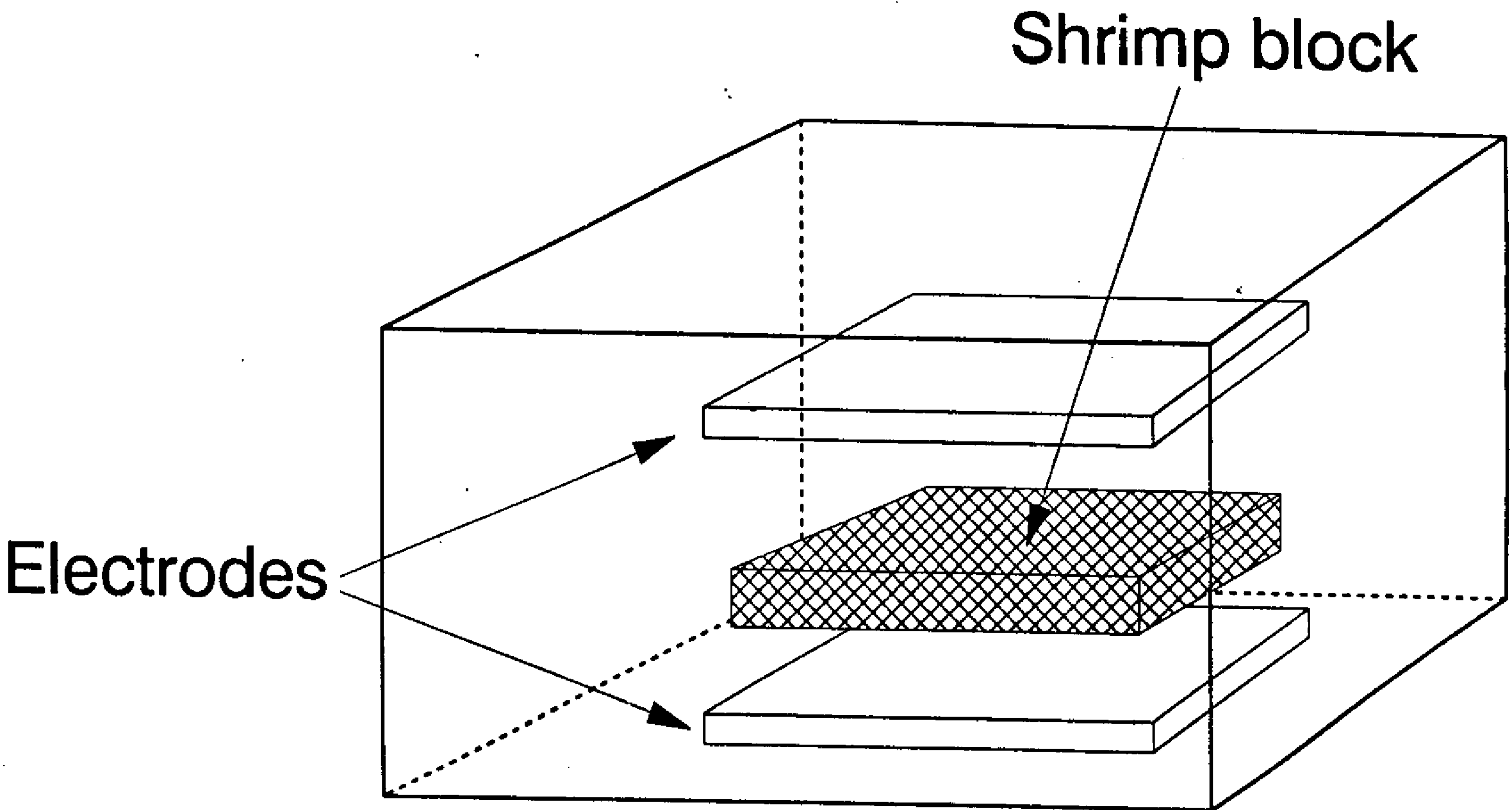


Figure 1. Experimental setup for ohmic thawing of frozen shrimp blocks.

V volts x A amps = W watts
W watts x t hrs = E watt-hrs
E watt-hrs x 3.415 = Q BTU, for that time interval

Table 1. Summary of experiments with different shrimp thicknesses, and different salt concentrations.

% Salt	SHRIMP LAYERS	%Na	FROZEN SAMPLE (g)	DRIP WATER (g)	CONTROL (g)	CALC. BTU	THEOR. BTU	TIME (min)
NONE	1	0.05	154.7	76.8		8.11	48.1	9
		0.10	153.3		139.5			
NONE	3	0.09	385.7	168.2		18.84	125	29.5
		0.07	382.3		389.2			
5.0	1	1.32	151.7	75.2		24.67	46.9	13
(1.97%Na)		1.25	153.8		147.2			
		1.20	153.7	75.9		14.9	48.5	8
		1.26	153.7		149.8			
5.0	3	1.24	382.4	149.4		50.32	116.7	20
(1.97%Na)		1.20	382.6		377.3			
		1.28	385.2	166.0		58.53	120.5	21
		1.31	384.0		381.1			
0.5	1	0.14	154.3	78.5		29.63	50.1	4
(0.197%Na)		0.15	155.0		152.7			
		0.15	154.2	70.8		8.24	48.3	6.5
		0.15	154.3		147.4			
0.5	3	0.15	380.7		372.0			
(0.197%Na)		0.13	385.3	166.2		22.74	120.9	29
		0.13	384.4	156.7		30.74	117.9	30.5
		0.16	384.9		372.9			

By repeating this calculation for all time intervals, we can calculate the "instantaneous" heat generation, as well as the cumulative heat generated by electricity for the entire thawing process.

2. Theoretical heat necessary for thawing the block : By knowing the thermophysical properties of water, ice, and shrimp in the frozen and unfrozen states and by knowing the initial and final temperatures of the block, we can estimate the heat necessary to thaw a block. The procedure is as follows :

a) Sensible heat of block below freezing point :

$$Q_{sb} = M_{water}\ (T_{init}-32)*Cp_{ice} + M_{shrimp}\ (T_{init}-T_{freezing})*Cp_{shrimp,b}$$

b) Latent heat of block :

$$Q_{l} = M_{water} \times 144 + M_{shrimp} \times L_{shrimp}$$

c) Sensible heat of block above freezing point :

$$Q_{sa} = M_{water} \times (T_{final}-32)*Cp_{water} + M_{shrimp}\ (T_{final}-T_{freezing})*Cp_{shrimp,a}$$

where :
M_{water} = Weight of water, lbs
T_{init} = Initial temperature of the block, F
Cp_{ice} = Heat capacity of ice, BTU/lb-F
T_{final} = Final temperature, F
Cp_{water} = Heat capacity of water, 1 BTU/lb-F
M_{shrimp} = Weight of drained shrimp, lb
$T_{freezing}$ = Freezing point of shrimp, taken as 28 F
$Cp_{shrimp,b}$ = Heat capacity of shrimp, below freezing, BTU/lb-F
L_{shrimp} = Latent heat of shrimp, taken as 119 BTU/lb
$Cp_{shrimp,a}$ = Heat capacity of shrimp, above freezing, BTU/lb-F

Then, $$Q_{th} = Q_{sb} + Q_{l} + Q_{sa}$$

Table 2. Thermophysical properties of shrimp and water, used in calculations.

Product	% water	T_{fp} °F	Cp_{below} BTU/lb-F	Cp_{above} BTU/lb-F	λ BTU/lb
shrimp	78.2	28	0.43	0.83	119
water	100	32	0.5	1.0	144

Siebel's equations : Cp_{above}(BTU/lb-F) = 0.008M + 0.2
Cp_{below}(Btu/lb-F) = 0.003M + 0.2

M = moisture content
λ : Latent heat of thawing

The thermophysical properties shown in Table 2 were used. The columns labeled "Calc. BTU" and "Theor. BTU" refer to the experimental heat generated, and the theoretical heat necessary for thawing, respectively. One interesting aspect of ohmic thawing performed in our experimental conditions was that the heat input from electricity was less than the theoretical heat necessary to thaw the block. There are two reasons for this :

1. During the experimental time, there was heat input to the block

from the ambient air, the parts of the equipment in contact with warmer materials, etc.. This is unavoidable in our conditions. In more rigorous experiments, the whole system could be placed in a Dewar container to eliminate all heat exchange with the environment, therefore isolating the heat coming from the electricity.

2. The end of the experiment was judged by the time at which shrimp thawed. In most cases, electricity preferentially passed through the shrimp, therefore heating it more than the ice. When all the shrimp thawed, there were still pieces of ice remaining. The heat contribution of this ice was not taken into consideration.

Thawing was quite rapid compared with the controls (columns labeled as "Frozen sample", and "control after thaw"). Samples of approximately the same composition and geometric properties were placed between two identical electrode plates and left at ambient temperature throughout the experiment. Thaw drip was allowed to separate from the control. The weight of the still frozen part of the control at the end of the experiment was recorded. It is apparent from Table 1 that ambient heat doesn't contribute significantly to the experiment during the relatively short durations of the experiments.

As expected, as the thickness of the sample increased, the time required for thawing was increased. Also, for similar weight and size blocks, 5% NaCl samples thawed faster than 0.5% NaCl samples. For example, experiments 2 and 7, with 3 rows of shrimp and with 5% NaCl required about 20 minutes to thaw, while experiments 4 and 8, 0.5%NaCl, took 30 minutes. This was expected. There was not a significant difference between the thaw times of no salt added, and 0.5% salt added samples. This could be due to the fact that the final Sodium levels in these were quite close. Therefore, they had similar electrical conductivities. These preliminary studies suggested that it was possible to thaw shrimp blocks with ohmic heating, and that salt levels could play a governing role in the thawing behavior. More study is needed in this area. Freezing shrimp in seawater may be advantageous in ohmic thawing.

Thawing of commercial shrimp blocks

Table 3 summarizes the results obtained by the thawing of commercial shrimp blocks. In data sets 2 and 3, the amps going through the block was maintained fairly constant at 1 amp, and the voltage was varied to achieve this. In data set 4, the amps were allowed to increase towards the end of the experiment. This increased the speed of thawing, as evidenced by the total thawing time of about 90 mins. It should be mentioned that electricity was periodically interrupted to check for possible hot spots, and to remove the thawed shrimp manually from the block. Therefore, in an optimized system, the total time is expected to be much shorter.

Table 3. Experiments with commercial shrimp blocks.

Set	time min	block wt(lbs)	shrimp (lbs)	H_2O (lbs)	Total BTU	calc. BTU	% elc.
2	101.5	4.1	3.55	0.5	526	627	83.9
3	179	5.98	4.78	1.02	538	907	59.3
4	86.35	5.68	4.94	0.58	736	812	90.6
5	40.83	5.64	4.6	0.88	904	874	96.7
6	50.17	5.54	4.59	0.84	849	895	94.9
7	62	5.18	4.14	0.9	909	909	99+
8	64	5.02	4.14	0.78	876	884	99+

Samples 5 and 6 were tiny shrimp, probably 500+ count, and samples 7 and 8 were 100-200 count. Our goal in these experiments was to reduce the thawing time without cooking the shrimp. Therefore, we increased the current going through the block. The results are shown in Table 3. Also, the graphs of voltage-amperage and BTU - total BTU are included (Figures 2-8). It should be noted that the cumulative heat vs. time is linear in most experiments. This is an advantage, since conventional heating operations have a decreasing heat input with time, as the temperature difference driving force decreases with time. Further experiments are planned to thaw different sizes of shrimp, and shrimp with shell on, to investigate these effects.

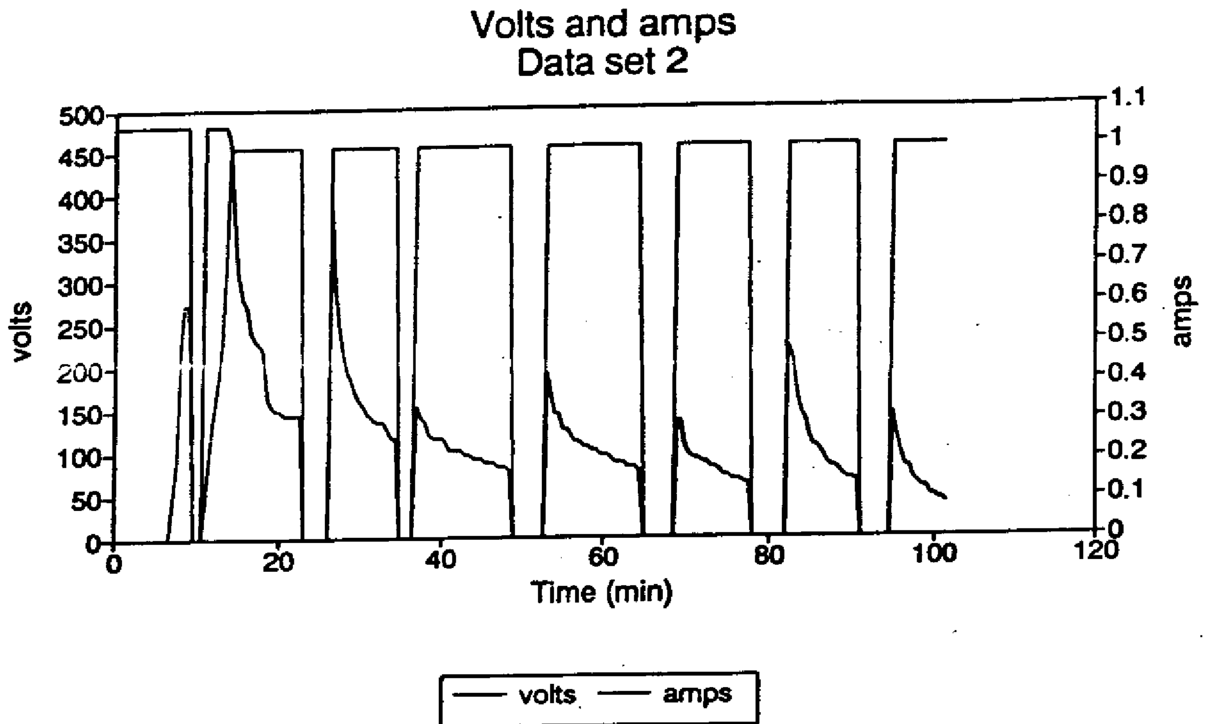
Freezing in seawater may be an advantageous alternative if combined with the ohmic thawing method. It was demonstrated that both frozen shrimp blocks, and frozen tuna steaks (data not shown) can be thawed quite effectively in this manner. There are still technical questions to be answered, the most important of which being the measurement of temperature during the experiments.

FUTURE WORK AND RECOMMENDATIONS

One shortcoming of these experiments was that temperature at different locations in the sample was not measured. Regular thermocouples are not suitable for this purpose, nor are glass thermometers. There are fiber optic based temperature measuring devices that are currently used for microwave environments. These would be suitable for use in an ohmic heating environment. This is a vital piece of information for the modeling and prediction of the maximum temperatures and thawing times of foods by this method.

Most of the time required during thawing using the ohmic unit came from checking the shrimp for hot spots and removing the thawed shrimp from the block, as can be seen from the Figures. If a method could be implemented to check and remove the shrimp more quickly and safely, the thaw time could be further reduced.

Figure 2. Experimental voltage - amp, and BTU - cumulative BTU plots vs. time for green, headless, shell off, butterflied shrimp, 50-60 count; data set 2.



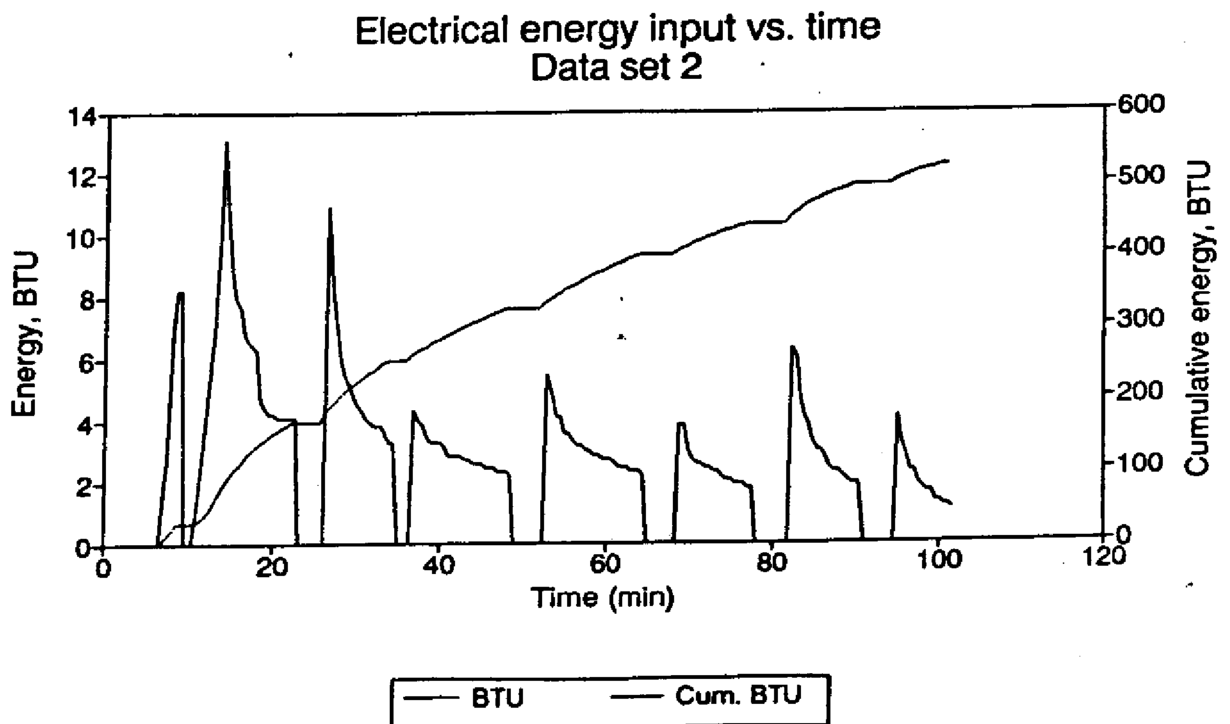
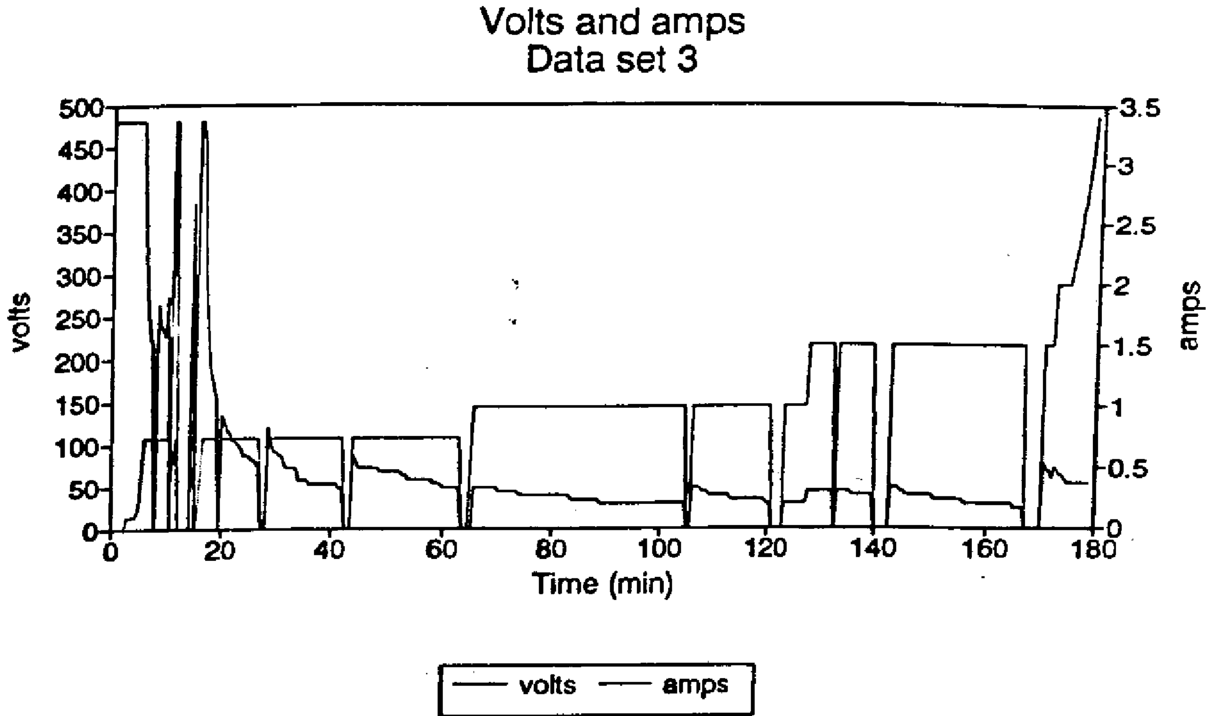


Figure 3. Experimental voltage - amp, and BTU - cumulative BTU plots vs. time for green, headless, shell off, butterflied shrimp, 50-60 count; data set 3.



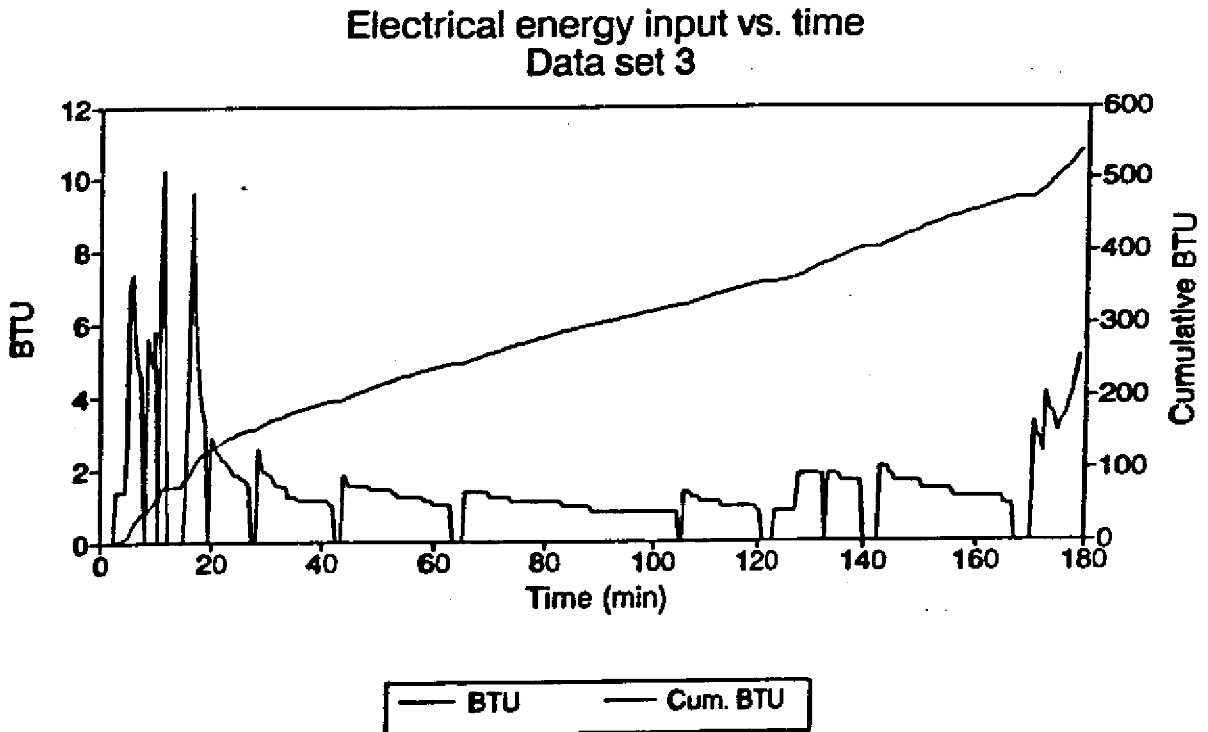
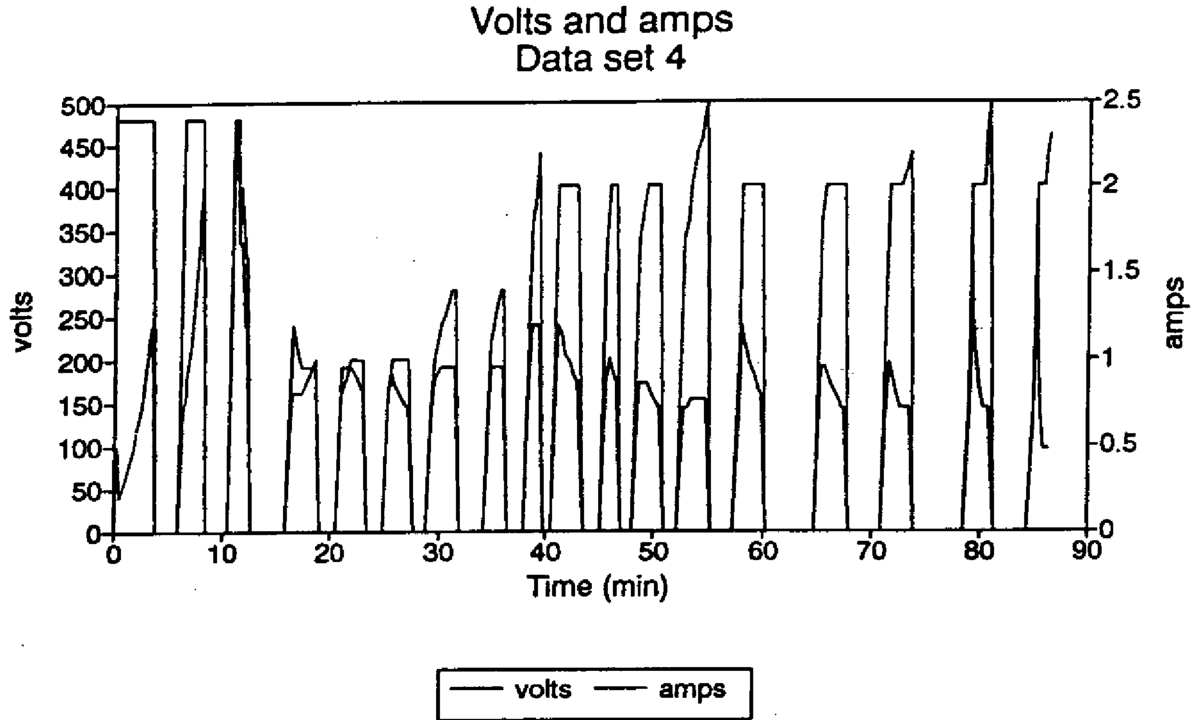


Figure 4. Experimental voltage - amp, and BTU - cumulative BTU plots vs. time for green, headless, shell off, butterflied shrimp, 50-60 count; data set 4.



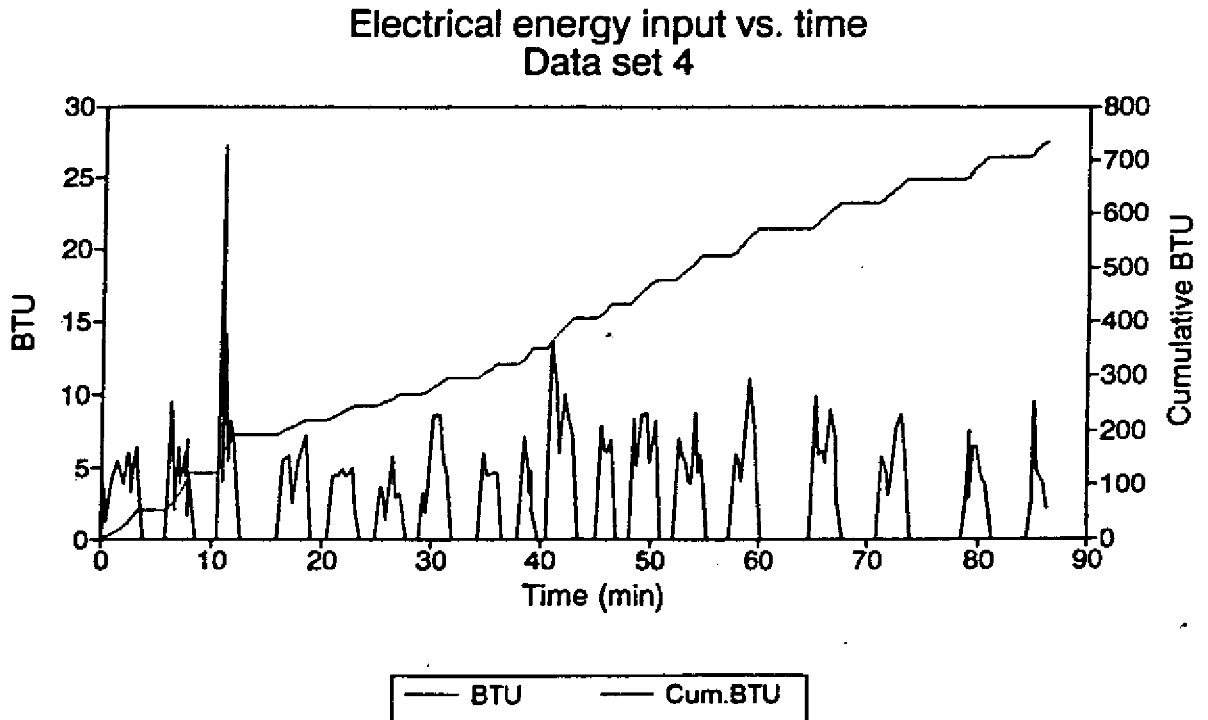
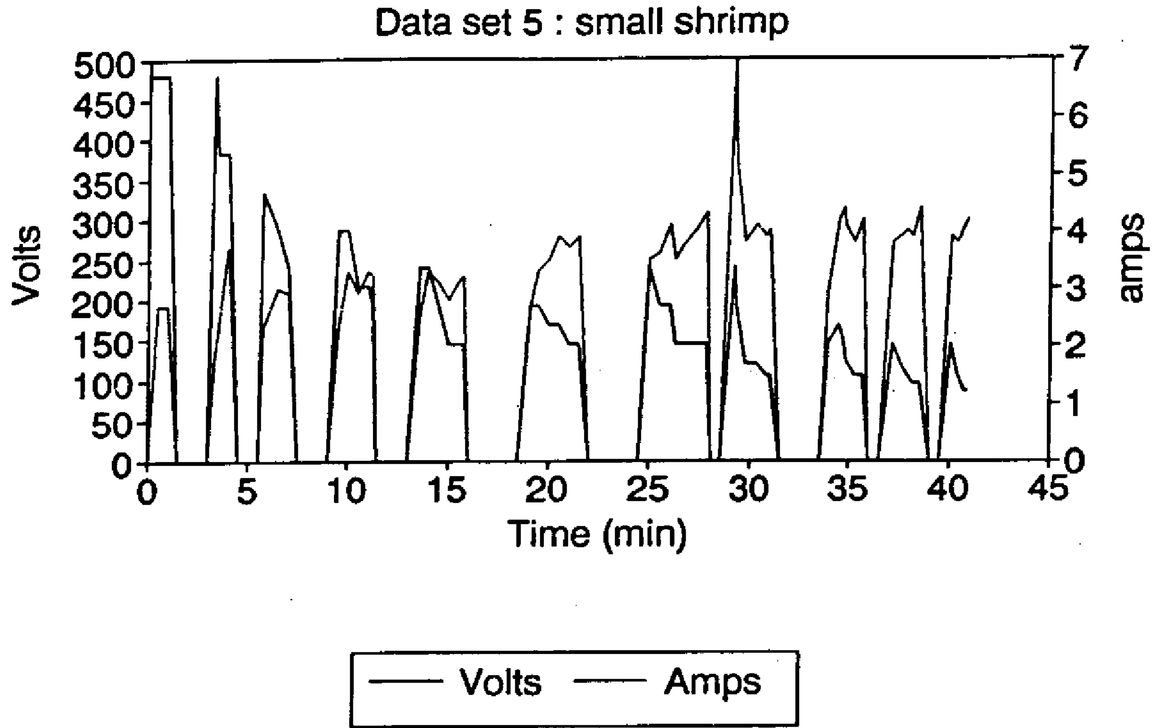


Figure 5. Experimental voltage - amp, and BTU - cumulative BTU plots vs. time for green, headless, shell off shrimp, 500 + count; data set 5.



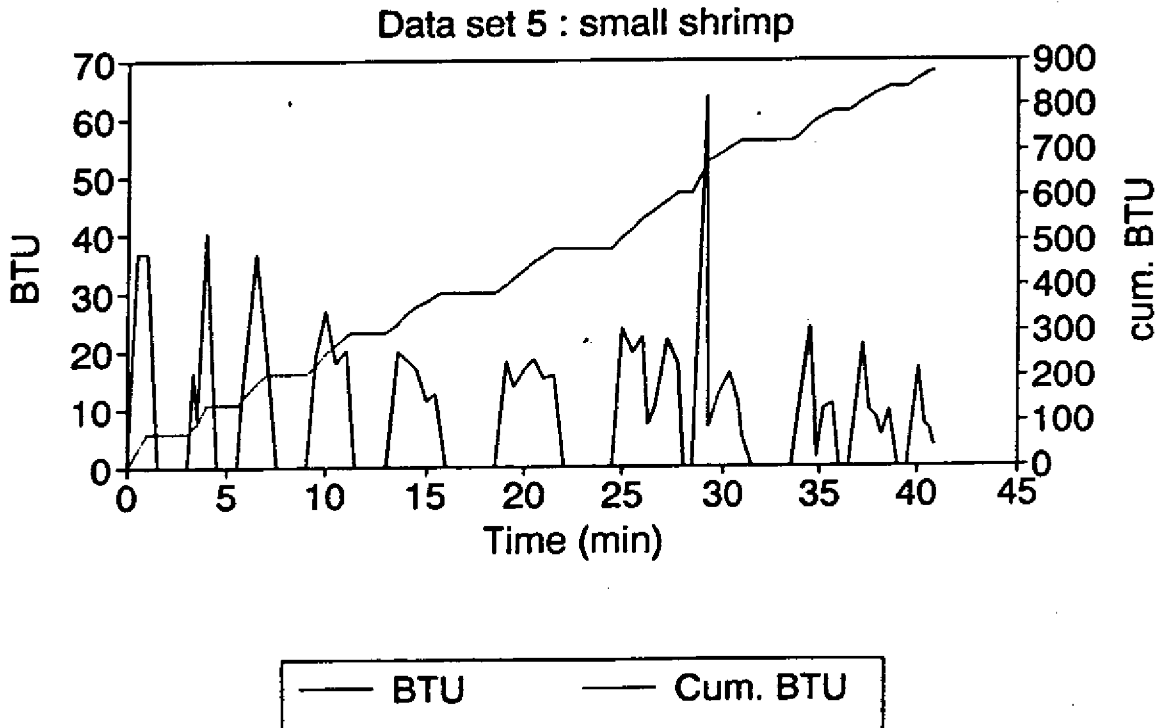
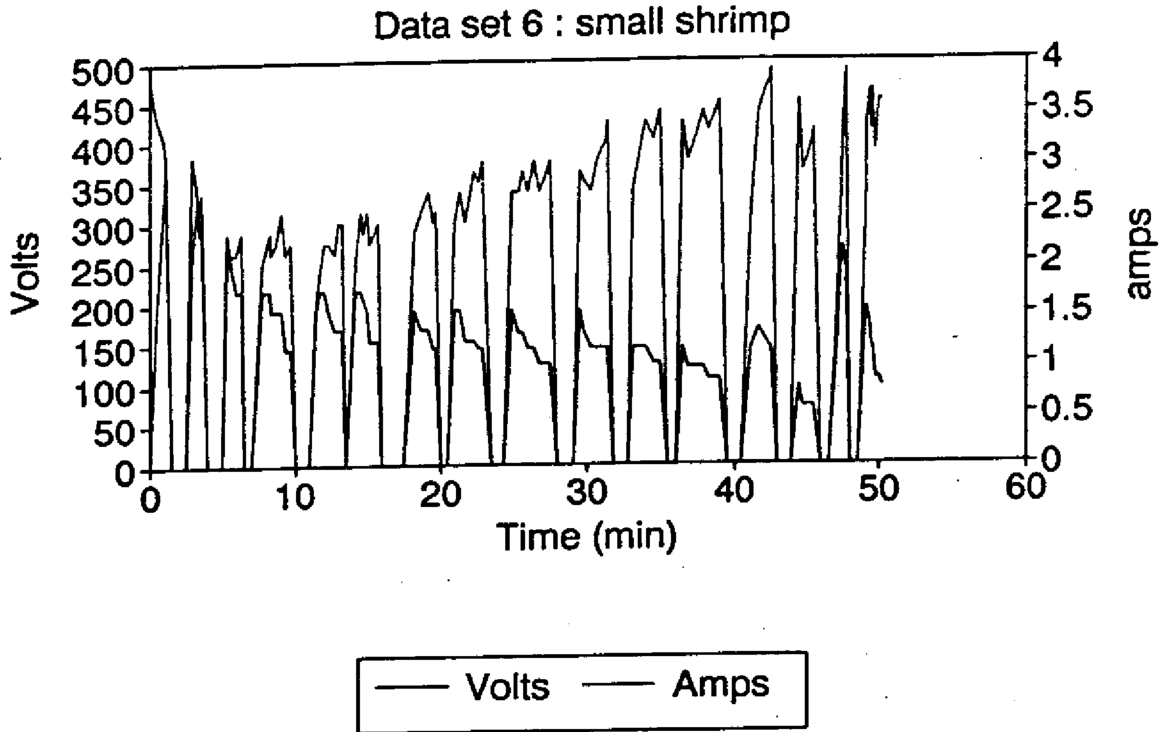


Figure 6. Experimental voltage - amp, and BTU - cumulative BTU plots vs. time for green, headless, shell off shrimp, 500 + count; data set 6.



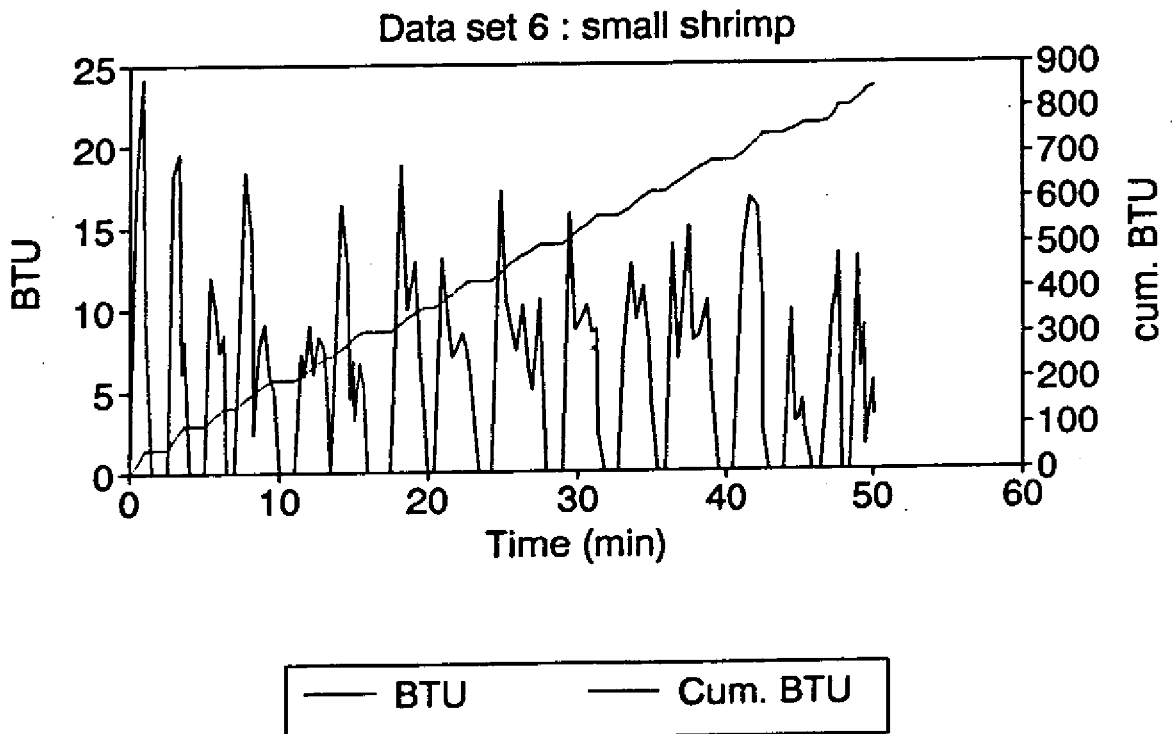
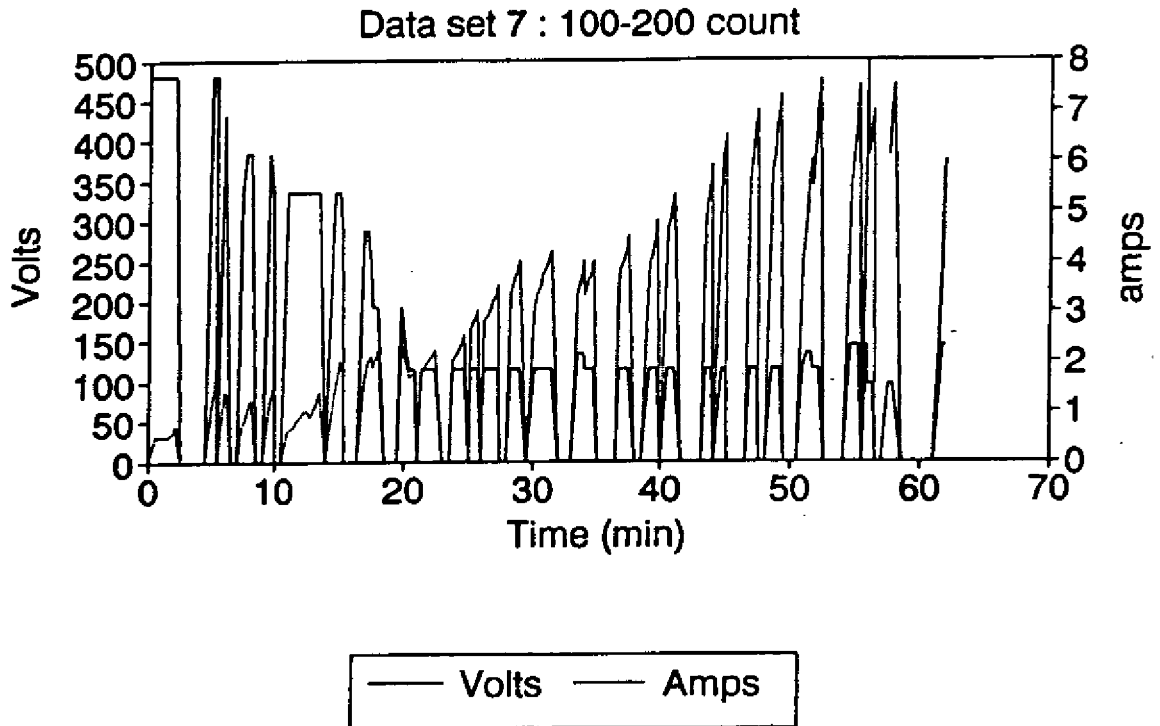


Figure 7. Experimental voltage - amp, and BTU - cumulative BTU plots vs. time for green, headless, shell off shrimp, 100 - 200 count; data set 7.



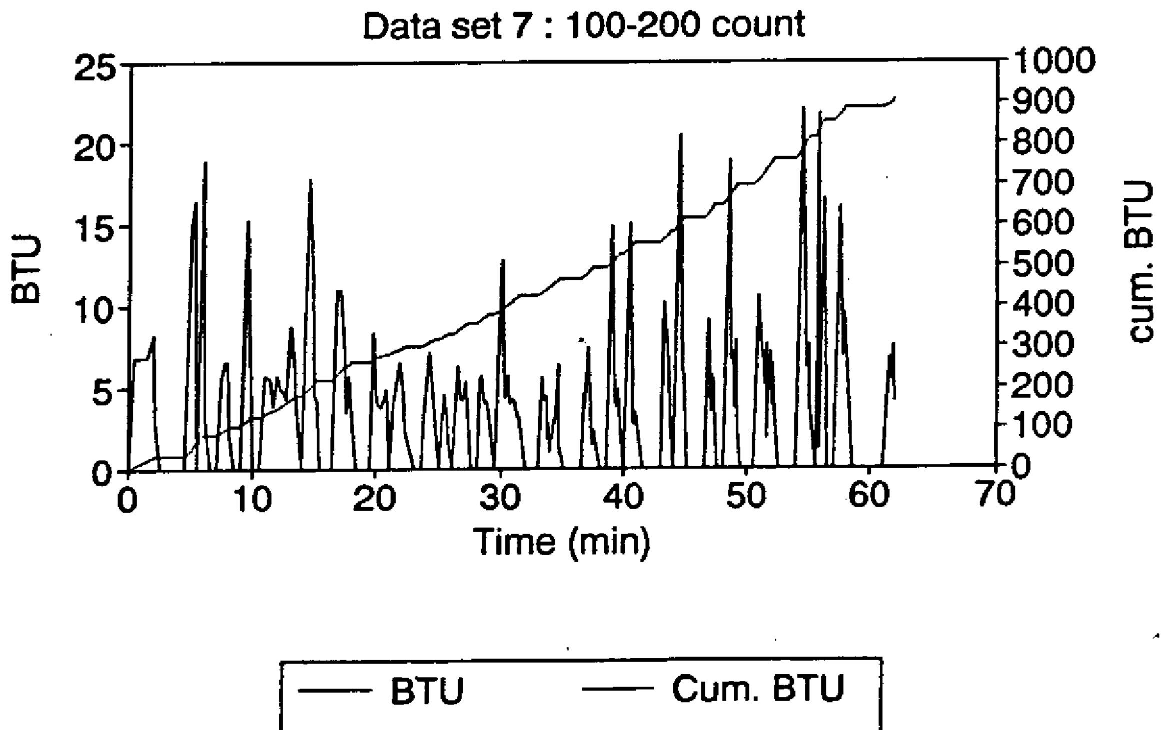
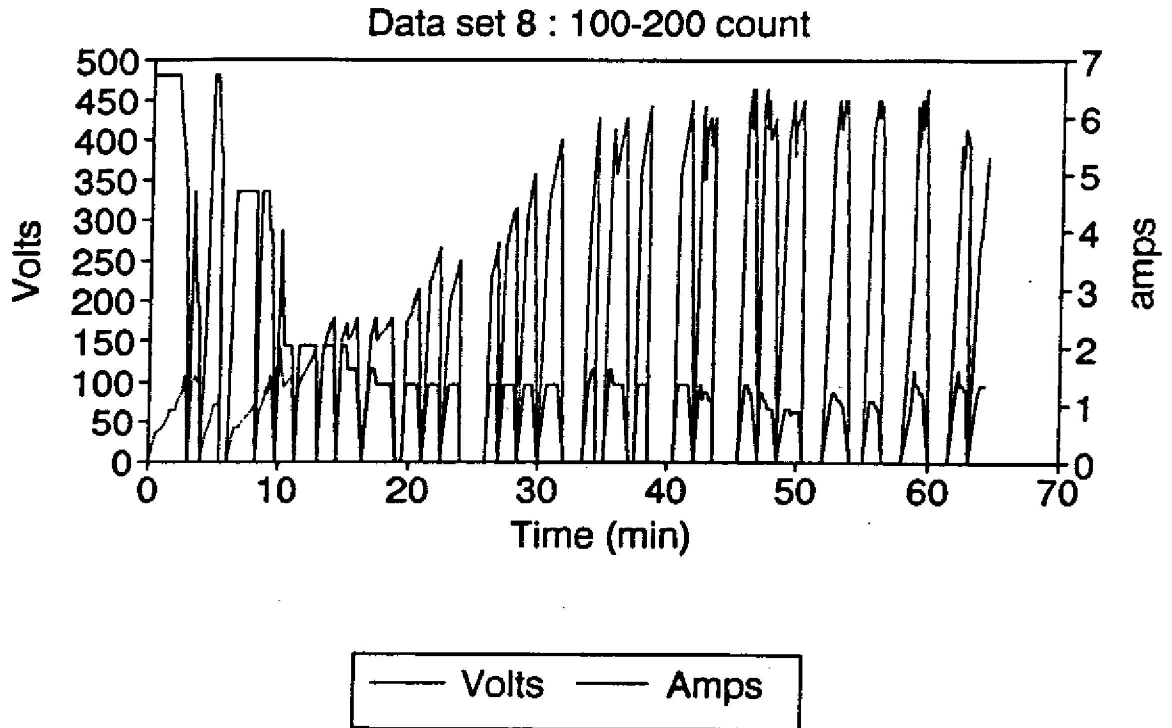
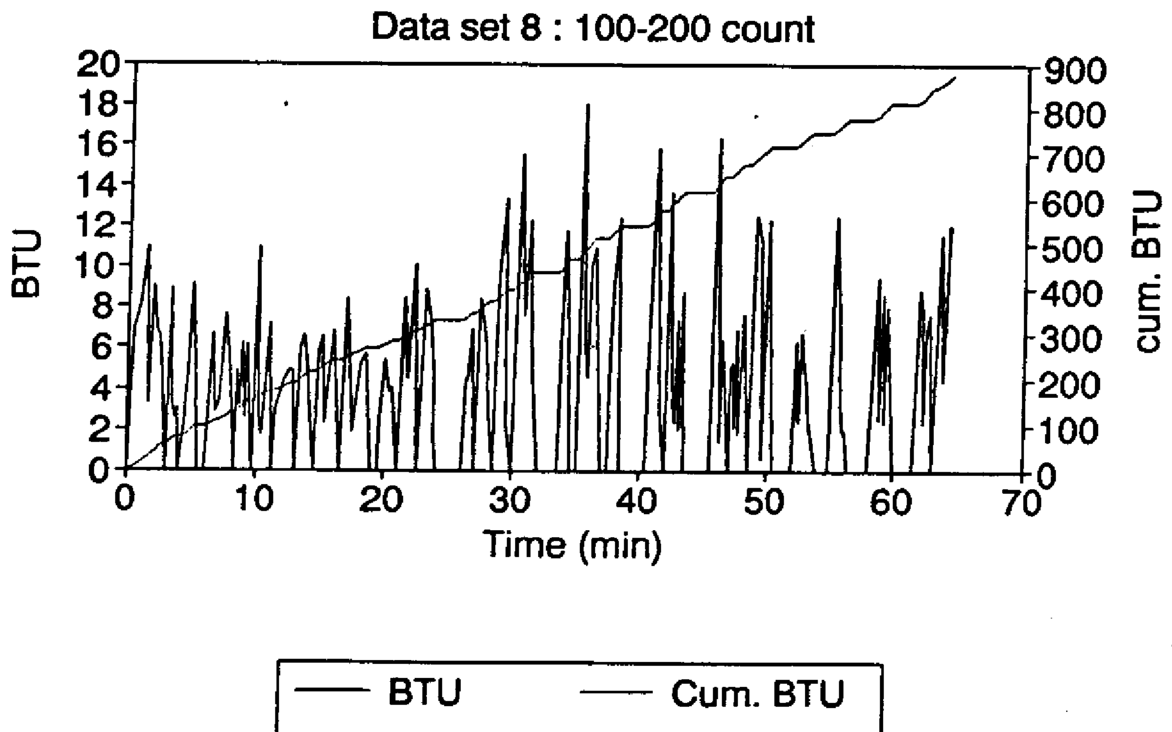


Figure 8. Experimental voltage - amp, and BTU - cumulative BTU plots vs. time for green, headless, shell off shrimp, 100 - 200 count; data set 8.





The effect of the shell, and shrimp size on the thawing and temperature distribution needs also be investigated. The electrical properties of shell-on and shell-off shrimp, as well as different forms of shrimp needs to be measured both in the frozen and non-frozen states, and as a function of temperature. Our next step is to build an apparatus to measure the frozen and non-frozen electrical properties of shrimp and other seafood.

The ultimate goal is to predict the behavior of frozen seafood blocks under ohmic thawing. This is a complicated process where changing electrical properties, variable heat generation, sensible and latent heat effects, and heat dissipation throughout the block need to be evaluated to predict the temperature and state (frozen or non-frozen) of the sample at any point, at any given time during the operation. The complexity of the model and the lack of experimental data suggest a doctorate level study.

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SURVIVABILITY OF TOXIGENIC VIBRIO CHOLERAE O1 FROM LATIN AMERICA

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In January, 1991, epidemic cholera appeared in Peru (2,3). Vibrio cholerae O1, biotype El Tor, serotype Inaba, was recovered from patients from Chancay and other towns along the northern Peruvian coast (2). This was the first time this century that epidemic cholera was identified in South America. The epidemic has spread to several countries in South and Central America and into Mexico.

In the Gulf of Mexico, an endemic focus of a Western Hemisphere strain of V. cholerae O1 exists along the Texas, Louisiana, and Florida coasts and possibly northern Mexico (9). The agent in the Latin American epidemic differs from the toxigenic strain endemic to the northern Gulf coast in its lack of hemolysin, lack of the VcA-3 vibriophage, and its genetic profile (12).

In July and September, 1991, toxigenic V. cholerae O1 resembling the Latin American strain was recovered from seafoods collected from closed oyster beds in Mobile Bay, Alabama (5). Recoveries were made independently by researchers at the Food and Drug Administration (FDA) and the Centers for Disease Control (CDC). This was the first report of the occurrence of this strain in U.S. coastal waters.

An investigation was initiated to determine possible means by which cholera could be introduced into U.S. waters. Primary emphasis was placed on foreign cargo vessels which might discharge contaminated ballast into local waters. A preliminary account of this work was presented elsewhere (10).

MATERIALS AND METHODS

Sampling and analysis

Samples (50 to 100 ml) were collected, placed in sterile containers, and maintained on ice until processing. Salinity and pH were determined for ballast and bilge waters. Tenfold serial dilutions (through 10^{-10}) of each sample were inoculated into six sets of alkaline peptone water (APW); three sets of enrichments were incubated at 35℃ and three sets were incubated at 42℃ for 18 to 24 h and were used for the estimation of most probable number (MPN). Inoculum from the pellicles was transferred to thiosulfate-citrate-bile salts-sucrose (TCBS) agar and incubated at 35℃ for 18 to 24 h. Suspect colonies were streaked to T_1N_1 agar (1% tryptone, 1% sodium chloride, 1.5% agar) for isolation and further testing. The membrane filter method (6) was used to determine numbers of fecal coliforms.

Characterization of bacterial isolates

Preliminary characterization of suspect V. cholerae isolates included oxidase reaction, "string" test (11), growth in 1% tryptone without salt, and API 20E (Analytab Products, Plainview, NY) biochemical reactions. Serotyping was done with V. cholerae antiserum poly (Difco Laboratories, Detroit, MI) and the V. cholerae AD Seiken kit (Denka Seiken Co., Ltd., Tokyo, Japan), which uses reversed passive latex agglutination (RPLA). Biotype was based on Voges-Proskauer (VP) reaction and resistance to polymixin B. V. cholerae enterotoxin was detected by RPLA (Oxoid VET-RPLA; Denka Seiken). The polymerase chain reaction (PCR) was used to detect the cholera toxin gene directly from APW enrichments with primers constructed at the Enteric Diseases Branch, CDC (7).

Survival studies

Studies were conducted to determine the organism's ability to survive in the environment and in ballast water aboard ship. We examined the combined effects of temperature and salinity on survival of V. cholerae O1 strain C6707, which is involved in the Latin American epidemic, and an isolate from one of the ships that was found to be the same as strain C6707. Strain C6707 was obtained from Elisa Elliot, FDA, Washington, DC.

These preliminary studies were made in the temperature gradient incubator; temperatures ranged from 5 to 55℃. The medium used was autoclaved, filter-sterilized (0.2 µm) seawater at 8, 16, and 32 ppt salinities; the lower salinities were obtained by dilution with distilled water. Seawater was collected 30 to 40 miles offshore from Dauphin Island, Alabama, and was aged at least 1 month in the dark at room temperature before use. At designated time intervals, cultures were inoculated into APW for enrichment, surface plated to T_1N_1 and TCBS agars, and incubated at 25℃ for 48 h for plate count.

Determination of metabolic activity

When V. cholerae became nonculturable by enrichment and plate count, cultures (2 ml) were incubated with yeast extract and nalidixic acid (8) and stained with 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride and acridine orange (13) to determine numbers of metabolically active cells. In addition, 10 ml was filtered through Fluoropore FGLP filters (Millipore Corporation, Bedford, MA) (1). The filters were boiled in 1 ml distilled water for 20 min; released DNA was subjected to PCR as above.

RESULTS AND DISCUSSION

Cargo ship surveys

Accompanied by U.S. Coast Guard authorities, FDA researchers collected samples of ballast, bilge, and sewage from 19 cargo ships docked in Gulf of Mexico ports at Mobile, Alabama, and Gulfport and Pascagoula, Mississippi. Sampling was begun in October, 1991. Initially, ships were sampled as they arrived in port with no particular reference to last ports of call (ships A through G; Table 1). Positive samples were then obtained from ships H, I, and J, which had last ports of call in Latin America (Brazil, Colombia, and Chile). Emphasis was then placed on Latin American vessels (ships K through Q) until December; however, no additional positive samples were obtained. As shown in Table 1, most of these ships had last ports of call in Venezuela, which was not contaminated with cholera at the time of sampling. In June, 1992, we sampled again and found two additional contaminated ships (ships R and S) that had last ports of call in Puerto Rico and Brazil.

Table 1. Last Ports of Call (LPC)

SHIP	LPC	SHIP	LPC
A	Japan	K	Venezuela
B	S. Africa	L	Honduras
C	Mexico	M	Venezuela
D	Nova Scotia	N	Venezuela
E	Venezuela	O	Venezuela
F	Louisiana	P	Venezuela
G	Puerto Rico	Q	Venezuela
H	Brazil	R	Puerto Rico
I	Colombia	S	Brazil
J	Chile		

Toxigenic V. cholerae O1, biotype El Tor, serotype Inaba, was recovered from sewage, bilge, and/or ballast from 5 of the 19 ships sampled (Table 2). Salinity ranged from 12 to 32; pH was 6 or 7. Counts were quite high, especially in the ballast and bilge from ships J, R, and S at $\geq 1.1^{11}$MPN/100 ml. The other positive ships had counts of $\geq 1.1^{6}$and $\geq 1.1^{8}$MPN/100 ml in ballast and bilge waters. The presence of cholera was not related to the presence of fecal coliforms. According to the ships' captains, some of the ballast had not been exchanged for 6 months, whereas others had what was called "permanent ballast," which was supposedly never exchanged.

Table 2. Fecal Coliforms (FC) and V. cholerae O1 (VC) in Nonpotable Waters

SHIP/SOURCE	pH	SALINITY	FC	VC
H Ballast	7	13	2.0^{1}[a]	$\geq 1.1^{6}$[b]
Fire Main	7	14	5.8^{2}	$\geq 1.1^{8}$
I Ballast	6	14	0	$\geq 1.1^{6}$
Bilge			0	$\geq 1.1^{8}$
Sewage			1.5^{5}	+
J Ballast	7	32	0	$\geq 1.1^{11}$
Bilge			0	$\geq 1.1^{11}$
R Ballast	6	20	0	$\geq 1.1^{11}$
S Ballast	6	12	0	$\geq 1.1^{11}$

[a]CFU/100 ml
[b]MPN/100 ml

These findings imply that ballast is a likely vehicle of transmission of cholera and that cargo ships may be responsible for the introduction of the Latin American strain V. cholera O1 into Mobile Bay. The findings also raise the question of the potential for global transfer of the organism, since ballast water is often discharged when a ship enters or docks at a port.

In 1973 the International Conference on Marine Pollution recognized the potential of ballast water discharges to cause harm. As a result, the International Maritime Organization adopted the resolution entitled "International Guidelines for Preventing the Introduction of Unwanted Pathogens from Ships' Ballast Water and Sediment Discharges" in July, 1991. Based on the results of our work, in November, 1991, the U.S. Coast Guard requested shipping agents to comply with these voluntary guidelines which recommend that ballast water be exchanged on the high seas before entry into a U.S. port. The guidelines were published in the Federal Register in December, 1991 (4).

As part of a joint nationwide effort with CDC and the U.S. Coast Guard, FDA conducted a nationwide sampling of nonpotable water from ships arriving in the United States from Latin America from November, 1991, through February, 1992. Six of the 110 ships tested, including those sampled by our laboratory, were confirmed as being contaminated with the Latin American strain.

Effects of temperature and salinity on survival

Studies were conducted to determine the effects of temperature and salinity on survival of this epidemic strain of V. cholerae. All cultures were inoculated at 10^2 CFU/ml. Results obtained were similar for both strains tested; those presented here are for strain C6707. Three temperatures (6, 18, and 30℃) were chosen for discussion. As seen in Figs. 1A-C, at 18℃ and 30℃ strain C6707 increased greatly in numbers during the initial incubation period; this increase was probably due to the presence of dissolved organic compounds in the seawater. Growth of the organism could be even more significant in an estuarine environment where the organic load would be higher. The strain survived for extended periods at 18℃ and 30℃ at all salinities. At 6℃ at 8 ppt salinity the organism was nonculturable by 14 days; however, growth and survival rates increased significantly as salinity increased. The cholera toxin gene was identified by PCR in both viable and nonviable cultures.

CONCLUSION

We have found that the Latin American strain of toxigenic V. cholerae O1 survives in large numbers and for extended periods of time in ballast water aboard cargo ships. Its association with ballast water is not related to the presence of fecal coliforms. It is a hearty organism that can survive and grow over a wide range of temperatures and salinities and, therefore, in various estuarine and marine environments. Survival appeared to be more dependent on temperature than on salinity. The organism can probably withstand exchange of ballast at sea and survive for several months in ballast tanks. Follow-up studies are needed to determine if ballast is actually being exchanged at sea as requested by the U.S. Coast Guard and what effect, if any, this has on a contamination problem aboard ship.

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LOW DOSE GAMMA IRRADIATION OF *PLESIOMONAS SHIGELLOIDES* IN LOUISIANA RANGIA CLAMS (*RANGIA CUNEATA*)

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Louisiana is a major site for molluscan shellfish and crustacea production, some examples are oysters, shrimp, and blue crab. In addition, the state of Louisiana has an extensive resource of estuarine water clams, commonly known as Rangia clams (*Rangia cuneata*). Rangia clams are abundant in the inland marshes of southern Louisiana (3). Over the years there has been an increasing interest in making the Rangia clams a viable seafood for market. Rangia clams are a popular seafood in local fishing communities, and the shells have been used as roadbed material and in the manufacture of several industrial materials (7).

Plesiomonas shigelloides has been a suspected pathogen for the past forty years. This organism has been suspected to be the causative agent in food and waterborne outbreaks of gastroenteritis, and recent information indicates that Plesiomonas is the responsible agent of many opportunistic infections. Several outbreaks of gastroenteritis have been linked to the consumption of raw oysters contaminated with *Plesiomonas shigelloides* (4,5). This organism is widely distributed in the environment, but it is mainly an aquatic species. It has been isolated from several mammals, birds, reptiles, water and environmental sediments, and seafoods like clams, crabs, shrimp and fish (1,6). This organism was originally described by Ferguson and Henderson in 1947 and named Paracolon C27 (2). Before its current classification, Plesiomonas was classified in a variety of genera including Pseudomonas, Eschericia, Aeromonas and Vibrio.

The purpose of this project was to determine the effect of low dose gamma irradiation on an inoculation of 10^7 cfu/g *Plesiomonas shigelloides* in sterile and non sterile Rangia clams stored at 0±2 °C for a period of 21 days.

MATERIALS AND METHODS

Organism

Plesiomonas shigelloides 7-1 isolated from Blue crab (*Callinectes sapidus*) was obtained from the Department of Food Science, Louisiana State University. The organism was stored at refrigeration temperature in Brain Heart Infusion Broth (BHIB), and was transferred periodically to fresh media. To prepare the inoculum, the organism was transferred to fresh BHIB and incubated at 35 °C for 14 hours to obtain an approximate concentration of $1x10^8$ cfu/ml of cells in the stationay phase. A known quantity of the starter culture was added to the clam samples in order to achieve a 10^7/g inoculum.

Substrate

Louisiana Rangia clams (*Rangia cuneata*) were collected from the marshes of the St. Bernard Parrish, Louisiana. The samples were processed as follows: the clams were washed, brushed and shucked. Clam meat and juice were collected and packed in plastic bags. The samples were kept frozen (-40 °C) until needed and thawed prior the experiment. Two treatments were performed: one set of samples was sterilized at 121 °C for 15 minutes prior inoculation and irradiation; the second set consisted of non sterile samples which were inoculated and then irradiated. Control samples were also prepared for both treatments. Approximately 20g samples, including meat and juice, were weighed into 60 ml nalgene bottles. Triplicate samples were prepared for each radiation dose, treatments, and controls.

Irradiation Treatment

A Cobalt-60 source was used for irradiation of the inoculated samples. The irradiation treatment was performed at the Nuclear Science Center of LSU. The samples were treated with 0, 1, 2, 3 and 4 kGy of gamma irradiation. Following irradiation, the samples were kept on ice and transferred to the Food Science building for microbiological analysis and storage at 0±2 °C. Control samples were treated exactly the same as irradiated samples except with no irradiation.

Microbiological Enumeration

Samples were examined for the presence of *Plesiomonas shigelloides* at 0, 7, 14, and 21 days of storage at 0±2 °C,

respectively. Two different media were used for the detection of the organism in the inoculated samples. Brain Heart Infusion Agar (BHIA) was used for total aerobic bacterial count, and a selective media, Plesiomonas (PL) Agar, was also used for the selective isolation of this organism. All samples were plated in duplicate and incubated at 35 °C for 48 hours. The number of surviving colonies in sterile and non sterile samples with or without irradiation was calculated by averaging the number of colonies recovered from the plating of triplicate samples for each radiation dose and time of storage respectively.

RESULTS AND DISCUSSION

In the nonsterile clam samples, with an inoculation of Plesiomonas shigelloides of 10^7 cfu/g added to the normal clam flora, there was an initial aerobic plate count of 10^8 of total microorganisms. After irradiation, the total numbers of microorganisms present in the clams were reduced with dosages of 1-4 kGy; however, this bacterial load was not reduced to zero after 21 days of storage (Fig. 1). After 7 days of storage, there is an apparent increase in the numbers of microorganisms in the samples treated with 3 and 4 kGy, while there is a decrease in the number of colonies recovered for the samples treated with 1 and 2 kGy. Between day 14 and day 21, the microbial load for the samples treated with a dose of 1 kGy increases reaching a concentration over 10^7 cfu/g, while with a dose of 2 kGy there was a slight decrease in the numbers. Samples treated with 3 and 4 kGy showed a a sharper decrease in the number of colonies recovered. These results appear to be contradictory since it is expected that higher dosages would be more effective against microbial growth than lower doses. A possible explanation could be the fact that the samples were inoculated individually and that their initial microbial load was much higher than other samples. A selective media, Plesiomonas agar, was used for the selective isolation of Plesiomonas shigelloides. The effects of low dose gamma irradiation on the recovery of this organism in nonsterile Rangia clams are shown in figure 2. With an irradiation dose of 4 kGy there is no recovery of the microorganism throughout the 21 days of storage. On day 0, 7, and 14 there were no colonies recovered, but on day 21 0.5×10^2 cfu/g were recovered with a dose of 3 kGy. Dosages of 1 and 2 kGy reduced the population of Pl. shigelloides by 4-5 log cycles on day 0; however, the growth seems to increase after 7 days of storage.

Sterile clam samples were also inoculated with an approximate concentration of 10^7 cfu/g Plesiomonas shigelloides. Figures 3 and 4 show the effects of gamma irradiation on the recovery of this organism. Samples irradiated and plated on Brain Heart Infusion Agar are shown on figure 3. A dose of 4 kGy reduced the population of this organism by 6 log cycles on day 0 in sterile clams, while there was no recovery from day 7 to 21. Gamma irradiation dosages

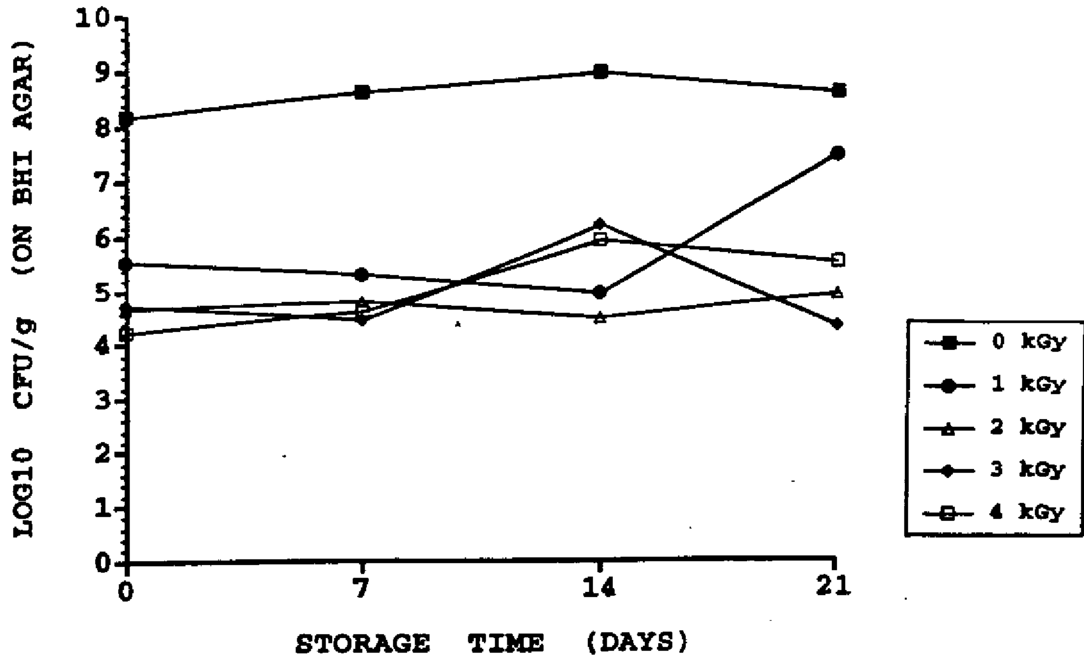

FIGURE 1. EFFECT OF GAMMA IRRADIATION ON THE RECOVERY OF NORMAL CLAM FLORA WITH AN ADDITIONAL INOCULATION OF 10^7 Pl. SHIGELLOIDES/g

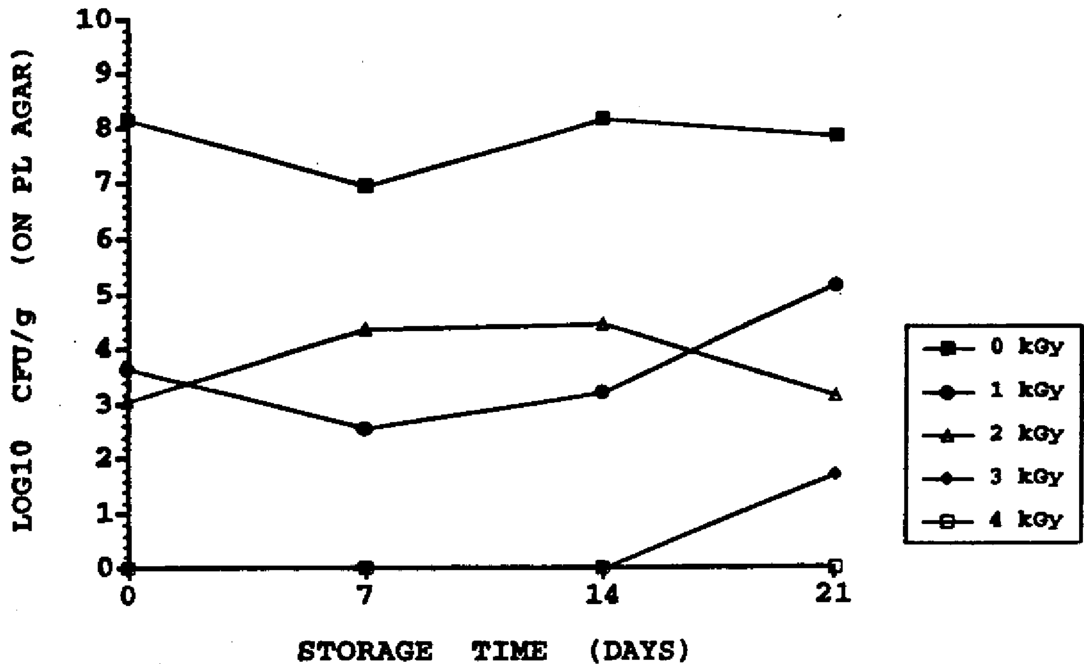

FIGURE 2. EFFECT OF GAMMA IRRADIATION ON THE RECOVERY OF Pl. SHIGELLOIDES IN NON STERILE RANGIA CLAMS

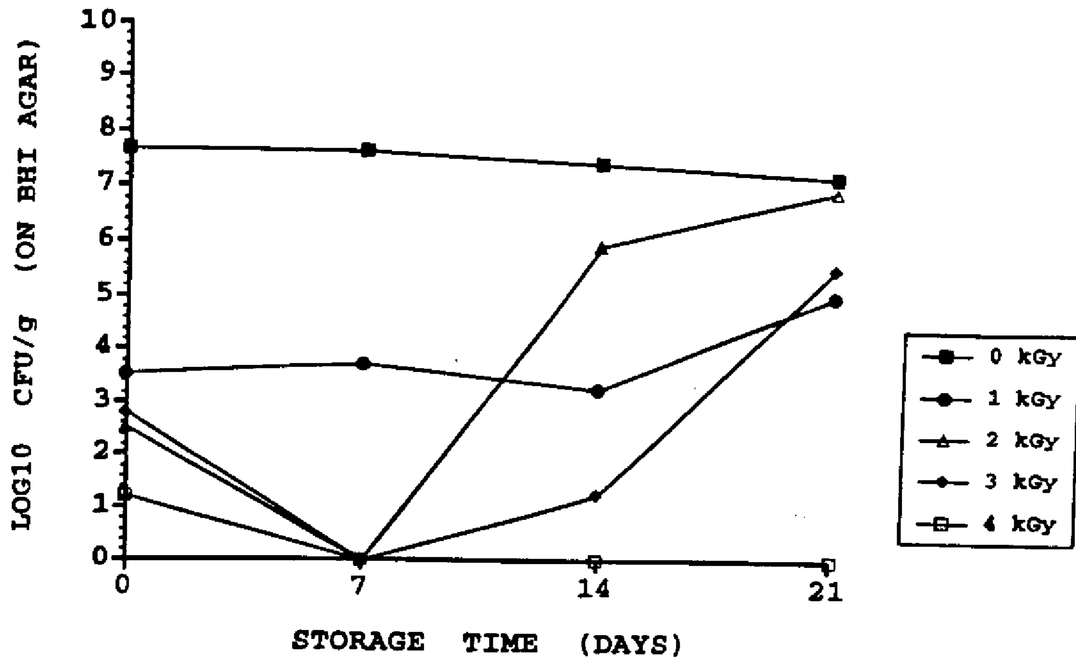

FIGURE 3. EFFECT OF GAMMA IRRADIATION ON THE RECOVERY OF Pl. SHIGELLOIDES IN STERILE RANGIA CLAMS

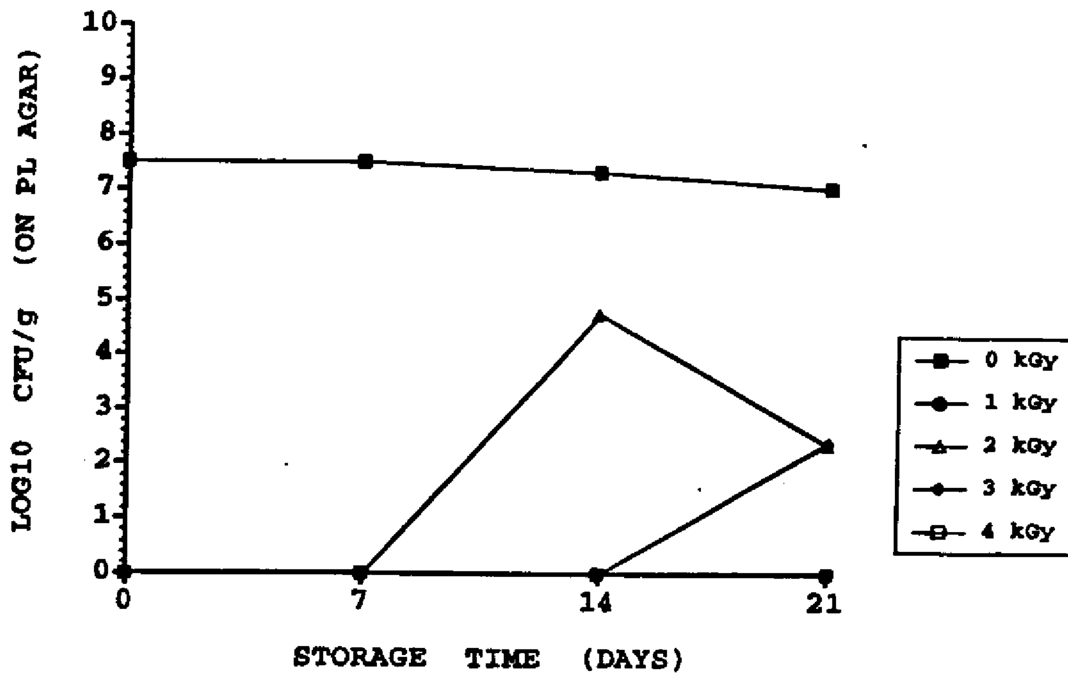

FIGURE 4. EFFECT OF GAMMA IRRADIATION ON THE RECOVERY OF Pl. SHIGELLOIDES IN STERILE RANGIA CLAMS

of 2 and 3 kGy show a reduction of the original inoculum by 5 log cycles on day 0. On day 7, the plated samples were negative for recovery; however, between day 7 and day 21, there was an increase in the growth of the organism. A 1 kGy dose appeared to be the least effective irradiation dose. When the selective PL media was used, similar results were obtained. There was no recovery of the organism for the entire storage time when the samples were subjected to a dose of 4 kGy.while the recovery was positive at day 14 and day 7 when 3 kGy and 2 kGy dose were used respectively. The percent recovery of the organism appears to be lower when the selective media is used when compared to the non selective. This can be due to the fact that irradiation has injured the cells making it more difficult for the organism to grow on the selective media.

CONCLUSION

The results of this experiment show that irradiation dosages lower than 4 kGy are not effective against *Plesiomonas shigelloides* at a concentration of 10^7 cfu/g. Lower gamma irradiation dosages do in fact reduce the number of colonies up to 7 days, but the organism is capable of recovering and multiplying after a week of storage. In non sterile samples, it appears that normal clam flora offers protection to the organism.

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GAMMA IRRADIATION OF Listeria monocytogenes IN CRAYFISH (Procambarus clarkii) TAIL MEAT

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The role of Listeria monocytogenes as an important food borne pathogen has been definitely established in products such as milk, cheese, and frozen dessert products. In the United States, the first cases of human listeriosis were reported in 1929. Human infections usually manifest themselves as septicemic infections in pregnant women, a fetus, or a newborn infant. In adults, the disease occurs most frequently in immunocompromised individuals and is characterized as septicemia, meningitis, or meningoencephalitis (Frazier and Westhoff, 1988). There has to date been no direct evidence linking the consumption of meat and seafood products to outbreaks of listeriosis. Recently, surveys of processed seasoned meats and freshly processed seafoods such as packaged crawfish tail meat have indicated the presence of measurable numbers of viable Listeria in these products(Farber et al.,1988) Listeria is an ubiquitous microorganism and is capable of growing in the intestinal tract and/or circulatory system of humans and other mammals until it causes septicemia or forms harmful toxin. Listeria is capable of growth over a wide range of temperatures (0.2 ^{o}C to 40°C) and is tolerant of highly salty conditions (<5%). The presence of these organisms in processed meats indicate that their mode of entrance into these meat products may be through contamination of processing equipment.

The purpose of this project was to determine the effect of low dose gamma irradiation (1.0 - 4.0 kGy) on known quantities of Listeria monocytogenes in processed crawfish tail meat. In addition, the effect of irradiation on the sensory quality of crawfish tail meat was evaluated by human sensory panel.

MATERIALS AND METHODS

Organism

Listeria monocytogenes (Scott A) was obtained from the microbioal collection of the Department of Food Science at LSU. To prepare the inoculum, the Listeria was transferred to fresh trypticase soy broth and incubated at 35°C for 18 hours to obtain an approximate 10^8 cfu/ml concentration of cells in the stationary growth phase. One ml of the starter culture was added to 10 g of fresh crawfish tail meat to obtain a 10^7 inoculum. Dilutions of the stock culture were made to obtain the 10^4 inoculum.

Substrate:

Fresh frozen crawfish tail meat was purchased from a local seafood market and kept frozen until the day of inoculation of 10 g aliquots.

Irradiation Treatment:

Following inoculation with Listeria (10^4 and 10^7cfu/g), 10 g samples (in whirl packs) [duplicate samples for each storage period] were processed using cobalt-60 source available in the basement of the Nuclear Science Center, LSU. The samples were treated with to 0,1,2,3, and 4 kGy of gamma irradiation. The samples were then maintained on ice and cultured for total aerobic bacteria and on Oxoid Agar, a selective media for Listeria at 0, 7, 14, and 21 days, respectively. The cobalt-60 gamma emission rate of 1 kGy/50min was determined using the Fricke Dosimetry Method.

Sensory Evaluation:

Unsterile fresh frozen crawfish tail meat with no microorganisms added (2-4 tails packaged in individual sealed polyethylene bags) was treated with 0,1,2,3 and 4 kGy gamma irradiation and stored on ice prior to panel evaluation. For panel evaluation, the bags of crawfish tail meat were cooked in boiling water for 10 min. Individual panelists were asked to evaluate odor, flavor, texture, and color as to acceptability and differences among samples. Two separate panels evaluated the samples: the first panel consisted of eight members of the Food Science department and the second panel consisted of six members of the Nuclear Science Department.

RESULTS AND DISCUSSION

Effects of Irradiation on Microorganisms:

In the nonsterile crawfish tail meat, with an inoculation of Listeria monocytogenes (10^7 cfu/g) added to the normal flora, there was an initial aerobic plate count of 10^8 total microorganisms. Irradiation processing reduced the total numbers of microorganisms present in the crawfish tail meat but at the maximum dose of 4 kGy still did not reduce to zero all of the bacteria after 21 days (Fig.1). The microbial load of this particular batch of crawfish seemed unusually high with the background count of 10^7 microorganisms before the listeria was added. However, even with an irradiation treatment of 1 kGy, the numbers of microorganism in the crawfish were reduced to acceptable levels. The concentration of Listeria that is believed to pose a health threat in otherwise healthy humans is unknown but estimated to be near 10^6cfu/g food and in primates it is higher (10^8). Enumeration of Listeria alone indicated an initial reduction of 1-2 log cycles with each 1kGy dose of irradiation, however, the 10^7cfu/g inoculum was reduced to near zero only in the initial subculture with 4kGy of gamma irradiation (Fig 2). After 7 days storage it was apparent that more of the Listeria cells were able to recover with 10^2-10^3cfu/g present on the 7 and 14 day subculture, respectively. The reduction of aerobic bacteria and Listeria by several log cycles with as little as 2 kGy gamma irradiaiton indicate that this process was successful in reducing microbial populations to a presumed safe level and maintain them at at this level for up to 3 weeks.

The second experiment using an inoculation of 10^4 cfu/g of Listeria was successful in reducing the Listeria population to zero following 2 kGy or greater dose of ionizing radiation(Fig 3). Huhtanen et al. reported similar results with chicken. This study indicated that a dose of 2 kGy was sufficient to destroy 1 x 10^4cells of L. monocytogenes. The greatest number of Listeria found in fresh seafood through market survey has been 10^2cfu/g(Dorsa, W.M. 1992. Department of Food Science, Louisiana State University). Therefore it was feasible to use a 10^4 inoculation as an

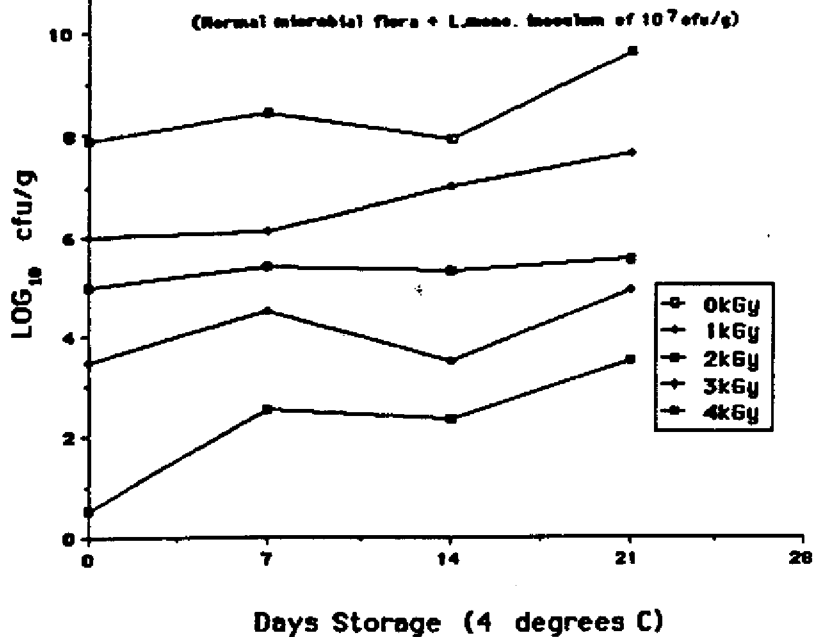
FIG.1: AEROBIC PLATE COUNTS FOR
FRESH FROZEN CRAWFISH TAIL MEAT
LOG10 cfu/g
0
2
4
6
8
10
12
0kGy
1kGy
2kGy
3kGy
4kGy
0
7
14
21
28
Days Storage (4 degrees C)

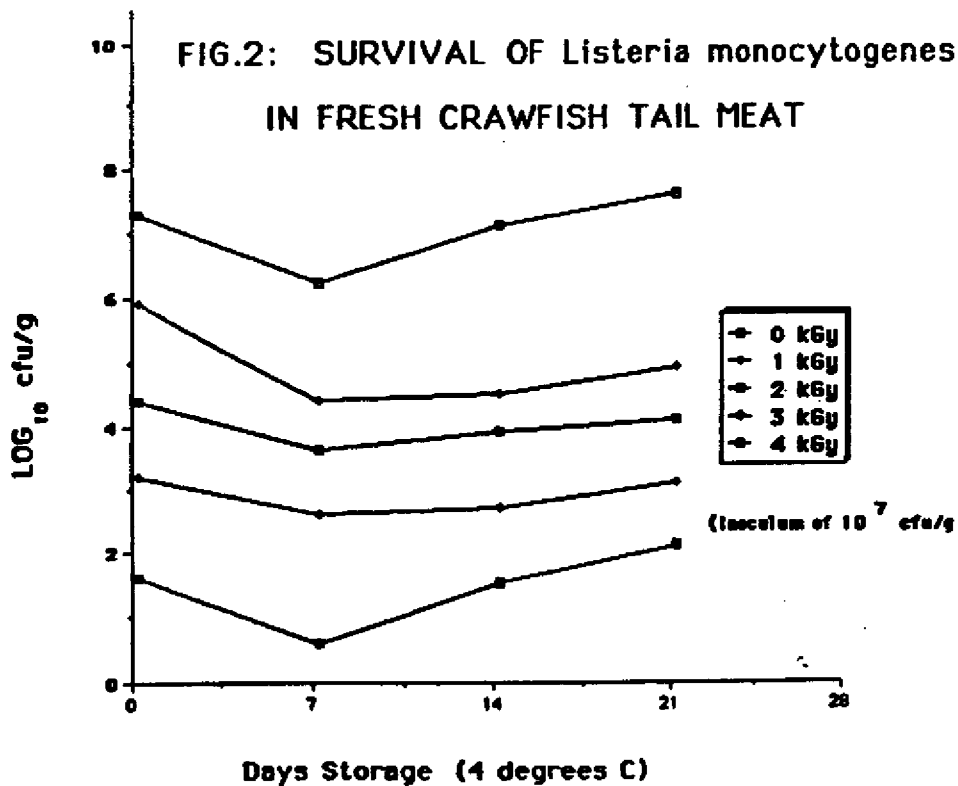
FIG.2: SURVIVAL OF Listeria monocytogenes
IN FRESH CRAWFISH TAIL MEAT
LOG10 cfu/g
0
2
4
6
8
10
0 kGy
1 kGy
2 kGy
3 kGy
4 kGy
0
7
14
21
28
Days Storage (4 degrees C)

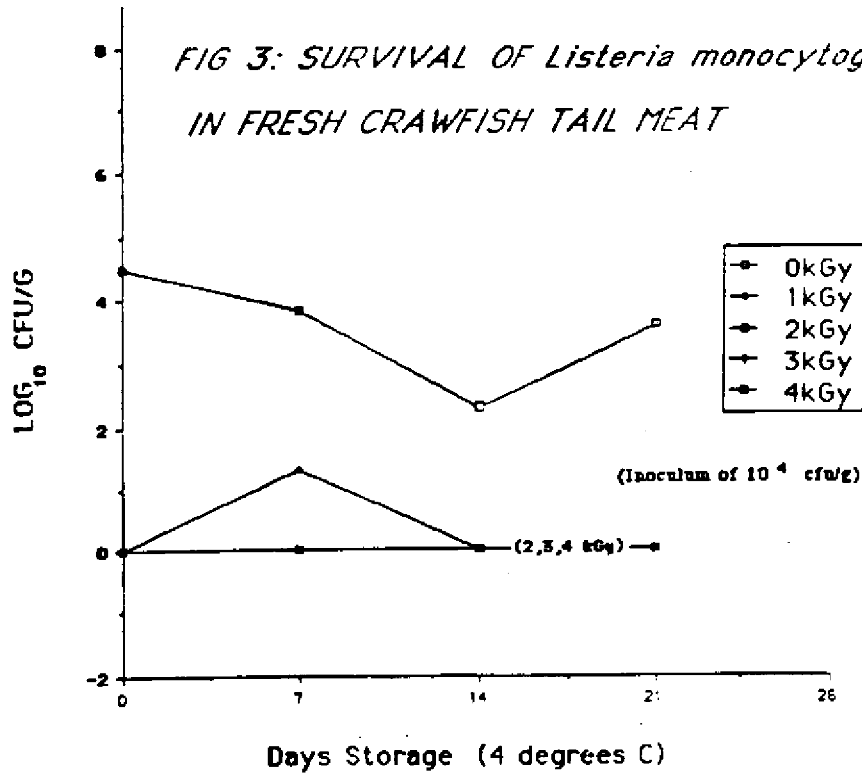
FIG 3: SURVIVAL OF Listeria monocytogenes
IN FRESH CRAWFISH TAIL MEAT
LOG10 CFU/G
0kGy
1kGy
2kGy
3kGy
4kGy
(Inoculum of 10 4 cfu/g)
(2,3,4 kGy)
Days Storage (4 degrees C)

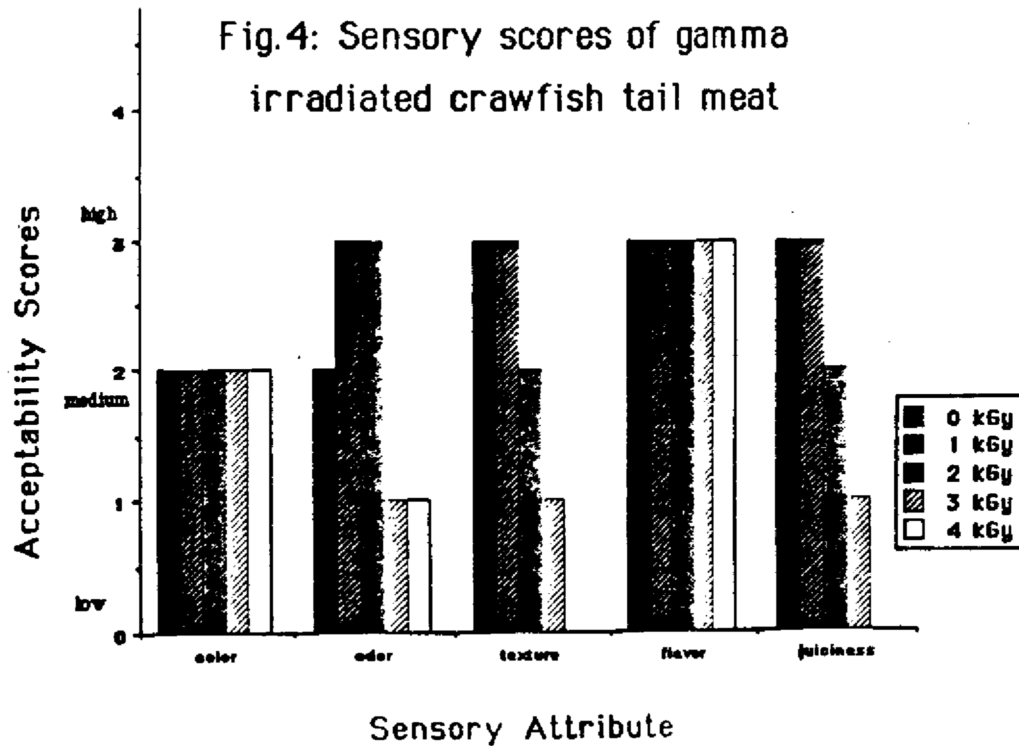
Fig.4: Sensory scores of gamma
irradiated crawfish tail meat
Acceptability Scores
high
medium
low
0 kGy
1 kGy
2 kGy
3 kGy
4 kGy
color
odor
texture
flavor
juiciness
Sensory Attribute

experimental level since it was greater than the actual probable risk. It was interesting to note that the Listeria was initially reduced to zero cfu/g with 1 kGy dose of irradiation at 0-day, however a few cells must have survived at this level because after one week of refrigerator storage a few colony forming units were recovered on the Oxoid Selective Media (Fig 3). No Listeria was recovered from irradiated crawfish after 2 and 3 weeks.

Effects of Irradiation on Sensory Quality

When evaluating the effect of a process on a food matrix, one of the important things to consider is the effect of the process on the sensory quality of that food. The levels of irradiation necessary to reduce or eliminate the more resistent bacteria like Listeria were shown to be very close to the threshold of sensory quality preference. Although color and flavor were evaluated the same for the control and all irradiation treatments (Fig.4), there was a marked reduction in acceptability of texture and juiciness as the irradiation dose passed 2 kGy. The fishy odor of crawfish was actually improved with 1-2 kGy but then was scored lower with higher doses due to the perceptability of browning from possible Maillard Reactions in the chemical matrix of the crawfish tail meat.

CONCLUSION

The results of these experiments indicate that gamma irradiation dosages at or below 2 kGy will be able to eliminate or reduce potentially harmful levels of Listeria monocytogenes to safe levels in seafoods. In addition, the extension of shelf-life of fresh seafoods, stored on ice, can be enhanced for up to 3 weeks.

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IMPROVING SEAFOOD SAFETY AND QUALITY IN PUERTO RICO AND THE US VIRGIN ISLANDS

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Most regulatory activity and legislative proposals to address the emerging concerns for seafood safety and quality have not addressed these issues as they occur in the most troublesome areas. A fundamental characteristic of the seafood industry in this region is, that with the exception of the tuna canning firms in Puerto Rico, these territories do not harbor any other important seafood processing operations. Since the local fisheries do not meet consumer demand, the seafood commerce on these islands depends mostly on imported products. While a significant portion of imports are transhipped from the mainland United States, others come directly from the country of origin. In 1989, Puerto Rico landed a total of 2,305,004 pounds of fish and shellfish (2) Matos et al., 1990). Total imports for that same year, excluding the tuna canneries imports, according to figures supplied by the Puerto Rico Planning Board, amounted to approximately 57,168,118 lbs (3).

Fishing in these islands is mostly artisanal and carried out, on the average, in small open dories that do not venture too far from shore. Larger boats are also being utilized, especially in the west coast of Puerto Rico. Processing of the harvest usually goes only as far as gutting and scaling. State and federal regulatory surveillance programs are typically based on inspection for processing, such as the Hazard Analysis Critical Control Points (HACCP) concept in the proposed National Model Seafood Surveillance Program. Local seafood production in Puerto Rico and the US Virgin Islands is usually not subject to inspection by current regulatory programs. Tuna canneries, aquaculture products destined for exportation and imported products coming directly from the country of origin get inspected by the Food and Drug Administration. However, the meaningful portion of transhipped products from US production or by prior clearance through US customs is not typically subjected to further surveillance as it enter these territories.

Ciguatera and scombroid poisoning add to the problems confronted by the seafood industry in these areas. Although in 1991 there were only 33 ciguatera's cases reported to the Puerto Rico Health Department (Anabel Santiago, 1992, Puerto Rico Department of Health, Epidemiology Division); the coverage that the news media gives to these cases is typically exaggerated and out of proportion. Reports stating that Puerto Rico, the US Virgin Islands, Guam and Hawaii are responsible for 49% of the cases of seafoodborne illnesses in the United States (1) negatively affect the industry. An additional seafood safety problem in these islands is the consumption of locally produced oysters and clams since there are no restrictions concerning the quality of the water from where they are harvested.

PREVIOUS RELATED WORK

A considerable amount of effort from the University of Puerto Rico's Sea Grant Advisory Service has gone into improving seafood quality and safety on the island. The emphasis has been directed to each link in the seafood distribution chain, from harvester to consumer, including restaurant and retailers personnel. Correct product preservation and handling, sanitary requirements, quality attributes, safety problems, selection and storage are among the topics that have been addressed. Special seminars emphasizing health related problems associated with eating seafood products and how to prevent them have been offered to the Puerto Rico Health Department's inspectors. Informative brochures about ciguatera, histamine poisoning and the risks of consuming raw seafood products have been prepared and are distributed free of charge. To deal with the problem posed by the consumption of local raw molluscan shellfish, the advisory service has recently submitted a proposal for S-K funds.

Assisted by the Sea Grant Program and the FDA, on November 1989 in San Juan, the Southeastern Fisheries Association of Tallahassee, Florida, conferred with the Puerto Rican's seafood industry as well as local and federal agencies on matters relating to seafood safety and quality. This forum recognized the limitations for regulations and identified immediate needs for additional education and inter-program communication (4). The current proposed project is an answer to the needs perceived at that gathering. Sea Grant's marine advisors helped coordinated and participated at a workshop given by the National Fisheries Institute and the National Marine Fisheries Service in San Juan on May 1990. Recommendations from this meeting, and an earlier one held in St. Thomas were later used in creating a Caribbean inspection program based on the HACCP concept.

PROJECT DESCRIPTION AND OBJECTIVES

This joint effort between the Sea Grant College Programs of the University of Puerto Rico and the University of Florida offers timely and innovative approaches to address the pertinent seafood safety and quality issues as they occur in Puerto Rico and the US Virgin Islands.

The proposed method will deal with the primary problems as they occur in PR and US Virgin Islands taking into consideration the traditional practices and economic realities of the geographic settings. The education/demonstration technique combines the established and experienced advisory expertise from Fl, PR and US Virgin Islands for immediate and continuing support in a manner that does not require additional regulatory imposition or significant industry restructure. The work intends to develop materials and specific programs for improvements that enhance the security and value of the local seafood industries and associated community commerce. Positive regional consequences for consumer welfare, industry values and community enhancement will likewise reflect on the national assessment for seafood safety and quality.

The suggested university-industry-regulatory partnership intends to resolve the major problems in seafood safety and quality in Puerto Rico and the U.S, Virgin Islands through buyer-supplier education. The task objectives will be: 1) to influence suppliers and buyers through an educational program and 2) to involve the regulatory agencies and the seafood industry in the development and creation of tools to be used in the educational program. The project will thus generate a 1) a seafood safety and quality advisory committee, 2) a buyer code to direct product procurement with safety and regulatory considerations, 3) a regulatory compendium for the seafood industry and 4) training for seafood buyers and respective local regulatory agencies.

METHODOLOGY

The methods chosen to address the prevailing seafood safety and quality to problems in Puerto Rico and the US Virgin Islands are based on proven approaches used in Florida and elsewhere, and modified to suit the unique situations in the respective territories. The premise is to make improvements through buyer education and align a partnership of concern between the principal industries, regulatory authorities and educational institutes. Unique factors which must be accounted for in planning or reviewing the project work are:

a) The main seafood industries with ability to affect seafood quality and safety in Puerto Rico and the US Virgin Islands are not processors or local fishermen. With the exception of tuna canneries based in PR and exporting, there are not other seafood processors in these territories. Local fishermen can indeed contribute to seafood quality and safety problems, i.e., ciguatera, histamine poisoning, raw molluscan-borne illnesses, etc., but due to their small numbers, responsiveness and limited production, they are best influenced through buyer specification.

b) The major portion of the seafood consumed in Puerto Rico (over 95%) and the US Virgin Islands comes from the outside. Prior lack of scrutiny and regulatory surveillance has created a partial reputation for the island territories as "dumping grounds" for inferior products.

c) Regulatory-Industry forums conducted in 1989 in Puerto Rico witnessed significant enthusiasm for a partnership in addressing their seafood quality and safety problems.

d) Main commercial outlets for seafood purchases are supermarkets and restaurants, the latter of which is linked to the economically important tourism.

Keeping these factors in mind the proposed methods are to generate and utilize educational tools to affect "buyer influence". Since buyers lack sufficient knowledge in order to screen quality and prevent safety problems; information in the form of an easy to use Product Code or Guide and a Regulatory Compendium will offer a uniform and long term educational approach.

Product Code

The code concept, as originally introduced by the Southeastern Fisheries Association has proven effective in influencing product quality in US supermarket and other retail settings. The same concept will be modified for PR and the US Virgin Islands but with more emphasis on product safety and inclusion of the specific products of major commerce in the territories. Development will proceed with initial drafts of each category by the project investigators, with reviews by the advisory committee and selected expertise, and finally an acceptance by the advisory committee affirmation. Consultation with SFA will assure complimentary efforts.

The code or guide will be formatted for the educational level of the intended audiences of buyers and will outline basic product quality and safety criteria for correct product selection and evaluation. The layout will be tabulated for easy reference and its format will include sections for fish, mollusk and crustaceans (imported as well as local) and a separate part for ciguatera and histamine poisoning. Under each entry, information such as product description, how it is fished, its market forms, sizes and grades, its quality attributes, as well as facts on packing and labeling will be given. Entries will be illustrated
when necessary, supplemented with pertinent regulations and referenced for substantiation with recommendations and additional reading.

Regulatory Compendium

The regulatory compendium will be developed in similar review and affirmations fashion as outlined for the product code. The intended design is a complete, yet condensed reference for all pertinent regulations and regulatory authorities affecting seafood safety and quality in PR and USVI. The scope will range from health and processing concerns to epidemiology and public advisories. The compendium will not address fisheries management unless a particular fisheries regulation is specifically related to a food safety and quality issue. The compendium will attempt to include pertinent and emerging issues with importation, aquaculture, and recreational harvest destined for commerce.

Advisory Committee

The vital element in this project will be the advisory committee which will be made up of representatives of the seafood industry and regulatory agencies. Composition of the committee will assure a balance of the represented sectors. One committee for Puerto Rico and another for the US Virgin Islands will be needed.

All work, including participation at the workshops, will occur with the advisement and some specific affirmations from each Regulatory-Industry Advisory Committee. Committee meetings will initiate the project work, monitor progress through continuous correspondence and biannual forums, and align with the final workshops.

Workshops

The educational materials, Product Code and Regulatory Compendium will be used in formal workshops for the industry and any regulatory professional dealing with seafood safety and quality. Projections suggest 2 to 4 workshops will be occurring during the second project year. The number will depend on audience size, composition, and location, so identified as the project progresses. Initial requests have called for open workshops for all industries and custom workshops for selected segments of the industry such as, larger supermarket chains, trade associations and regulatory agencies.

The workshops will consist of 1 to 2 days in classroom instruction complimented with actual hands on training. The major emphasis will be hands-on and demonstrations. Instructors will include project staff and representatives from the industry and regulatory agencies.

An essential element of the workshops will be the "certifications". Each participant will receive some form of certification to denote their completion of further seafood specific training. This certification will be offered in an attempt to encourage more long term utility and appreciation of the educational approach; this approach could evolve into an established university/regulatory based seafood quality and safety certification program.

ANTICIPATED BENEFITS

The most obvious users and beneficiaries will be the primary buyers of seafood. The educational materials and training sessions, by generating a better educated buyer, will positively affect the **quality** and **safety** of local and imported seafood products reaching the consumer. Reduction in the cases of seafoodborne illnesses will in turn impact the tourism and local retail trade. The ultimate beneficiary, the consumer, will influence the value of the seafood industry and related commerce in this region and the associated commerce based in the continental United States. As with all educational approaches, immediate benefits are difficult to quantify. Expectations are, 1) incorporation and increased use of product specifications, 2) altered commercial practice in product choice, handling and marketing, 3) more industry and regulatory

participation and alignment, 4) increased opportunities for emerging products of aquaculture, and 5) an improved quality and safety reputation that can impact on tourism as well as local retail trade. These developments will not be evidenced until the project is complete. The potential continuation of the "certification" workshops can document participants and monitor the development through these participants.

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APROVECHAMIENTO INTEGRAL DE LA LANGOSTA *Panulirus argus* EN QUINTANA ROO.

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Chetumal, Quintana Roo. México.

El ser humano consume, de la mayoría de los pescados, principalmente los músculos. Las porciones restantes, al igual que cantidades enormes de especies no comerciales, se destinan a alimentos para animales o bien, se tiran al mar. Esto representa más del 50 % del peso de la pesca capturada (15).

La langosta del Caribe contribuye con el 32% del total de la producción en México, haciendo del país el octavo productor mundial (19). La carne extraída de las langostas que se maneja en las plantas de procesamiento se vende congelada, generalmente de las colas. El rendimiento promedio de carne en una operación comercial es aproximadamente 25 %, aunque se considera que el 40 % de la langosta es comestible (8). Hasta la fecha no se ha utilizado integralmente a la langosta en el Sureste; los caparazones casi siempre se descartan al final de la jornada de pesca, representando éstos las dos terceras partes del crustáceo.

Con base en la información proporcionada por la Secretaría de Pesca, la producción de las Sociedades Cooperativas Pesqueras a lo largo de las costas de Quintana Roo fue de 220 782.95 Kg de colas de langosta en la temporada 1989-1990. La más alta producción se observa en la Cooperativa Pesquera "Vigía Chico" de Punta Allen, por lo tanto la cifra de desperdicio de cabezas más alta. El presente estudio está basado en la producción de la mencionada cooperativa, cuya producción de cabezas de langosta podría ser utilizada por la industria y obtener de este recurso alimentos para el consumo humano: harina de langosta y pasta o paté de langosta. Se considerarán las cooperativas del Norte del Estado como ampliación futura a fin de que todas sean beneficiadas con los resultados aquí expuestos. El auge y estabilidad de producción de materia prima está en función directa a las fluctuaciones de la pesquería (22).

MATERIALES Y METODOS

1.- MATERIA PRIMA

Las cabezas de langosta se obtuvieron en Punta Allen, Quintana Roo donde fueron tratadas con bisulfito de sodio al 1 % durante 15 minutos, colocadas en hielo y transportadas.

2.- PRODUCTOS ELABORADOS

a).- HARINA DE LANGOSTA.

Para la elaboración de harina de langosta se utilizaron cabezas de langosta enteras cocidas, que seguidamente fueron deshidratadas y molidas. Se prepararon siete muestras de esta harina (tabla 1), a las que se adicionaron en diferentes proporciones carne de langosta seca y molida obtenida de otras cabezas. Se empleó pulpa de langosta por estar disponible. Sin embargo, ésta puede sustituirse por carne de especies de bajo valor comercial.

TABLA 1. Composición de las mezclas de harina de cabezas de langosta y pulpa seca molida de langosta.

MUESTRA	COMPOSICION	PULPA ADICIONADA (%)
A	100 g harina *	0
B	90 g harina 10 g pulpa	10
C	80 g harina 20 g pulpa	20
D	70 g harina 30 g pulpa	30
E	60 g harina 40 g pulpa	40
F	50 g harina 50 g pulpa	50
G	40 g harina 60 g pulpa	60
H	100 g harina **	0
I	100 g pulpa	100

* harina de cabezas de langosta enteras
** harina de cabezas de langosta sin pulpa

También se prepararon muestras de la harina de cabezas de langosta mezclada con harina de soya (tabla 2) con el fin de comparar la fuente de proteína vegetal con la animal.

TABLA 2. Composición de las mezclas de harina de cabezas de langosta y harina de soya.

MUESTRA	COMPOSICION	SOYA ADICIONADA (%)
I	100 g harina *	0
II	50 g harina 50 g soya	50
III	30 g harina 70 g soya	70
IV	10 g harina 90 g soya	90
V	100 g soya	100

* harina de cabezas de langosta enteras

La harina de soya que se utilizó fue un derivado del procesamiento industrial de la soya, en el cual ésta es tratada para extraer el aceite.

b).- PATE DE LANGOSTA.

Para elaborar un producto como el paté, se utilizó la carne extraída de las cabezas cocidas, la que se mezcló con otros ingredientes y se esterilizó. Se llevaron a cabo cuatro formulaciones diferentes para paté (9, 18); en todas se utilizaron como base 300 g de pulpa de langosta, entonces todos los ingredientes se pesaron en función a dicha cantidad:

FORMULA 1
Pulpa de langosta
Harina de trigo
Agua fría
Mantequilla
Leche
Manteca de cerdo
Sal
Pimienta blanca

FORMULA 2
Pulpa de langosta
Arroz cocido
Aceite vagetal
Leche
Sal

FORMULA 3
Pulpa de langosta
Fécula de maíz
Leche
Sal
Azúcar

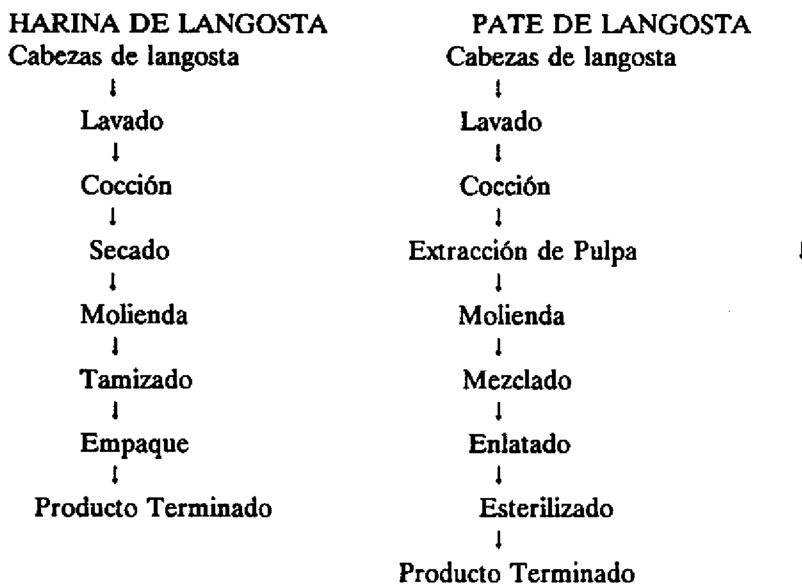
FORMULA 4
Pulpa de langosta
Harina de trigo
Pan molido
Agua fría
Mantequilla
Leche
Pimienta negra
Sal con ajo
Sal con cebolla

c).- APLICACIONES DE LA HARINA DE LANGOSTA.

La harina de langosta puede utilizarse para elaborar diversos alimentos, tanto industriales como caseros. Algunas opciones son: frituras de maíz (churritos) y tortitas fritas (5). Ambos productos se elaboraron utilizando diferentes mezclas con harina de langosta.

3.- PROCESO PRODUCTIVO

Las operaciones básicas (7, 14, 17) que se llevaron a cabo para la elaboración de los productos derivados del cefalotórax de langosta, tienen la siguiente secuencia general:



4.- METODOS DE EVALUACION Y ANALISIS

a).- Harina de Langosta

Tanto a la harina de langosta como a las mezclas de ésta con pulpa de langosta y/o soya se le aplicaron los siguientes análisis:
Humedad, Cenizas Totales, Extracto Etéreo, Proteínas y Fibra Cruda. Todas las técnicas se realizaron de acuerdo a los métodos descritos por la A.O.A.C. (1).

b).- Paté de Langosta

Se sometió a los siguientes análisis (17):
pH, Fuerza de Gel y Organolépticos.

c).- Churritos y Tortitas de Langosta

Organolépticos.

Las propiedades organolépticas de estos productos fueron evaluadas aplicando una prueba panel (8) a un grupo de personas elegidas al azar. Las escalas de evaluación (12) se muestran en las tablas 3 y 4 que a continuación se detallan:

TABLA 3. Escala de evaluación para el análisis organoléptico objetivo.

CALIFICACION	NOTA	DESCRIPCION
7	Excelente	Olor a mantequilla
6	Muy bueno	Leve olor a pescado
5	Bueno	Olor a pescado
4	Regular	Fuerte olor a pescado
3	Menos regular	Leve olor a descomposición
2	Malo	Olor a putrefacción
1	Muy malo	Olor insoportable

TABLA 4. Escala de evaluación para el análisis sensorial de textura aplicado al paté.

NOTACION	FUERZA DE GEL	DESCRIPCION
7	Muy firme	Cuesta romperlo
6	Firme	Elástico fuerte
5	Algo firme	Elástico
4	Mediano	Poco elástico
3	Débil	Inelástico
2	Muy débil	—
1	No produce gel	—

5.- METODOS ESTADISTICOS

Análisis de varianza de una vía fueron aplicados para determinar diferencias en la composición de la harina de cabezas de langosta y las mezclas de ésta con pulpa de langosta. Los análisis se realizaron para Proteína, Grasa, Cenizas y Humedad, con un nivel de probabilidad de 95% (23). Una prueba de contrastes múltiples de Student-Newman-Keuls (test S-N-K) evaluó los

grupos homogéneos entre los tipos de harina, tanto como para Proteínas como en Cenizas Totales; para muestras con número igual de repeticiones y con α = 0.05 (2). Las diferencias entre medias para las mezclas de harina de langosta y harina de soya, con respecto a Humedad, Cenizas, Grasa, Proteínas y Fibra cruda, se hallaron aplicando análisis de varianza de un factor, con diferente número de repeticiones en cada grupo y con α = 0.05 (16). Para este caso, las comparaciones múltiples en los ANOVA se realizaron con las pruebas siguientes: Test de S-N-K con número igual de repeticiones y α = 0.05; Test de Scheffé para todas las comparaciones posibles con diferente número de repeticiones por grupo y con α = 0.05 (2, 16).

RESULTADOS

Las cabezas de langosta no se comercializan, ocasionalmente hay quienes se dedican a extraer la pulpa de las cabezas y la venden a diversos precios por kg (11). Para obtener un kilo de pulpa se deben procesar 28 a 30 cabezas aproximadamente, con talla y peso promedio de 84.5 mm y 280.4 g respectivamente. El rendimiento en esta operación es de 11.57 %, ya que una cabeza de langosta tiene en promedio 32.45 g de carne. La extracción de pulpa se realiza en forma manual, ya que no existe tecnología apropiada, el proceso es difícil y se desecha el caparazón.

El rendimiento de harina es aproximadamente 33.9 %. Siendo un proceso sencillo que aprovecha íntegramente el cefalotórax. Para la temporada de producción langostera 1989-1990 se hubieran tenido 441 565.95 Kg de cabezas de langosta, por lo tanto 149 690.85 Kg de harina de cabezas de langosta.

En la tabla 5 se presentan los valores medios obtenidos del análisis bromatológico de la harina de cabezas de langosta y de las mezclas de ésta con pulpa de langosta. Cada muestra fue analizada por triplicado, excepto para proteína, donde el análisis se realizó por duplicado.

TABLA 5. Resultados del análisis bromatológico de las mezclas de harina de cabezas de langosta y pulpa de langosta.

CLAVE	HUMEDAD (%)	CENIZAS TOTALES (%)	EXTRACTO ETEREO (%)	PROTEINAS (%)	CHOs. (%)
A	8.83	34.83	2.39	40.46	13.49
B	11.33	34.16	1.75	41.14	11.62
C	11.00	29.66	2.32	39.78	17.24
D	9.83	26.18	2.26	42.51	19.22
E	14.16	23.66	3.56	42.20	16.42
F	14.33	23.00	2.93	49.53	1.21
G	11.83	22.50	1.47	50.96	13.24
H	8.50	36.66	0.66	37.46	16.72
I	3.00	10.33	3.75	52.05	30.87

Los ANOVA aplicados a las muestras de harina de langosta y pulpa, no mostraron diferencias significativas para Humedad y Grasa Cruda en un nivel de probabilidad de 95%. Sin embargo, para Cenizas Totales y Proteínas, se hallaron diferencias significativas entre las mezclas. De acuerdo a los valores encontrados en los ANOVA, se realizó una prueba de contrastes múltiples de S-N-K tanto para Cenizas como para Proteínas, obteniéndose los siguientes resultados:

a) Cenizas Totales
H A B C D E F G I

b) Proteínas
I G F D E B A C H

donde los grupos homogéneos están subrayados con la misma línea.

En la tabla 6 se presenta el bromatológico de las muestras de harina de langosta mezclada con harina de soya, asimismo la composición nutricional de la harina de soya. Las muestras fueron analizadas por duplicado.

TABLA 6. Análisis bromatológico de las muestras de harina de langosta mezclada con harina de soya.

MUESTRA	HUMEDAD (%)	CENIZAS (%)	EXTRACTO ETEREO (%)	PROTEINA (%)	FIBRA (%)	CHOs. (%)
I	8.83	34.83	2.39	40.46	0	13.49
II	7.58	18.89	1.57	49.00	3.45	19.51
III	7.30	13.96	1.08	49.06	3.78	24.82
IV	7.24	9.36	0.94	48.13	5.18	29.15
V	6.84	7.05	1.01	46.25	6.45	32.40

El estadístico aplicado se analizó con un nivel de confianza de 95 %, demostrando ser significativo en la mayoría de los nutrientes, excepto para Humedad. En función de los datos obtenidos en los ANOVA, se efectuaron pruebas de contrastes múltiples para cada nutriente; los grupos homogéneos se subrayan con la misma línea:

- Test de S-N-K para número igual de repeticiones por tratamiento.
  a) Proteínas: III II IV V I
  b) Fibra Cruda: V IV III II

- Test de Scheffé con diferente número de repeticiones por grupo.
  c) Cenizas Totales: I II III IV V
  d) Extracto Etéreo: I II III IV V

En la tabla 7 se presentan los resultados organolépticos del paté de langosta, en la cual se observan calificaciones similares en las 4 fórmulas respecto al olor. Para el sabor, la fórmula 1 obtuvo la calificación más baja, del mismo modo esta fórmula tiene el valor más bajo para fuerza de gel. La fórmula 3 mantiene para todos los análisis las calificaciones más aceptadas.

TABLA 7. Características organolépticas del paté de langosta en cuatro fórmulas diferentes.

TIPO	COLOR	OLOR	SABOR	FUERZA DE GEL
1	pardo-rojizo	7	3	3
2	marrón	6	5	5
3	marrón-crema	7	6	6
4	pardo-rojizo	8	5	4

Los valores de pH obtenidos en los cuatro tipos de paté:

FORMULA	pH
1	6.5
2	6.5
3	6.4
4	7.1

DISCUSION

La extracción de pulpa de las cabezas de langosta tiene un rendimiento muy bajo y además de ser un proceso largo y meticuloso, se desecha el caparazón en la producción de paté. Para esta etapa del proceso no existe en el país ni en el extranjero tecnología apropiada disponible. Consecuentemente, la obtención de la pulpa representa un costo elevado en tiempo y mano de obra. Sin embargo, la elaboración de harina de cabezas de langosta representa un proceso sencillo, económico y fácil de aplicar en la dieta humana. No obstante, la producción de paté no puede descartarse, tomando en cuenta que es un producto de mayor aceptación con relación a la harina. Los desechos o carapachos podrían subsanar el costo del paté elaborando con ellos una harina debidamente enriquecida con proteína de fuente animal o vegetal. O bien, podría emplearse como fuente de minerales en alimentos balanceados para animales.

Del análisis proximal de la harina de cabezas enteras (clave A, tabla 5) se observa un porcentaje aparentemente alto de proteína, así como de cenizas totales. De acuerdo a las normas oficiales de calidad establecidas por la SECOFI (20) para harinas de productos del mar destinadas al consumo humano (proteína 50% mín. y cenizas 19% máx.), la harina de langosta se califica como deficiente en contenido proteínico y elevado contenido de cenizas. Por lo tanto, no puede utilizarse directamente. Para las mezclas de esta harina con pulpa de langosta, muestras B, C, D, E y F, se observa que aún permanecen bajos los valores de proteína con respecto al valor mínimo establecido; del mismo modo los valores para cenizas se mantienen elevados. Sin embargo, la mezcla G presenta los valores aparentes más cercanos a los límites establecidos.

De acuerdo a la prueba de S-N-K; para proteína la mezcla G es igual a I y ambas representan el mayor contenido proteico y el más próximo al valor mínimo establecido en las normas de calidad. F es menor que éstas y mayor que el resto de las mezclas, pero no alcanza el valor mínimo para proteína. La muestra H representa el contenido más bajo o similar a C, lo que indica que esta harina ha sido desprovista de la pulpa. Con respecto a cenizas totales, las mezclas E, F y G son iguales y/o similares a D. Estas mezclas podrían emplearse bien, ya que se encuentran dentro del límite establecido para cenizas en harinas de productos del mar, no así para proteínas. Por lo tanto, la mezcla G, con 60 % de pulpa de añadida, es la única aceptable para el consumo humano.

Los valores de humedad y extracto etéreo para todas las muestras se encuentran dentro de los límites dictados por las normas oficiales de calidad (humedad 9% máx. y extracto etéreo 6% máx.).

De acuerdo al análisis de la harina de soya y de las mezclas de ésta con harina de langosta (tabla 6), se puede observar que para humedad no existen variaciones significativas. Respecto al extracto etéreo, las muestras I y II son estadísticamente mayores a las demás; la muestra I corresponde a la harina de cabezas enteras, la cual tiene un contenido de grasa mayor que la harina de soya; se hace hincapié en que la harina de soya utilizada ha sido procesada para extraer el aceite. El contenido de grasa en todas las muestras se encuentra dentro de los límites establecidos. Para cenizas totales o minerales, se aprecia que los valores descienden significativamente al sustituir la harina de langosta por harina de soya (Test de Scheffé); la mezcla II con 50 % de harina de soya, ya se encuentra en el límite máximo establecido para cenizas.

Los valores del contenido proteico entre ambas harinas (langosta y soya) son significativamente diferentes (Test de S-N-K), donde la harina de langosta presenta el valor más reducido. Esto se debe a que las cabezas de langosta contienen sólo el 11.57 % de carne, en consecuencia su composición es mayor en otros compuestos que para proteína; en tanto la soya es una leguminosa de alto valor proteínico (8).

Las mezclas II y III exiben valor similar y significativamente mayor al presentado por alguna de las dos harinas independiente; lo que indica que una mezcla proteica puede incrementar el valor nutritivo de los productos alimenticios (13). Respecto al contenido de fibra cruda, la harina de soya tiene el mayor valor, de igual modo la mezcla IV. Pero las muestras II y III, estadísticamente con igual contenido, tienen valores más bajos. Las mezclas II y III dan lugar a productos con valor biológico bastante alto, aunque el contenido proteínico es el mismo en ambas mezclas, puede elegirse la mezcla II porque contiene mayor cantidad de harina de langosta (50 %), lo que redunda en una proteína de mejor calidad. La calidad de una proteína está dada por la cantidad y proporciones de aminoácidos esenciales que contenga (3). En este caso, la soya es limitante en metionina, por lo tanto, la carne contenida en las cabezas de langosta proporciona un mejor balance de aminoácidos al producto.

Comparando las mezclas a base de harina de cabezas de langosta G (60 % de pulpa) y II (50 % de soya), tablas 5 y 6 respectivamente, observamos que ambas cumplen con los requerimientos nutricionales especificados. A pesar de que la harina G aporta proteína de mejor calidad, el porcentaje añadido de sustituto para establecerse dentro de las normas de calidad es mayor que en II.

Es necesario indicar que la fuente proteica para enriquecer la harina de cabezas de langosta deberá provenir de fuentes más económicas, como la harina de soya. La harina II por lo tanto, cumple con los requisitos señalados; a la vez que reduce el contenido de minerales proporciona un valor notable de fibra cruda, la cual no se encuentra en alimentos de orígen animal, pero que es necesaria para la dieta humana (3).

Con respecto al paté (tabla 7), los cuatro tipos presentan un olor agradable ligero a marisco y es en la fórmula 4 donde se acentúa el olor a mantequilla y se disfraza con aditivos el olor característico del marisco. En las fórmulas 1 y 4 se presentó el color menos agradable, ya que éstas contienen alto nivel de grasas y otros componentes como pimienta, ajo y cebolla. Los lípidos son principalmente los que contribuyen al color rojizo de las mezclas (3). Para el sabor, la fórmula 1 tiene una baja calificación porque la grasa de cerdo añadida predomina deformando los sabores de marisco y de mantequilla. La fórmula 3 presentó la calificación más alta para este concepto. Para textura o fuerza de gel las fórmulas 1 y 4 calificaron bajo, la 2 regular y la 3 calificó alto. La fuerza de gel depende del tipo de ligador utilizado; en las formulas 1 y 4 se emplearon como ligadores harina de trigo y pan molido, los que no contribuyen a la formación de gel tanto como la fécula de maíz empleada en la fórmula 3 (8).

Por lo tanto, la fórmula 3 es la pasta de langosta más aceptable de acuerdo a los mencionados análisis organolépticos. En su elaboración se emplea un mínimo de ingredientes, lo que resulta económico. La fórmula está basada en la composición del kamaboko japonés que utiliza pulpa de pescado de bajo valor comercial
(9).

La pasta puede envasarse en frascos de vidrio esterilizados adecuadamente. No requiere de conservadores ni antioxidantes dado su bajo contenido de grasa (7). La fécula de maíz puede ser sustituida por un ligador de menor valor económico, pero de igual o mayor capacidad gelificante; como fécula de papa u otros almidones modificados (10).

Los productos a base de harina de langosta (churritos y/o tortitas fritas) deben tener un contenido bajo de la misma, entre 10 - 15 % aproximadamente, ya que el sabor resulta demasiado fuerte para porcentajes mayores añadidos. Se obtuvieron mejores productos empleando la mezcla G en su elaboración.

CONCLUSIONES

La harina de cabezas de langosta no puede ser aplicada directamente al consumo humano, ya que no cumple con los requisitos de calidad establecidos. Esta harina puede incluirse en la dieta humana si es debidamente adicionada con proteína de otra fuente, como harina de soya, la que a tiempo de incrementar el nivel proteico reduce notablemente el contenido de minerales. Del mismo modo proporciona una cantidad adecuada de fibra. La composición óptima se obtuvo con la siguiente mezcla:

-Harina de cabezas de langosta 50 %
-Harina de soya 50 %

Este tipo de harina resulta ideal en la elaboración de diversos productos alimenticios como son tortitas fritas y frituras de maíz. El paté de langosta puede elaborarse adecuadamente a menos que se utilice la tecnología apropiada para extraer la carne; el cefalotórax se puede aplicar en la fabricación de piensos, como fuente de minerales o bien, adicionarse con pulpa de pescado de bajo valor comercial y/o soya hasta obtener la composición óptima para el consumo humano.

Los resultados obtenidos permiten iniciar la planificación del aprovechamiento integral de la langosta, dando las bases tecnológicas para el desarrollo eventual de una industria con estos objetivos. La puesta en marcha de esta industria involucrará el desarrollo tecnológico-pesquero en el Estado; la generación de empleos y la utilización de alimentos no convencionales, entre otros indicadores de impacto social y ambiental.

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ANEXO

INVESTIGACION DE MERCADO.

Dentro de este paquete se analizaron los siguientes puntos: Mercado de Consumo, Mercado de Abastecimiento y Aspectos T écnicos.

De acuerdo a las investigaciones de campo y de gabinete realizadas tanto en la ciudad de Chetumal, Q. Roo como en Mérida, Yuc., se obtuvo información al respecto. El producto tendrá un canal de comercialización directo, ya que reduce al mínimo costos para la industria. La forma de pago será a crédito con períodos de 30 días y la comercialización se llevará a cabo por pedidos. Analizando la demanda y la oferta de los productos competitivos se llegó a la conclusión que el producto tiene cabida definitiva en el mercado proyectado. Se espera obtener un margen de utilidad entre el 15-20%.

La harina de langosta no tiene competencia en el mercado regional. Salvo los productos de pescado seco-salado que se comercializan esporádicamente en algunas tiendas aisladas. Para este producto se requerirá un doble esfuerzo en promoción y propaganda, y se considera que su introducción a la dieta popular en la Península de Yucatán será lenta y a largo plazo.

La materia prima para abastecer a la procesadora de cefalotórax de langosta se encuentra disponible en un 95% aproximadamente. Se consideran algunos altibajos para ciertos productos como: cabezas de langosta cuya adquisición se debe ajustar al período de pesquería de langosta, ya que cada año se extiende la veda para este recurso durante cuatro meses.

La planta procesadora de cabezas de langosta se ubicará en la Sociedad Cooperativa P. de "Vigia Chico" en Punta Allen, Q. Roo. El poblado tiene vías de comunicación accesibles para el transporte de materia prima y producto terminado. Los trabajadores de la planta serán gente del mismo poblado, obteniendo mayores beneficios los pescadores al recepcionar el producto que antes tiraran al mar.

La maquinaria podrá encontrarse totalmente en la ciudad de Mérida, Yuc., no siendo necesaria la adquisición de equipo auxiliar. El programa de ejecución y puesta en marcha contempla 16 semanas a partir de la cotización de maquinaria hasta la etapa de operación.

CHEMICAL, MICROBIOLOGICAL, PHYSICAL, AND SENSORY CHANGES IN ICED AND FROZEN WRECKFISH (*POLYPRION AMERICANUS*)

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INTRODUCTION

The wreckfish, *Polyprion americanus*, has a sea-bass like body with a large rough head. The fish is strongly flattened sideways, with a large mouth. Wreckfish reach lengths of 1.4 m to 1.5 m and can weigh more than 45 kg. Wreckfish range widely on both sides of the North and South Atlantic Ocean. Small fish are normally found under floating logs and wreckage, as the name implies. Larger fish move to the bottom in deeper water (2).

Portugal, France, and Spain accounted for the largest 1989 commercial landings of the fish, 535 mt. Malta and Angola landed an additional 65 mt. New Zealand landings of the related Hapuku Wreckfish (*Polyprion oxygeneios*) were 669 mt in 1989 (7). U.S. commercial landings of *Polyprion americanus* have increased rapidly over the last two years totaling 874 mt in 1991. This seasons landings through August 15, 1992 were 337 mt. Current U.S. fishing regulations limit the yearly catch of wreckfish from U.S. waters to 907 mt or 2,000,000 lb (6).

The crew of the R/V GEORGIA BULLDOG collected 640 kg of wreckfish over a 10 d period in the spring of 1991. Vertical fishing lines attached to hydraulic reels were used to catch the fish. The exploratory fishing grounds were over the Charleston Bump portion of the Blake Plateau, approximately 240-290 km east of Brunswick, GA in 418 m to 538 m of water. Iced storage characteristics of gutted wreckfish and the frozen storage characteristics of packaged fillet portions were investigated.

MATERIALS AND METHODS

Forty-four fish, 28 male and 16 female, were gutted and iced at the time of harvest. Lengths ranged from 800 mm to 1130 mm. Weights ranged from 10.4 kg to 26.8 kg. Mean lengths and weights were 958 mm and 14.5 kg, respectively. Fish were transferred to the University of Georgia Marine Extension Service facility in Brunswick for further study. Newly captured fish reached our facility 4 d after harvest. Gutted wreckfish were monitored through 21 d of iced storage. Random samples were collected at 4, 5, 6, 9, 10, 13, 16, 18, and 21 d after harvest. Sub-samples of whole fish caught on the

same day were evaluated for changes in physical, sensory, chemical, and microbiological characteristics.

Gutted fish were evaluated by the following characteristics: Torrymeter readings (5, 9, 10), sensory analyses (using the Torry Score system) (1, 13), ammonia levels (determined with specific ion electrodes) (16), pH (17), TBA levels (8, 14, 15, 18), plate counts, and psychrotrophic plate counts (8, 14). The Torrymeter was used to objectively measure wreckfish quality during the iced study. The operating principle of the Torry Meter is based on the dielectric constant of the flesh. Electrical properties of finfish change as solutes leak from the cells during product deterioration. The Torrymeter reads the phase angle and converts it into a numerical freshness scale from 0 to 16. The meter was originally developed for whitefish and absolute readings tend to be species specific (1, 12, 13).

Iced Storage

A trained five member sensory panel used the Tory Score system to rate fish quality and to estimate shelf life. Panelists evaluated four major sensory components: general appearance, flesh appearance, odors, and texture (1, 3, 13). The general appearance of the fish were evaluated by the panel on a scale from 5 to 0. A pristine fish rated a score of 5 (Table 1). Fish with a mean rating ≥3 were considered to be of acceptable quality.

Table 1. Torry Scale general appearance ratings for iced fish.

General Appearance	Score
Eyes fresh, convex flat pupil, translucent cornea; bright red gills; no bacterial slime; outer slime water white or clear; bright opalescent sheen; no bleaching	5
Eyes flat, very slight greyness of pupil; slight loss of color in the gills	4
Eyes slightly sunken, grey pupil, slight opalescense of cornea; some discoloration of gills and some mucus; outer slime opaque and somewhat milky; loss of bright opalescene; some bleaching	3
Eyes sunken, milky white pupil, opaque cornea; thick knotted outer slime with some bacterial discoloration	2
Thick knotted outer slime with some bacterial discoloration; eyes completely sunken; shrunken head covered with thick bacterial slime; gills showing bleaching or dark brown discoloration and covered with thick bacterial mucus; outer slime thick yellow-brown; bloom completely gone; marked bleaching and shrinkage	0

Table 2. Torry Scale flesh appearance ratings for iced fish.

Appearance of Flesh	Score
Bluish translucent flesh, no reddening along backbone and no discoloration of belly flaps; kidney blood bright red	5
Waxy appearance, no reddening along backbone, loss in original brilliance of kidney blood, some discoloration of belly flaps	3
Some opacity, some reddening along backbone, brownish kidney blood and some discoloration of the flaps	2
Opaque flesh, very marked red or brown discoloration along the backbone, very brown to earthy brown kidney blood, and marked discoloration of the flaps	0

Flesh appearance was monitored by the panel on a scale from 5 to 0. A 5 rating indicated the best quality flesh (Table 2). Flesh appearance was acceptable when the mean panel score was ≥3.

The panel evaluated iced storage odors on a scale from 10 to 0 (Table 3). Prime quality fish have seaweedy odors that gradually disappear. The minimum acceptable raw odor score was a mean value ≥5.

Table 3. Torry Scale raw odor ratings for iced fish.

Odors	Score
Fresh seaweedy odors	10
Loss of fresh seaweediness, shellfish odors	9
No odors, neutral odors	8
Slightly musty, mousy, milky or caprylic acid like odors, garlic, peppery odors	7
Bready, malty, beery, yeasty odors	6
Lactic acid, sour milk, or oily odors	5
Some lower fatty acid odors, grass, "old-boots"	4
Stale cabbage water, turnipy, sour sink, wet matches, phosphine-like odors	3
Ammoniacal (Trimethylamine) with strong byre-like odors	2
Hydrogen sulphide and other sulphide odors, strong ammoniacal odors	1
Indole, ammonia, fecal, nauseating, putrid odors	0

Table 4. Torry Scale texture ratings for iced fish.

Texture	Score
Firm, elastic to the finger touch	5
Softening of the flesh, some grittiness near tail	3
Softer flesh, definite grittiness, scales easily rubbed off	2
Very soft and flabby, retains finger indentations grittiness quite marked and flesh easily torn from backbone	1

Fish texture was rated by the panel on a scale from 5 to 0 (Table 4). A 5 rating indicated firm and elastic flesh. Minimum acceptable flesh texture was defined as a mean score ≥2.

Ammonia and pH levels were determined with specific ion electrodes (16, 17). Samples for total plate counts and psychrotrophic plate counts were incubated on standard method's agar for 48 h at 37°C and at 7°C for 10 d, respectively (8, 14). Oxidative changes and rancidity development were estimated through TBA determinations. Distillation, color development, and spectrophotometric analyses were used to determine TBA levels (15, 18).

Frozen Storage

For the frozen packaging part of the study, portions of fresh fillets were packaged in the following materials: (i) Saran[R] wrap (PVDC, polyvinylidene chloride film, Dow Chemical Co., Indianapolis, IN); (ii) film-to-film with Trigon ISPF (oxygen-barrier Intact[R] Skin Packaging Film, ISPF, using a Trigon RM331 Mark III Mini Intact[R] machine, Koch Suppliers Inc., Kansas City, MO), (iii) film-to-film with Cryovac surlyn (Cryovac Corporation, Duncan, SC), and (iv) barrier pouches (Cryovac P640B with nylon base, Saran[R] barrier, and low density polyethylene sealant, Cryovac Corporation, Duncan, SC). Fish used in the frozen study were filleted 4 d after harvest and gutting. The fillets were cut into smaller portions, packaged, and then frozen for 9 mo at ≤-17°C. Cryovac P640 barrier pouches were used in the study because they are readily available and their competitive pricing makes them a popular commercial choice for packaging frozen seafood. However, Cryovac does not recommend their use for fish packaging because they can be punctured by bones (4).

Frozen wreckfish were evaluated at 3, 6, and 9 mo. The study was ended at 9 mo because of freezer failure and subsequent loss of samples. Torrymeter readings were dropped from the fillet analyses and sensory evaluations of cooked flesh were added. Sensory panelists evaluated both odor and flavor attributes using the Torry Scale for cooked fish. Fish with a score of 10 have strong seaweedy odors that are gradually lost (Table 5). Sensory ratings range from 10 to 0. Cooked odor was no longer acceptable when the mean panel rating was <5.

Table 5. Torry Scale cooked odor ratings for iced fish.

Cooked Odor	Score
Strong seaweedy odors	10
Some loss of seaweediness	9
Lack of odors, neutral odors	8
Slight strengthening of odor but no sour or stale odor, wood shavings, vanillin or terpene-like odors	7
Condensed milk, caramel or toffee-like odors	6
Milk jug odors, boiled potato or boiled clothes-like odors	5
Lactic acid and sour milk or byre-like odors	4
Lower fatty acids, some grassiness or soapiness, turnipy or tallowy odors	3
Ammoniacal (trimethylamine) odors	2
Ammoniacal (trimethylamine) with some sulphide odors	1
Strong ammonia, fecal, indole, and putrid odors	0

Table 6. Torry Scale flavor ratings for iced fish.

Flavor	Score
Fresh sweet flavors characteristic of the species	10
Some loss of sweetness	9
Slight sweetness and loss of flavors characteristic of the species	8
Neutral flavor, definite loss of flavor, but no off-flavors	7
Absolutely no flavor, as if chewing cotton wool	6
Trace of off-flavors, some sourness, but no bitterness	5
Some off-flavors and some bitterness	4
Strong bitter flavors, rubber-like flavor, slight sulphide-like flavors	3
Strong bitterness but not nauseating	1
Strong off-flavors of sulphides, putrid, tasted with difficulty	0

Cooked flavors were rated on a descending scale ranging from 10 to 0 (Table 6). Fresh sweet flavors that are characteristic of the species receive a rating of 10. Cooked flavor was not considered acceptable if the mean sensory value was <5.

RESULTS AND DISCUSSION

Iced Storage

Microbiological and sensory shelf life limits for iced fish are summarized in Table 7.

Table 7. Sensory shelf life limits for iced fish.

Microbiological and Sensory Attributes	Shelf Life Limits
Aerobic Plate Counts	10^7CFU/g
General Appearance	<3
Flesh Appearance	<3
Odors	<5
Texture	<2

The log of aerobic plate counts for iced fish samples showed an estimated shelf life in excess of 21 d, the length of the study. Elevated psychrotrophic plate counts were detected by the tenth day of storage. Panel members determined that shelf life based on flesh appearance spanned 16 d. Sensory analyses using the Torry Scoring System indicated a 16 d shelf life based on the overall appearance and odor of wreckfish. Psychrotrophic populations then decreased on day 12, only to increase through day 18 (Fig. 1). Flesh texture was acceptable for 18 d (Fig. 2).

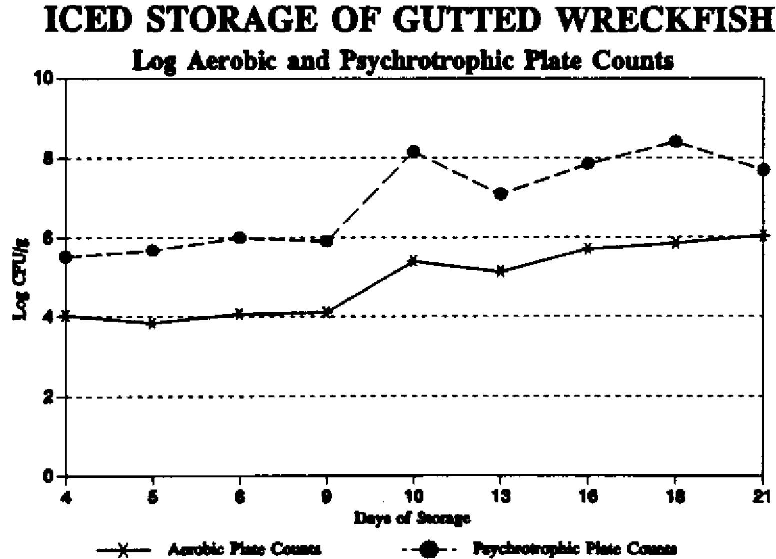

Figure 1. Microbiological levels in iced wreckfish during 21 days of iced storage.

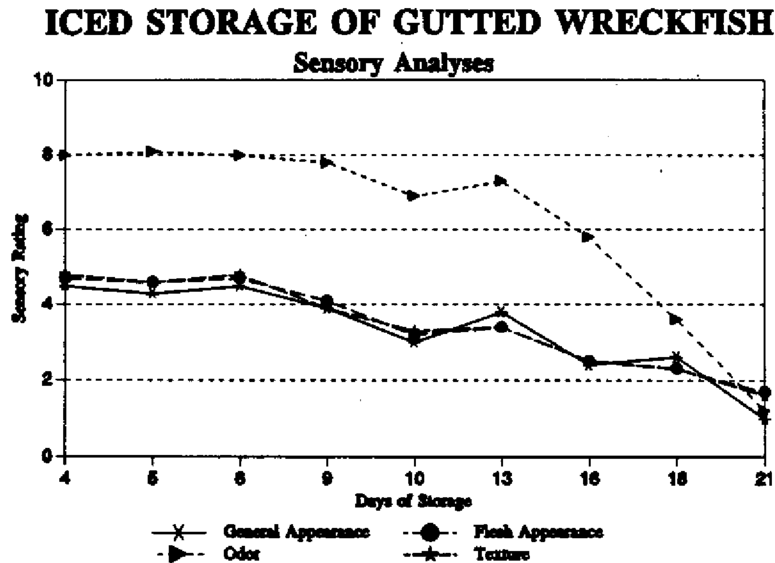

Figure 2. Torry Score sensory ratings of iced wreckfish during 21 days of iced storage.

Ammonia and pH measurements showed no consistent changes over 21 d of storage (Fig. 3). The first Torrymeter reading was taken at 4 d of iced storage. The initial reading was 6.8. Torrymeter scores declined slowly through 13 d of iced storage. Readings decreased much more rapidly following 13 d of storage, reaching a minimum value of 2.3 on day 18. The initial mean Torrymeter reading of 6.8 was low compared to readings obtained from the literature for several other species of fish found off the southeastern coast of the United States (Fig 3). In 1977 Gates and Miller (9) monitored Torrymeter readings for croaker, gray trout, bluefish, and menhaden held on ice for 20 d. Croaker and trout maintained definitely higher scores than wreckfish throughout the 20 d period. Bluefish

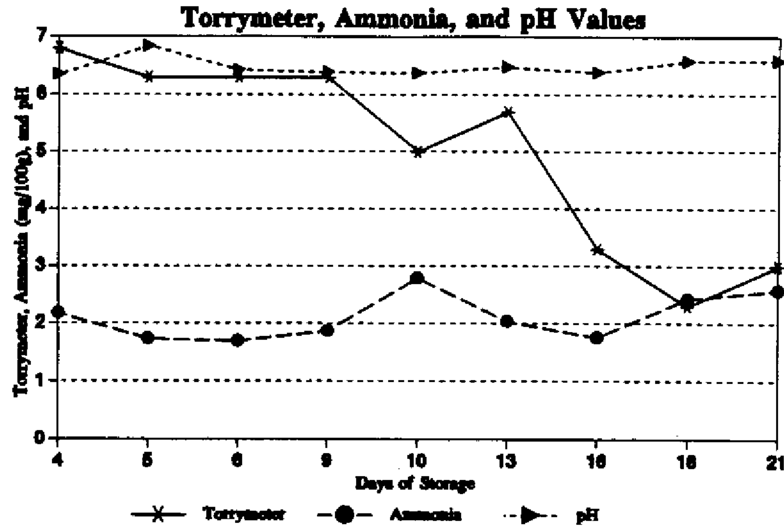

Figure 3. Torrymeter, ammonia, and pH levels determined for wreckfish during 21 days of iced storage.

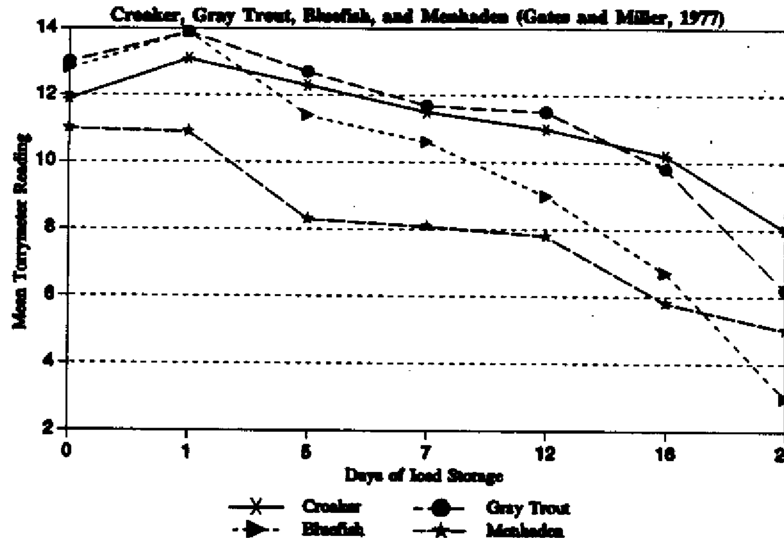

Figure 4. Torrymeter ratings determined for croaker, gray trout, bluefish, and menhaden during 20 days of iced storage (9).

and menhaden torrymeter readings approached wreckfish levels at the end of 20 d (Fig. 4). In 1979 Constantinides and Jhaveri (5) conducted a 15 d iced storage study of flounder, whiting, scup, and butterfish. Current Torrymeter readings for wreckfish were similar to results obtained for whiting and butterfish (Fig. 5). Hegen's 1981 (10) study of trout, red drum, black drum, sheepshead, and flounder produced higher Torrymeter readings for all investigated fish than those determined for wreckfish (Fig. 6).

The Torrymeter was developed to evaluate whitefish, with the readings correlated to the raw odor or appearance scores determined by the Torry Score System. Howgate, 1982, (11) compared Torrymeter

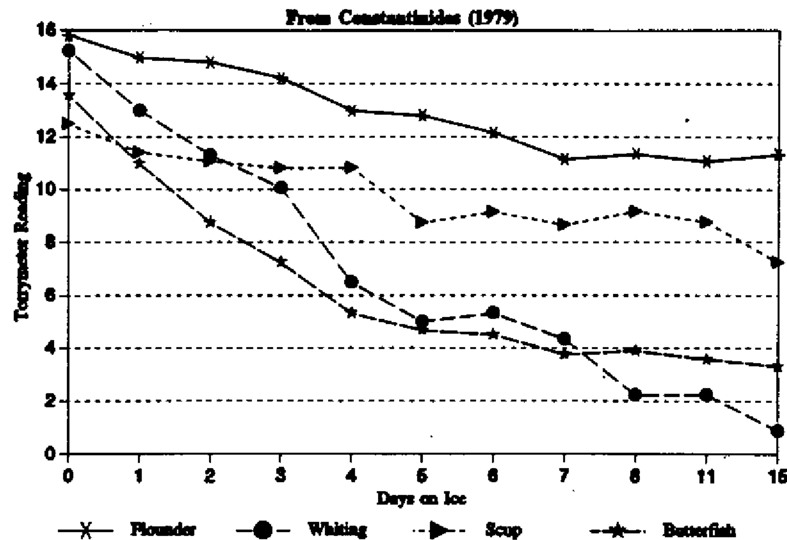

Figure 5. Torrymeter ratings determined for flounder, whiting, scup, and butterfish during 15 days of iced storage.

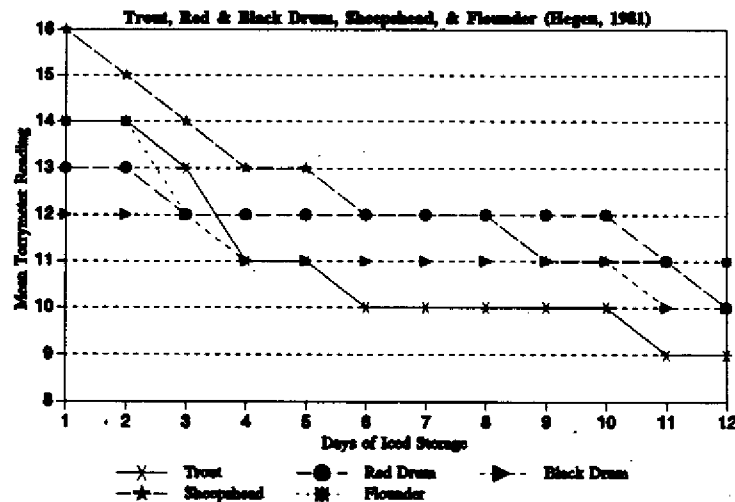

Figure 6. Torrymeter ratings determined for trout, red drum, black drum, sheepshead, and flounder during 12 days of iced storage (10).

readings with cod sensory data. Iced storage for 20 d produced a straight line relationship. Wreckfish from the current study produced less predictable results. The slopes of wreckfish and cod Torrymeter vs Torry Sensory results were 1.22 and 1.68, respectively. Ammonia and pH levels did not show any significant trends.

Several of the monitored parameters developed statistically significant correlation coefficients when compared to iced storage day. Log values of both plate counts and psychrotrophic plate counts had the highest correlation coefficients with storage time.

The correlation values were 0.970 and 0.935, respectively. Flesh texture and flesh appearance sensory ratings had the next highest correlation coefficients at -0.908 and -0.896. Torrymeter ratings correlated well with storage day, producing a coefficient of -0.861. General appearance and odor correlations were -0.858 and -0.801, respectively.

No significant differences were determined for fillets held in the 4 packaging types during 9 mo of frozen storage.

CONCLUSIONS

The panel sensory ratings limited the shelf life of iced wreckfish to 16 d. The following parameters had high correlation coefficients with iced storage day: Torrymeter readings, plate counts, psychrotrophic plate counts, and sensory analyses.

ACKNOWLEDGEMENTS

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Use of Computer Simulation in Aquatic Food Science and Technology

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INTRODUCTION

One major concern in experimental research is the problem of choosing the right components for the experiment. This is an extremely hard task for the researchers; the main difficulty is the large number of possibilities involved. For example, a problem with N components would yield N! (N factorial) possibilities.

If each of these components is a variable then the number of possibilities becomes so large that it would be impossible in many cases to select the right variables for a particular problem using the conventional techniques - most of these techniques are essentially based on trial and error strategy.

Due to factors such as wrong choice of components, variables, and tools, a large subset of these experiments do not yield any useful results. The work outlined in this paper shows how costs can be reduced by SELECTING only those
experiments that have the highest potential of success. With todays advanced software and hardware technology, this selection can be done with the aid of Computer Simulation.

We describe our approach together with a typical problem in Food Science which is undergoing at The University of Georgia. However, the strategy described is as applicable (and in many cases more so) to other problems including the study of different diets on human, determining the side effects of particular drugs on different parts of human organs, and feasibility studies and validity tests of engineering designs.

AN EXAMPLE - A RESEARCH PROBLEM IN FOOD SCIENCE

Farm-raised catfish have recently gained popularity in USA market. The per capita consumption of catfish in 1989 was 0.3 kg. This level of consumption placed channel catfish as the fourth most popular fish in the United States ranked behind tuna, cod, and Alaska pollock.

The increase in consumption of catfish emphasizes that production of catfish with good storage characteristics throughout the delivery system is important to the industry. However, in recent years, the increase in fat content in fish coming from production ponds has been identified as a trend. It has been reported that the amount of body fat in processed farm-raised channel catfish averaged 8% in the early 1970's and 12% in th early 1980's. Researchers subsequently found that body fat in catfish could be affected by feed composition. (refer to Huang et al. (1991, 1992) for a more comprehensive description of this problem).

One problem in farm-raised catfish of interest is to findthe components that affect the storage quality of iced catfish by evaluating chemical, microbial, and organoleptic components.

However, a more fundamental problem is to reduce the fat content of catfish; this has to be investigated before the first problem since experiments have shown that the fat content of catfish has a major impact on the storage quality of iced catfish.

The solutions to the two problems mentioned above can only be found by experiments. In order to set up the experiments, all the components and variables need to be defined so that a subset of these could be chosen for the experiments. As an example, consider the problem of reducing the fat content of catfish. The major components that may affect the fat content of catfish include: the percentage of protein in each feed (P), the quantity of each feed (Q), the temperature of the water in the pond (T), the number of fish in the pond (F), and the duration of experiments (D). Notice that each of the components, P, Q, T, F, and D is itself a variable.

For example, P could be any number within the range min and max where min is the smallest and max is the largest numbers that could be used for P (e.g., theoretically 0-100% but in practice 20-40%). Similarly, each of the components Q, T, F, and D has a predefined valid range and so each can have a value within its allowable range.

Now the researcher is faced with the difficult problem of selecting a value for each of the components of the experiment. The number of possible choices is enormous. For example, if there are 20 possible choices in selecting a value for P, 10 possible choices for Q, 5 for T, 1000 for F and 100 for D, then the total number of choices that the researcher is faced with is 100,000,000 (i.e., 20X10X5X1,000X100 = 100,000,000). In practice, because of economical reasons only a very limited number of experiments can be performed. This means that only a small number of choices can be selected; referring to the example, if only 3 experiments are to be performed then only 3 choices can be selected out of 100,000,000. In the next section, we show how a computer simulation software can help the experiment designer to select the components and values that have the highest potential of success.

A COMPUTER SIMULATION FOR EXPERIMENT DESIGNERS - OUR APPROACH

Simulation is one of the most powerful analysis tools available to those responsible for the design and operation of complex processes or systems. To simulate is "to obtain the essence of, without the reality" according to Webster's Collegiate Dictionary.

Simulation involves the modeling of a process or system in such a way that the model mimics the response of the actual system to events that take place over time (Pegden et al. 1990). Generally, simulation is considered to include both the construction of the model and the experimental use of the model for studying a problem.

Our proposed simulation system can be used to predict/ESTIMATE future behavior, i.e., the effects produced by changes in the system or in its method of operation.

Figure 1 show the steps involved in our simulation system. The following is the general description of our approach (the letters on the left refer to the corresponding boxes in the figure):

(a) The problem is defined as precisely as possible and then it is formulated by observing the relationships of the components to one another.

(b) A data base is compiled by collecting data from the ACTUAL experiments (typically, the data is collected from related published papers or alternatively by performing the actual experiment with

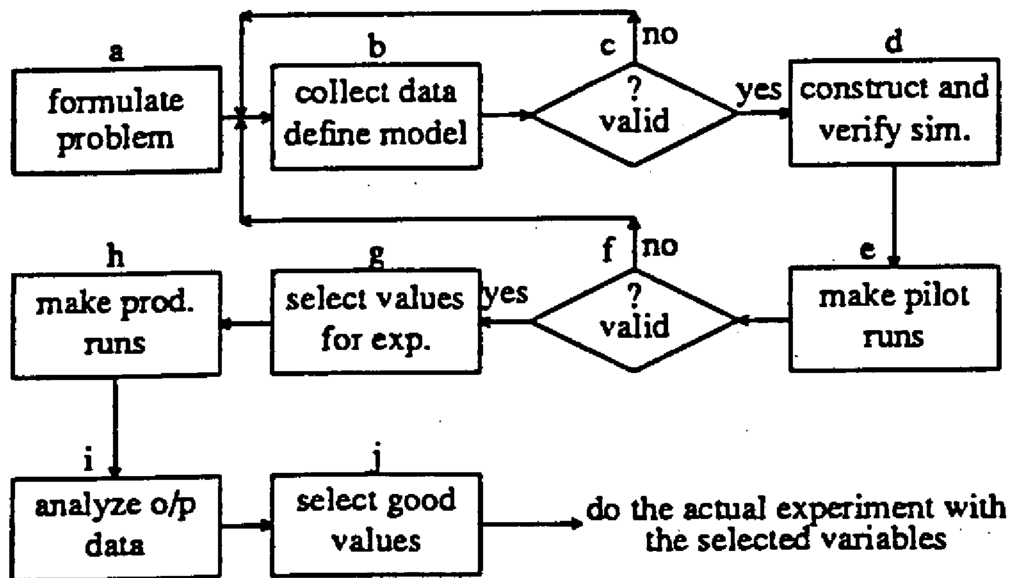

Figure 1. The steps involved in the simulation system

only a few components to obtain some data). These are used to specify operating procedures and probability distributions for the random variables. A model is defined that is only moderately detailed - later the model will be made more sophisticated if necessary.

(c) In building the model, it is imperative for the modelers to involve people in the study who are intimately familiar with the operations of the actual system. Therefore, interaction with the model's intended user is essential at this phase. This will increase the validity of the model and its credibility. In addition, the adequacy of the probability distributions specified for generating input random variates is tested using goodness-of-fit tests (Law and Kelton, 1991).

(d) A computer program is constructed and verified. Although a general-purpose high level language can be used to simulate the system, we have chosen the simulation language, SIMSCRIPT, to implement this project.

(e) Pilot runs are made with different values for each component of the experiment/study.

(f) Data generated from the pilot runs for the MODEL of the system is compared with those from the ACTUAL EXISTING system (collected at step b). If the agreement is good enough then the simulation is good enough; otherwise go to step b.

(g) Large sets of values are selected for the components of the experiment (e.g., 1,000,000 randomly selected out of 100,000,000 possible values).

(h) The values selected (at step g) are fed into the simulator; i.e., production runs are made.

(i) Statistical techniques are used to analyze the output data (obtained at step h); e.g., confidence intervals and goodness-of-fit analysis. At the end of this phase, a table is generated which contains the input data and corresponding output data together with other useful information.

(j) A subset of the input values that yielded desirable results is selected. These values have the highest potential of success when used in the actual experiment.

The most time consuming part of building the simulation software for the farm-raised catfish problem was the data collection phase (step b) which is now complete. The actual experiments were carried out in five different experimental ponds in Alabama. The catfish in the ponds were fed with five different protein levels. The fat content of each catfish was measured after six months; the data obtained forms the basis of our data for the simulation software (more will be gathered from published papers). We believe that step h of our approach will also be quite time consuming. We estimate that the production runs will take between 1/2 a day to many weeks depending on the number of variables selected (at step g) if a single processor computer is used.

With the use of a parallel processing system (a computer with more than one processor) this can greatly be speeded up. We already have purchased 20 transputer boards which when connected together will form a powerful parallel computer (potential power will be about 400 Million Instructions Per Second - RISC based).

The details of the parallel system suitable for this project can be found in Arabnia (1990a, 1990b) - it is described in a different context. We estimate that the implementation of the simulation software described in this paper will be completed within a year.

REMARKS

The task of selecting a set of values for the components of an experiment to be performed is one of the most difficult problems (sometimes impossible) faced by experiment designers. The difficulty is the careful selection of a small set of values that would yield the desired results from a very large number of possible choices. For example, the number of possibilities in the problem of finding a catfish feed that would not result excessive fat content in the catfish is enormous. Since it is not feasible (for economical reasons) to test all the possible choices, the researcher selects only a small number of components; he hopes that his selection yields the desired results (e.g., adequate fat content in the catfish). Unfortunately, the probability of choosing the desired variables/components is very small. With the proposed simulation software, the user can make more intelligent choices. One important advantage that our design has over conventional simulation systems is that as more data is gathered (i.e., more experiments are performed), the system becomes more reliable (i.e., generates more accurate results).

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AN ECONOMIC ANALYSIS OF THE SOUTHEASTERN U.S. BLUE CRAB PROCESSING INDUSTRY, 1973-90

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Southeast[1] blue crab (Callinectes sapidus) landings generally represent in excess of 50% of the nation's total production of this species. The species is highly estuarine dependent and, as such, landings fluctuate widely from year to year, independent of fishing pressure.

Unlike some species, the majority of blue crab landings, particularly those that are reported by the National Marine Fisheries Service, are processed upon arrival at dock.[2] As noted by Ward (2), the technology used in processing blue crabs has changed little since the turn of the century. Picking of the crab is still generally done by hand and, as such, it is a very labor-intensive operation. Upon picking, other value-added activities may be conducted including pasteurizing, breading of cakes/ patties, and the preparation of stuffed crabs, gumbos, and soups.

The purpose of this paper is to provide an economic assessment of blue crab processing activities in the Southeast United States for the 1973-90 period. National Marine Fisheries Service confidential annual interview data with the Southeastern U.S. seafood processors provided the information needed to achieve this objective.[3] To this end, the paper begins by analyzing total Southeast blue crab in total and by three specific forms; fresh meat, breaded products, and "other products".[4] Then, discussion turns to analysis of processing activities by region, i.e., Gulf and South Atlantic.

RESULTS

SOUTHEAST ACTIVITIES

Blue crab processing activities in the Southeast are first evaluated in aggregate. Then, attention is turned to processing of specific product forms.

Aggregate Activities

As indicated in Table 1, the number of firms engaged in blue crab processing activities in the Southeastern United States increased during the 1973-90 period. For example, the 162 firms processing blue crabs, on average, during 1988-90 represented a seven percent increase over the 151 firms reported during 1982-84 and a 20% increase over the number of firms reported annually during the 1973-75 period. Overall, expansion in the number of firms was evident during each three-year period considered in the analysis except during 1979-81 when the number of firms declined by four from the previous three-year period, i.e., 1976-78.

The quantity processed, as provided in Table 1, is reported on both a product-weight basis and an edible-meat-weight basis. The product weight, as reported here, includes the meat weight of crabs used in the processing activity, plus any additional ingredients that may be added such as the breading materials, plus some shell weight if appropriate (such as in the case of stuffed crabs and cocktail claws). The edible-meat weight, as reported, is equal to the product weight excluding any additional ingredients or shell.[5] In other words, the edible-meat weight is equal to the amount of blue crab meat that is used in the processed product.

As indicated in Table 1, the processed quantity, expressed on either a product weight or edible-meat-weight basis, tended to increase during the period of analysis, with the exception of a relatively sharp drop during the latest three year period. Overall, the growth in blue crab processing activities, measured by weight, tended to reflect an overall increase in the Southeast blue crab landings, though the decline in processing activities during the most recent three-year period of analysis was not related to a decline in landings. Despite the recent decline in processing activities, 1988-90 average annual production of 18.7 million pounds (edible meat weight basis) represented an increase of almost 35% when compared to 1973-75 average annual processing activities of about 14 million pounds. When evaluated on a product weight basis, the increase was in excess of 40%, suggesting an increase in value-added activities, such as breading, that contributed proportionately more to the product weight.

The current value of blue crab processing activities in the Southeast expanded from an annual average of about $40 million in 1973-75 to $108 million in 1988-90 (Table 1). When adjusted for inflation, however, the value of processing activities exhibited only minimum growth (excluding the 1985-87 period), despite significantly higher processing activities when measured by poundage. This is the result of a significant decline in the deflated price per pound of the processed product. As indicated in Table 1, the deflated price of the processed product (expressed on an edible-meat-weight basis) peaked at $8.35 per pound in 1976-78 and the 1988-90 average price of $6.06 per pound equalled less

Table 1. Blue Crab Processing Activities in the Southeastern United States, 1973-90.

		PROCESSED QUANTITY		VALUE		
Time Period	No. of Firms	Product Weight	Edible Meat Weight	Current	Deflated[a]	Deflated Price Per Pound[b]
		------- 1,000 lbs. -------		-------- $1,000s --------		$/lb.
1973-75 avg.	135	18,939	13,953	39,778	106,206	7.61
1976-78 avg.	136	17,754	12,342	48,086	103,067	8.35
1979-81 avg.	132	19,540	13,873	61,539	98,240	7.08
1982-84 avg.	151	25,336	17,396	87,464	113,836	6.54
1985-87 avg.	160	32,603	21,460	109,227	129,425	6.03
1988-90 avg.	162	26,950	18,709	107,942	113,493	6.06

[a] The 1990 Consumer Price Index, i.e., 1990 = 100, was used to deflate value and price.
[b] The deflated price per pound was calculated on the basis of edible-meat weight. The deflated price per pound on a product weight basis generally followed that on an edible meat weight basis but at about a two-dollar discount.

Source: Unpublished data provided by the National Marine Fisheries Service, Fisheries Statistics Division.

than 75% of this peak price and only 80% of the 1973-75 average annual price of $7.61 per pound. The observed decline in the deflated price could be the result of any number of factors including, but not limited to (1) increased production of processed blue crab, (2) competition from other fish and shellfish products, (3) declining input costs, and (4) a change in consumer tastes and preferences. Additional research must be conducted, however, before specific causes for the reduction in price can be adequately addressed.

The size distribution of Southeast blue crab processing firms during 1973-90, evaluated on the basis of the deflated value of processed blue crab sales, is presented in Table 2. As indicated, approximately 35%-45% of Southeast blue crab processing firms had less than $250 thousand in annual processed blue crab sales (adjusted for inflation to 1990 dollars) when evaluated on a three-year basis. Another 35%-45% of the firms generally had processed blue crab sales in the $250 thousand to $1.0 million range. Finally, from 17% to 23% of the Southeast blue crab processing firms had sales in excess of $1.0 million annually. Overall, the information contained in Table 2 suggests that the size distribution of blue crab processing firms, measured in sales of processed blue crab products, remained stable during 1973-90.

Table 3 provides an analysis of 1990 blue crab processing activities in the Southeast in relation to the firm's age. One-hundred-and-forty-nine blue crab processors operated in the Southeast in 1990 according to National Marine Fisheries Service unpublished data. Fifty-four of these firms, or slightly more than one-third of the total, began blue crab processing operations before 1976. These firms each produced, on average, 199 thousand pounds of processed blue crab products (edible-meat weight basis) in 1990 valued at $1.1 million per firm. By comparison, 35 firms have initiated blue crab processing operations since 1988, or 23% of the total. These firms each produced, on average, 76 thousand pounds of blue crab product valued at $420 thousand per firm.

At least two factors can be highlighted from the information contained in Table 3. First, the oldest blue crab firms, i.e., those beginning operations before 1976, are clearly larger than the more recently started firms, on average. Second, entry by firms into blue crab processing activities in the Southeast has been significant in recent years, e.g., 57 firms since 1985, and production among these firms is relatively large, rivaling all but the oldest firms. Among these 57 firms, pounds processed averaged 90 thousand per firm in 1990 and the value of processed blue crab sales among these firms averaged $551 thousand.

Table 2. Size Distribution of Southeastern United States Blue Crab Processing Firms, 1973-90.

	ANNUAL DEFLATED VALUE OF PROCESSED BLUE CRAB SALES (1990 BASE YEAR)						
	Less Than $250,000		$250,000 to $1 Million		$1 Million or More		Total
Time Period	No. of Firms	Percent of Total	No. of Firms	Percent of Total	No. of Firms	Percent of Total	No. of Firms
1973-75 avg.	50	37%	55	41%	30	22%	135
1976-78 avg.	49	36%	62	45%	25	19%	136
1979-81 avg.	46	35%	64	48%	22	17%	132
1982-84 avg.	65	43%	53	35%	33	22%	151
1985-87 avg.	56	35%	67	42%	37	23%	160
1988-90 avg.	69	43%	58	36%	35	22%	162

Source: Unpublished data provided by the National Marine Fisheries Service, Fisheries Statistics Division.

Table 3. Relation Between Blue Crab Processing Firm Size in 1990 and the Number of Years in Operation.

		Processed Blue Crab Sales Per Firm (1990)		
Time Period	No. of Firms	Pounds[a]	Value	$/lb.[b]
≤ 1975	54	198,643	$ 1,130,769	5.69
1976-78	10	89,546	$ 577,833	6.45
1979-81	14	79,149	$ 481,901	6.08
1982-84	14	86,395	$ 558,510	6.46
1985-87	22	112,815	$ 761,456	6.74
1988-90	35	75,997	$ 420,101	5.52

[a] Pounds are provided on an edible-meat weight basis.
[b] Expressed on an edible-meat weight basis.

Source: Unpublished data provided by the National Marine Fisheries Service, Fisheries Statistics Division.

Activities by Product Type

As noted, blue crab processing activities were analyzed on the basis of three product forms - fresh meat, breaded products,and "other" products. Discussion of these product forms is provided below.

Fresh Meat Activities. An overview of blue crab meat processing activities in the Southeastern United States is provided in Table 4. As indicated, the majority of the firms engaged in blue crab processing activities in the Southeast process fresh meat. For example, of the 135 firms engaged in processing activities during 1973-75 (see Table 1), 108 of the total, or 80%, produced fresh-meat products. This approximate share held throughout the period of analysis, with a peak of 86% during the 1979-81 period and a low of 79% during the 1988-90 period.

The quantity of processed fresh meat increased from an annual average of 9.6 million pounds during 1973-75 to 13.4 million pounds during 1988-90, or by almost 40%. The deflated value of processed meat, however, increased by less than 20% during 1973-90, though the 1985-87 average annual meat production exceeded the 1973-75 comparable figure by 30%. The difference between increases in poundage and the deflated value reflects the sharp decline in the deflated price per pound of processed meat. After peaking in excess of nine dollars per pound during 1976-78 (expressed in 1990 dollars), the price of processed meats declined to a low of $6.65 in 1985-87 before increasing marginally to $6.62 in 1988-90. Overall, the 1988-90 average price was less than 75% percent of the peak 1976-78 price and was only 85% of the 1973-75 price. On a per firm basis, meat processing activities increased from about 90 thousand pounds in 1973-75 to 105 thousand pounds in 1988-90.

Breaded Product Activities. The number of blue crab breaders in the Southeast remained relatively stable at about 20 since the sharp decline after the first three-year period of analysis (Table 5). Despite the stability in the number of breaders, the poundage of breaded product increased significantly. During 1973-75, for instance, almost 2.2 million pounds of breaded product (expressed on an edible-meat weight basis) was processed annually in the Southeast. Roughly twice that amount, i.e., 4.4 million pounds, was processed during the most recent three year period. Almost 25% of the total Southeast blue crab processing activity, evaluated on an edible meat weight basis, was comprised of breading activities during 1988-90 (i.e., 4.4 million of the total 18.7 million pounds) compared to only 15% during 1973-75 (i.e., 2.2 million of the total 14.0 million pounds). On a per firm basis, breading activities increased from an annual average of 80 thousand pounds (edible meat weight) in 1973-75 to 222 thousand pounds in 1988-90.

Table 4. Meat Processing Activities by Blue Crab Processors in the Southeastern United States, 1973-90.

Time Period	No. of Firms	Edible Meat Weight[a]	VALUE		Deflated Price Per Pound
			Current	Deflated[b]	
		1,000 lbs.	---------- $1,000s	----------	$/lb.
1973-75 avg.	108	9,640	28,038	74,873	7.76
1976-78 avg.	111	7,768	32,866	70,588	9.08
1979-81 avg.	113	9,015	42,816	68,119	7.55
1982-84 avg.	129	11,430	62,748	81,692	7.14
1985-87 avg.	134	14,854	82,249	97,371	6.55
1988-90 avg.	128	13,412	84,615	88,887	6.62

[a] In the case of blue crab meats, product weight and edible meat weight are identical.
[b] The 1990 Consumer Price Index, i.e., 1990 = 100, was used to deflated value and price.

Source: Unpublished data provided by the National Marine Fisheries Service, Fisheries Statistics Division.

Table 5. Breaded Processing Activities by Blue Crab Processors in the Southeastern United States, 1973-90.

Time Period	No. of Firms	PROCESSED QUANTITY		VALUE		
		Product Weight	Edible Meat Weight	Current	Deflated[a]	Deflated Price Per Pound[b]
		------- 1,000 lbs. -------		-------- $1,000s --------		$/lb.
1973-75 avg.	27	6,261	2,179	7,080	18,884	8.67
1976-78 avg.	21	7,616	2,928	9,586	20,502	7.00
1979-81 avg.	17	8,133	3,256	12,190	19,738	6.06
1982-84 avg.	22	11,918	4,515	18,623	24,168	5.35
1985-87 avg.	21	16,180	5,478	23,063	27,403	5.00
1988-90 avg.	20	12,224	4,433	19,622	20,742	4.68

[a] The 1990 Consumer Price Index, i.e., 1990 = 100, was used to deflate value and price.
[b] The deflated price per pound was calculated on the basis of edible meat weight.

Source: Unpublished data provided by the National Marine Fisheries Service, Fisheries Statistics Division.

While the quantity of Southeast blue crab breading activities more than doubled between 1973-75 and 1988-90, the deflated value of these processing activities increased by only 10%, from $18.9 million to $20.7 million (Table 5). This is because the deflated price of the breaded product fell by almost 50% during the period of analysis. On a deflated value basis, breading activities per firm expanded from $700 thousand annually during 1973-75 to slightly more than $1.0 million during 1988-90.

"Other" Product Activities. While the number of firms processing "other" blue crab products increased significantly during 1973-90, particularly after the 1982-84 period, the quantity and deflated value of "other" products declined significantly (Table 6). The increase in the numbers of firms in recent years appears to reflect increased claw production. The large decline in the quantity and value of "other" processing activities, however, appears to reflect the demise of the blue crab canning industry throughout the Southeast. Overall, the share of total Southeast blue crab processing comprised of "other" products declined from about 12% during the first period of analysis to less than four percent during the latest period of analysis, when measured in terms of value.

Discussion. A comparison of the information on different product types allows some useful findings to be drawn from the data. First, it is noteworthy that the price per pound of breaded crab products (edible-meat weight) has been significantly below that of meat products in recent years, suggesting that the less expensive blue crab meat (e.g., claw meat) is used in the production of breaded products. A second finding is that a relatively few number of firms produce more than one crab product. As indicated in Table 1, a total of 162 firms produced crab products on an annual basis in 1988-90. Summation of numbers of firms by product type leads to 172 firms. This suggests that at a maximum, only ten firms produce more than one of the three product forms. The actual member may even be less than ten if some firms are producing all three products.

REGIONAL ACTIVITIES

Aggregate Activities

Of the 135 firms processing blue crabs in the Southeast during the 1973-75 period, an average of 53 (39%), were located in the South Atlantic Region (Table 7). The other 82 (61%) were located in the Gulf Region (Table 8). These proportions remained remarkably stable throughout the period of analysis.

Overall, little growth was evident in South Atlantic blue crab processing activities during the 1973-90 period, especially when measured in terms of deflated value. For example, the deflated value of South Atlantic blue crab processing activities peaked during 1985-87 at $63.4 million annually (Table 7). This is only about five percent above the 1973-75 average annual

Table 6. "Other" Processing Activities by Blue Crab Processors in the Southeastern United States, 1973-90.

Time Period	No. of Firms	PROCESSED QUANTITY		VALUE		
		Product Weight	Edible Meat Weight	Current	Deflated[a]	Deflated Price Per Pound[b]
		------- 1,000 lbs. -------		-------- $1,000s --------		$/lb.
1973-75 avg.	12	3,038	2,133	4,660	12,450	5.84
1976-78 avg.	11	2,370	1,645	4,634	11,977	7.28
1979-81 avg.	9	2,392	1,602	6,533	10,383	6.48
1982-84 avg.	11	1,988	1,451	6,093	7,976	5.50
1985-87 avg.	18	1,569	1,128	3,915	4,651	4.12
1988-90 avg.	24	1,313	864	3,705	3,865	4.47

[a] The 1990 Consumer Price Index, i.e., 1990 = 100, was used to deflate value and price.
[b] The deflated price per pound was calculated on the basis of edible meat weight.

Source: Unpublished data provided by the National Marine Fisheries Service, Fisheries Statistics Division.

Table 7. Blue Crab Processing Activities in the South Atlantic, 1973-90.

		PROCESSED WEIGHT		VALUE		
Time Period	No. of Firms	Product Weight	Edible Meat Weight	Current	Deflated[a]	Deflated Price Per Pound[b]
		------- 1,000 lbs. -------		-------- $1,000s --------		$/lb.
1973-75 avg.	53	9,872	7,329	22,476	60,094	8.20
1976-78 avg.	51	7,895	5,745	24,254	51,846	9.02
1979-81 avg.	56	9,500	7,090	34,154	54,473	7.79
1982-84 avg.	58	12,312	8,517	45,448	59,254	6.96
1985-87 avg.	66	17,029	10,064	53,514	63,448	6.30
1988-90 avg.	63	11,888	7,742	48,287	50,801	6.56

[a] The 1990 Consumer Price Index, i.e., 1990 = 100, was used to deflate value and price.
[b] The deflated price per pound was calculated on the basis of edible meat weight.

Source: Unpublished data provided by the National Marine Fisheries Service, Fisheries Statistics Division.

Table 8. Blue Crab Processing Activities in the Gulf Region, 1973-90.

		PROCESSED WEIGHT		VALUE		
Time Period	No. of Firms	Product Weight	Edible Meat Weight	Current	Deflated[a]	Deflated Price Per Pound[b]
		------- 1,000 lbs. -------		-------- $1,000s --------		$/lb.
1973-75 avg.	82	9,067	6,623	17,302	46,112	6.96
1976-78 avg.	85	9,859	6,597	23,832	51,213	7.76
1979-81 avg.	77	10,040	6,783	27,385	43,768	6.45
1982-84 avg.	93	13,024	8,880	42,016	54,582	6.15
1985-87 avg.	94	15,574	11,396	55,713	65,977	5.79
1988-90 avg.	99	15,062	10,967	59,655	62,692	5.72

[a] The 1990 Consumer Price Index, i.e., 1990 = 100, was used to deflate value and price.
[b] The deflated price per pound was calculated on the basis of edible-meat weight.

Source: Unpublished data provided by the National Marine Fisheries Service, Fisheries Statistics Division.

deflated value of $60.1 million. The 1988-90 deflated value of South Atlantic blue crab processing activities, averaging $50.8 million annually, was 15% below the comparable 1973-75 figure. The edible-meat weight of blue crab processing activities in the South Atlantic during 1988-90, averaging 7.7 million pounds annually, was only six percent greater than the 7.3 million pounds processed annually during 1973-75.

In contrast, the deflated value of blue crab processing activities in the Gulf Region increased from an average of $46.1 million during 1973-75 to $62.7 million during 1988-90, or by about 35%. Pounds processed in the Gulf Region expanded from an average of 6.6 million pounds annually (edible-meat weight basis) during 1973-75 to almost 11 million pounds annually during 1988-90, or by 65%. It is, thus, evident that most of the expansion in the Southeast blue crab processing activities during the 1973-90 period was Gulf Region based.

Edible-meat price per pound was higher in the South Atlantic throughout the period of analysis. Several hypotheses can be advanced to explain this price differential. First, it is thought that more steaming occurs in the South Atlantic than in the Gulf, and steaming commands a higher price for the processed products. Furthermore, a greater amount of pasteurizing is thought to occur in the South Atlantic than in the Gulf. Pasteurization results in a higher price for two reasons: (1) the process of pasteurization adds value to the processed product, ceteris paribus, and (2) pasteurization allows for storage of the crab meat until off-season, when prices rise. Finally, prices in the South Atlantic may exceed those in the Gulf reflecting markets of sale.

Activities by Product Type

Fresh Meat Activities. Selected information on the processing of fresh meat distinguished by South Atlantic and Gulf Region blue crab processors is presented in Table 9. The number of blue crab meat processors in the South Atlantic increased from an annual average of 44 in 1973-75 to 53 in 1985-87 before declining somewhat to 49 in 1988-90. In the Gulf Region, the number of blue crab processors increased from an average of 64 annually during 1973-75 to an average of 80 during 1985-87 and 79 during 1988-90.

In terms of pounds of meats processed, production in the South Atlantic expanded from an average of 4.9 million pounds annually during 1973-75 to 7.1 million annually during 1985-87, before declining sharply to 5.7 million pounds annually during 1988-90 (Table 9). Expansion in the production of processed blue crab meats in the Gulf Region exceeded that reported for the South Atlantic Region. Overall, the 7.7 million pounds of fresh meat produced annually in the Gulf Region in 1988-90 represented about a 65% increase over annual production of 4.7 million pounds in 1973-75.

Table 9. Meat Processing Activities by Blue Crab Processors in the South Atlantic and Gulf Region, 1973-90.

	SOUTH ATLANTIC REGION			GULF REGION		
Time Period	No. of Firms	Weight	Deflated Value[a]	No. of Firms	Weight	Deflated Value[a]
		1,000 lbs.	$1,000s		1,000 lbs.	$1,000s
1973-75 avg.	44	4,940	40,355	64	4,700	34,518
1976-78 avg.	43	3,726	35,208	69	4,042	35,379
1979-81 avg.	47	4,997	39,350	66	4,018	28,769
1982-84 avg.	50	6,124	44,344	79	5,305	37,349
1985-87 avg.	53	7,086	47,456	80	7,768	49,915
1988-90 avg.	49	5,718	39,401	79	7,694	49,486

[a] The 1990 Consumer Price Index, i.e., 1990 = 100, was used to deflate value.

Source: Unpublished data provided by the National Marine Fisheries Service, Fisheries Statistics Division.

The majority of increased value of fresh meat has been Gulf Region oriented. As indicated in Table 9, the deflated value of processed meats in the South Atlantic Region in 1988-90, averaging $39.4 million annually, was slightly below the 1973-75 figure of $40.4 million. In the Gulf Region, on the other hand, the deflated value of processed meat sales in 1988-90, $49.5 million annually, exceeded the 1973-75 average annual sales of $34.5 million by more than 40%.

Breaded Product Activities. The member of blue crab breaders in the South Atlantic has consistently averaged eight annually since the mid-1970's (Table 10). The edible meat weight of blue crab breaded products from the South Atlantic, however, has increased from an average of 650 thousand pounds annually during 1973-75 to 1.4 million during 1988-90 and peaked at more than two-million pounds during 1985-87. Despite the significant increase in quantity processed, the deflated value of these activities during 1988-90 was essentially unchanged from the 1973-75 period.

Breading activities in the Gulf Region, measured in pounds, consistently exceeded similar activities in the South Atlantic (Table 10). On average, 1.5 million pounds of breaded products (edible meat weight basis) were produced in the Gulf Region during 1973-75 compared to 3.0 million in 1988-90. The deflated value of these activities increased from $9.5 million annually to $11.5 million annually.

"Other" Product Activities. The production of "other" processed blue crab products in the South Atlantic and Gulf Region for the 1973-90 period is presented in Table 11. The production of "other" blue crab products in the South Atlantic fell significantly during 1973-90 and most of the decline has been since 1984. The deflated value of these activities declined from an average of $10.3 million annually in 1973-75 to $2.2 million annually in 1988-90. All of the decline in total South Atlantic blue crab processing activities throughout the period of analysis can be traced to the reduction in "other" blue crab processing activities.

Production of "other" blue crab processing activities in the Gulf Region is relatively minor, averaging less than 300 thousand pounds (edible-meat weight basis) annually during 1973-90. The value of this production, expressed in 1990 dollars, has averaged less than $2.0 million annually.

SUMMARY

Analysis of previously unavailable Southeast processing data provided unique insight to a generally seldom researched industry. Noteworthy elements of the findings are:

Table 10. Breaded Processing Activities by Blue Crab Processors in the South Atlantic and Gulf Regions, 1973-90.

	SOUTH ATLANTIC REGION			GULF REGION		
Time Period	No. of Firms	Edible Meat Weight	Deflated Value[a]	No. of Firms	Edible Meat Weight	Deflated Value[a]
		1,000 lbs.	$1,000s		1,000 lbs.	$1,000s
1973-75 avg.	11	650	9,433	16	1,529	9,450
1976-78 avg.	8	473	5,584	12	2,456	14,918
1979-81 avg.	8	535	5,104	9	2,721	14,634
1982-84 avg.	8	1,070	7,781	14	3,446	16,387
1985-87 avg.	8	2,068	12,799	14	3,410	14,604
1988-90 avg.	8	1,419	9,233	12	3,014	11,509

[a] The 1990 Consumer Price Index, i.e., 1990 = 100, was used to deflate value.

Source: Unpublished data provided by the National Marine Fisheries Service, Fisheries Statistics Division.

Table 11. Other Processing Activities by Blue Crab Processors in the South Atlantic and Gulf Region, 1973-90.

	SOUTH ATLANTIC REGION			GULF REGION		
Time Period	No. of Firms	Edible Meat Weight	Deflated Value[a]	No. of Firms	Edible Meat Weight	Deflated Value[a]
		1,000 lbs.	$1,000s		1,000 lbs.	$1,000s
1973-75 avg.	4	1,739	10,305	8	395	2,144
1976-78 avg.[b]	*	*	*	9	100	916
1979-81 avg.[b]	*	*	*	6	45	364
1982-84 avg.[b]	*	1,475[c]	9,403[c]	9	129	846
1985-87 avg.	10	910	3,193	8	218	1,458
1988-90 avg.	12	606	2,167	12	259	1,697

[a] The 1990 Consumer Price Index, i.e., 1990 = 100, was used to deflate value.

[b] The 1976-84 period has been aggregated in the South Atlantic in this table to preserve confidentiality of individual companies processing "other" blue crab products. A total of five firms processed "other" products in the South Atlantic during the 1976-84 period.

[c] Reflects annual average during the 1976-84 period.

Source: Unpublished data provided by the National Marine Fisheries Service, Fisheries Statistics Division.

a. The number of Southeastern U.S. firms engaged in blue crab processing activities has gradually increased during the 1973-90 period when examined in three-year increments.

b. The quantity of processed blue crab products in the Southeast, while largely varying in relation to landings, trended upwards during 1973-90 .

c. Because of a sharp decline in the deflated price per pound of the processed product, the deflated value of blue crab processing activities in the Southeast increased only marginally during the period of analysis.

d. The size distribution of Southeast blue crab processors remained extremely constant during the period of analysis when examined in three-year increments.

e. The more established Southeast blue crab processing firms, i.e., those in operation before 1976, had consistently higher processed blue crab sales than the rest of the industry.

f. Almost 40% of blue crab processors in operation in 1990 have been established since 1985. However, these processors had lower average annual sales than those established prior to 1975.

g. Production of blue crab meats in the Southeast increased by about 40% between the 1973-75 and 1988-90 periods. Because of a deflated price per pound of the processed product, however, the deflated value of these activities increased by less than 20%.

h. Production of breaded processed blue crab products roughly doubled between the 1973-75 and 1988-90. Because of a nearly 50% decline in the deflated price per pound of breaded products, however, the deflated value of these activities increased by only 10%.

i. Production of "other" processed blue crab products declined sharply during the period of analysis. Overall, the 1988-90 average annual deflated value of "other" activities was less than a third of annual 1973-75 activities.

j. Total processed blue crab product volume in the South Atlantic expanded very little during 1973-90. While meat and breading production increased, the production of "other" processed blue crab products declined significantly.

k. Most of the observed expansion of blue crab processing activities in the Southeast was Gulf Region based. Both meat products and breading activities in the Gulf Region expanded significantly during the period of study.

ENDNOTES

1 For purposes of this study, the Southeast Region is defined as the coastal states from North Carolina through Texas. The South Atlantic Region extends from North Carolina through the East Coast of Florida. The Gulf Region extends from the West Coast of Florida through Texas.

2 It is generally recognized that National Marine Fisheries Service reported blue crab landings significantly underestimate actual production. In a recent study by Keithly et al. (1), for instance, the authors contend that Louisiana's 1986 landings were at least 50% greater than those reported by NMFS.

3 The information requested by the National Marine Fisheries Service from processors is considered voluntary but compliance rate is quite large.

4 Fresh meat includes both pasteurized and non-pasteurized product. Breaded products include items such as cakes, patties, stuffed, croquettes, deviled, and burgers. "Other" products include items such as canned specialties (soups, bisques, and stews), canned meats (not pasteurized), claws, and other specialty items (eg., cocktails, and cooked gumbos).

5 For a complete list of conversion factors used, see Keithly et al. The Southeastern Seafood Processing Industry: An Economic Assessment for Private and Public Management Decisions, National Marine Fisheries Service, Saltonstall-Kennedy Project # NA90AA-H-SK053 (forthcoming).

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QUALITY AND SAFETY CONSIDERATIONS FOR THERMALLY PROCESSED BLUE CRAB MEAT

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INTRODUCTION

Blue crabs comprise the largest crab fishery in the United States. U.S. landings in 1991 were 89.9 million kg (222.1 million lb), with a dock value of $73.3 million (9). Much of that supply was commercially cooked and marketed as fresh or pasteurized product. Crab meat is highly perishable and can serve as the vector for several serious food pathogens. Every crab plant must establish an effective quality control program.

Thermal processing requirements must be understood and made an integral part of any blue crab processing quality control program. The National Marine Fisheries Service (NMFS) in cooperation with the National Fisheries Institute (NFI) has developed a Hazard Analysis Critical Control Point (HACCP) quality control and inspection model for the blue crab industry. Four of seven critical control points listed by the model involve thermal processing steps or product temperature control (14).

In 1984, The National Blue Crab Industry Association (NBCIA) adopted thermal processing guidelines for the blue crab industry. NBCIA in cooperation with FDA and the state Sea Grant Advisory Programs is upgrading the guidelines to reflect HACCP principles, new processing and packaging technologies, and emerging threats from newly uncovered pathogens and spoilage organisms. Although NBCIA has delineated minimum pasteurization requirements in terms of calculated F-values, individual processing needs can result in adjusted cooking times and temperatures to achieve a given shelf life, reduce bluing, or process in newly available packaging (12, 13, 14).

The University of Georgia Marine Extension Service recently completed a study to evaluate the effects of several commercially available packaging materials on the quality of pasteurized crab meat. Stored pasteurized lump meat was held at refrigerated temperatures for 15 mo in the

following containers: steel cans, aluminum cans, plastic cans, non-barrier pouches, and barrier pouches (6). Packaging options for pasteurized meat will be discussed later in the paper.

Minimal thermal processing has been proposed as a pre-packaging or post-packaging treatment to control pathogens in freshly picked crab meat. Blue crab processors through NBCIA have established the development of proper time/temperature treatments that will destroy *Listeria monocytogenes* in freshly-picked crab meat as a leading priority.

MATERIALS AND METHODS

The methods followed for the study of commercial container options for the refrigerated storage of pasteurized crab meat are explained below. Freshly picked meat obtained from a cooperating Georgia processor was packed under commercial conditions into experimental and control pasteurization containers for a 15 mo refrigerated storage study. Meat was cooked at 83.3°C (182°F) to a target F^{18}_{85}-value of approximately 40 min for all treatments. Lump meat was pasteurized in the following: (i) 453.6 g (16 oz) in #401 steel cans which served as the industry control (Steeltin Can Company, Baltimore, MD); (ii) 226.8 g (8 oz) of crab meat in plastic cans with aluminum easy-open ends (#307 copolymer polyethylene cans with 283.5 g (10 oz) capacity, King Plastic Corporation, Orange, CA); (iii) 226.8 g (8 oz) in #307 aluminum cans (Central States Can Company, Massillon, OH); (iv) 226.8 g (8 oz) in non-barrier pouches (P640 with nylon base and low density polyethylene sealant, 16.5 cm x 22.9 cm, Cryovac Corporation, Duncan, SC); and (v) 226.8 g (8 oz) in barrier pouches (Cryovac P640B with nylon base, Saran^{R}barrier, and low density polyethylene sealant, 16.5 cm x 22.9 cm) (6).

RESULTS AND DISCUSSION

Blue crab thermal processing requirements and critical control points can be illustrated by following the major operational steps found in a typical Southeastern U.S. crab plant. Specific details may vary from plant to plant, but the general process is the same.

Crabs are commonly harvested by traps and brought to the processing plant the same afternoon. Live crabs should be cooked within 1 - 2½ h of delivery or transferred to coolers. Live crabs that must be cooked the following day should be refrigerated at between 7.2°C (45°F) and 10°C (50°F). Minimum time/temperature cooking requirements must be met. To prevent cross contamination, cooked and raw crabs should not be stored in the same cooler (12, 13, 14).

Crabs are normally cooked by pressurized steam or boiled in water. Achieving proper time/temperature cooks for live crabs is the first critical control point in the NMFS model HACCP program. The dominant method is steam retorting at 1.03 bar (15 psi). Crabs are cooked for 10 min after the retort reaches 121.1°C (250°F). Boiled crabs are usually cooked for 15 min after the water resumes boiling. Ulmer (19) found that a 15 min boil produced bacterial levels comparable to 10 min of steam retorting. Internal temperatures of steamed crabs usually range from 90.5°C (195°F) to 100°C (212°F). Boiled crabs yield more meat because of their higher moisture content, but they have a shorter shelf life (12, 13, 14, 18, 19).

Retorting criteria are not uniform throughout the United States, but usually require the internal temperature of the crab to reach between 112.8°C (235°F) and 115.5°C (240°F). In 1964 Ulmer (19) determined the average internal temperature of steamed crabs reached 119.4°C (247°F) after 10 min. A record of time/temperature conditions achieved during retorting of each batch of crabs should be maintained by the plant management. Crabs are cooled for several hours after cooking. NBCIA recommendations require cooked crabs to be refrigerated at ≤4.4°C (40°F). Along the Georgia and Gulf Coasts, crab backs are removed and the claws and cores are placed in refrigerated storage at between 0.6°C and 4.4°C (33°F to 40°F) before picking. The crabs are

picked the following morning. In other states, whole crabs are refrigerated before they are picked (13, 14, 18, 19).

Crab meat is removed or picked by hand in most operations. Several grades of white meat based on meat size are marketed. Market grades include: Jumbo, Lump, and Special. Claw meat and cocktail claws are also picked by hand (10). Some plants use a Quik-Pik machine (Crane Research and engineering, Hampton, VA) to remove white meat. Crab cores are placed on metal racks and pre-warmed. The meat is shaken onto a belt by the rapidly vibrating machine. Only Special meat is recovered. The Harris Machine uses a hammer mill and salt brine flotation to separate claws from claw meat. The picking room is included by the second critical control point. Good sanitation methods must be maintained for both hand and machine picked meat. Fresh meat should be inspected, weighed, packaged, and iced without delay to complete the third critical control point of the crab processing operation. Fresh meat has a shelf life of 6 to 14 d. The shelf life of refrigerated crab meat can be extended to 6 mo or more by pasteurization. The product maintains the characteristics of fresh crab meat (10, 12, 13, 14, 18).

The fourth critical control point is confirmation that the pasteurization container is hermetically sealed. The traditional process would inspect the can seam of a 16-ounce steel can. Current pasteurization container options include aluminum cans, plastic pouches, and plastic cans (12, 13, 14).

NBCIA recommends that the following information be displayed on each container of pasteurized crab meat:

(1) a code indicating the day, month, and year of processing
(2) the words "PASTEURIZED CRAB MEAT" should appear on both the individual and shipping containers
(3) the word "Pasteurized" should appear with each use of the words "Crab Meat"
(4) "Perishable--Keep Under Refrigeration" should be prominently displayed on each can

At least one individual should be trained to complete can seam or container seal evaluations and in the adjustment of the seaming/sealing equipment. The plant manager should keep seam or seal records for at least 2 years (12).

Use of safe and approved time/temperature parameters for the pasteurization process comprise the fifth critical control point.

Clostridium botulinum has traditionally been the organism of concern for canned or other hermetically sealed foods. Thermal process requirements are usually designed around a target organism. Blue crab pasteurization requirements were developed empirically to achieve a desired refrigerated shelf life with no specific target organism. The process was designed to achieve an internal meat temperature of 85℃ (185℉) for 1 minute at the geometric center of a 0.45 kg or 1 lb (401 X 301) steel can. The original empirical process requirements for 1 lb cans have been redefined by thermal lethality or total F-value to expand the concept to other container types and sizes. A z-value of 8.9℃ or 16℉ was picked arbitrarily in the absence of a specific target organism. A reference temperature of 85℃ or 185℉ was chosen. NBCIA adopted a minimum commercial pasteurization process of F_{16}^{185} = 31 min for their pasteurization guidelines. The process provides a wide margin of safety for the destruction of *C. botulinum* Type E spores (2, 12, 13, 14, 18). Cockey and Taitro (3) estimated that a typical commercial pasteurization process could provide an 8-D reduction in the number of *C. botulinum* spores.

D_{85} values determined for Type E spores have ranged from 0.2 - 0.32 min, confirming a 96-D process at F[illegible] = 31 min.

Each pasteurization system should have a time/temperature recording thermometer with a temperature controller and an indicating thermometer. The system should be calibrated annually.
An automatically regulated steam valve is required when steam is used as the heat source for the pasteurization tank. Baskets, dividers, and cover plates should be perforated to permit circulation within and around the pasteurization baskets. The water in the bath should be mixed or agitated to achieve a uniform temperature. Compressed air or recirculating pumps are effective (12).

The pasteurization process should be standardized by qualified individuals. Subtle variations in the size and shape of the water bath, steam source, and water circulation patterns make each processing plant unique. In-plant process standardization and batch monitoring are required for any pasteurization operation. Processing boundaries should be set. Any variation in the following parameters would require restandardization of the pasteurization process:

(1) Process time (both heating and cooling)
(2) Water bath temperatures (both heating and cooling)
(3) Initial crab meat temperature
(4) Container size, shape, and material

Rapid cooling of the crab meat is as important to the final quality, safety, and shelf life of the product as the heating portion of the pasteurization process. Slow cooling rates may allow injured bacteria to recover and multiply before refrigeration temperatures are reached within the can (7, 17, 20).

The sixth critical control point requires cans to remain in an ice-water bath capable of cooling the meat at the geometric center of the can to 12.8°C (55°F) within 180 min. Cooling water should be break-point chlorinated or treated with another acceptable sanitizer. The cooled meat should be moved to refrigerated storage that is maintained between 0°C (32°F) and 2.2°C (36°F). The geometric centers of the cans must cool to 2.2°C (36°F) within 18 h or less (12, 14, 17, 18).

The seventh critical control point addresses storage temperatures. Pasteurized crab meat must be kept between 0°C (32°F) and 2.2°C (36°F) throughout the wholesale/retail distribution system. Cooling below 2.2°C (36°F) is important for both maximum shelf life and safety. *C. botulinum* does not produce toxin below that temperature. Accidental freezing will toughen the meat and cause drip and flavor loss. The plant manager should maintain heating and cooling records covering each batch of pasteurized meat that is processed by the crab plant. Storage temperatures should be monitored and documented throughout the wholesale and retail distribution chain (12, 13, 14, 18).

One potential problem is variation in meat temperature before pasteurization. Often crab meat is packed into pasteurization cans directly from the picking table, at temperatures approaching 21°C (70°F). At other times meat may be placed in cans and held overnight in the cooler before pasteurization. A process based on an initial meat temperature of 21°C (70° F) would under process meat with a starting temperature of 0°C (32°F) or 1°C (33.8°F). We recommend that the pasteurization process be standardized with meat at the lowest initial temperatures that are expected in the plant. This method is fail-safe. A second approach would be to measure the initial meat temperature of each batch and adjust the process time accordingly (17).

Moody (11) developed a hardware and software system that utilizes a personal computer and a Strawberry Tree (Strawberry Tree Incorporated, Sunnyvale, CA) data acquisition board to calculate F-values in real time for each batch of pasteurized meat. The system will allow processors to adjust process times and temperatures to daily changes in meat quality, bacterial loads, starting temperatures, and package types.

Many processors exceed the minimum $F_{85}^{8.9}$-value level of 31 min recommended by NBCIA. Rippen of VPI has compiled industry data that associates achieved lethalities with commercial shelf life (Table 1) (16). Some processors routinely reach F-values of 60 to 120 min.

Table 1. Achieved $F_{85}^{8.9}$ values and estimated commercial shelf life of pasteurized blue crab meat (16).

$F_{85}^{8.9}$-Value (Minutes)	Shelf Life (Months)
10 - 15	1.5
15 - 20	2 - 4
20 - 25	4 - 6
25 - 30	6 - 9
30 - 40	9 - 18
>40	12 - 36

Rippen's study determined what shelf life range should be expected for a given pasteurization $F_{85}^{8.9}$-value if can seam integrity and proper cooling schedules are maintained. Crab plant owners can tailor processing parameters to meet their marketing needs.

Pasteurized meat spoilage has been at much higher than normal levels over the last 3 years. Most of the problems have been traced to poor can seams, however a more insidious problem has presented itself. Webster et al. (23) at VPI & SU have uncovered a thermoduric, psychrotrophic, anaerobic, non-pathogenic *Clostridium* that has been connected with early spoilage of pasteurized crab meat. Preliminary data indicates a D_{85}value of 9 min compared to 0.2 - 0.32 min for *C. botulinum* Type E. Fortunately the new isolate does not appear to be widely distributed. Its true impact on the crab industry is not known.

Heat processing to a F-value of sufficient lethality can provide a safe product with acceptable microbiological shelf life. However, there are other quality factors associated with blue crab meat thermal processing. Pasteurized crab meat can turn blue. Crab blood, copper-based hemocyanin, may form light grey to blue-black complexes. The discoloration is harmless, but it is not aesthetically pleasing (1). Bluing greatly reduces the meat's marketability and value. Bluing occurs during pasteurization and intensifies with storage. Bluing is temperature dependent. Meat processed above 88°C (190°F) frequently discolors. Previous studies have shown that pasteurization temperatures between 79.4°C (175°F) and 85°C (185°F) have reduced the incidence of bluing. Pasteurization at 83.3°C (182°F) to achieve a F-value of approximately 36 min and storage at -0.5°C to 0°C (31°F - 32°F) has reduced bluing levels in meat at two cooperating Georgia plants. A temperature of 83.3°C was chosen as a compromise between anticipated bluing reduction and the practical need for processors to limit increased cooking times required for lower pasteurization temperatures. Contamination of picked meat with metals, particularly iron, accelerates and intensifies bluing. The addition of

and phosphates can retard or reduce bluing levels (4, 5, 21, 22). Additional quality control steps that can help control bluing include:

(1) Reducing free liquid formation by steaming raw crabs and not washing or fluming cooked crabs.
(2) Maintaining an even circulation pattern in the pasteurization tanks. Turbulence in one area of the tank may trigger bluing.
(3) Reducing meat contact with any source of iron, including corroded steel and aluminum.
(4) Trying different package types, styles, and manufacturers until the most satisfactory container is found.

Product dryness is usually caused by cooking longer than 2 h. Dryness is not sensitive to process temperature. Dryness develops between the meat and the can's headspace. Periodic inversion of the cans during storage can help the problem. Rapid heating and cooling reduces drying.

Moody (11) has traced the presence of small crystalline grains that are sometimes found in pasteurized crab meat to struvite, a form of magnesium ammonium phosphate. The addition of sodium acid pyrophosphate can control the problem.

Thermal processing can be used as a final treatment for "Fresh Crab Meat". Vegetative cells of pathogenic or spoilage organisms can be targeted. Plate counts can be reduced to achieve market specifications or extend shelf life in packaging that is not hermetically sealed. A specific pathogen such as *Listeria monocytogenes*, with a zero tolerance level enforced by FDA, can be controlled with steam or microwave heating. Greater consumer awareness has led to increased pressure to deliver pathogen free crab meat. A process to control *Listeria monocytogenes* would need to meet the following criteria, an average F[illegible] value $\geq$1.0 second with a minimum value of 0.5 seconds (8, 15).

Gates et al. (6) conducted a 15 mo refrigerated storage study to determine the storage characteristics of the following previously described pasteurization containers: (i) steel cans, (ii) polyethylene cans, (iii) aluminum cans, (iv) non-barrier pouches, and (v) barrier pouches. Figures 1, 2, and 3 present heating and cooling rates for the cans. Figure 4 shows the heating and cooling curves for the pouches. Total heating times and mean F-values obtained for each type of container are shown in Table 2. Notice that the process times and shapes of the curves vary with each package type.

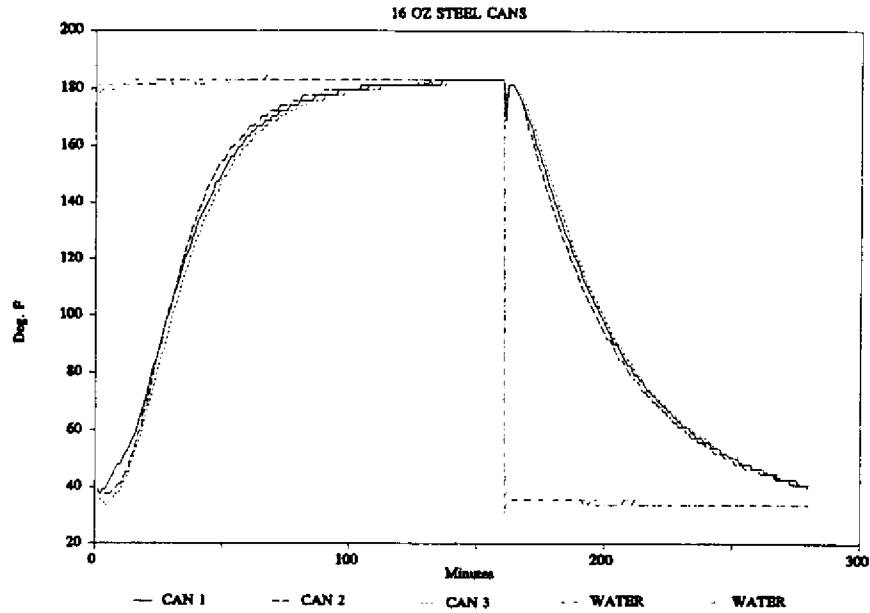

Figure 1. Time/temperature pasteurization curves showing both heating and cooling of crab meat in steel cans.

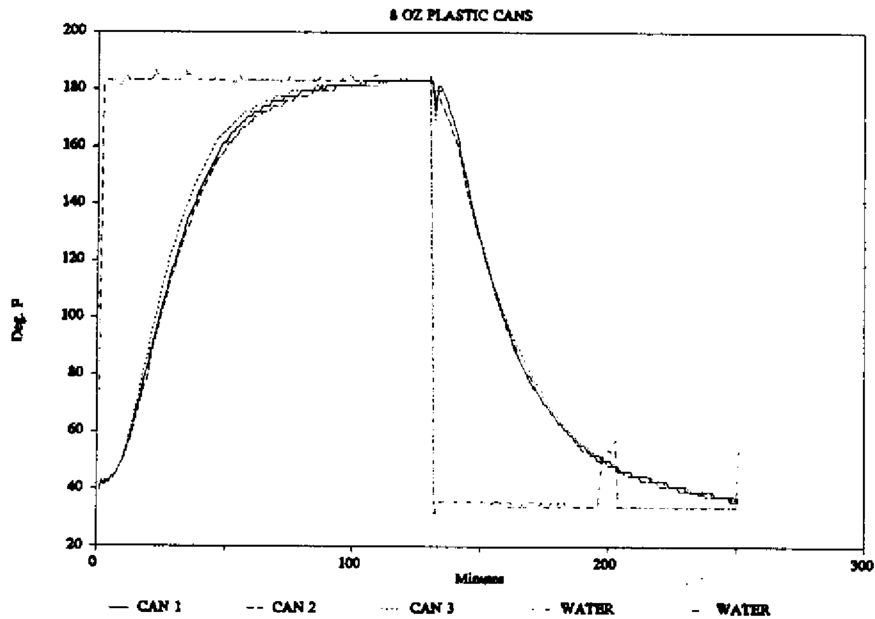

Figure 2. Time/temperature pasteurization curves showing both heating and cooling of crab meat in co-polymer polyethylene cans.

CRAB MEAT PASTEURIZATION

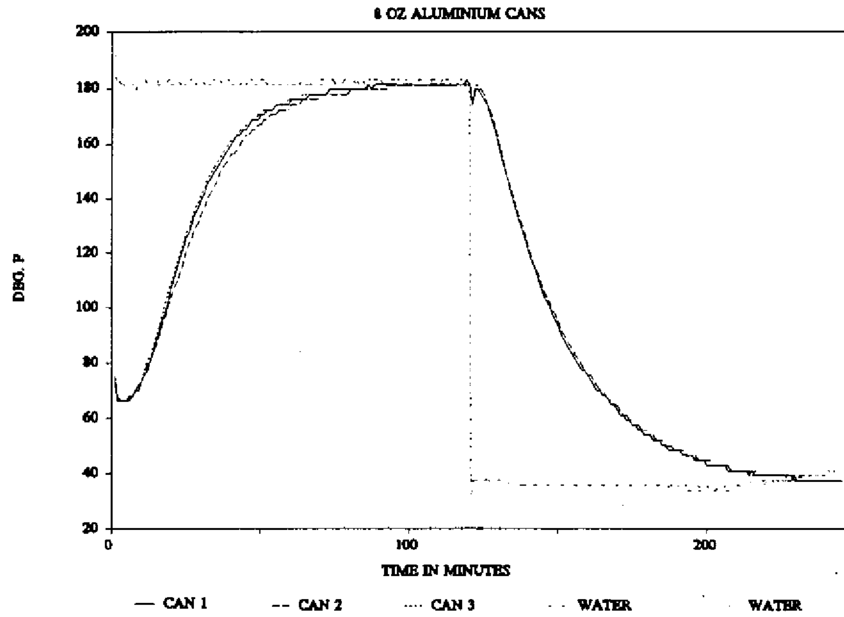

Figure 3. Time/temperature pasteurization curves showing both heating and cooling of crab meat in aluminum cans.

CRAB MEAT PASTEURIZATION

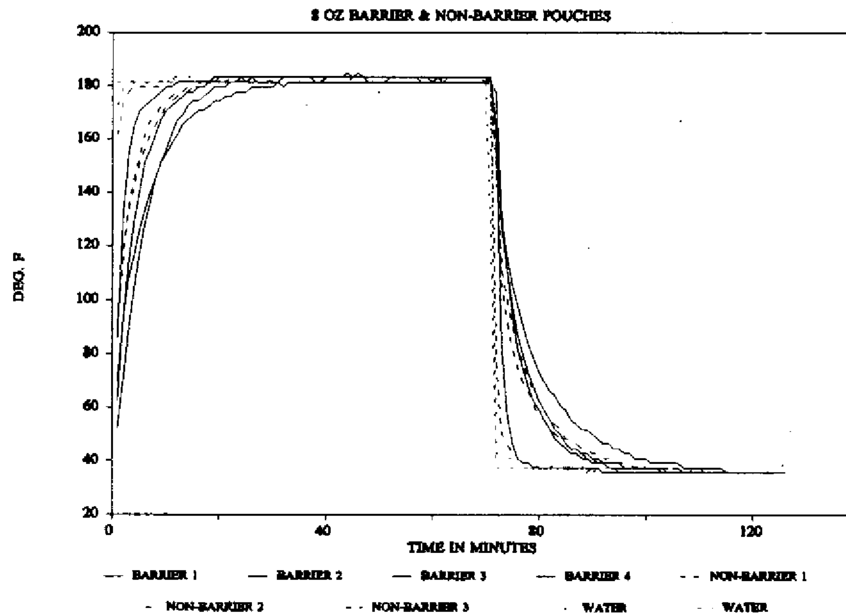

Figure 4. Time/temperature pasteurization curves showing both heating and cooling of crab meat in barrier and non-barrier pouches.

Table 2. Total heating times and achieved F_{85}^{9}-values for steel cans, plastic cans, aluminum cans, non-barrier pouches, and barrier pouches.

Container Type	Cook Time (Minutes)	F_{85}^{9}-Value (Minutes)
Steel	163	53.8
Plastic	130	43.8
Aluminum	120	39.7
Non-barrier Pouch	70	45.2
Barrier Pouch	70	42.8

Pasteurized meat in plastic containers had higher sensory color and appearance scores than meat from other evaluated containers through 8 mo of refrigerated storage. Barrier pouches were the least effective package, scoring below other containers for sensory quality and whiteness. Microbiological shelf life was limited to 10 mo. Aluminum and plastic containers scored the highest sensory color and appearance ratings at 10 and 13 mo of storage. Meat from steel cans was microbiologically and chemically spoiled following 15 mo of storage. Meat in plastic and aluminum cans and non-barrier pouches maintained acceptable sensory and microbiological quality through 15 mo. Meat pasteurized in less expensive plastic and aluminum containers had better sensory and microbiological quality than meat packed in steel cans.

CONCLUSIONS

Thermal processing is an integral part of the blue crab industry. Adoption of HACCP quality control procedures, the introduction of new packaging materials, and the use of computer processing technology can provide improved quality and safety for a traditional seafood industry.

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EXTENDING SHELF LIFE OF FRESH BLUE CRAB MEAT WITH LACTATES

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Both real and perceived public health concerns about the quality and safety of seafood products may soon force the seafood industry to modify some of its traditional practices. Of special concern are previously cooked, ready-to-eat foods such as fresh blue crab meat (8). Early attempts to enhance quality and safety with additives during processing of fresh (11,13), frozen (21,22), canned (5) and pasteurized crab meat (17) were either unsuccessful (21,22,17) or not beneficial enough (11,5,13) to be adopted by seafood processors. Consequently, fresh blue crab meat is primarily sold in the traditional way, which means refrigerated with no preservatives added.

In recent years, natural lactic acid and sodium lactate have been successfully used in food processing. They are known antimicrobial agents and provide inhibitory effects on most pathogens in poultry (14,15,20,6) and red meat (18,1). Lactates are recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA) and their use in food processing is restricted to good manufacturing practices (GMP)(9). Seafood technologists have recently focused their attention on the effect of lactates on shrimp (3,4), fish and frozen crab meat (7).

The primary objective of this study was to demonstrate shelf life extension by lactic acid and sodium lactate in blue crab meat, and secondly, to determine their effects on physical and sensory qualities.

MATERIAL AND METHODS

Crab Meat

Atlantic blue crab meat (*Callinectes sapidus*) was purchased from a commercial processor (Luther Lewis & Son, Davis, NC). The crabs had been pressure cooked, and the meat had been directly picked into standard one pound plastic containers. The crab meat containers were stored in crushed ice and removed only for treatment and analyses procedures.

Treatments

Fifty-five pounds of crab meat was mixed under sanitary conditions to ensure uniformity. It was divided into five equal portions. Control samples were immediately repacked in sanitized containers. Treated samples were dipped for 30 min in either (i) water, (ii) 1% lactic acid, (iii) 4 % sodium lactate or (iv) a combination of 1% lactic acid and 4% sodium lactate. They were allowed to drain for 10 min. The crab meat of all treatments, including the control, was subdivided and stored in separate containers for microbiological, physical and sensory analyses. The experiment was replicated three times.

Analyses

Physical, microbiological and sensory analyses of treated crab meat were performed during a 19-day period. Moisture content, pH value, color and texture were measured. Oven moisture and the pH values were determined, using standard procedures (24). The pH values were determined after the treatments. The crab meat (10 g) was blended in distilled water to achieve a final dilution of 1:3 (w/v) and the pH was measured, using a Fisher Accumet pH meter model 620. The texture grades were measured with an Instron universal testing machine equipped with a Kramer shear cell. The maximum force prior to rupture of triplicate 10 g samples was recorded. The color of the crab meat was measured with a Spectrogard colorsystem. Measurements, described on the Hunter Lab scale, were taken with a tungsten filament and an average daylight source. The observer angle was 10°. Triplicate 15 g samples were prepared, and two measurements in different areas were taken.

Aerobic plate count at 35°C and the confirmed test for E. coli were performed, according to standard AOAC procedures (2).

The consumer acceptability of treated crab meat was evaluated by an in-house consumer panel of 35 panelists on days 1, 5 and 8 (16). Panelists were asked to rank appearance, odor, flavor, texture and overall acceptability. The ratings were given on a 9 point hedonic scale from dislike extremely ('1') to like extremely ('9'). All ratings were converted to numerical values.

A rating below 5 (neither like nor dislike) was considered a consumer rejection.

Statistical analyses on the means of physical, microbiological and sensory data were performed, using the GLM procedure (19). To determine significant differences ($p<0.05$) between storage time and treatments, the least-square-means or the Waller-Duncan k-ratio t-test was computed.

RESULTS AND DISCUSSION

Physical Parameters

Physical effects of treatments on moisture, pH, color and texture were analyzed. Control samples had an average moisture content of about 76%. Dipping procedures elevated moisture levels in all samples (Figure 1). Water dips resulted in a 5% moisture increase while all other treatments showed an overall increase of about 2%.

The pH value of fresh handpicked crab meat was 7.8 (Figure 2). Neither distilled water nor 4% sodium lactate treatments

Figure 1: Oven moisture of fresh blue crab meat following lactate treatment.
LA = lactic acid, NaL = sodium lactate.

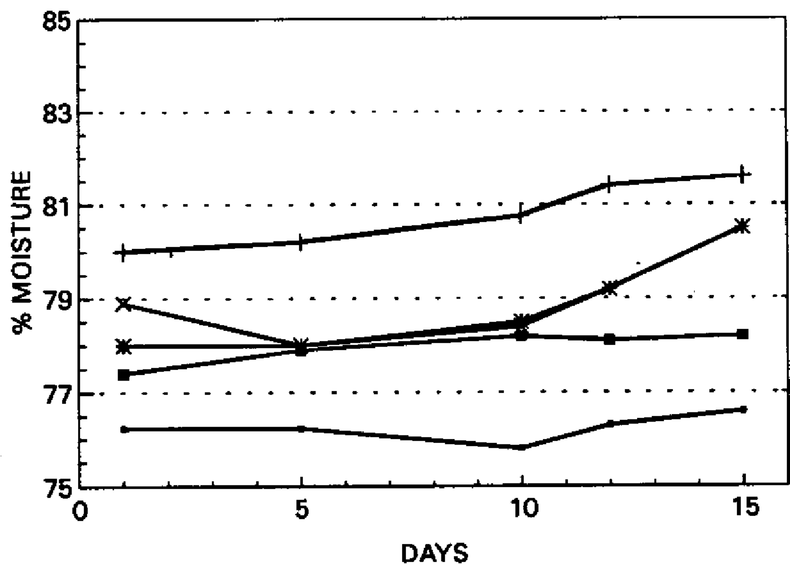
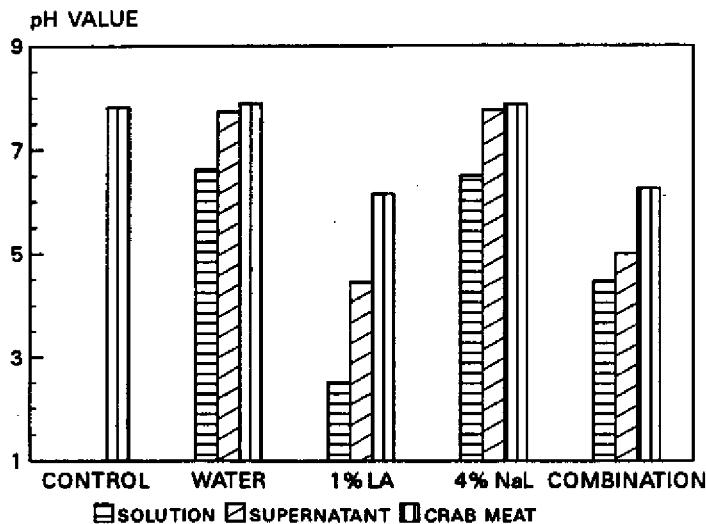


Figure 2: pH-values of fresh blue crab meat, dipping solutions and supernatants during lactate treatments. LA = lactic acid, NaL = sodium lactate, combination = 1%LA + 4%NaL.

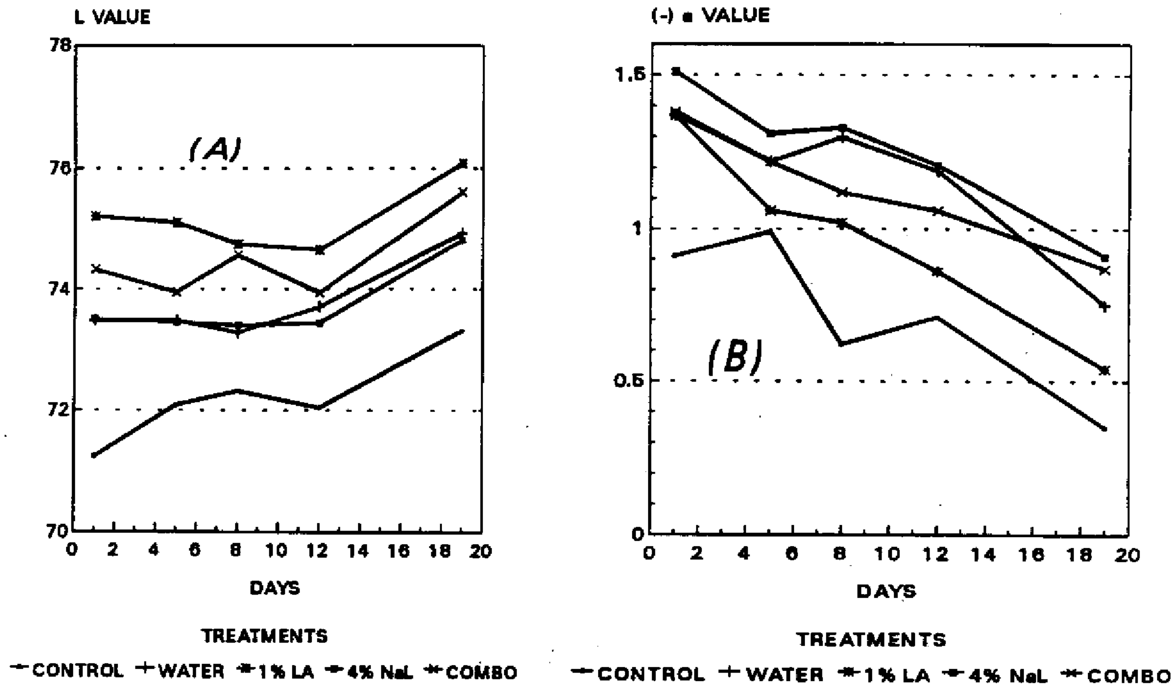


affected the pH of the crab meat. The pH of 1% lactic acid solution alone was 2.5 and increased to 4.4 when in combination with 4% sodium lactate. Both treatments containing lactic acid lowered the resultant meat pH to 6.2.

Visual observations of the crab meat indicated a lightening in color where lactic acid was used. This was confirmed with colorimetric measurements using a Hunter color scale. Both treatments containing lactic acid had significantly higher L values than the control (Figure 3a). The water and the sodium lactate dipped samples were lighter than the control, but significantly darker than the lactic acid samples. The a-value of all treated samples shifted to the green spectrum. This color shift was more pronounced in samples containing sodium lactate in their treatment solutions (Figure 3b). No significant differences were observed measuring the b-value (data not shown).

No significant differences in texture measurements with the Instron universal testing machine were found between lactate samples and control sample (data not shown).

Figure 3: Hunter color L-values (A) and a-values (B) of fresh blue crab meat following lactate treatments
LA = lactic acid, NaL = sodium lactate, Combo = 1%LA + 4%NaL.



<u>Bacteriological Quality</u>

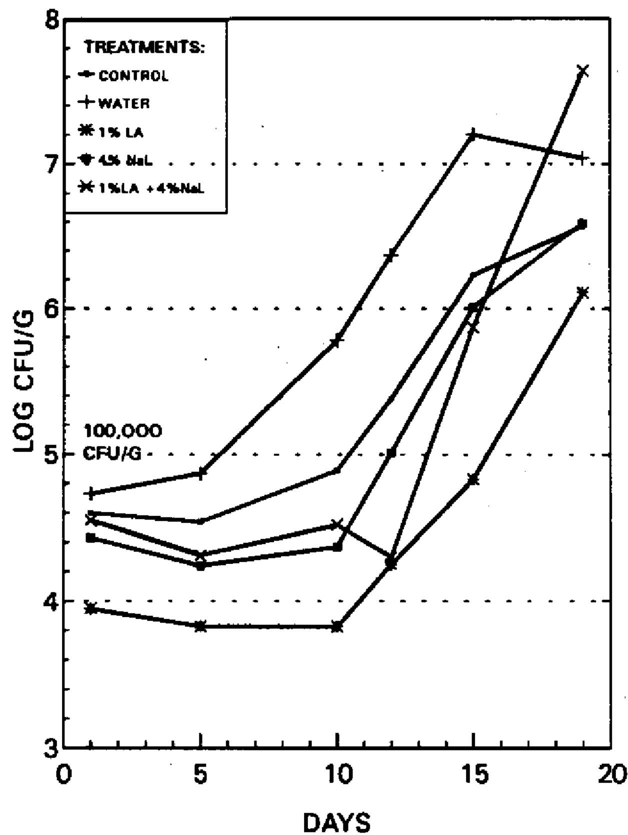
The classification of the crab meat followed similar guidelines described by Gates (12). Therefore, crab meat samples with aerobic plate counts (APCs) less than 100,000 cfu/g were considered good quality (10). Plate counts between 100,000 and 1,000,000 cfu/g were classified as acceptable quality. Samples with APCs greater than 1,000,000 cfu/g were judged to be spoiled.

The initial quality of the non-treated control sample averaged <60,000 cfu/g (Figure 4), with bacterial growth following the natural spoilage pattern for fresh crab meat on ice. After a lag phase of 5 days, bacterial growth increased slowly. The crab meat continued to be of good quality until 10 days and was regarded as unacceptable after 14 days. The microbial quality of water-dipped crab meat, one day after treatment, indicated that

the handling process during treatment did not significantly increase the bacterial numbers. Because of the rate of spoilage, good quality shelf life was reduced to 6 days and acceptable quality to 11 days.

Bactericidal properties of the 1% lactic acid solution (1,14,15) were demonstrated (i) by lowering the initial APCs to less than 10,000 cfu/g and (ii) by extending the lag phase to 10

Figure 4: Log of aerobic plate count (35°C) of fresh blue crab meat following lactate treatments and storage on crushed ice.
LA = lactic acid, NaL = sodium lactate



days. Crab meat remained in good quality (<100,000 cfu/g) for 16 days and spoiled after 19 days of storage on ice (>1,000,000 cfu/g). Sodium lactate is known to have a bacteriostatic effect (6,20). Like lactic acid, sodium lactate prolonged the lag phase in blue crab meat to 10 days before bacterial growth commenced. The treatment extended shelf life an average of 20%, to 12 days. The lower spoilage rate was consistent over 15 days but was not found to be statistically significant.

The combination of sodium lactate with lactic acid did not offer an advantage over the sodium lactate treatment in retarding microbial spoilage. The spoilage followed a pattern similar to the sodium lactate samples, with a good quality shelf life of 12 days. However, these data were not found to be significantly different ($p<0.05$) from the control.

Once bacterial growth began, growth patterns were similar for all samples, as indicated by the slope of the curves. What advantage did lactic acid treatment offer? There were two effects, which led to a shelf life extension of 60%, to 16 days. First, initial plate count was reduced and second, the lag phase was extended. Lactic acid treated crab meat was still in good condition (<100,000 cfu/g) 2 days after the control samples were spoiled (APC>1,000,000 cfu/g). The differences were significant at a 0.05 level.

Finally, tests for E. coli in control and treated samples were negative during the first 10 days of storage .

Sensory Evaluations

All treatments improved the sensory appearance rating of crab meat (Figure 5a). This may correlate to the higher moisture content and lighter color of the crab meat after the treatments. Differences were more pronounced over time.

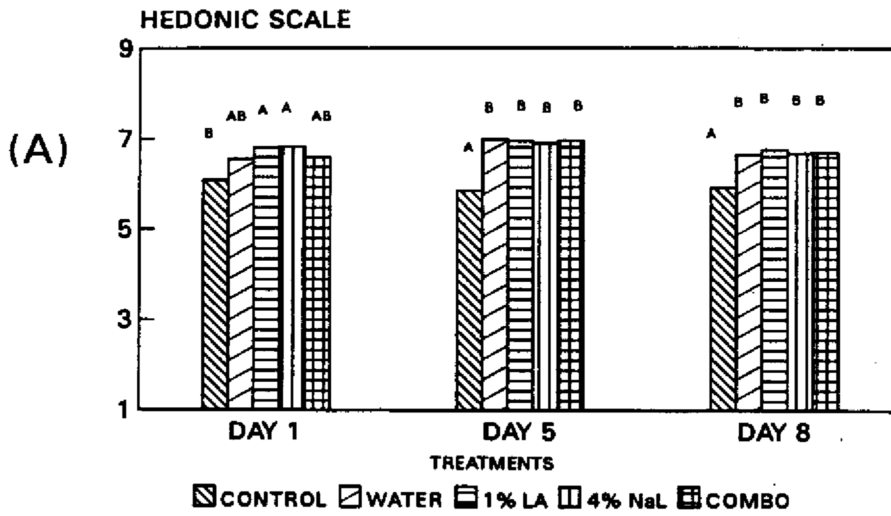
A slight off odor was detected by the panel in lactic acid samples and in the combination samples at the beginning of the storage time (day 1). This resulted in a lower rating for this test period (Figure 5b). However, overall odor for all treatments, including the lactic acid containing samples, was not found to differ significantly ($p<0.05$) from the control. The only exception was the sodium lactate sample, which was preferred by the panelists.

Panelists accepted the flavor of all samples and rated them above the rejection level of 5 (Figure 6a). Panelists rated both treatments containing lactic acid significantly less than the other treatments. Sodium lactate alone is known to enhance flavor. Panelists found a positive effect on crab flavor and preferred sodium lactate samples over control samples on day 5. The differences in flavor between all samples diminished with storage time and lost any significance after 8 days.

In terms of sensory texture ratings, no significant differences between treatments could be detected by the panelists (data not shown). This confirmed the physical data obtained with the Instron testing machine.

When asked to judge overall acceptability, panelists rated both lactic acid containing treatments lower than the control (Figure 6b). The 4% sodium lactate treatment was judged equal or superior to the control ($p<0.05$).

Figure 5: Sensory evaluations of appearance (A) and odor (B) of fresh blue crab meat following lactate treatments. Means of the same day marked with different letters were significantly different at a 0.05 level.
**)F-value too small.*
LA = lactic acid, NaL = sodium lactate, combo = 1%LA + 4%NaL,
9 = like extremely
1 = dislike extremely



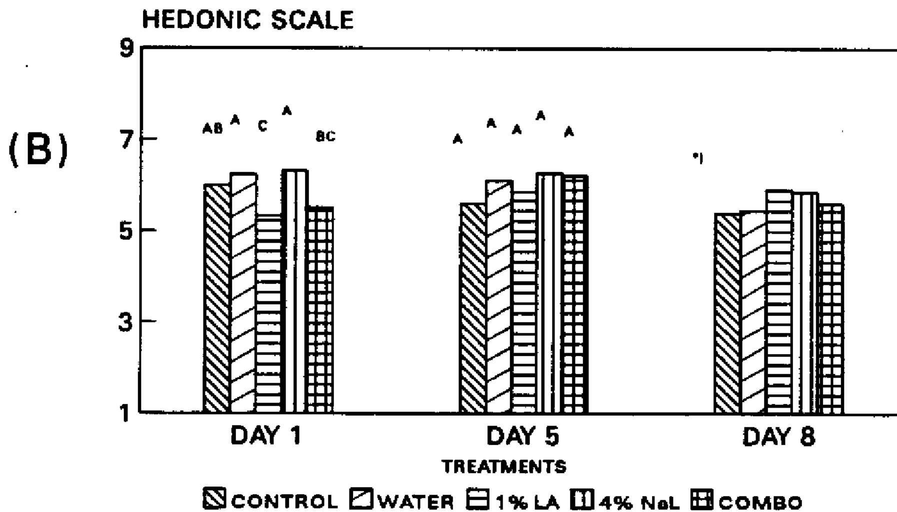
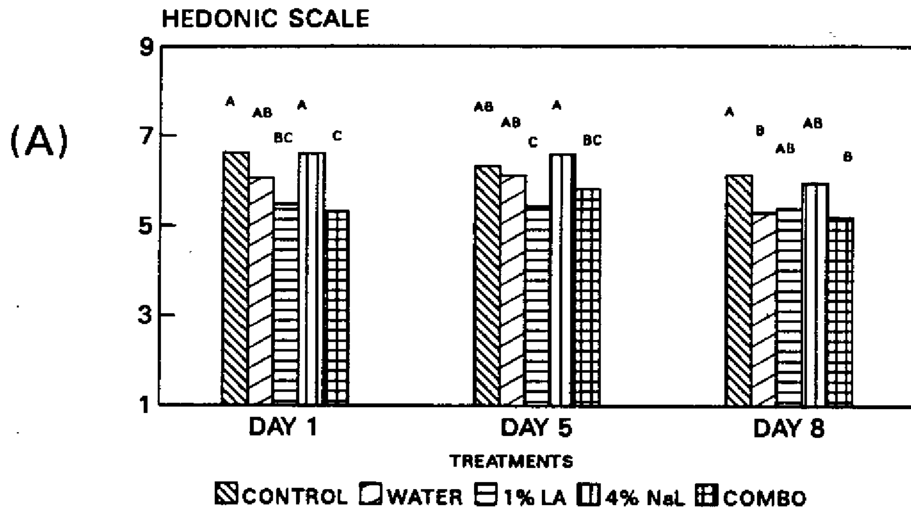
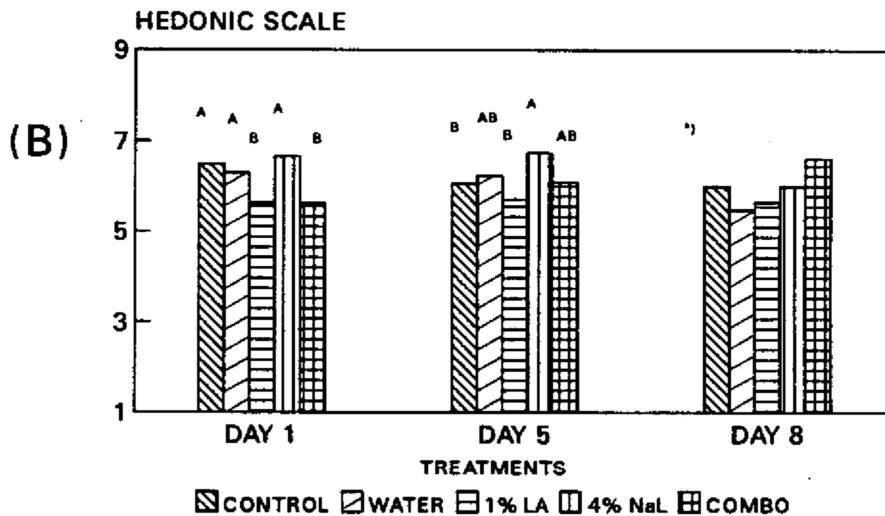


*Figure 6: Sensory evaluations of flavor (A) and overall acceptability (B) of fresh blue crab meat following lactate treatments. Means of the same day marked with different letters were significantly different at a 0.05 level. *) F-value too small.*
LA = lactic acid, NaL = sodium lactate, combo = 1%LA + 4%NaL
9 = like extremely
1 = dislike extremely

BLUE CRAB MEAT
SENSORY EVALUATIONS
FLAVOR



BLUE CRAB MEAT
SENSORY EVALUATIONS
OVERALL ACCEPTABILITY



CONCLUSIONS

Shelf life of fresh blue crab meat was extended 20% by sodium lactate (4%), 60% by lactic acid (1%) and 30% by the combination of both (1% lactic acid and 4% sodium lactate) treatments.

Elevated moisture content and an increase in lightness were observed in all treated samples. Lactic acid containing treatments showed reduced pH values. No treatment effects on texture were detectable with either sensory or physical evaluations.

Panelists judged lactate samples better in appearance with no differences found overall in odor and texture. Panelists found both lactic acid and sodium lactate treated crab meat to be acceptable with sodium lactate judged best in flavor and overall acceptability.

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EXTENDING SHELF LIFE OF REFRIGERATED SEAFOOD PRODUCTS

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INTRODUCTION

Seafood products are widely known for their high degree of perishability. For the most part, U.S. seafood processors have relied on good sanitation and proper time and temperature controls for slowing decomposition and spoilage in refrigerated seafood products. Other food processing industries have employed traditional food preservation concepts for extending shelf life of refrigerated foods (29). These concepts (Table 1) include control of water activity (a_w), pH, irradiation (ultraviolet/ionizing), preservatives and altered atmospheres (MAP, CAP or vacuum). More recently, new technologies such as ohmic heating, ultra high pressure, microwave pasteurization and natural and innovative preservatives have become available to food processors. This paper examines current technologies used in processing blue crab, health regulations impacting the U.S. blue crab industry and opportunities to apply new technologies for increasing value and extending shelf life of refrigerated blue crab products.

THE U.S. BLUE CRAB PROCESSING INDUSTRY

Current technologies used in processing blue crab involve traditional time and temperature controls and sanitation programs. Cooked, ready-to-eat refrigerated and pasteurized crab meat are the primary product types. Frozen crab meat has been a traditional type for use in value-added products (e.g., crab cakes and deviled crabs). Market interest in both live and frozen (cooked) whole crab, crab portions and vacuum-packed crab meat has increased recently (32). This interest, coupled with natural fluctuations in blue crab supply, has made for volatile market conditions.

A critical point in the processing of blue crab is cooking. Cooking times, temperatures and methods affect yield and subsequent shelf life of refrigerated products (6,9). Traditional time and temperature controls for cooking blue crab were based on industry practice and research during the 1950s to 1970s (7,20,33). The products fall under Current Good Manufacturing Practices (CGMPs) as required of all U.S. food processors engaged in interstate commerce (5). Specific time and temperature controls in production of refrigerated (24,34) and pasteurized (12,21,27) crab meat are available.

Still, good sanitation programs are required for the low bacterial levels necessary to protect the processor from losses due to spoilage and to ensure the safety of the product to the consumer (18,30,31). During processing, crab meat can be exposed to a wide variety of microorganisms. Therefore, it is vitally important that sanitation programs be rigorously applied, processes carefully managed to eliminate cross-contamination and refrigerated storage routinely monitored.

Table 1. Food Preservation Concepts for Extended Refrigerated Foods

Traditional Technologies	New Technologies
* Water Activity (a_w)	* Ohmic Heating
* pH (acidity)	* Ultra High Pressure
* Irradiation	* Microwave Pasteurization
- Ultraviolet	* Natural Preservatives
- Ionizing	* Innovative Preservatives
* Preservatives	
* Altered Atmosphere	
- MAP (modified)	
- CAP (controlled)	
- Vacuum	
* Time and Temperatures	
* Sanitation (prevention)	

HEALTH REGULATIONS IMPACTING BLUE CRAB PRODUCTS

All cooked, ready-to-eat blue crab products will be affected by new health regulations, including HACCP-based inspection, microbiological criteria, adulteration and economic fraud and labeling. Industry along with the U.S. Food and Drug Administration (FDA) and the U.S. National Marine Fisheries Service (NMFS), has worked cooperatively to implement a Hazard Analysis Critical Control Point (HACCP) inspection program. This approach will improve the quality and safety of cooked, ready-to-eat blue crab products.

Critical steps for control of microbial levels in refrigerated blue crab products are shown in Figure 1. Cooking, picking and packing are identified as critical control points under the blue crab HACCP regulatory model (26). Current industry practices for cooking of blue crab include steam pressure, atmospheric steam (partial cook) and boiling (Table 2). Specific process schedules have been incorporated into most state health regulations in the United States. Picking and packing represent additional critical control points (Table 3) where potential bacterial contamination of product is prevented through good manufacturing practices and proper sanitation.

Microbiological criteria (Table 4) for cooked, ready-to-eat crab meat and shrimp have been recommended for process verification (3,25). They include specific tolerance levels for Salmonella, Listeria monocytogenes, Staphylococcus aureus and thermal tolerant coliforms.

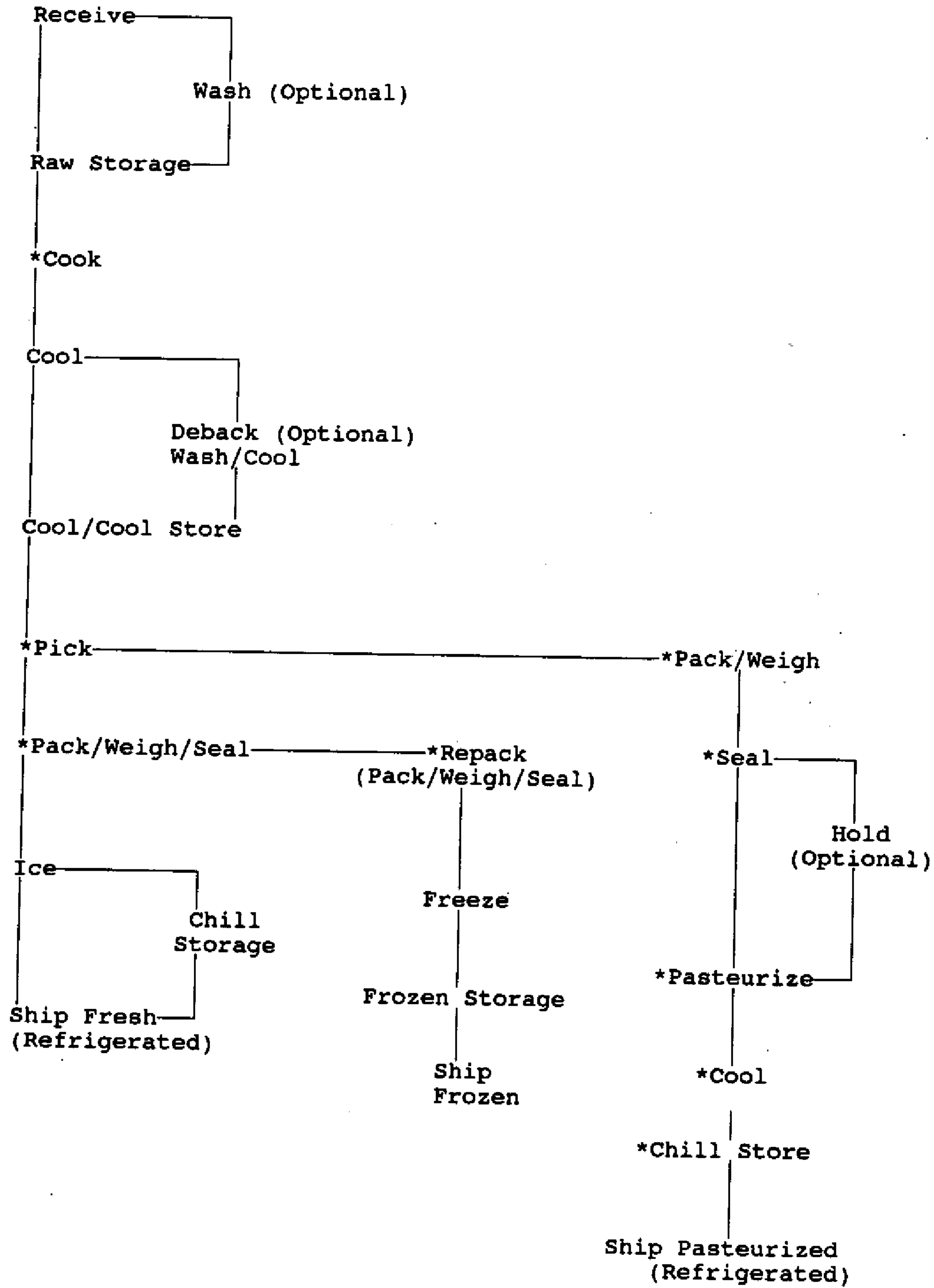

Figure 1. Flow chart for processing of blue crab products.

Table 2. CRITICAL CONTROL POINT (COOKING) IN BLUE CRAB PROCESSING

METHOD	HAZARD	PREVENTIVE MEASURES	MONITORING	RECORDS
Steam pressure	Microbial survival	Adequate time and temperature	Pressure monitoring	Annual cooker retort certification
	Decomposition (short shelf life)	Approved scheduled process	Temperature monitoring	
Steam atmospheric (partial cook only)	Microbial survival	Adequate steam distribution and venting	Time in retort Venting	Annual cooker retort certification
	Decomposition (short shelf life)	Approved scheduled process	Check of and construction	Approved scheduled process
Boiling	Microbial survival	Adequate time and	Cooking time	
	Decomposition (short shelf life)	Approved scheduled process	Boiling of water	Approved scheduled process

Table 3. CRITICAL CONTROL POINTS (PICKING AND PACKING) IN BLUE CRAB PROCESSING

STEP	HAZARD	CONTROL POINTS	PREVENTIVE MEASURES	MONITORING	RECORDS
Picking (hand and machine)	Bacterial contamination	Picking station	Good manufacturing practice	Supervisory checks	
	Foreign material	Machine	Personal hygiene	QA checks	
	Excessive shell		Clean and sanitize equipment		
	Decomposition		Pest control		
			Short hold time		
			Immediate icing		
Pack/weigh and seal fresh meat	Incorrect weight	Packing weighing station	Scale check	Supervisory checks	Annual scale certification
			Employee training		
	Foreign material			Scale calibration	
			Sanitation		
	Bacterial contamination			QC checks	
			Time/temperature control		

Table 4. RECOMMENDED MICROBIOLOGICAL CRITERIA FOR PROCESS VERIFICATION IN COOKED, READY-TO-EAT SHRIMP AND COOKED, READY-TO-EAT CRAB MEAT

MICROORGANISM	CRITERIA		EXPLANATION
	SHRIMP	CRABMEAT	
Salmonella	n = 30 c = 0 m = M = 0	n = 30 c = 0 m = M = 0	Analytical unit = 25 g
Listeria monocytogenes	n = 5 c = 0 m = M = 0	n = 5 c = 0 m = M = 0	Sample unit = 50 g; analytical unit = 25 g through compositing of 5-g portions from 5 sample units
Staphylococcus aureus	n = 5 c = 2 m = 50/g M = 500/g	n = 5 c = 2 m = 100/g M = 1000/g	
Thermal Tolerant Coliforms	n = 5 c = 2 m = 100/g M = 1000/g	n = 5 c = 2 m = 500/g M = 5000/g	

n number of sample units examined from a lot to satisfy plan
c maximum allowable number of defective units (class 2) or marginally acceptable units (class 3)
m microbiological limit in class 2, separates good from defective quality
in class 3, separates good from marginally acceptable quality
M microbiological limit in class 3, separates marginally from unacceptable quality

One criteria, zero tolerance for Listeria monocytogenes, has proven to be quite a challenge for industry. The organism is widespread in nature and the primary concern is for its growth under refrigerated storage conditions (13,14). Of significant interest has been detection of Listeria in processing plants generally recognized as having better designed sanitation programs, i.e., a cleaner working environment (1).

Another issue affecting refrigerated blue crab products is adulteration and economic fraud. Concerns have led U.S. processors to petition the FDA for increased surveillance of imported products and use of tamper-evident packaging in domestic markets (15). The fraudulent mixing of domestic and foreign meats and slacking out of frozen product to be sold as fresh have added to the economic instability of traditional blue crab markets. In an effort to thwart fradulent practices and address health concerns for refrigerated crab products in distribution, North Carolina processors have agreed with state health authorities to use tamper-evident packaging for all North Carolina produced crab products. This new state health regulation (28) will take effect April 1, 1993.

Another area impacting refrigerated blue crab products is labeling. Historically, use of the descriptor "fresh" has referred to cooked blue crab meat that has not undergone any further processing or freezing. Industry anxiously awaits the FDA's decision on continued use of this descriptor for packaging of refrigerated blue crab products. In addition, mandated nutrition labeling will force blue crab processors to redesign packaging materials and deplete present stocks of packaging as early as May of 1993.

OPPORTUNITIES FOR NEW PRODUCTS AND NEW MARKETS

Now that we have reviewed current practices and listed challenges facing blue crab processors, let's discuss some opportunities for applying new technologies to refrigerated blue crab products. Broadly speaking, chilled foods are extended shelf life products that exhibit shelf lives longer than those traditionally associated with the food and rely mainly on refrigeration for safety (29). Examples of extended shelf life products are Oscar Mayer's Lunchables with cured cooked chicken, sodium lactate and MAP, a Contadina pasta that uses A_w MAP and heat treatment after extrusion to destroy psychrophiles and Sara Lee's Bilmar deli meats process that packages, pasteurizes and chills without headspace, i.e., sous vide.

What about seafood products? Ionizing irradiation is approved for use in over 30 nations to extend shelf life of a variety of foods (10). Research on seafood products, including blue crab, has demonstrated positive effects on quality and freshness (11,16,17). Presently, low-dose gamma irradiation is not approved for use in the U.S. by the FDA in processing of refrigerated seafood products. Its approval would greatly enhance the value, safety and consumer confidence for pathogen-free fresh seafood in the marketplace (10).

Altered atmospheres are traditional technologies used to extend shelf life of a wide variety of refrigerated foods including fresh fish and shellfish (19). Its use in the blue crab industry has been limited primarily to vacuum packaged, frozen hand-picked meats. Currently, MAP does not have U.S. FDA endorsement for seafoods although several European countries have accepted this technology for commercial use (4). Limited use of MAP under specified conditions was approved under the previous voluntary U.S. NMFS inspection program.

What about extended shelf life blue crab products? Natural and innovative preservatives represent a new technology so far untapped by traditional seafood processors. An example is use of the natural preservative sodium lactate to extend the shelf life of blue crab meat (22). Lactates are just one of a variety of GRAS approved substances that may be used as direct or indirect additives in seafood products.

BENEFITS OF INCREASED VALUE AND EXTENDED SHELF LIFE

What are the benefits for industry to incorporate new technologies into current practice? Short term benefits are increased value and greater consumer confidence in refrigerated products. Longer term, the benefits are new forms and markets for extended shelf life products. The blue crab industry traditionally has been a commodity-based industry at the mercy of supply and demand economics. Application of new technologies in development of refrigerated blue crab products would alleviate restraints on growth and open the door to the modern food era.

Any new development must be properly planned and cautiously scrutinized to ensure its safety. For industry, this development usually is in cooperation with university and regulatory personnel. One approach is the barrier concept which employs a combined technologies approach to shelf life extension and safety (23). Under this approach, all new products should be abuse tested and challenged with various bacteria to check for the effectiveness of the barrier system. Protocols for verifying the effective control of specific pathogens have been proposed (2,8).

SUMMARY

In summary, regulatory guidelines for processing of cooked, ready-to-eat crab meat are well defined. The successful implementation of the HACCP-based inspection program for blue crab will greatly enhance the quality and safety of blue crab products. While the U.S. blue crab industry is undergoing some significant changes, opportunities do exist for applying innovative approaches to extend the shelf life of blue crab products. Establishment of HACCP-based inspection and microbiological criteria may provide industry with the proper mechanism to apply new technologies, add value and develop new markets. The challenge before all of us here today is to develop a new generation of extended shelf life seafood products.

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TECNOLOGIA DE LAS PESQUERIAS CUBANAS Y ASEGURAMIENTO DE SU CALIDAD

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Las pesquerías cubanas se realizaban inicialmente en la plataforma insular; a partir de la institucionalización del sector (1959), éstas aumentaron su capacidad mediante el desarrollo de flotas de mayor porte dirigidas a pesquerias internacionales y más recientemente comenzó a desarrollarse la acuacultura como una nueva forma de amplias perspectivas de producción.

La primera industria se estableció antes de 1959 y su unico objetivo era la elaboración de conservas de langosta. Se carecia además de las condiciones adecuadas para la conservación y procesamiento de las capturas.

Con el desarrollo pesquero cubano se ha fomentado tanto el procesamiento en tierra y mar de los recursos pesqueros como el aseguramiento de su calidad; ello se ha visto recompensado por la aceptación de los productos tanto a nivel nacional como internacionalmente, todo lo cual ha sido posible por el programa de inversiones desarrollado en el país.

Este desarrollo tecnológico nacional, fue acompañado por la formación de una infraestructura cientifico-técnica, dada por el aumento de la exigencia por parte de los consumidores y debido adicionalmente a la incorporación a la dieta de la población de un mayor volúmen de productos pesqueros elaborados, fundamentalmente a partir de especies de agua dulce cuyo consumo no era tradicional para la población cubana.

La presente ponencia tiene como objetivo central, mostrar los resultados obtenidos en los aspectos de caracterización y procesamiento de los recursos pesqueros, así como en el aseguramiento de su calidad relacionados con la propia evolución que ha sufrido la industria de pesquera en el país.

Industria Procesadora.

En Cuba la industria procesadora se caracteriza por estar dirigida hacia dos objetivos fundamentales:

- El procesamiento de crustáceos y mariscos de alto valor comercial con destino al mercado exterior

- La obtención de productos pesquerosproducción dirigidos al consumo interno (2).

En la Fig. 1, se muestra el crecimiento de la producción industrial en los ultimos 20 años, el que provocó una transformación cualitativa y cuantitativa de la industria procesadora, la cual a partir de la decada del 60, estuvo caracterizada por un fuerte proceso inversionista; dirigido a establecer las capacidades de procesamiento y la producción de hielo necesarias para este desarrollo y permitió la diversificación de la oferta tanto en el mercado nacional como el extranjero.

De igual forma, el programa nacional para la producción de especies de la acuacultura, requirió nuevos esquemas de almacenamiento y conservación, dadas las características de estas pesquerias.

La producción pesquera constituye una de las fuentes protéicas de origen animal mas importantes en la dieta de la población cubana, la que tiene hábitos arraigados de consumo de organismos acúaticos, En la Fig. 2, se muestra la evolución del consumo percapita de pescado entre 1958 y 1990, donde se aprecia que éste creció significativamente en la década del 80, con un ligero descenso en 1990 debido a la disminución del volumen de importaciones no suplidos aún con la producción acuícola.

Entre los surtidos más importantes destinados a las exportaciones se encuentran la langosta entera precocida congelada, cola de langosta congelada y camarón entero o cola congelada; y en menor medida las capturas de túnidos que se procesan como conservas en aceite vegetal.

La forma organizativa más generalizada en la industria pesquera cubana es la conocida como Combinados Pesqueros Industriales, de los cuales existen 8 dedicados a la langosta y 3 al camarón de la plataforma marina. Estos complejos industriales se encargan integralmente de las capturas, el traslado a la fabrica y su transformación hasta productos terminados, contando además para ello, con laboratorios de control de la calidad (2).

Igualmente ya están instaladas en el país 4 plantas industriales para el procesamiento del camarón de cultivo en los principales polos productores nacionales, mientras para las especies dulceacuicolas lo más generalizado es su beneficio y oferta a la población en forma de enteros eviscerados, pulpas o filetes, para lo cual existen plantas de procesamiento ubicadas en las principales áreas.

Para asegurar el proceso productivo, y la diversificación necesaria con incremento en la calidad, se fomentó el desarrollo de un programa de investigaciones del cual el Centro de Investigaciones Pesqueras, del Ministerio de la Industria Pesquera, es su máximo responsabe. Participan en menor medida en estas investigaciones el Instituto de Investigaciones de la

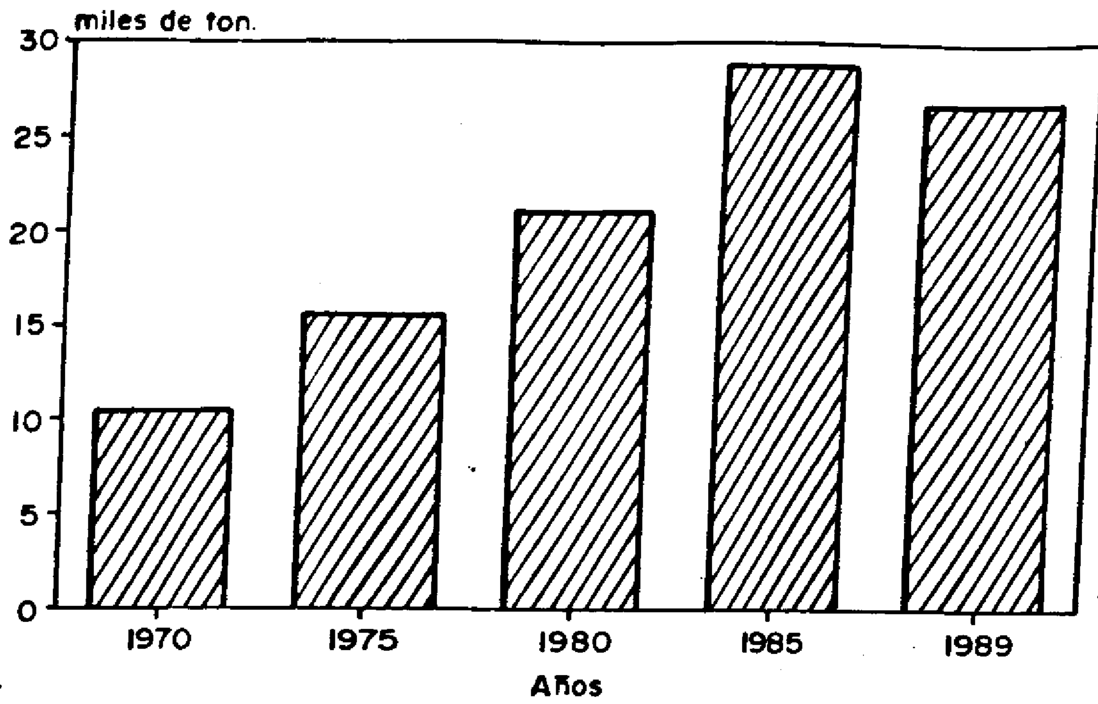

Fig.1 Comportamiento de la producción industrial de productos pesqueros.

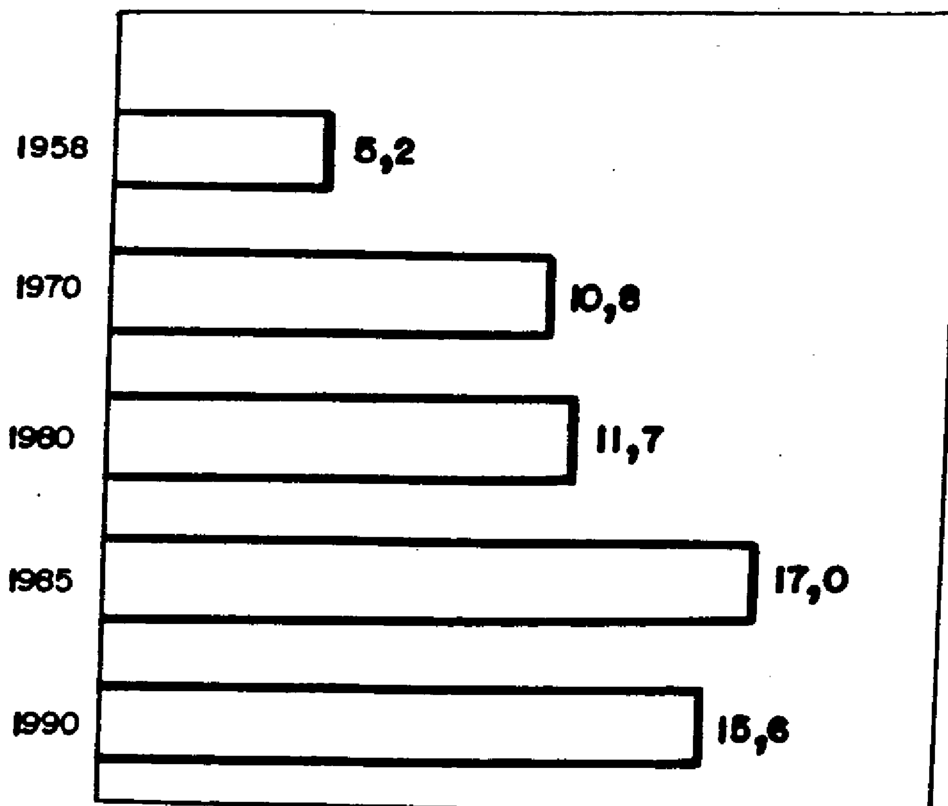

Fig.2 Consumo percápita de pescado en Cuba.

Industria Alimenticia y el Instituto de Farmacia y Alimentos entre los más representativos.

La actividad de investigación se inició a partir de la década del 70, lo que significó en consecuencia la base científico técnica necesaria para este desarrollo perspectivo.

Caracterización de los Recursos Pesqueros.

De las especies utilizadas como materia prima en la Industria Procesadora, se han caracterizado quimicamente 27, de ellas 17 pertenenecen a la ZE (Zona Económica), 4 provenien de capturas internacionales y 6 de la acuacultura; obteniendose que el bonito y la carpa plateada resultaron ser los de mayor contenido proteico mientras el primero es el de menor contenido graso (Tabla 1).

Adicionalmente se caracterizaron, física, química y nutricionalmente el bonito (Katsuwonus pelamis), la carpa plateada (Hypophthalmichthys molitrix) y la carpa comun (Cyprinus carpio).

Los resultados demostraron que las 3 especies presentan un elevado valor nutricional dado por su elevado contenido de proteinas de alto valor biológico y excelente composición aminoacídica, asi como un bajo contenido de lípidos (Tabla 2), en cuya composición se encuentran los acidos grasos esenciales para el hombre y otros de la serie W-3 con importancia terapéutica. Estas especies resultan favorecidas además por un alto contenido de macro y micronutrientes esenciales para el metabolismo humano.

Características microbiológicas de algunas especies de importancia económica.

Existen estudios para determinar la flora de microorganismos en los productos pesqueros, tanto en su medio natural como una vez capturados y almacenados, los que se han realizado con el objetivo de conocer su flora deteriorante y el riesgo de contaminación humana por su ingestión.

Estos resultados han sido registrados en diversos artículos científicos y en lineas generales, se corresponden con las características de estas especies cuando son capturadas en aguas no contaminadas y procesados higiénicamente.

A consecuencia del desarrollo acuícola , con especies de agua dulce y el camarón se intensificaron estos estudios dado que las propias condiciones de cultivo constituyen un riesgo potencial desde el punto de vista microbiológico

Estos permitieron precisar la microbiota de la tilapia, principal especie cultivada en Cuba, la carpa común, la carpa plateada y el camarón blanco (Tabla. 3) donde se hallaron microorganismos

Tabla 1. Composición quimica de especies comercializadas en Cuba.

ESPECIES	% HUMEDAD	% PROTEINA	% GRASA	% CENIZA
Jurel	74.75	19.74	3.79	1.65
Mintay	80.16	17.28	0.52	1.08
Calamar	76.6	18.35	1.5	1.72
Bonito	68.71	28.17	0.38	1.29
Merluza	81.15	16.06	1.19	1.10
Machuelo	75.96	17.97	0.66	1.75
Sardina de ley	75.89	22.19	1.43	1.92
Lisa de Abanico	74.00	20.40	3.60	1.20
Sardina Escamuda	76.75	19.21	1.23	1.81
Biajaiba	78.75	16.70	1.59	1.60
Mojarra	79.60	16.50	0.21	2.29
Casabe	79.50	17.0	0.82	1.85
Besugo	79.25	15.3	1.55	1.29
Pez Volador	80.50	15.5	0.63	1.40
Lagarto	80.30	15.8	0.90	1.75
Clarin	80.0	17.0	0.46	1.20
Cangrejo de Tierra	77.32	18.07	0.37	1.87
Almejas	77.58	11.57	3.44	2.36
Langosta	76.93	19.88	0.71	1.5
Ostión	87.40	7.8	0.60	3.3
Ronco	87.40	19.9	2.1	1.3
Tilapia	77.50	18.50	1.60	1.18
Carpa	77.40	18.60	2.34	1.25
Tenca Blanca	75.84	20.20	2.53	1.35
Pez Gato	75.53	18.06	4.76	1.40
Amura Blanca	78.21	16.46	3.87	1.18
Pez Bufalo	72.56	13.81	11.31	0.72

Tabla 2. Características nutricionales de especies de importancia económica para Cuba (6).

	Bonito	Carpa plateada	Carpa común
Calidad protéica			
Relación a.a.esenc/no esenc.	0.97	0.95	0.94
Total a.a.azufrados g/100 g	4.12	3.79	3.97
Total a.a.aromáticos g/100 g	13.37	7.60	7.74
A.a. primer limitante FAO/WHO 1989	Triptófano	Histidina	Ninguno
Cómputo Químico Corregido	0.70	0.99	1.00
Digestibilidad *in vitro* (%)	99.30	99.60	99.70
PER(Razón eficiencia proteíca)	3.40	3.10	-----
Valor Biológico (calculado)	81.00	79.00	-----
Indice Osser-Mitchel	78.00	76.00	-----
Fracción lipídica			
Ac.gr.esenc. y semiesenc. (%)	25.80	14.70	19.40
Colesterol mg/100	68.80	76.10	71.60
Triglicéridos mg/g	-----	51.40	-----
Fosfolípidos mg/g	-----	33.00	-----
Total ac.grasos saturados (%)	39.10	35.36	27.10
Total ac.grasos insaturados(%)	64.00	64.64	69.50
Rel ac.gras.insat/sat	1.60	1.82	2.50
Macro y Micronutrientes			
Fosforo (mg/100 g)	140.51	100.10	103.10
Potasio (mg/100 g)	448.40	281.30	289.50
Sodio (mg/100 g)	98.90	33.20	43.60
Calcio (mg/100 g)	-----	18.90	-----
Cinc (mg/100 g)	-----	0.59	0.63
Cobre (mg/100 g)	0.09	0.04	0.04
Hierro (mg/100 g)	1.10	0.51	0.56
Vitaminas			
Vitamína B_1 (µg/100 g)	-----	28.00	16.00
Vitamína B_2 (µg/100 g)	-----	-----	47.00
Vitamína E (µg/100 g)	-----	1391.50	629.00

comunes a estos sistemas de cultivo.

Conjuntamente se ha establecido como linea de trabajo la determinación de los puntos críticos de control desde el inicio del cultivo hasta la salida industrial del producto terminado, resultados no concluidos aún. Se estudia también, la distribución espacial de las baterias del género **Vibrio** dada su significación en los productos marinos.

Determinación de otros contaminantes químicos en productos pesqueros.

El desarrollo industrial favorece la contaminación del medio acuático con metales pesados, pesticidas u otros compuestos químicos, e igualmente hay otras suatancias tóxicas como la histamína que consituyen un riesgo potencial a la salud publica y por tanto son importantes en el establecimiento de la calidad de los productos pesqueros.

De ahí que en el programa de investigaciones se haya considerado los mismos segun la zona y especies de riesgo, considerando las especies provenientes de la acuacultura y aquellos con una cadena alimenticia larga debido a sus características migratorias.

En la Tabla 4, se muestra el resultado del estudio de mercurio total en bonito para las dos plataformas de pesca de la especie, donde se aprecia que esta presenta valores muy inferiores a los límites permisibles en pescado según la OMS.

A su vez, fue evaluada la presencia de histamina en la materia prima como jurel y bonito empleados en la preparación de picadillo de pescado, o en el proceso tecnológico de aquellas especies utilizadas en el país para estos fines y que poseen histidina en una proporción elevada, determinándose los puntos de riesgo lo que permitió a su vez, arribar a conclusiones y brindar recomendaciones concretas que aseguren un producto final con niveles inferiores a los establecidos por las regulaciones correspondientes.

Desarrollo y Aprovechamiento de los Recursos Pesqueros.

El Programa Nacional de Investigaciónes, ha permitido que en la actualidad se hayan desarrollado las formulaciones de 215 productos, de ellos 43 con especies provenientes de la acuacultura. Los mismos se han agrupado de la siguiente forma, según diferentes tipos de tecnologías:

- Conservas 47%.
- Preelaborados, conformados y embutidos 25%.
- Congelados 10%.
- Ahumados y preservados 3.2%.
- Salados 1.2%.
- Otros 10%.

Tabla 3. Microbiota de especis de interés economico.

Especie	**Generos bacterianos que predominan**
Carpa común (3)	*Aeromonas* *Acinetobacter* Enterobacterias Cocos *Bacillus*
Carpa plateada (8)	*Aeromonas* *Alcalígenes* *Acinetobacter* Enterobacterias Estafilococos *Pseudomonas* *Moraxella*
Tilapia (7)	Enterobacterias *Pseudomonas* Micrococos *Acinetobacter* *Aeromonas* *Moraxella* *Alcalígenes* Flavobacterias
Camarón de cultivo (9)	*Bacillus* *Aeromonas* *Acinetobacter* Flavobacterias Micrococos *Vibrio* *Moraxella* *Alcalígenes*

Tabla 4. Contenido de mercurio total (Hg) en bonito (mg/Kg) (10).

Z.E. de la plataforma cubana	**Hg Total media**	**D.S.**	**Rango**
Nororiental	0.37	0.18	0.06-0.7
Suroccidental	0.31	0.17	0.08-0.79

En la actualidad el trabajo se ha encaminado hacia la preparación de nuevos productos y el aprovechamiento de subproductos y residuos de alto valor nutricional no considerados con anterioridad.

En relación con el primer aspecto se trabaja en la preparación de alimentos de alto valor nutricional (13 %) a base de pulpas de pescado, empleando especies provenientes de la acuacultura tales como carpa común, carpa plateada, tilapia y especies marinas de menor aceptación como el ronco y el jurel. En este sentido la disminución del indice de insumo de materia prima ha sido posible incorporando extensores con los que se logran: mayores volúmenes de producción y disminución en los costos de elaboración.

También (y dada la importancia del desarrollo de una infancia adecuadamente alimentada), se desarrollaron conservas para la preparación de alimentos infantiles que cubren los requerimientos establecidos para este grupo poblacional por la OMS.

En Cuba, el aprovechamiento de subproductos de la industria ha cobrado interés por la posibilidad de disponer de una nueva fuente de alimentación humana y/o animal, asi como por la disminución considerable de los contaminantes ambientales que esto significa.

Partiendo de estos objetivos centrales se determinó el potencial que ello representa en la industria langostera y camaronera, la captura incidental en las pesquerias de camarón, los peces de pequeña talla, el aceite de hígado de tiburón y las aguas residuales de la industria, entre otros.

En este sentido se caracterizó el aceite del hígado de 13 especies de tiburón, encontrandose que ha sido común entre ellas su contenido de ácidos grasos polinsaturados de la serie W-3, y su elevada concentración de Vit. A. Con ello se obtuvo un concentrado de ácidos grasos con un 70% de estos compuestos con fines medicinales.

Las aguas de cocción del proceso de langosta entera precocinada han demostrado su aplicabilidad en la elaboración de extractos aromáticos naturales, igualmente el carapacho se emplea en la fabricación de harina para la alimentación animal, quitina y quitosana; y las masas residuales del cefalotorax en la obtención de pulpas para el consumo humano (4 y 5).

Con las huevas de pescado y la carne roja de los túnidos se han desarrollado formulaciones de conservas, pastas y embutidos de gran aceptación y contenido proteíco, mientras las aguas residuales de la industria hoy pueden ser depuradas en lagunas de oxidación de alta velocidad mediante su empleo en el cultivo de microalgas (i.e. **Chlorella**) con una producción adicional de proteínas de origen vegetal u otros compuestos bioactivos a partir de la cepa cultivada.

El Aseguramiento de la Calidad en la Industria Pesquera.

El aseguramiento de la calidad de los productos pesqueros, en el período anterior a la decada del 60 era practicamente inexistente. En los mercados y en las mismas industrias se aplicaban practicas engañosas para enmascarar los defectos de calidad de algunos productos(1).

Las actividades en esta linea comenzaron en 1962 y su desarrollo ha estado caracterizado por las siguientes etapas generales (1):

- 1962: Control de productos en conserva.
- 1967: Control de productos congelados.
- 1970: Creación del laboratorio central.
- 1974: Desarrollo de inspecciones.
- 1975: Actividad de normalización.
- 1977: Actividad de metrología.
- 1981: Creación de la primera organización básica en Normalización, Metrología y Control de la Calidad en el país.

En la actualidad todos los parámetros de calidad estan registrados en documentos técnico-normalizativos de carácter legal, los cuales al igual que los métodos de ensayo se encuentran en la espiral ascendente de la tendencia actual a nivel mundial.

En este programa ha sido de gran importancia el establecimiento de indices de calidad y tiempos de vida útil de cada materia prima y producto terminado, según las condiciones de conservación.

Para nuestras condiciones climáticas, el tiempo de durabilidad de las especies dulceacuícolas fundamentales, conservadas en hielo, se encuentran entre 15 y 21 días, siendo la tilapia la más resistente a este tipo de almacenamiento.

CONCLUSIONES

La Industria Pesquera Cubana se ha desarrollado sobre la base de un amplio proceso inversionista a nivel nacional que consideró, el establecimiento de centros de procesamiento a lo largo del país, enmarcado como parte integral de las Empresas Pesqueras de la Plataforma y paralelamente por el desarrollo de la industria productora de hielo, y una infraestructura para la conservación y distribución de estas producciones basada en 3 Empresas Mayoristas, responzabilizadas con ello a nivel nacional.

El aseguramiento de la calidad ha sido determinante a nivel nacional, y hoy se cuenta para ello con una amplia red de laboratorios en todas las plantas procesadoras asi como con un centro de referencia para ensayos en el Centro de Investigaciones Pesqueras.

La actividad de aseguramiento de la calidad se realiza de forma integral, ejecutandose el control de la materia prima desde su captura, a lo largo del proceso tecnológico, así como de la producción terminada y existe una retroalimentación en cuanto a los criterios y requerimientos de los compradores y productores.

En todo ello ha jugado un importante papel el trabajo científico-técnico como fuerza calificada capaz de asegurar todo este programa de desarrollo.

Las inversiones previstas para el futuro inmediato responden basicamente a la modernización de las técnicas de refrigeración de modo que la Industria Pesquera Cubana esté a la altura del mercado, asi como a la creación de nuevas capacidades industriales que amparen el crecimiento de la camaronicultura y el desarrollo de la acuacultura .

Finalmente debemos significar que esta industria se encamina hacia un desarrollo tecnológico armonico para afrontar de forma eficiente la creciente demanda nacional de alimentos nutritivos y el aumento de las exigencias de los paises importadores.

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Prevención del riesgo de infeccion por la ingestion de productos pesqueros contaminados en Cuba, con particularidad en *Vibrio cholerae* .

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La contaminación humana por la ingestión de alimentos portadores de patógenos es conocida, y las bacterias del género **Vibrio** su máximo exponente en los productos marinos, ya que a pesar de que el número de bacterias en el agua de mar es generalmente menor que en el agua dulce, el predominio del género **Vibrio** en ella, hace necesario considerar este riesgo potencial a la salud pública.

A esta realidad se ha sumado en la última década el riesgo de contraer el cólera dado el surgimiento de la epidemia en Perú y su propagación hacia otros países de la región en los últimos años.

Existen actualmente por lo menos 9 especies de **Vibrio** asociadas con la enfermedad, siendo **V. cholerae** la especie más importante y conocida, aunque no deben excluirse otras especies del género dado que causan enteritis en humanos.

En Cuba, el programa de aseguramiento de la calidad ha conllevado a la determinación sistemática de bacterias del genero **Vibrio** en los productos marinos y el agua de la plataforma, así como de otros géneros importantes. En la actualidad el programa se mantiene y se le incorporó la determinación de **V. cholerae**, así como recomendaciones a los manipuladores, dadas las formas de trasmisión de la enfermedad.

El objetivo de la presente ponencia es brindar una panorámica general sobre las investigaciones que se realizan para el conocimiento de las especies del género **Vibrio** presentes en aguas marinas y productos pesqueros, su prevalencia y distribución así como el programa de vigilancia para la prevención de la introducción del cólera en el país.

Cuba es reconocida internacionalmente por la calidad de sus exportaciones y por el sistema nacional de vigilancia epidemiológica en el programa de Salud Publica. Ello lleva implícito el trabajo de monitoreo a los alimentos que se consumen nacionalmente o se exportan, de los cuales el Ministerio de la

Industria Pesquera, en particular el Centro de Investigaciones Pesqueras, se responsabiliza con este trabajo en relación a los productos pesqueros.

Sobre el cólera, se considera que Cuba no esta exenta de verse afectada por la enfermedad, lo que está condicionado a tres factores fundamentales:

1. Cercanía a los paises que ya han reportado la misma,

2. Velocidad y características de las corrientes marinas que pueden arrastrar el gérmen hasta nuestras costas y,

3. Desarrollo de un programa de turismo internacional intenso.

Tomando en consideración estos aspectos, se han realizado estudios sistemáticos sobre la distribución espacial de bacterias del género **Vibrio** y se han trazado los lineamientos encaminados a la prevención de la entrada de **V. cholerae** al territorio cubano en el cual participan todos los organismos de la Administración Central del Estado relacionados con ello, siendo responsabilidad del Ministerio de Salud Publica la detección de presuntos casos asi como el pesquizaje a alimentos con la participación del Ministerio de la Industria Pesquera en el caso de los productos pesqueros.

DISTRIBUCION ESPACIAL DE BACTERIAS DEL GENERO Vibrio EN LA PLATAFORMA CUBANA

Se han realizado estudios en 3 de las plataformas pesqueras cubanas sobre la prevalencia y densidad de especies del género en langosta (Panulirus argus), ostión (Cassostrea rizophorae) y agua de mar.

En las 3 plataformas (Nor-occidental, Sur-occidental y Sur-oriental) se pesquizó el agua de mar, mientras que la langosta (masa y exoesqueleto) solamente en las zonas suroccidental y suroriental; así mismo el ostión (masa y concha) en la zona noroccidental y suroriental, dando como resultado el aislamiento de especies del género cuyo porciento relativo según la localización se muestra en las figuras 1,2,3,4 y 5.

El estudio de ecología cuantitativa realizado mostró las especies predominantes en cada zona, siendo Vibrio alginolyticus y Vibrio anguillarum para las zonas nor y suroccidental, mientras que para la zona suroriental fué Vibrio marinus.

El conteo total de vibrios se encuentra en el orden de 10^2 - 10^3 ufc/ml en el agua constituyendo aproximadamente el 50% de la microflora heterótrofa marina de las zonas en estudio.

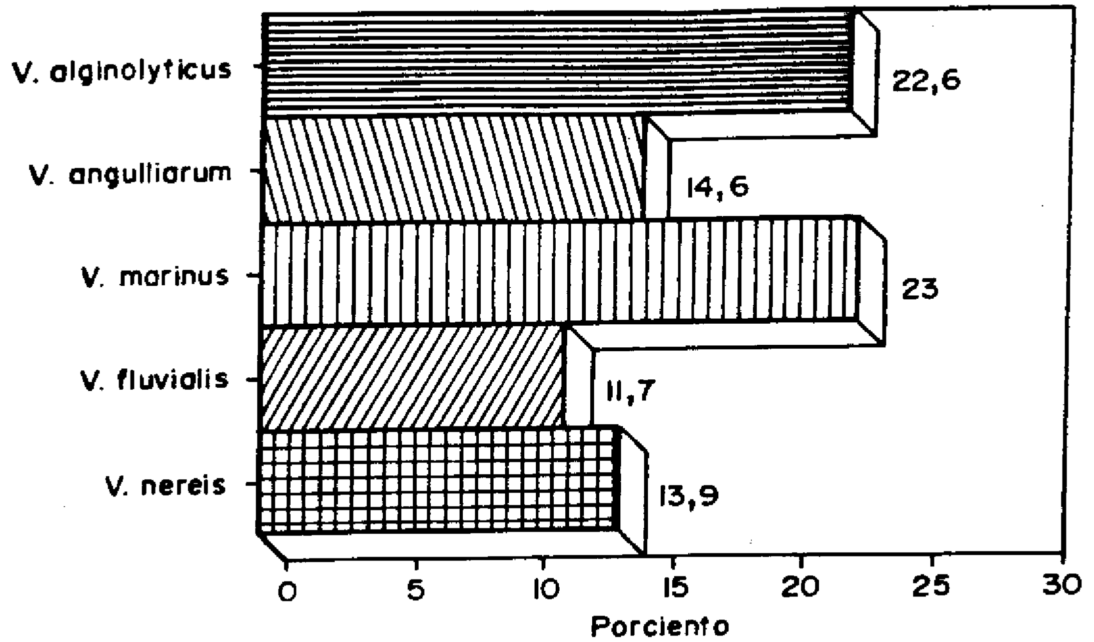

Fig.1 Porciento relativo de las principales especies de Vibrio aisladas en la musculatura de langosta.

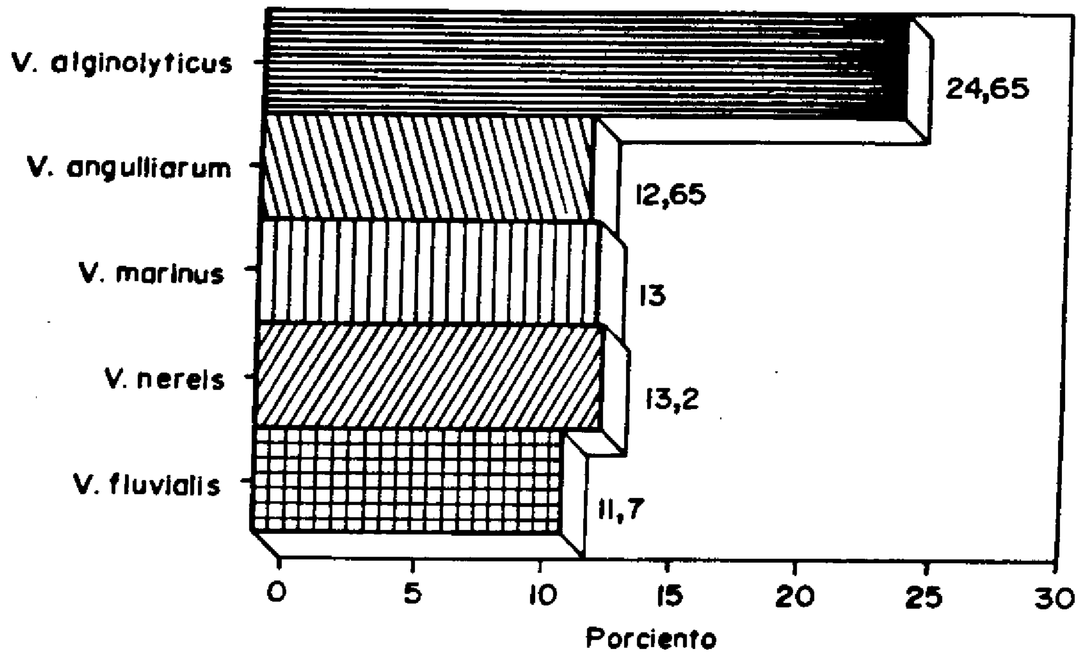

Fig.2 Porciento relativo de las principales especies de Vibrio aisladas en exoesqueleto langosta.

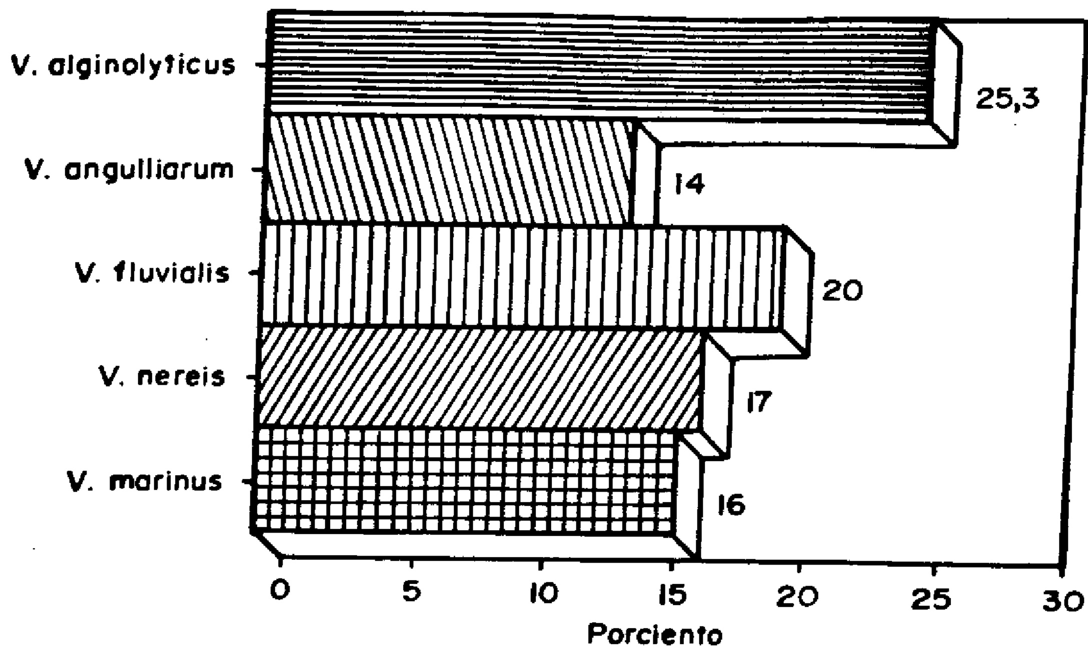

Fig.3 Porciento relativo de las principales especies de Vibrio aisladas en concha de ostión.

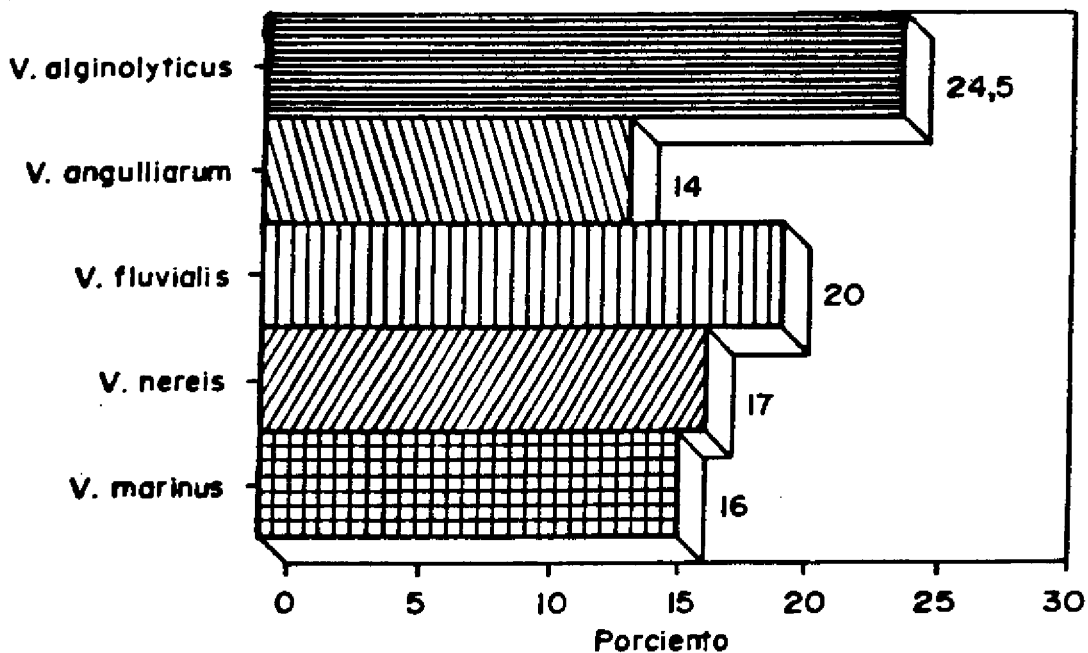

Fig.4 Porciento relativo de las principales especies de Vibrio aisladas en masa de ostión.

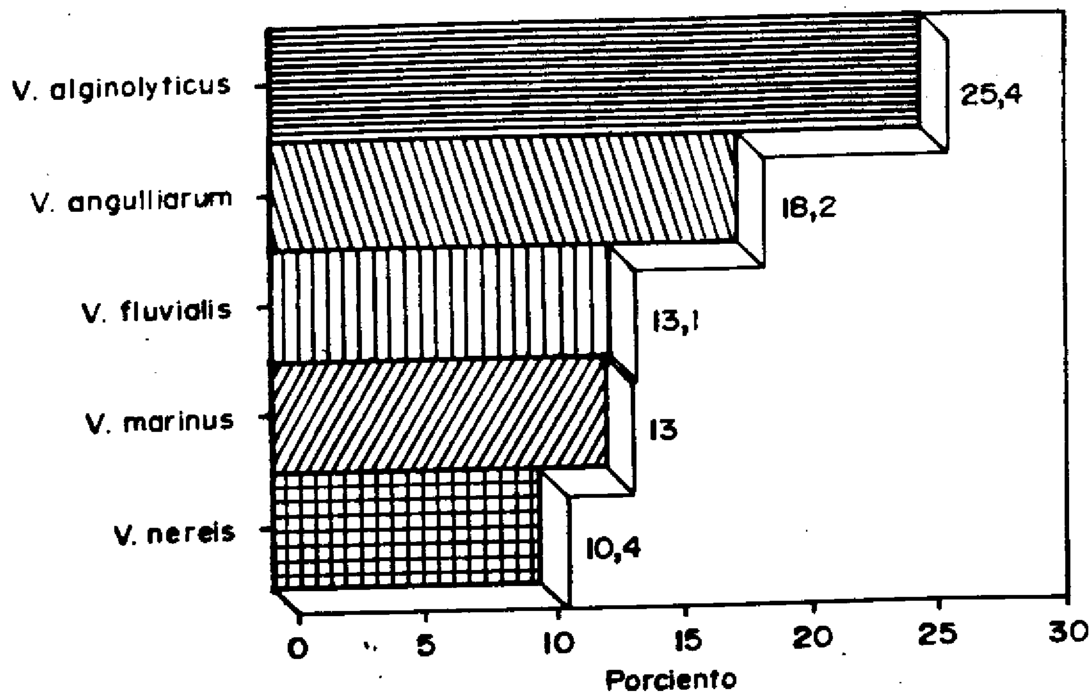

Fig.5 Porciento relativo de las principales especies de Vibrio aisladas en agua de mar.

PROGRAMA DE PREVENCION DE V. cholerae

En este caso el trabajo ha estado encaminado a:

1. Certificación de ausencia del gérmen en los alimentos,

2. Adiestramiento del personal profesional y técnico en los métodos de diagnóstico,

3. Control sanitario de frontera

4. Pesquizaje de aguas y alimentos no acuáticos mediante los centros municipales de Higiene y Epidemiología con referencia en los Institutos de Higiene, Epidemiología y Microbiología, así como de Nutrición e Higiene de los alimentos y el Instituto de Medicina Tropical para las muestras clínicas, y

5. Pesquizaje de productos pesqueros con el Centro de Investigaciones Pesqueras como centro de referencia.

Los muestreos relacionados con los productos marinos o de agua dulce tienen los siguientes objetivos:

AGUA - Abasto público en zonas de riesgo
- Residuales y áreas aledanas
- Puntos de vertimientos de residuales en rios, embalses y zonas costeras.

ALIMENTO - Pescado, moluscos y crustáceos procedentes de las pesquerías nacionales y las importaciones
- Pescado procedente de áreas de pesca internacional con riesgo potencial de contaminación.

Paralelamente, se desarrolla una labor de divulgación encaminada a orientar a tripulantes y personal que labora en la industria sobre las características de la enfermedad, formas de contraerla y por tanto como prevenirla; para ello se establecen normas sanitarias para naves pesqueras y tripulaciones que realicen sus faenas en aguas cercanas a paises afectados por la enfermedad así como centros de procesamiento de alimentos.

Con estos fines han sido realizados 453 muestreos en 1991 y 381 hasta septiembre de 1992 a productos pesqueros. Las técnicas empleadas son las convencionales con el caldo de peptona alcalina como enriquecimiento y TCBS como medio selectivo; aquellas cepas consideradas presuntos **Vibrios** se someten a estudios de caracterización que incluyen:

- Tinción de Gram,

- Motilidad
- Oxidación y fermentación de la glucosa
- Sensibilidad al agente vibriostático Ø/129
- Reducción de nitratos a nitritos

- Utilización del citrato como única fuente de carbono
- Decarboxilación de L-lisina y L-ornitina, y dehidrolización de L-arginina
- Reacción de Rojo metilo y Vogues Proskauer
- Producción de Indol
- Fermentación del manitol, inositol, sacarosa, L-arabinosa y manosa
- Confirmación serológica de presuntos **Vibrio** por aglutinación en lamina con antisuero polivalente de **V. cholerae Ø1**.

El muestreo incluye branquias e intestinos, así como las partes comestibles en algunos casos.

El resultado alcanzado hasta la fecha arroja la presencia de bacterias del género como era de esperar, pero en ningún caso se ha aislado **V. cholerae** en ninguno de sus serotipos y permite concluir que hasta la fecha Cuba se mantiene libre de riesgo de contraer la enfermedad por los productos pesqueros.

ABSTRACTS

Fish Packaging Benefits and Trends

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Packaging can provide seafood processors with extended shelf life, ease of distribution and merchandising appeal. Numerous packaging options are available for frozen fish because packaging is highly species and market dependent. Packaging of consumer sized frozen fish allows of ease of distribution and is an effective means of retarding microbial spoilage and development of rancid off-flavors resulting from lipid oxidation. The variety of Cryovac systems being used throughout the world for packaging fresh fish are described.

Options for fresh fish packaging for the U.S. market are limited by concerns over food safety and U.S. inspection regulations. USDC has placed a moratorium on fresh fish packaging because of concerns over botulism. Voluntary inspection in many U.S. plants precluded the export of fresh packaged fish from Latin America. Expected changes in U.S. Government regulations could open up new opportunities for packaged, branded fresh fish in the U.S.

Application of the HACCP Model to the Retail Seafood Industry

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FMI maintains a commitment to retail training in Hazard Analysis Critical Control Point Surveillance. Their most recent efforts are composed in a manual written for department managers and seafood associates not fully versed in the subject of HACCP. The intent of the manual is to provide seafood retailers with an understanding of the seven basic principles of HACCP and the benefits to both retailer and consumer. The extension of the HACCP principles includes not only food safety hazards, but also hazards associated with food processing and food handling hygiene, sanitation of the retail seafood operation, and economic malpractice. The seafood associate is guided through a series of nine easy-to-follow steps necessary for the development of an in-house HACCP-based plan. Additionally, generic examples are given for fresh fish, frozen fish, molluscan shellfish, and ready-to-eat seafood products. The user should understand that the generic plans presented do not attempt to cover all possible preventive measures, critical limits, monitoring procedures, or corrective actions. They are for illustrative purposes to guide seafood retailers of different size and complexity. [Manuals can be obtained from FMI.]

Effect of Washing on Menhaden Surimi

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Surimi was prepared from Gulf menhaden caught off the Florida coast. Fish was iced until processing which was usually within 24 hours post catch. Fresh menhaden was eviscerated, rinsed with water, and mechanically deboned. Minced fish muscle was washed up to seven times with 4°C water at a ratio of 1:4 (1 part fish to 4 parts water w/w). Each washing cycle consisted of 10 min of stirring followed by 10 min. of settling. The washed fish was partially drained in rotary rinser and the slurry was passed through a refiner. The refined meat was dewatered using a screw press, blended with cryoprotectants, and stored at -20° C. Results indicate that menhaden surimi gels prepared after two washings were significantly superior in textural properties (Instron) and Hunter color values over the other treatments.

Functions and Applications of Phosphates in the Seafood Industry

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When applied to seafoods, food-grade phosphates enhance the retention of moisture and flavor; inhibit lipid oxidation; aid in shelf-life extension and have cryoprotective effects on functional proteins. Condensed phosphates are most often used. Information on the uniform and consistent application of phosphates is generally lacking and the process protocol in many seafood plants is often derived empirically. Abuse of phosphates leads to dramatic increases in water retention, objectionable (soapy) flavors and a glassy appearance. This presentation will summarize those research results related to the responsible application of phosphates to seafood products and the benefits.

Thermal Inactivation of Vibrios Associated with Shellfish-borne Disease

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Estuarine vibrios including *Vibrio vulnificus*, *V. parahaemolyticus*, *V. cholerae*, *V. mimicus*, and *V. fluvialis* have been documented to cause shellfish-borne illness. Recently a mild thermal process (50 °C for 10 minutes) has been reported to destroy *V. vulnificus* in raw oysters. In order to determine if this thermal process would be sufficient to destroy other potentially pathogenic vibrios, thermal inactivation studies using pure cultures of environmental strains of the above mentioned vibrios were conducted.

Decimal reduction times at 50 °C (D_{50}) for 18 strains of *V. vulnificus* ranged from 22 seconds to 69 seconds with a mean D_{50}-value of 39.8 seconds. The D_{50}-values for most of the other vibrios tested are higher than those for *V. vulnificus* indicating that the mild thermal process developed to destroy *V. vulnificus* in raw oysters may not be sufficient to destroy other pathogenic vibrios.

The Application of HACCP in Reducing the Incidence of *Listeria* in Smoked Salmon

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Listeria monocytogenes is widely distributed in the environment and has been isolated from several types of seafood products including fish, shrimp, lobster, crabmeat and smoked salmon. Government regulatory agencies have specified that no detectable levels of *L. monocytogenes* be present in ready-to-eat food products, which includes cold and hot smoked salmon. A HACCP management program was implemented in a smoked salmon processing plant to manage the incidence and level *L. monocytogenes*. The incidence of *L. monocytogenes* was 29% before HACCP implementation, and decreased to 1.6% with HACCP initiation. Details of this HACCP program will be presented.