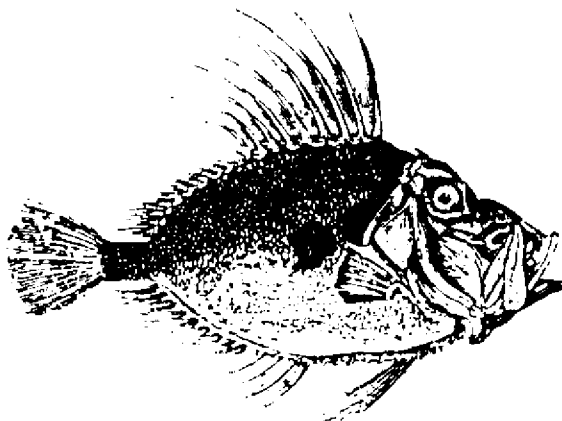


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**Annual Conference
Tropical and Subtropical Fisheries
Technological Conference of the Americas**

in second joint meeting with:

Atlantic Fisheries Technology Conference



Program and Abstracts

**August 29-September 1, 1993
Williamsburg, Virginia**

**Florida Sea Grant College Program
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ANNUAL CONFERENCE
**TROPICAL AND SUBTROPICAL FISHERIES
TECHNOLOGICAL CONFERENCE OF THE AMERICAS**

in second joint meeting with

ATLANTIC FISHERIES TECHNOLOGY CONFERENCE

August 29 - September 1, 1993

Williamsburg, Virginia

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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, and utilization of tropical and subtropical fishery species be they harvested, cultured or imported. The sister organization, Atlantic Fisheries Technological Society has maintained the same meeting objectives in the North through Mid-Atlantic coastal regions of Canada and the U.S. since 1957.

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FDA SEAFOOD HACCP INSPECTION

Philip Spiller
Food and Drug Administration
Office of Seafood, HFS-401
Washington, DC

(Text based on actual oral presentation)

Thank you. I appreciate the opportunity to talk to you today about the application of HACCP to seafood. Last march, Commissioner Kessler announced an intention on the part of FDA to require something called HACCP of the seafood industry. This requirement is to be initiated through the issuance of a federal regulation, first as a proposal for public comment, then as a final document. Neither of these documents has published yet, although we hope the proposal will shortly, so my remarks will intentionally be long on general policy considerations and short on detail.

Dr. Kessler's announcement of last March has been commonly characterized as involving "HACCP inspection" of seafood. That is true in part. Actually, HACCP is something that the industry would do, while FDA would examine how well these establishments were doing it, among other things, during the course of FDA's inspections of seafood processors and establishments.

As an aside, it is fair to say that Dr. Kessler's speech generated a considerable amount of interest. We in the FDA office of seafood immediately found ourselves in great demand by the media, and if it is true that we all have been allotted 15 minutes of fame in this life, we quickly ran through our allotment thanks to HACCP.

One morning, for example, I found myself with less than 30 seconds at my disposal to try to explain over the radio to the morning commuters in Tampa, Florida, what HACCP is all about and why they should be pleased that it was coming. I'm still not quite sure what I said.

But I remember what the commissioner said last March. The essence of it is worth reiterating today because it forms the basis for our emerging policy in this area. He said the following:

- First, food safety will be a major issue that will occupy federal policymakers over the next several years.
- Second, that while the public does not expect absolute assurances that food is perfectly safe, they do expect that a system is in place to ensure that food is as safe as we can possibly make it.

- Third, that the current system of food safety regulation is too reactive and places too much of the burden on the taxpayer to find problems. What is needed is a system that is built on preventing problems in the food supply and that gives regulatory agencies the tools to know that the system is working.
- Fourth, that the best way to do that is to institutionalize basic preventive controls to ensure safety. And the best way to do that is by companies developing and following state-of-the-art quality assurance plans and by the government verifying that the plans are being successfully carried out.

The commissioner then used seafood as an example of both how and why such a preventive system could and should be applied.

As I am sure we all know by now, HACCP, or hazard analysis critical control point, is a system of preventive controls that can be applied by a food producer to ensure, to the maximum extent possible, the safety of the food being produced.

HACCP was first applied to food by Pillsbury in the late 1950's during that company's effort to develop food for astronauts, after it occurred to them that the amount of end-product testing necessary to ensure safety would use up most of the food. So they tried an opposite approach that was designed to prevent hazards from occurring in the first place rather than to catch hazards at the end through testing. The result is a system that has become recognized throughout the developed world.

Briefly, a processor operating a HACCP system develops and implements a HACCP plan that

- ID's likely hazards that could cause the product to be unsafe to consumers;
- ID's what have been termed critical control points in the process where a failure would likely cause the hazard to occur;
- ID's critical limits, or proper operating parameters, for each critical control point;
- Includes monitoring procedures for tracking the performance of critical control points and recordkeeping procedures for recording the results of that monitoring.

As envisioned by Pillsbury, HACCP could be applied to hazards that could occur in the plant and those that could occur before a processor takes possession of raw materials. The latter types of hazards could include those with an Environmental origin, such as toxins in seafood. For these types of hazards, the receiving dock becomes figuratively, and in many cases literally, the first critical control point in a HACCP system.

To the extent that it is reasonable and appropriate for the federal government to mandate HACCP controls within the food industry, seafood is, in our view, a good place to start. For the reasons that

I will discuss. (Although, as an aside, FDA first applied HACCP principles to the regulation of low acid canned foods in the 1970's to control botulism, so seafood would really be number two.)

The philosophical underpinning for government-mandated HACCP is the view that the industry should be responsible for the safety of the food it produces. This responsibility should be exercised by design and not by happenstance. And for those who appreciate an alliteration, that design must be demonstrable. The role of the government should be that of verifier that the industry is meeting its responsibility.

Now lets apply that philosophy to seafood.

- Since seafood is still predominantly wild-caught, it is at least potentially exposed to a wide range of natural hazards and hazards from human pollution.
- It is the most perishable of all flesh foods.
- It is consumed raw much more than any terrestrial flesh food.
- It consists of literally hundreds of species from all over the globe, many having little in common other than an aquatic origin.
- No other flesh food is imported in such quantity or from so many places -- over 135 countries. Some of these countries have advanced regulatory structures for seafood safety, but many do not.
- It has a far flung distribution system that can affect both safety and shelf life.
- Some recreational activity finds its way into commercial channels.
- The seasonal nature of the business, sometimes at very remote locations, presents the industry with special challenges in terms of training and facility upkeep.

Given these realities, it is imperative that those who handle and process seafood for commerce know the potential likely hazards and demonstrate that the likely hazards are being controlled to the extent possible.

It is not unusual, however, for FDA to receive inquiries about safety requirements and related matters from individuals who wish to process or import seafood, or who already do, that indicate a lack of awareness of hazards specific to their products.

For the most part, such knowledge is not a legal prerequisite to doing business. HACCP would go a long way toward making it so.

HACCP is not a zero risk system, but it is a system for preventing problems that are preventable, or at least significantly reducing the likelihood of their occurrence. Moreover, its use, in our view,

constitutes an appropriate alignment of industry and government responsibilities for food safety. Put another way, HACCP provides an opportunity to link the food industry's system for producing safe food with the government's system of regulatory oversight.

The adequacy of the government's system of regulatory oversight of seafood has been seriously questioned in recent years, as many of you probably know. Seafood, and the regulatory program for seafood, have been the target of considerable media scrutiny as well as congressional attention since the late 1980's. Both the number of bills relating to seafood regulation and the number of hearings held in the congress on the adequacy of the federal program are cumulatively in double figures over the past three congresses. That is truly staggering. Seafood sales stopped growing around the same time. There are many probable reasons why; consumer concern over safety probably contributes to some degree. The industry itself went to the congress looking for additional legislation in order to regain consumer confidence. (Incidentally, the overwhelming majority of seafood safety bills introduced over the past several years would have authorized HACCP, either explicitly or implicitly.)

These in and of themselves are not reasons to institute HACCP, but they are not irrelevant either.

FDA's inspection program for seafood is mandatory, but it is far from continuous. In the absence of cause, FDA inspects at most once a year for products that it considers relatively high risk and at a lesser frequency for relatively low risk products. To significantly increase the frequency of inspection would require resources that may well not exist any time soon.

These inspections provide us with a "snapshot" of conditions at the moment that the inspector is in the plant. Assumptions have to be made about conditions during the bulk of the time that the plant is operating. The reliability of these assumptions over the intervals between inspections raises questions about the adequacy of the system.

Moreover, current federal inspection and surveillance strategies place a heavy burden on the government to prove that a problem exists rather than on the firm to establish for itself, for the regulator, and for consumers, that adequate controls exist to ensure safety by design.

A similar situation exists for imports. Nearly 60 percent of the seafood consumed in the U.S. is imported. The number of U.S. customs entries for seafood is approaching 200,000 annually. FDA is committed to ensuring that imported seafood products meet the same standards that are required of domestic products.

Just as the adequacy of our domestic inspection frequencies have been questioned, we have been frequently asked whether we physically examine enough import entries. While our import examinations are highly targeted and there are a number of other mitigating factors, the fact remains that most imported seafood is not physically sampled or examined by a federal health official. Increasing the physical sampling and analysis of seafood to statistically significant levels would cost substantial additional health resources.

FDA is pursuing two ways of increasing the scope of coverage for imports and they are highly interrelated. The first involves the development of memoranda of understanding, commonly referred

to as "MOU's," with countries that export seafood to the United States and have inspection programs we can rely on. The purpose of a MOU would be to establish that the regulatory system of an exporting country and the regulatory system in the U.S. are equivalent in their ability to ensure safety. An MOU would provide for regular verification by both countries. Products from a MOU country would not require as much examination by FDA as those from other countries.

The second approach is HACCP. HACCP should provide the basis for MOU's. MOU's based on HACCP could ensure that foreign processors are applying systems of preventive controls equivalent to those being required of domestic processors. But even in the absence of a MOU, we have tentatively concluded that all seafood, both domestic and imported, should be subject to HACCP controls, and we contemplate that our regulatory proposal will reflect that tentative conclusion.

The rest of the developed world appears to be heading in that direction. In some respects, we're playing catch up.

There are several additional points I would like to make about HACCP and our HACCP initiative for seafood.

First, HACCP is a system that can "go with the flow" in terms of technology. We do not expect processors to implement controls that are not technologically feasible, but we do expect processors to be aware of what is reasonable and feasible and to integrate such measures into their HACCP controls. For example, there is no rapid test yet available for ciguatera so the controls that can be reasonably put in place to prevent the presence of ciguatera are limited in that respect. However, if and when an inexpensive, rapid test does become available, it should constitute a HACCP control where appropriate.

Second, we do not want HACCP to constitute an undue regulatory burden on the industry. In our view, HACCP is really the systematic application of common sense measures to ensure safety, and thus should be an extension of what most people are or should be doing now. It does involve a certain amount of paperwork and plan development, but FDA is working to mitigate the burden through the development of extensive guidelines on hazards and controls that will be made available to the industry and through the development of fill-in-the-blank type plans to get people started. FDA cannot do that sort of thing alone. We look forward to working with and through trade associations, sea grant colleges and others to help develop and provide as much technical assistance as possible to the industry to facilitate the transition to HACCP.

One important aspect of this technical assistance involves basic training to the industry in HACCP principles and how to apply them. The National Academy of Sciences concluded in 1985 the success of our regulatory approach for low acid canned foods, which, as I indicated earlier is based on the application of HACCP principles, has been due to the training requirement in those regulations for each processor. We are contemplating extending the low acid canned food approach to training for HACCP. Under that approach, training is conducted by the private sector after being approved by FDA. We are pleased that trade associations and academia have both shown an interest in conducting HACCP training for the seafood industry.

Third, I am aware that there is some nervousness within the consumer community that HACCP is deregulatory in nature because inspections under a HACCP-based system would involve little more than a quick look at industry-generated records of the history of critical control points. We do not expect that to be the case. While we do expect that inspections under HACCP will be more effective and efficient, they should provide the legitimate basis for increased, not decreased consumer confidence. HACCP will build upon the existing system. It will be an additional tool for both the regulator and the industry, and will not simply be a wholesale replacement for of the current system.

Fourth, we hope that HACCP will provide the basis for additional federal/state cooperative activity. We continue to believe that the states have a crucial role to play in seafood safety. The federal government cannot even begin to do that job alone. Ideally, to the extent that states adopt HACCP programs of their own, a federal/state team approach to inspection can be greatly enhanced. We urge the states to join with us in our HACCP initiative.

Fifth, after saying all that I have, I do not want to suggest that we believe that HACCP is the end-all and be-all of seafood safety. It isn't and we know it. It only works in conjunction with many other things, such as a strong research base, public education, a reasonable resource commitment, adequate training of federal and state officials, and so on. But it can and should be an important part of the core of the food safety system.

Finally, we are truly looking forward to the public input part of the regulatory process. We urge people to read the proposal carefully and we promise that we will read the public's comments back to us just as carefully.

**THE CANADIAN DEPARTMENT OF FISHERIES AND OCEANS'
QUALITY MANAGEMENT PROGRAM**

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During the past 5 years the Department of Fisheries and Oceans and the Canadian fish processing industry have worked together to develop the Quality Management Program (QMP) and on February 1, 1992, it became mandatory and is now a condition of federal registration for fish processing plants.

The Inspection Branch Mandate

The Inspection Branch of the Department of Fisheries and Oceans (DFO) is mandated through federal legislation to inspect all fish and fish products intended for export from Canada or for inter-provincial trade, and all fish and fish products imported into Canada. Through this mandate DFO provides assurance that both domestic production and imported products meet Canadian and/or foreign country standards for grade, handling, identity, process, quality and safety.

For fish and fish products produced in Canada, DFO has a dual concern, the health and safety of Canadian consumers and the overall quality of Canadian fish and fish products and their acceptability in international markets.

Canada is a major exporter of fish products and exports 84% of its fish products. The Inspection Branch plays an important role in facilitating the trade of these Canadian fishery products through its product inspection and certification programs.

To achieve its mandate the Inspection Branch has developed over the years a multi-faceted program that focuses on the strategic steps of the fish processing industry to ensure safe and acceptable fish products. This involves a variety of inspection activities which include the inspection of:

- * domestically produced fish products to determine the acceptability of these products for sale in Canada or on foreign markets;
- * domestic fish processing establishments to determine the degree of compliance with construction, equipment and operating regulatory requirements;

- * domestic fishing vessels, unloading sites and transport vehicles to determine compliance with the applicable construction and operating requirements;
- * imported product and the offshore processing operations to determine the acceptability of these products for sale in Canada; and
- * the monitoring of shellfish growing waters through the Canadian Shellfish Sanitation Program.

Approximately 100,000 fishermen and plant workers earn their living directly from fishing and fish processing and Canada is one of the world's largest exporters of fish.

There are approximately 1,300 fish processing plants operating in Canada and 40,000 fishing vessels.

In 1991 fish landings amounted to 1.2 million metric tonnes of fish worth approximately 1.4 billion dollars. The production of this fish amounted to approximately 3 billion dollars worth of final product.

The Canadian Fish Inspection Program is a federal program delivered in 6 regions across Canada. They are as follows:

Newfoundland, Scotia-Fundy, Gulf, Quebec, Central & Arctic, and Pacific. Regional offices are responsible for the delivery of the inspection program and direction of the resources.

The Inspection Branch employs approximately 500 individuals.

320 Inspectors

80 Laboratory personnel

Across Canada there are 14 fish inspection laboratories and 120 Inspection offices.

New Challenges

In the 1980s it became apparent that present DFO resources dedicated to the Inspection Program were stressed and unable to deliver effectively all of the elements of the program. DFO was being forced to direct its resources at the activities of final product inspection and certification. The other areas of the program were being neglected and the Department was not able to respond to new challenges.

One of the key challenges will be to endure the scrutiny of the informed consumer and public media. Because of the increase in contaminants, pollution and threats to the environment, there has

been an increase in media and public concern regarding the safety of the food supply in general and fish products in particular.

Current trends lead us to believe that there will be no let up in media attention in the 1990s. Today's consumers are better educated, better informed and concerned about the safety of the food they eat. In all probability the workload of all food inspection agencies will continue to increase.

The rapid pace of changing technologies is also presenting an additional challenge to industry and food inspection agencies. As the Canadian fish processing industry develops new products and processes the Fish Inspection Program must adapt its inspection methods to continue to meet its mandate.

Another major challenge for the 90s will be responding to trade issues. The movement towards Hazard Analysis Critical Control Point systems (HACCP) in the United States and the developments in the European Community are already indicating additional demands on the Canadian fish processors and the Fish Inspection Program. These countries and others are requiring more assurances from the Canadian Government that standards are being met.

The above factors are all external factors that will affect both the Inspection Branch and the Canadian fish processing industry; but there is also another key factor internal to government that will have an impact on all of the Canadian Food Inspection Agencies. That is the question of resources.

The Canadian Government, as well as other western governments, are under constant pressure to limit spending and inspection programs such as the Department of Fisheries and Oceans and Agriculture Canada's cannot expect to have ever increasing resources to meet the challenges of the future. We must find smarter and more cost effective ways to carry out our mandate.

The challenges of the 90s make it necessary for government and the food processing industries to find, develop and implement innovative and cost effective approaches to food inspection. These new approaches must be flexible and sensitive to the needs of the industry and permit industry to adapt and remain competitive in the changing markets.

The Department of Fisheries and Oceans' Quality Management Program (QMP) is a key component of our strategy for responding to the demands of the future marketplace and addressing both consumer and industry concerns. The Program has been jointly developed by the Canadian fish processing industry and the Department of Fisheries and Oceans and became mandatory February 1, 1992. The QMP is based on the HACCP philosophy. QMP is, as HACCP is, a system designed to prevent instances of public health significance. However, QMP has been designed to also prevent instances of unacceptable quality and economic fraud from occurring.

This is a fundamental difference with the QMP and a pure HACCP Program.

QMP is closely linked to the Canadian Fish Inspection Regulations and the application of a QMP by a processing plant will assist the processor in operating within the regulatory requirements

which mean the fish produced will be safe, of acceptable quality and they will be marketed fairly. By implementing the Quality Management Program the fish processing industry will be able to demonstrate that they are operating on a day to day basis with controls that ensure compliance with the regulations. The development of an individual Quality Management Program for a fish processing operation incorporates all of the basic steps involved in developing a HACCP system for a specific food product. A hazard assessment of the process operation is performed. Critical control points are identified. Defect definitions and tolerances, monitoring procedures, record keeping criteria, corrective action systems, and company verification measures are established for each critical control point.

As of February 1, 1992 each Fish Processing Plant is required by regulation to have in place and be operating under a QMP specific to it's fish processing operations. The Department has developed the QMP Submission Guide to assist the industry in developing their programs. The Guide helps the processor identify the Critical Control Points in the process and the associated hazards and sets out for the fish processing industry the minimum requirements for a plant's Quality Management Program. Under QMP a fish processing plant is required to address 12 elements that are applicable to their operation. Potential hazards will be prevented through the application of controls at each of the elements in the process operation:

1. Incoming Fish
2. Other Ingredients
3. Packaging Material
4. Labelling
5. Chemicals (cleaning agents, sanitizers, lubricants, and pesticides)
6. Construction and Equipment
7. Operation and Sanitation
8. Process Control
9. Storage
10. Final Product
11. Recall Procedures
12. Employee Qualifications

"Critical Control Point" is defined as a point in time or a physical location in the process at which failure of preventive measures will expose the customer to unacceptable risks related to tainted, decomposed, or unwholesome fish or to economic fraud.

At each Critical Control Point the fish plant must:

- identify the standard that is being applied to ensure compliance with regulatory requirements;
- identify the monitoring procedures and inspection frequencies that will be followed to ensure that the standard is being met during production;

- identify the reporting mechanism that will be used at each Critical Control Point to document the results of the inspections; and
- the fish plant will be required to develop contingency plans or corrective action plans that will be followed if and when the monitoring procedures identify an instance where the standard is not being met.

The fish processing plant will be required to have available for inspection their documented QMP that provides a written description of the program being implemented in the processing plant. The plant will also be required to retain records of all inspections performed as part of their QMP for 3 years. These records must be made available to DFO Inspectors when requested.

The Department of Fisheries and Oceans inspects the fish processing plant against the Quality Management Program requirements. Individual Inspectors perform QMP Inspections that entail:

- * the verification of the written QMP to ensure the documented standards, monitoring procedures, record keeping systems and guidelines for corrective action meet the minimum requirements as set by the Department of Fisheries and Oceans;
- * the confirmation that the written QMP is being followed in the plant. This will require the inspector to observe the processor's QMP activities at each critical control point in the plant; and
- * the verification that the processor's records are accurate. This will require the inspector to withdraw and inspect parallel samples of the processor's products and compare the results with those of the company's.

The completion of the QMP Inspection will result in the process operation being rated as either Excellent, Good, Satisfactory, or Fail. These QMP ratings represent the degree of confidence that DFO has in the company's ability to operate within compliance of the regulations and will determine the inspection coverage to be directed at the operation in subsequent weeks. Fail rated plants will be asked to voluntarily correct the deficiencies and improve their rating to at least a "Satisfactory". Refusal to deal with the problems voluntarily will jeopardize the federal certificate of registration and therefore the ability of the processing plant to export its products. Plants which receive a "Satisfactory" rating will be inspected on a frequent basis until they gain greater control over their process and obtain a higher rating.

Processing operations that are successful in meeting all but a few of the QMP requirements will receive an "Excellent" or "Good" rating. These plants will be qualified to apply for the use of the "CANADA INSPECTED" logo on their product labels. Also the product certification process will be streamlined and provided without delay, and the company will have more autonomy in their day to day processing operation.

The Quality Management Program - Industry's Role

The major change for industry under QMP is that they must accept more responsibility and accountability in monitoring their own performance. The processing plants will be required to perform inspections of the plant and products and initiate corrective actions when they identify a problem and records of all these QMP activities must be maintained so that they are able to demonstrate that they consistently operating in compliance with the regulations.

The Role of Government in Regulating Under QMP

The implementation of the Quality Management Program will mean a change in the relationship between the fish processing industry and the Department. Under QMP the Department of Fisheries and Oceans' role will shift from solely an inspection function to include an auditing function. The Inspector will continue to perform random inspections of the process operation and products but the focus will not be on individual lots of product or on a day of plant operation as now is the case, but rather on the overall QMP system. The Inspector's decisions will be based upon a compilation of inter-related inspection results gathered over time by both the Inspector and the processor.

Conclusion

The Inspection Branch of DFO is confident that the Quality Management Program will provide the Canadian fish processing industry and the Department of Fisheries and Oceans with an effective mechanism to ensure the protection and assurance needed in today's demanding markets. The price of this assurance is change.

DFO will have to change. Industry will have to change. But this approach should realize more impact from each inspection. The number of inspections we do in total may be somewhat reduced for some plants, but each inspection will count for more. We will be able to focus our effort on areas of higher risk and apply our resources in a more cost effective manner.

In summary, the Department of Fisheries and Oceans' new approach to quality management is a joint industry/government system which is aimed at preventing problems before they occur. Working together, through the Quality Management Program the Canadian Fish Processing industry and the Federal Government will be able to provide Canadian consumers and our international customers even better assurance than in the past that the high standards Canadian fish products have been known for will be met in the future.

POLYPHOSPHATES: RATIONALE FOR USE AND FUNCTIONALITY IN SEAFOOD AND SEAFOOD PRODUCTS

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Food Grade Phosphates

Among the legitimate functional goals for the use of phosphates in seafoods are retention of natural moisture and flavor, inhibiting fluid losses during shipment and prior to sale, emulsification, inhibiting oxidation of flavors and lipids by chelation of heavy metals and cryoprotection, thereby; extending shelf-life. Properly used, phosphates impart no flavor.

Recently, the use of phosphates in some segments of the seafood industry has been subject to government scrutiny. When improperly used, excessive absorption of moisture may lead to charges of economic fraud by the U.S. Food and Drug Administration. It is important to note, however, that seafood myofibrillar proteins readily denature at refrigeration temperatures (5°C) and may lose up to 80% of their water-binding capacity within five days (10) while similar changes to beef muscle take in excess of 45 days at >20°C (8). Failure to protect these delicate proteins leads to significant overpack to meet net stated weight and negative economic consequences to seafood processors.

Phosphates are refined from calcium phosphate which is mined. Through varying degrees of neutralization of phosphoric acid with either alkali metal ions (i.e., sodium or potassium) or alkaline earth metals (i.e., calcium), two general classes of phosphates (simple and condensed) are formed (7). Simple phosphates consist of a phosphorous atom surrounded by four oxygens and valences that can be filled by metal ions or hydrogen. Condensed or combined phosphates are short to long chains or rings, the latter forms have the broadest applications in the seafood industry.

Application of Phosphates

Phosphates are generally applied by dipping in, spraying with or tumbling in a phosphate solution. Injector needle systems may also be used with and without added tumbling. Dry addition is used in comminuted meat systems, e.g., surimi and sausage formulations.

The most predictable way to apply phosphates is through vacuum tumbling, if done properly and the structure of the flesh can withstand mechanical action. Contrary to some practices, tumbling in an excess of solution results in protein extraction rather than absorption of solution. This uniform and rapid means of treating the muscle offsets the inefficiency of protracted holding in phosphate-based solutions (soaking).

It has been demonstrated that treating finfish prior to smoking requires different phosphate concentrations depending on the dimensions of the fillets and/or pieces. For example, with the same size pieces of flesh (within selected species), a 5% phosphate dip requires 24 hours treatment time while a 25% phosphate dip requires only two seconds (16) to reach equal processing effects, i.e., inhibition of surface curd formation and reduced cook-cool losses. This is especially valuable when delicate muscle structure eliminates tumbling as an option. Caution should be exercised when applying phosphates to fish of different muscle thickness, muscle types (e.g., interspecies variation) and initial moisture content (spawning).

Methods to Determine Phosphate Application

Some methods to monitor phosphate use are based upon total moisture content of the muscle. One example would be the French HP (9) method which is used to monitor the ratio of protein to water within muscle. In scallops, the ratio is considered to be between 4.0 and 4.9: 1.0 (water: protein). The moisture content of commercially harvested seafood muscle is 80% or greater in species including, but not limited to, soft-shell blue crab, some mollusks and post-spawned finfish. Webb et al., (21) determined that the moisture content of bay scallop meats was significantly different at the 5% level between harvest years, sounds, locations within the sounds and among months and within locations. These researchers (21) also determined that the moisture content (monthly sampling) of land-shucked bay and Calico scallops ranged between 74.15 to 83.66 and 76.12 to 81.86%, respectively.

The HP ratio then would not be realistic for many species or at certain times of year. This value is based upon Kjeldahl protein to moisture (overnight drying at 100 to 105°C).

In theory, determination of total phosphorous in seafoods might be a useful marker of phosphate treatment; however, it is not necessarily accurate. For example, Crawford (4) determined that the natural level of phosphorous in fresh shrimp (*Pandulus jordani*) ranged from 537 to 727 mg/100 gm. Shrimp of the same history showed increases of 81 ± 39 and (base not given) ± 110 mg of phosphorous, respectively after treatment with either 1.5 or 6% phosphate solutions for five minutes. In shrimp (*Pandulus jordani*), the natural variation in phosphorous exceeded that added by responsible treatment.

Total natural phosphorous has also been reported to vary in lobster, blue mussels, squid, anchovies, carp, capelin, catfish, Atlantic cod, eel, hake, herring, yellow leatherjacket, European pilchard and albacore tuna (13). Penetration of phosphate, and therefore phosphorous content, will also vary according to concentration of solution used, variations in muscle thickness, subsequent processing, etc.

Other methods to screen for added phosphates include high pressure liquid chromatography (HPLC), ion chromatography and thin layer chromatography. Wood and Clark (22) have reviewed the difficulties associated with these phosphate determinations.

Biochemical decomposition of condensed phosphates necessitates assaying immediately after treatment of the seafood species. Hydrolysis of condensed phosphates occurs due to muscle alkaline phosphatase activity during the post-treatment (lag) time prior to cooking. Sutton (15) determined that sodium tripolyphosphate is rapidly hydrolyzed to pyrophosphate (phosphate dimer) and orthophosphate (phosphate monomer) in cod muscle at either zero or 25°C.

It has also been determined that after two weeks of frozen storage (-26°C), only 12% of the total phosphorous in raw shrimp muscle corresponded to the originally added sodium tripolyphosphate. By ten weeks, phosphorous levels corresponded to 45% orthophosphate (17). Clearly, in treated seafood muscle, the condensed phosphates were unstable over time.

Mechanism of Action

Offer and Trinick (11) determined that pyrophosphate [(10 mM), (from beef myofibrils)] in combination with reduced levels of sodium chloride, extracted the A-band completely beginning at both ends. This effect was confirmed by Voyle et al. (1984) with pork. In the absence of pyrophosphate, however, only the center of the A-band was extracted. Lewis et al. (1986) determined from 5 gm pork, beef, chicken and cod samples that an A/I overlap composed of denatured actomyosin and connectin was formed while unassociated myosin and actin were probably dispersed (sol) through the meat structure in the form of a water-holding gel (post heat treatment).

Trout and Schmidt (18) concluded that at high ionic strengths (>0.25), pyrophosphate affected hydrophobic interactions which stabilize the protein structure, and thus, the thermal stability of the protein. Elevating pH (1M NaOH), in combination with pyrophosphate, increased the temperature (from 70 to 87°C) for, and the extent of, protein aggregation. Yagi et al. (25) confirmed that inorganic polyphosphate offered a high degree of protection (to carp myofibrils) from thermal denaturation.

Water retention is correlated with increased pH and normally associated with the use of alkaline polyphosphates such as sodium tripolyphosphate. Orthophosphates have virtually no effect on water-binding (12). Pyrophosphates are associated with improved protein solubility (myosin) and water binding. Consequently, water binding is dependent upon the type of phosphate used and specific physicochemical reactions may require the use of blends.

Phosphates as Processing Aids

This area will be given only a cursory mention since it is a topic of a later presentation. Crawford (4) was instrumental in developing a protocol for the treatment of Pacific shrimp (*Pandulus jordani*) to be mechanically cooked and peeled. By the responsible use of phosphates in treating Pacific shrimp to be mechanically cooked and peeled, meat yield increased an average of 12%. There was no significant uptake of moisture, and there was an added ex-plant income (in Oregon alone) of greater than 65 million dollars in the first eight years of use (1).

Preservation of Freshness

A process for using low concentrations (1 to 2%) of sodium tripolyphosphate in either flaked or crushed ice was patented by Stone (14). Use of this ice increased the yield of shrimp and effectively reduced moisture and nutrient loss. Shrimp stored in phosphated ice could be over-exposed to polyphosphates if treated again during further in-plant processing which could cause either off-flavor, >0.5% residual phosphate or both.

Specialty Blends

Among products for extending the shelf-life of fish fillets, Crawford (2, 3) developed a patented blend consisting of sodium tripolyphosphate, sodium hexametaphosphate, citric acid and potassium sorbate (FISH-PLUS™, BK Ladenburg Corporation). Fish fillets were dipped into either distilled water or (ca.) 12% treatment solutions. The shelf-life (aerobic plate count $\leq 1 \times 10^6$ CFU/g) for treated samples was 12.4 days and that of the control (water-dipped) was 6.8 days. Both control and treated fillets increased in weight by 4% after 60 seconds of immersion. Those dipped in the patented blend remained at their stated package weight throughout the 14 days of storage at 5°C, while the controls, dipped in water, dropped below the initial weight within four days of chill storage. Shelf-life extension would most likely be increased due to, first, the antimicrobial activity contributed by the sorbic acid, and second, the sequestration by phosphates of enzyme (metal) co-factors.

Frozen Seafoods

Researchers at Texas A&M University reported that sodium tripolyphosphate dissolved slowly in seawater (6). In addition, fresh, shell-on and peeled shrimp (Gulf of Mexico) became translucent and slippery to the touch after dipping in solutions of phosphate-sea water. This led to subsequent treatments which included five minute dips in water and 2, 4 or 5% condensed phosphates. Using a blend of sodium tripolyphosphate and hexametaphosphate (BRIFISOL™ 512, BK Ladenburg Corporation) resulted in rapid solubilization of the condensed phosphate, and more desirable sensory (touch) properties. The dipped shrimp were frozen and stored at -26°C for two weeks. Upon thawing and cooking (four mins), those shrimp dipped in the 4% blend for five minutes lost 0.8% weight after frozen storage (control, 2.0% loss) and 19.8% after cooking (control, 25.3% loss). It was concluded that addition of these phosphate blends imparted a cryoprotective effect.

Woyewoda and Bligh (24) dipped Atlantic cod fillets into 12% solutions of sodium tripolyphosphate, sodium metaphosphate blends or no solution (FREEZ-GARD™ FP-19, FP-65 [Rhône-Poulenc] and a control, respectively) for 45 seconds and stored each treatment at either -12°C or -30°C for up to 26 weeks. Phosphate-treated cod showed decreased thaw, and cooked drip loss and resulted in higher moisture content in both raw and cooked product. After 26 weeks (at -30°C), all phosphate treated fillets were judged the most tender and highly acceptable by sensory evaluation. The use of tripolyphosphate significantly reduced expressible water after holding at -30°C up to 26 weeks and up to 24 days at -12°C.

Thermally Processed Seafoods

Struvite, or magnesium ammonium phosphate, may be formed in thermally processed seafoods (e.g., canned tuna and crab). Sodium acid pyrophosphate can be used to sequester magnesium ions and thus, inhibit struvite crystals, which resemble broken glass.

Salmon may develop a surface curd (denatured protein) if either held on ice for a protracted length of time and/or frozen prior to canning. The curd may constitute up to four percent of the pack, by weight, and may be considered questionable by many consumers.

Curd was significantly ($P \leq 0.05$) reduced by dipping sockeye salmon steaks for 2 to 120 seconds in 15 to 20% solutions of condensed phosphate (BRIFISOL™ 512 [sodium tripolyphosphate and sodium hexametaphosphate], BK Ladenburg Corporation) and by dipping for 30 to 120 seconds in 5 to 10% solutions (19). To avoid dipping, Wekell and Teeny (19) verified that there was a 68% reduction in curd formation by dry addition of the phosphate blend prior to sealing the can. Although it was estimated that 1.0% polyphosphate would be needed to completely inhibit curd formation, this would exceed the legal limits for phosphate in canned salmon.

Domestically, phosphate is not uniformly allowed in canned salmon except for a temporary allowance granted to several processors. Its use in canned salmon has, however, been given provisional approval by the Canadian government.

Phosphates provide significant benefit to the seafood industry when there is a large harvest within close proximity, and conversely, there are limited quotas (i.e., freezing fillets to extend wholesale/retail availability). Spawning salmonids may represent one of the most important applications since the muscle has been physico-chemically altered. Such finfish contain reduced levels of myofibrillar proteins which lead to impaired muscle water holding capacity. This is parallel with elevated levels of sarcoplasmic proteins and total moisture, a combination conducive to curd development.

Troubleshooting

Often when phosphates are added in excess, a glassine look develops. This is particularly noticeable on shrimp. There are regulatory constraints to the use of polyphosphates along with organoleptic problems (a soapy taste) if the phosphates are used in excess. The glassine appearance probably occurs more in error than through intentional overuse of phosphates since there are no standard or defined procedures for their application. Most industrial protocols have been developed by trial and error and/or have been based upon far more resilient terrestrial muscle.

Combining sodium tripolyphosphate with sea water will frequently promote the formation of a "floc" on the surface of certain species. Mineral content and pH of the muscle will exacerbate the formation of this crystalline precipitate.

Polyphosphate insolubility is related to water quality and to the individual type of condensed phosphate. Minerals in hard water will compete with some types of polyphosphate for solubility. In addition, not all forms of polyphosphate are readily soluble in water.

Erratic functionality of phosphates also may be caused by either heating phosphates to promote solubility or using old solutions. Many of the polyphosphates are prone to hydrolysis, and the monomeric forms will not perform the same as the polymers.

The maximum permitted legal level in processed meat and poultry is 0.5% by weight of the final product and serves as the current guideline where their use is permitted. Polyphosphates are not allowed in breaded shrimp and in certain other species (5). They are, however, self-limiting. If much more than 0.5% of the high pH phosphates, such as sodium tripolyphosphate, is used, flavor and appearance will be adversely affected.

Summary and Conclusions

Phosphates are valuable to maintenance of the functional properties of seafood myofibrillar proteins which preserve the natural muscle juices. Inhibiting drip loss in the fresh state, while thawing and in cooking is important to prevent economic loss. Phosphates increase the thermal stability of proteins which, in seafoods, are normally lower than that of terrestrial muscles. Improper use of phosphates leads to sensory defects and the potential for charges of economic fraud.

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FUNCTIONALITY OF POLYPHOSPHATES

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Polyphosphates are used extensively in food processing. Many studies have shown the benefits of polyphosphates in beef, pork, poultry, and seafood. It was found that these compounds have a profound effect on the functional properties of the food products of which they become a part (Deman, 1970). The first patent on the use of polyphosphates to inhibit thaw drip and cook losses in frozen fish were issued in the United States to John H. Mahon in 1962. In recent years, polyphosphates have gained widespread acceptance as additives in the fish and seafood industry. The functional goals when applying polyphosphate to seafood are to retain moisture and flavor, to prevent oxidation and to extend shelf-life. Appropriate applications of polyphosphates result in the retention of natural juices which in turn slow down normal fish deterioration. Excessive (or abusive) use of polyphosphates, however, is linked to excessive water uptake (i.e., economic fraud), which has given rise to tensions between the seafood industries and regulatory agencies like the FDA. The FDA fears that polyphosphates might be used to improve the quality of damaged food products and/or would be used to "add" water to foods. However, if excessive polyphosphates are added, the fish develops a soapy taste and a glassine look which are obviously not desirable. This probably occurs more in error than through intentional overuse of polyphosphates. The problem exists because there are no treatment standards. Standards are difficult to develop since many variables influence polyphosphate uptake in the fish muscle, among these variables are: thickness of the muscle, muscle type, type of polyphosphate used, concentration of the polyphosphate solution, dipping time, post-mortem age, etc. The purpose of this study is to determine the relationship between polyphosphate uptake and water retention in fish in different seafood models as a function of type of polyphosphate (including blend of polyphosphates in

concert with microbial inhibitors), concentration of the polyphosphate solution, and time of dipping. The water retention properties after the different treatments were evaluated by water uptake ability (WUA) and expressible moisture (EM) in order to determine the interaction between protein and polyphosphate.

Traditionally, a number of different methods were used to measure the water retention properties in muscle food systems. According to Regenstein (1984), two of the major types of the water retention methods, WUA and EM, respond to different properties of flesh. WUA refers to the ability of a material to hold added water while under the influence of an external (centrifugal) force. Therefore, WUA represents the maximum water retention of insoluble material in the presence of excess moisture at a particular force (Jauregui, 1981 & Regenstein, 1984). EM refers to the amount of water squeezed out of a material by the application of a force (Centrifugal). Both EM and WUA should be measured at the same centrifugal force when they are to be compared. The results of these studies showed that for WUA, the different cations gave similar results while the anions gave very different results. WUA is then found to be a anion-distinguishing technique. The EM, however, is a cation-dependent effect. It divides the salts into two classes of behaviors: with and without changes, e.g., EM of Mg changes with the salt concentration while with Ca it doesn't change.

MATERIALS AND METHODS

Sodium tripolyphosphate (STPP) (FMC Corp., Industrial Chemical Groups, Philadelphia, PA), sodium hexametaphosphate (SHMP) (Sophadex, FMC Corp., Industrial Chemical Groups, Philadelphia, PA) and FishPlus® (BK Ladenberg Corp., Cresskill, NJ) were used to prepare the dip solutions. STPP is the most popular polyphosphate used as a food additive. SHMP is probably the second most popular polyphosphate used commercially with flesh foods. It is broken down to orthophosphate more slowly than STPP. FishPlus® contains a blend of generally-recognized as safe (GRAS) ingredients: sodium, phosphates, citric acid and potassium sorbate (Technical Bulletin, 1982).

Commercial cod and mackerel of 3 to 5 days age were used as seafood models. Fresh cod fillets and whole mackerel were obtained from the P&C supermarket (Ithaca, NY) as soon as they arrived. After gutting the mackerel, they were cut into fillets. These fillets were skinned and the white muscle was cut into 4 to 8 pieces which were usually between 4 cm x 2 cm x (0.7 to 1.5) cm. Cod skinless fish fillets were cut into pieces (4 cm x 3 cm x 2 cm). Ground samples of cod muscle were prepared by grinding the fillet in a HC20 Handychopper™ Mincer/Chopper (Black & Decker Inc., Shelton, CT). The muscle pieces were then dipped in the polyphosphate solutions at specified concentrations for specific length of times. In the case of mackerel, the

muscle pieces were dipped in 0, 1, 3, or 6% (w/v) STPP for 0.5, 1.5, or 3.0 min. The cod blocks were dipped in 0, 1, 2, 5, 10% (w/v) polyphosphate solution for 0.5, 1, 2, 3, 5 min. The polyphosphate added to the minced sample were 0, 0.1, 0.3, 0.5 and 0.7%. The treated samples were packed and stored on ice for different length of time. In the case of cod, treated samples (fillets and polyphosphate added minced cod) were stored for 1, 4, 7, 10, 15 days. Water retention properties were measured at certain storage periods.

Four different methods were used to measure the water retention properties: EM, WUA, thaw (drip) loss and cook loss. Polyphosphate uptake and, in the case of mackerel, thiobarbituric acid (TBA) were also measured.

Expressible moisture

To determine EM, 3 pieces of Whatman (Whatman International Ltd., Maidstone, England) #3 filter paper, 5.5 cm in diameter, and 1 piece of Whatman #50, 7.0 cm in diameter were folded into a thimble shape over the outside of an inverted 16 x 150 mm test tube with the #50 filter paper as the internal surface. The filter paper was weighed on a Mettler H20T balance (Mettler Instrument Corp., Hightstown, NJ) before and after addition of 1.5 ± 0.3 g sample of treated ground fish. The sample in the thimble was then centrifuged in a 50 mL polycarbonate centrifuge tube (Nalgene Co., Rochester, NY) at 16,000 rpm ($30,900 \times g$, measured at the bottom of the tube) in a refrigerated centrifuge (Sorvall RC2b, SS34 rotor) at about 2°C for 15 min. The filter paper and sample were then removed from the tube with tweezers, the fish cake was removed from the filter paper, and the paper was reweighed. EM was reported as the percent weight lost of the original sample (Jauregui, 1981).

Water uptake ability

According to the method of Jauregui (1981), 30 mL of water was pipeted into 50 mL polycarbonate centrifuge tubes. The tubes were placed in ice while the treated samples were grounded and 1.7 g was weighed on a piece of wax paper using a Mettler H20T balance. The samples were added to the water and the mixture was blended in an Omni-mixer (Sorvall Omni-mixer Model 17105, Dupont Co., New Town, CT) for 30 sec at the midpoint setting (5). The blades of the Omni-Mixer were then rinsed with 4 more mL of water, to make the ratio of fish to solution 1:20. The tubes were stored in ice for 30 min before centrifugation. The samples were centrifuged in a refrigerated centrifuge at 16,000 rpm for 15 min at 2°C. After centrifugation, the supernatants were decanted and their volume determined. The pellets were placed in aluminum pans, weighed and dried overnight at 105°C in an air oven. The protein concentration of the supernatant and the original sample were determined by the Lowry method (1951).

WUA was calculated by dividing the mg of water in the pellet by the mg of protein in the same pellet. The protein content of the pellet was calculated by difference: total initial protein minus total protein in the

supernatant. The WUA can also be expressed as a percent of the WUA of the control which was run at the same time. In addition, the total bound water based on the original sample weight can be calculated. This can be used to compare WUA values since the amount of insoluble protein in different samples were different.

In addition, the effect of the pH on the WUA was examined. The actual pH of the solution was measured immediately after centrifugation. NaOH and HCl were used to adjust the pH of the system.

Thaw, drip and cook loss

The thaw loss determination method was modified from Santos (1990). Frozen samples (kept at -10°C) were weighed and placed in a glass funnel on top of a flask, wrapped tightly with a plastic bag and incubated in a cold room (1 to 4°C) for 24 hr. The thawed samples were weighed and the percent of thaw drip was expressed as the percent of weight loss during thawing.

The drip loss of fresh treated samples were measured using the thaw loss method after different storage time.

After the thaw loss and drip loss determination, the samples were placed in a covered microwaveable baking dish and cooked in a microwave oven at its maximum energy (750 Watt) for 1 min (Amana Touchmatic II Radarange® microwave oven, Amana Refrigeration, Inc., Amana, IW), and air-cooled. The cooked fish blocks were weighed while the liquid remained in the dish. Cook loss was expressed as the percentage of weight loss after cooking (Santos, 1990).

Phosphate determination

The Quimociac method was used to measure total phosphate uptake by measuring the concentration of phosphorus (or phosphorus pentoxide) in the treated samples (Anonymous, 1977 & Jauregui, 1981)). The sample is digested in nitric acid to solubilize the orthophosphate and to hydrolyze condensed phosphates to the orthophosphate form. The orthophosphate is precipitated as quinolium phosphomolybdate which contains 3.207% P_2O_5 .

Small amounts of dipped sample were added into 300 or 150 mL Erlenmeyer flasks containing 50 or 25 mL concentrated HNO_3 , respectively, and boiling chips. The flasks were heated on a hot plate until all the samples were dissolved and the solutions were clear. The flasks were left on the hot plate for 4 hr just below boiling. After this, 100 or 50 mL water was added to each flask and the solution was allowed to boil for about 45 min. After diluting the solution to about 100 or 50 mL, 50 or 25 mL of the Quimociac Reagent, respectively, was transferred to the hot solution while stirring and was allowed to boil for two or three min before cooling. The bright yellow precipitate obtained was filtered through a dried pre-weighed Gooch crucible (Coors No 3, Coors Porcelain Co., Golden, CO) containing a glass fiber filter (Reeve Angel No 934AH, 2.1 cm, Whatman Inc., Chifton, NJ) and washed 5 times with 25 mL distilled water, allowing each portion to pass through the filter completely before another was added. The crucible and contents were

dried in an oven for 30 min at 250°C, cooled in a desiccator and weighed. The weight of precipitate was calculated by subtracting the weight of the empty crucible from the weight of the crucible and precipitate. The amount of phosphorus pentoxide (P_2O_5) in mg was calculated by multiplying the dried weight of the precipitate (g) times a 32.07 factor.

Thiobarbituric acid (TBA) value

The TBA determination was adapted from Hwang (1988). A 1.9 to 2.0 g portion of ground sample was weighed, added to a 50 mL centrifuge tube and blended with 25 mL of extracting solution for 30 sec in an Omni-mixer at a low setting (3). The mixture was filtered through Whatman #1 filter paper. Five mL of the extract was mixed with 5 mL of the TBA reagent and added into a 20 mL screw cap test tube. The test tube was capped tightly and heated in boiling water for 40 min. The test tubes were cooled under running tap water. The absorbance of the samples were measured and the μ moles of malonaldehyde (MA) were calculated from the standard curve of 1, 2, 3, 3-tetraethoxypropane, which is quantitatively hydrolyzed to MA when heated, and multiplying this value by the dilution factor (5.0). The TBA value was expressed as μ mol MA/g of sample.

RESULTS AND DISCUSSION

Measurement of EM:

The effect of STPP and FishPlus® on cod fillets were studied by dipping in different concentration of polyphosphate solutions for different time periods (Fig. 1 & 2).

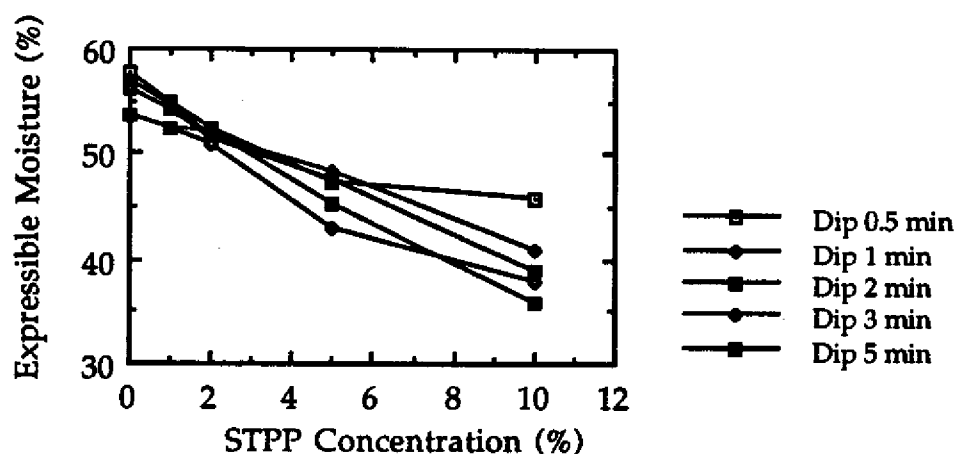


Fig. 1 Effect of STPP concentration and dipping time on EM

Increasing the STPP or FishPlus® concentration and the dipping time decreased EM. Compare to STPP, FishPlus® was not as effective. The differences might be due to the different polyphosphate content and pH of the dipping solutions. At the same total concentration level, polyphosphate

content is higher in the STPP solution than in the FishPlus® solution. In addition, the pH of the STPP solutions were around 9 while the pH of the FishPlus® solutions were around 6. From Fig. 1, we found that dip times over 1 min had a minimal effect. For those samples dipped in FishPlus®, there was little change in the EM when the concentration was above 5% and dip time had no effect.

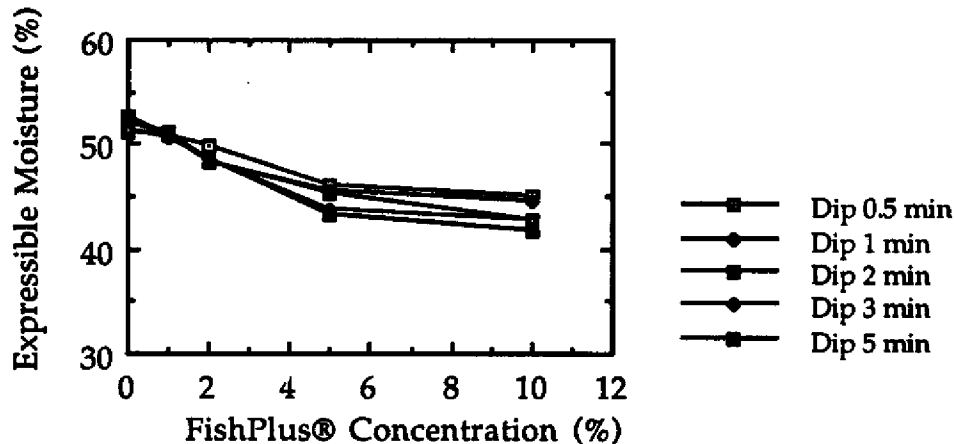


Fig. 2 Effect of FishPlus® concentration and dipping time on EM

Measurement of WUA:

The effect of STPP concentration and dip time on WUA is shown in Fig. 3. Longer dipping times led to increased WUA. Fig. 4 shows the effect of STPP concentration and dip time on the total amount of soluble protein in the supernatant. With the increase of the STPP concentration, the amount of protein in the supernatant increased although the trend is not apparent at low concentration. Since the protein content in the pellets were different with different dip concentrations and dip times, we also calculated the percent water absorbed based on the original sample weight as shown in Fig. 5. Compared to Fig. 3, the total amount of water absorbed in the sample decreased, especially for the sample dipped for 5 min, because the protein content in the pellet decreased with dipping.

Ground cod was used to measure the effect of dip solution pH on the WUA. The pH of the homogenized cod was adjusted after blending. However, since the pH of the solution was not stable (the pH of the solution before and after centrifugation were different), the actual pH of the solution was measured immediately after centrifugation. The sample with the lowest WUA, i.e., at pH 5.5 was used as the control. Fig. 6a show the effect of pH on cod from pH 3 to 11. Since the WUA change was largest at the extreme pHs

and we are more interested in the middle pH range, we replotted the data for pH 4 to pH 9 (Fig. 6b), and from pH 5 to 7 (Fig. 6c). From these results, i.e., a large pH dependence, we would expect that STPP and FishPlus® would have different effects on WUA since their solutions have different pHs. pH change had an important effect on fish WUA. Around pH 5.5, WUA is at its lowest point. And at this pH, there is the least amount of soluble protein in the supernatant and the least amount of water absorbed in the pellets.

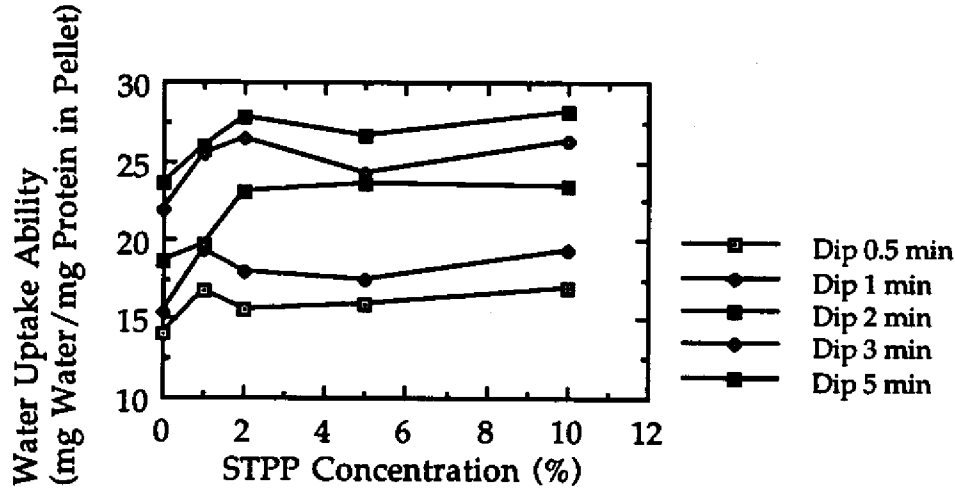


Fig. 3 Effect of STPP concentration and dipping time on WUA

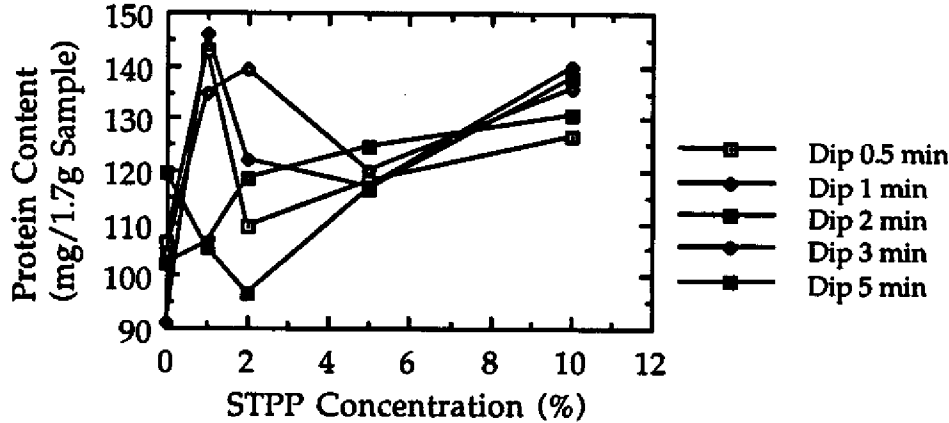


Fig. 4 Effect of STPP concentration and dipping time on the amount of soluble protein in the supernatant

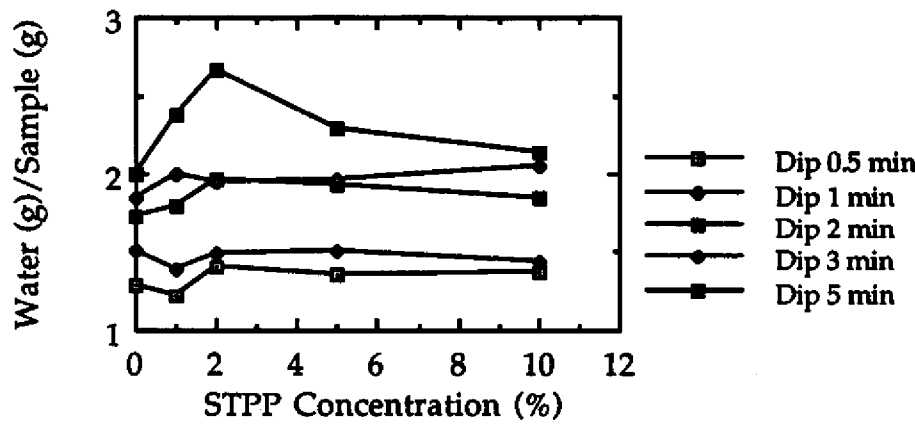


Fig. 5 Effect of STPP concentration and dipping time on the water absorbed based on the original sample weight

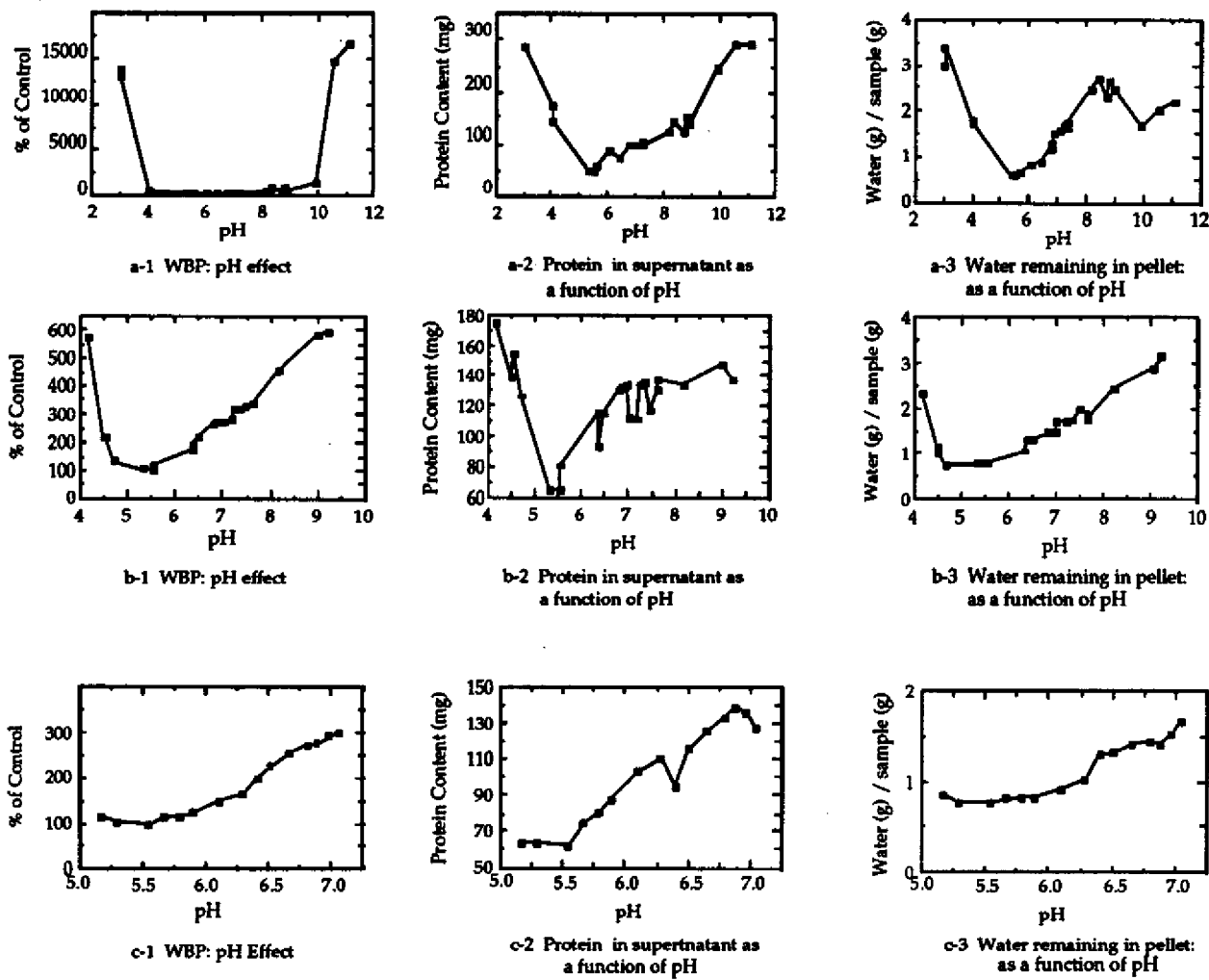


Fig. 6 Effect of pH on WUA

Thaw and cook loss:

The effect of STPP and FishPlus® on the thaw and cook loss are shown in Fig. 7a and Fig. 7b respectively. The period of storage had a greater effect on thaw drip than the dip concentration. STPP and FishPlus® concentration were more effective in preventing cook loss than in preventing thaw loss. STPP was more effective over intermediate storage times in preventing total moisture loss.

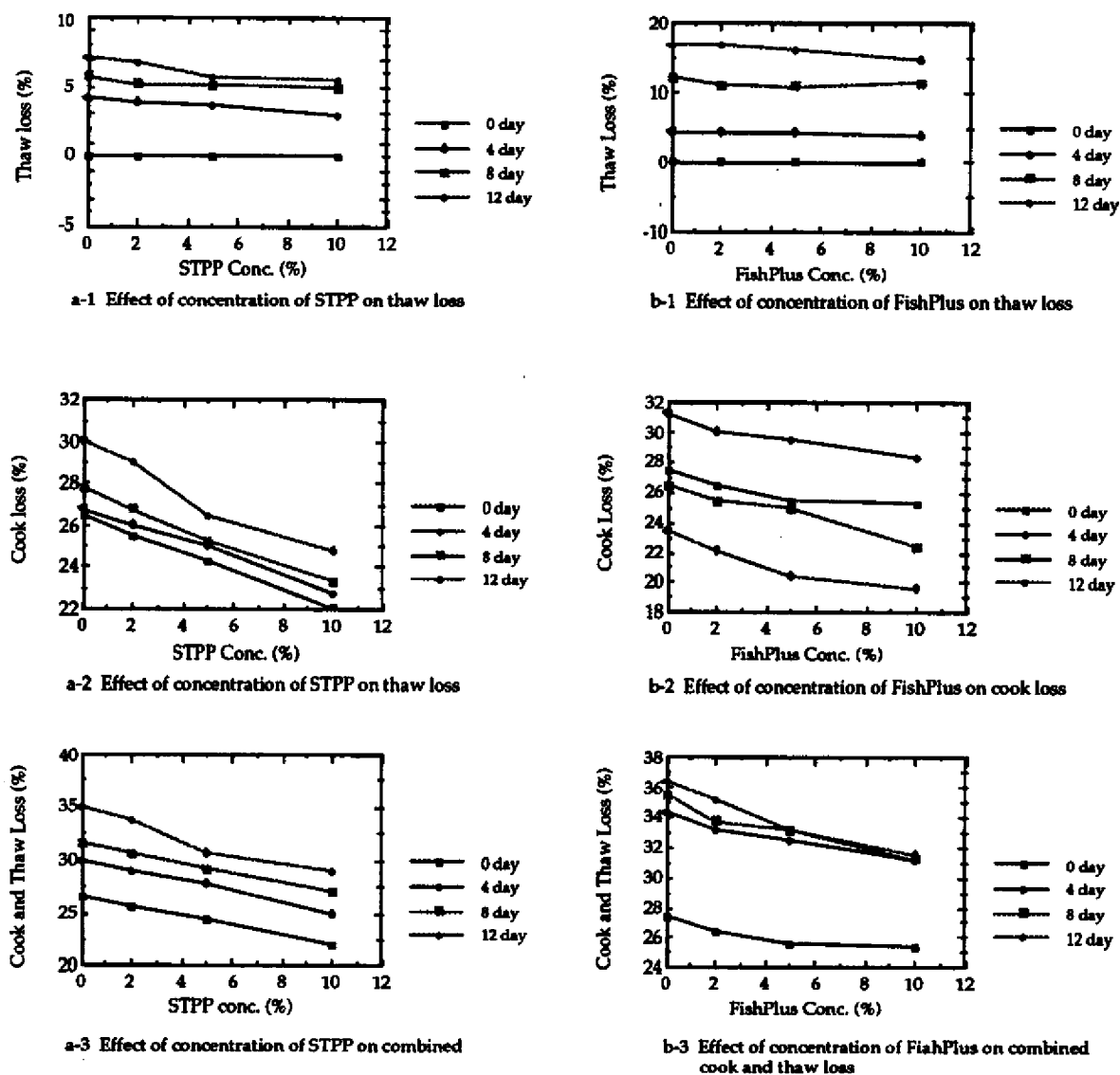


Fig. 7 Effect of STPP and FishPlus on thaw and cook loss

Phosphate determination:

Increasing dip time and polyphosphate concentration increased the absorption of polyphosphate in fresh cod (Fig. 8). Since the sample size was small, the amount of polyphosphate picked up in the cod blocks was higher than expected. According to the observed changes in phosphate levels, we

expect to be able to control the phosphate uptake by changing dip time and dip solution concentration.

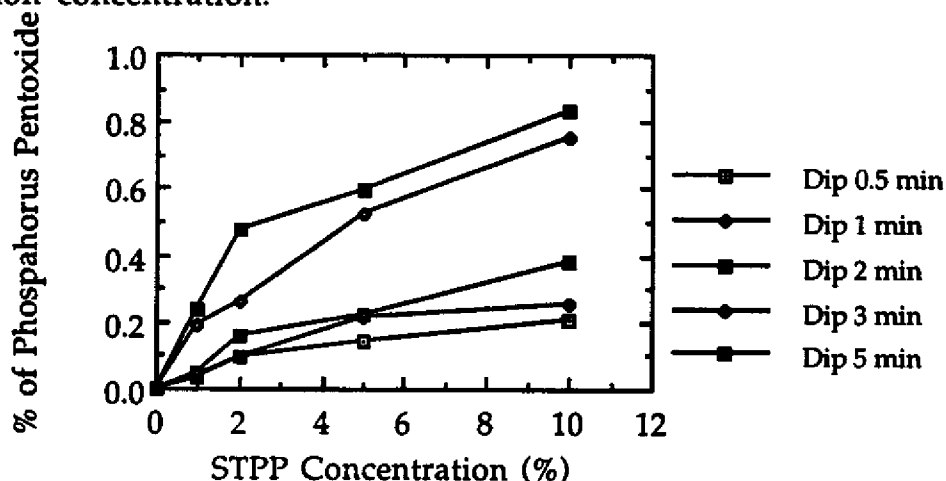


Fig. 8 Effect of dip time and STPP concentration on the % of phosphorus pentoxide pick-up in cod blocks

Storage study:

The effect of storage time on the EM of Sophadex treated fresh cod fillets

Fig. 9a -9e show the EM as a function of dipping time and concentration. Each figure represents a different storage time. On day 1 after dipping (Fig. 9a), the EM did not change much with different dipping times and concentrations. The samples dipped in water showed an increase in the EM with dipping time. This is probably due to the increased water uptake. However, in all cases with Sophadex, the EM decreased. The decrease in EM of the samples dipped in 2% and 5% solutions was slight with increased dipping time; the EM decreased about only 1% from 0.5 min to 5 min. At 10%, dipping time had a greater effect, the EM decreased about 3% from 0.5 min to 5 min. The EM increased with Sophadex concentration when the dipping time was the same and concentration had a greater effect as time went on. On day 4, notable changes occurred (Fig. 9b). The EM of the samples dipped in water showed large increases with dipping time (4.5%), while the samples dipped in different concentrations of Sophadex showed similar decreasing water loss with dip time. Higher concentrations gave lower EM. The difference of the samples dipped in Sophadex versus that in water was significant. On day 7, 10 and 15, the difference continued and the EM changes were similar to day 4 although the curves all shifted upward without a slope change (Fig. 9c-9e). On the other hand, the EM of the samples dipped in Sophadex kept on increasing. The lowest EM sample (dipped 5 min in 10% solution) changed from 42.4% to 47.0% over 15 days. Sophadex was helpful in lowering EM.

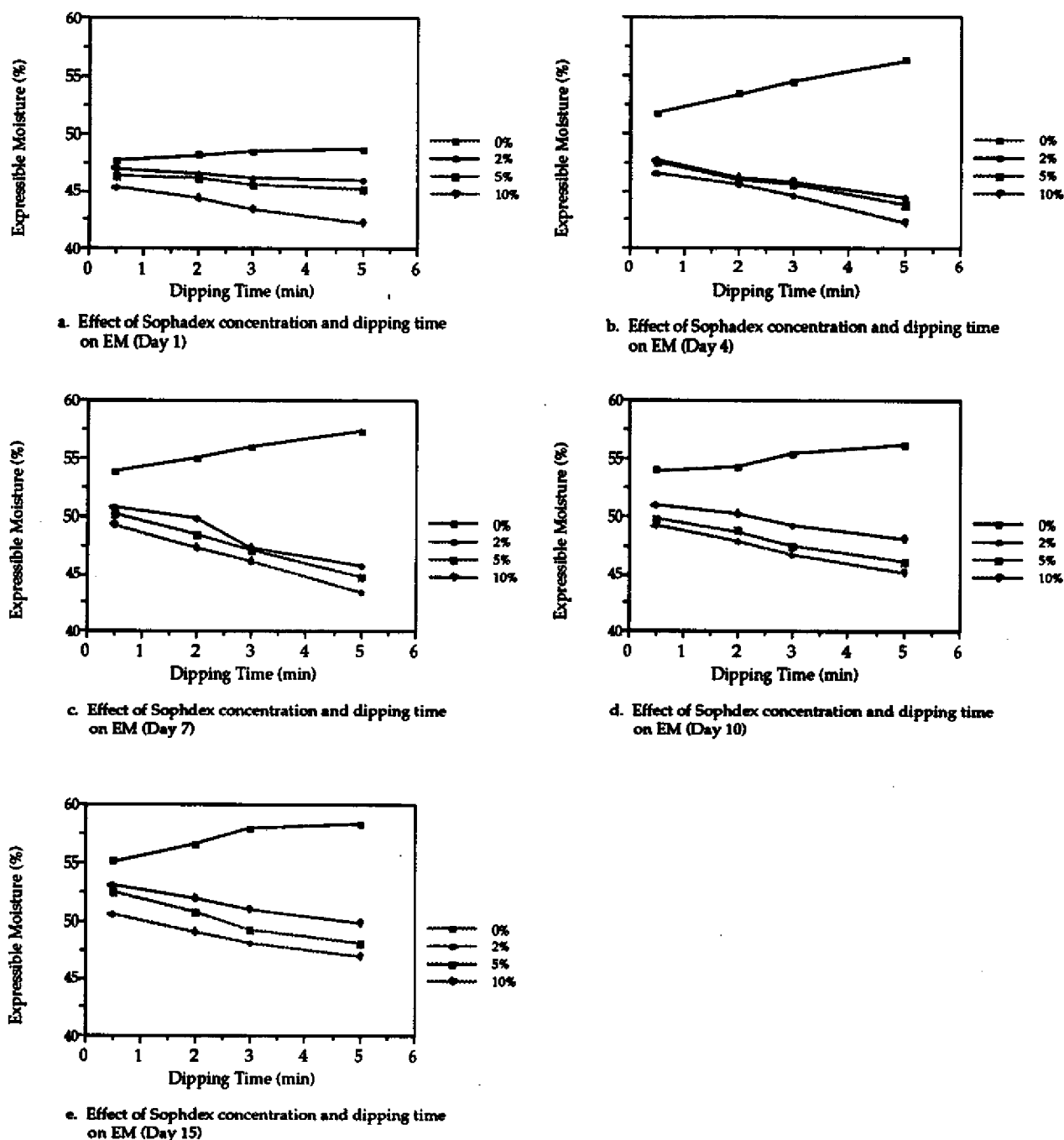


Fig. 9 Effect of storage time on the EM of Sophadex treated cod fillets

The effect of storage time on the WUA of Sophadex treated fresh cod fillets

Fig. 10a-10e show the WUA with the same conditions as for EM. In Fig. 10a, WUA of the fillets dipped only in water did not change with dip time, whereas the WUA of the fillets dipped in Sophadex increased from 0.5 min to 2 min and then showed no change from 2 min to 5 min. The higher the dipping concentration, the higher the WUA. On day 4 (Fig. 10b), the water dipped and the 10% Sophadex had similar WUA as day 1. The other 2

samples were different, especially the 5% solution, where WUA decreased after 2 min dipping, and was lower overall than day 1. From day 7, the WUA decreased in all cases (Fig. 10c) and on day 10 (Fig. 10d) the decrease of the 10% dipping sample was notable. By day 15 (Fig. 10e), the WUA of all samples was lower and more similar. This might be caused by the spoilage of the fish.

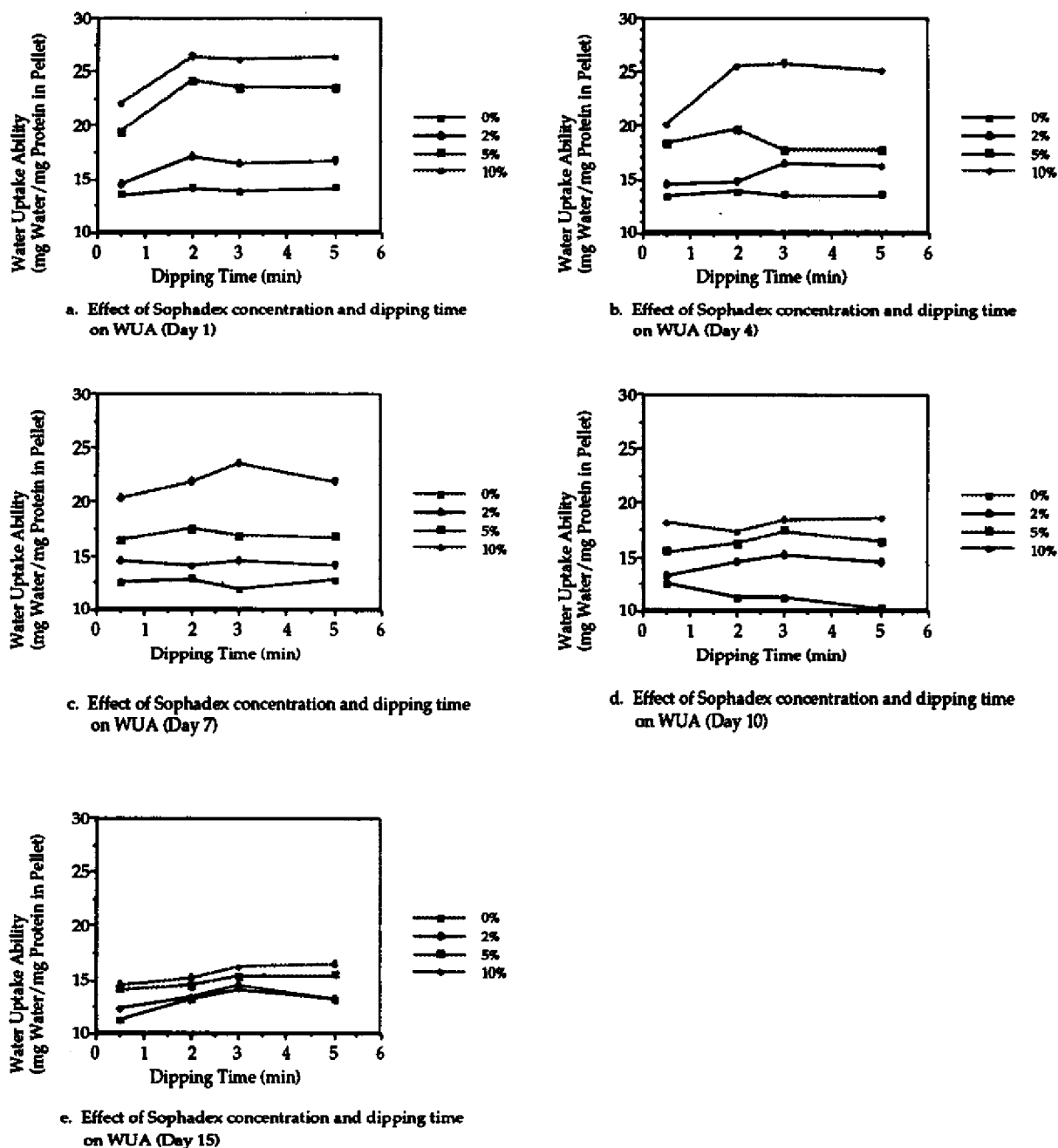


Fig. 10 Effect of storage time on the WUA of Sophadex treated cod fillets

The effect of storage time on the drip loss of Sophadex treated fresh cod fillets

The drip loss after different treatments is shown as the effect of storage time (Fig. 11). The values of the drip loss were very close at the beginning of the storage (day 1), from 2.0% to 2.2%. With the increase of the storage time, the differences became greater. On day 15, the values had a range of 10.0% to 12.5%. The lower the Sophadex concentration, the higher the drip loss; the shorter the dipping time, the higher the drip loss. However, the drip loss was mostly affected by the storage time.

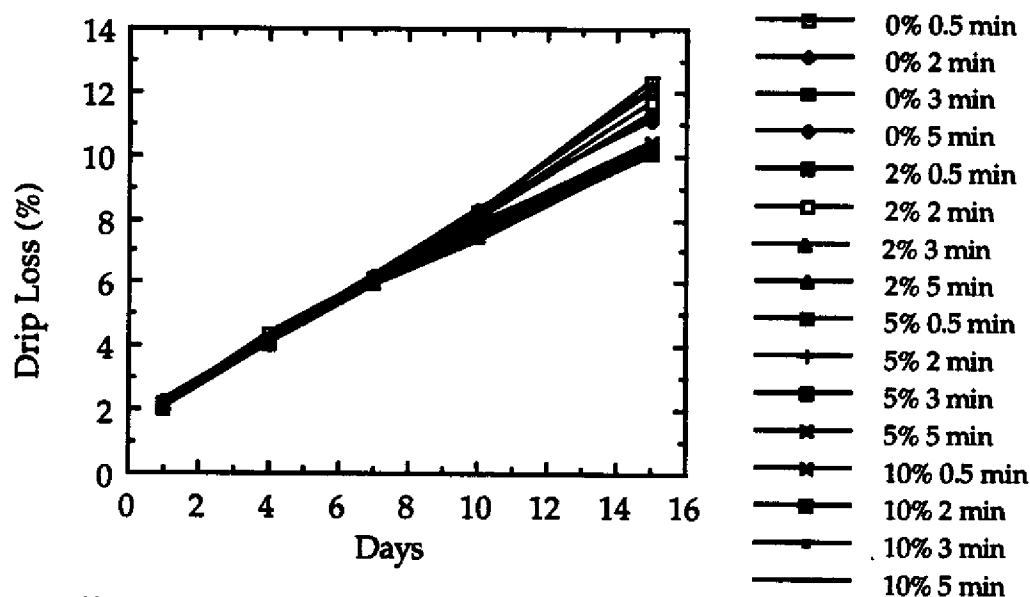


Fig. 11 Effect of storage time on drip loss of fresh cod fillet which dipped in Sophadex

The effect of storage time on the cook loss of Sophadex treated fresh cod fillets

Fig. 12 shows the change of the cook loss as the effect of storage time. Although there was no similar pattern, the cook loss, generally decreased with the addition of Sophadex, and there was a trend that increased concentrations and dipping times, decreased cook loss.

The effect of storage time on the EM of STPP treated fresh minced cod

The effect of STPP concentration and storage time on EM of minced cod is shown in Fig. 13. The EM decreased with increased STPP. The decrease was greatest on day 1 with more than a 10% decrease. The changes on day 4 and day 8 were less, around 4-5%. Increase storage time increased the EM. However, on day 15, an unusual change occurred, the EM dropped again. The values of every sample with different STPP concentrations were lower than those of day 4 and day 8, and closer to day 1. The change was unexpected and might be due to the spoilage of the fish.

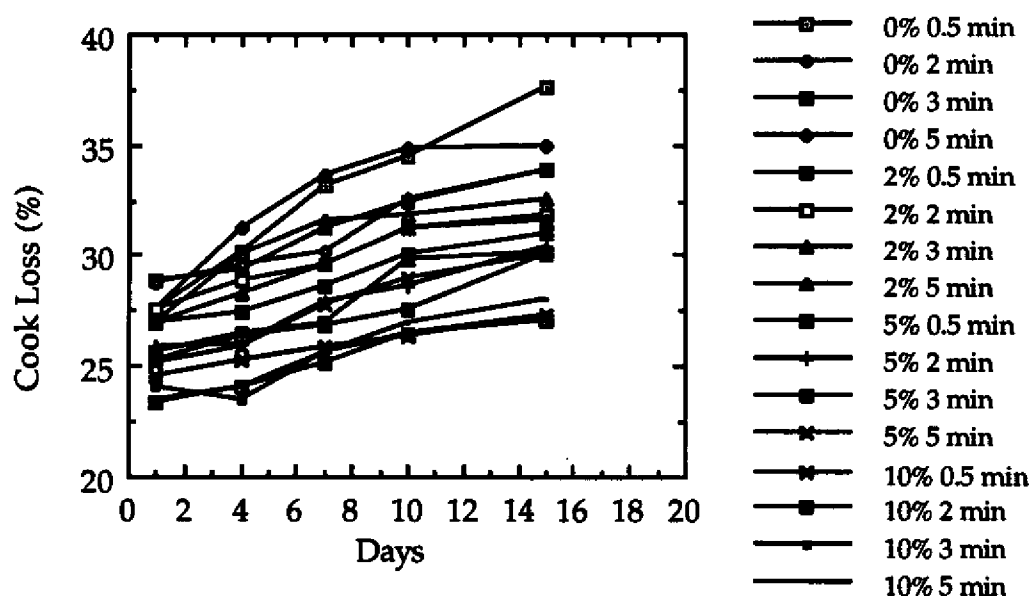


Fig. 12 Effect of storage time on cook loss of fresh cod fillet dipped in different Sophadex

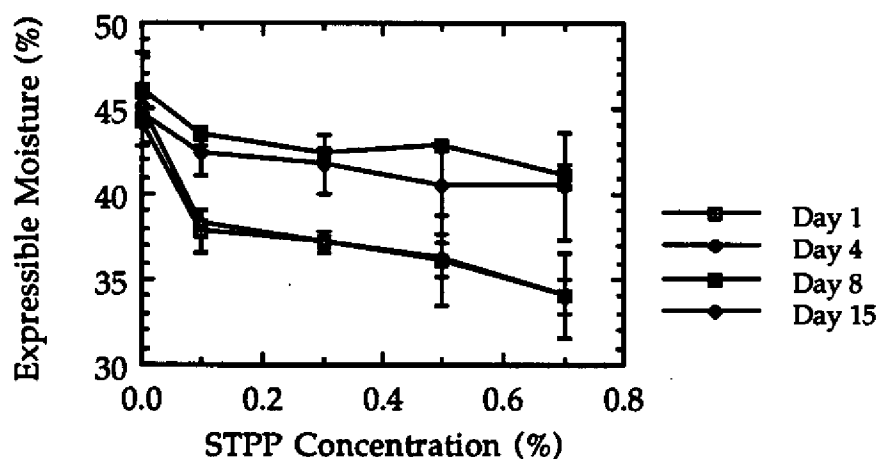


Fig. 13 Effect of storage time on EM of minced fresh cod with the addition of STPP

The effect of storage time on the WUA of STPP treated fresh minced cod

The effect of STPP concentration and storage time on WUA of minced cod is shown in Fig. 14. On day 1 the WUA decreased slightly and smoothly with the increase of the STPP concentration, from 22 to 18 (mg water/mg protein in pellet). On day 4, the WUA increased for all the samples with different STPP concentration with the exception of the samples that had no STPP added. The decrease of the samples with no STPP added was 0.8 (mg water/mg protein in pellet). For the other samples, the values increased, especially for the one with the highest STPP concentration (from 18 to 27 (mg

water/mg protein in pellet)). On day 8, the values decreased again and were all close to the values on day 1. On day 15, the values decreased. The value of the sample with no STPP added dropped to 11 (mg water/mg protein in pellet).

Other changes observed during the WUA test were in the protein in the supernatant and the water absorbed as a function of total sample weight (Fig. 15 & 16). The protein content in the supernatant increased with storage. The effect of STPP on protein solubility was modest, storage time had a greater effect. The increase on day 15 was greater with the highest point at 141.3 (mg protein/1.7 g sample). This might again be caused by the spoilage of the samples. It was interesting that the protein content in the supernatant dropped with the increased STPP concentration on day 1 and increased on day 4 and day 8. For the water absorbed by the samples, the notable changes also occurred on day 15.

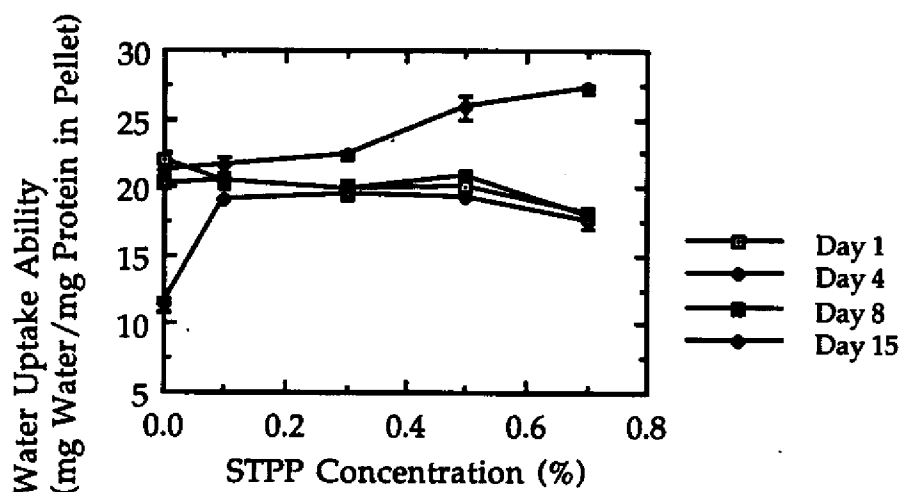


Fig. 14 Effect of storage period on WUA of minced fresh cod with the addition of STPP

Since pH had a larger effect on WUA than the concentration of polyphosphates, the pH of the homogenized samples was monitored before centrifugation (Fig. 17). The pH of all the samples increased with storage time and STPP. However, the pH of the samples with the same STPP concentration did not change much from day 1 to day 4, whereas the change from day 4 to day 8 is large. Again, the change on day 15 was unexpected and may be due to the spoilage of the fish. The WUA of the sample increased with the increase of pH in the range of 7 to 9 as suggested.

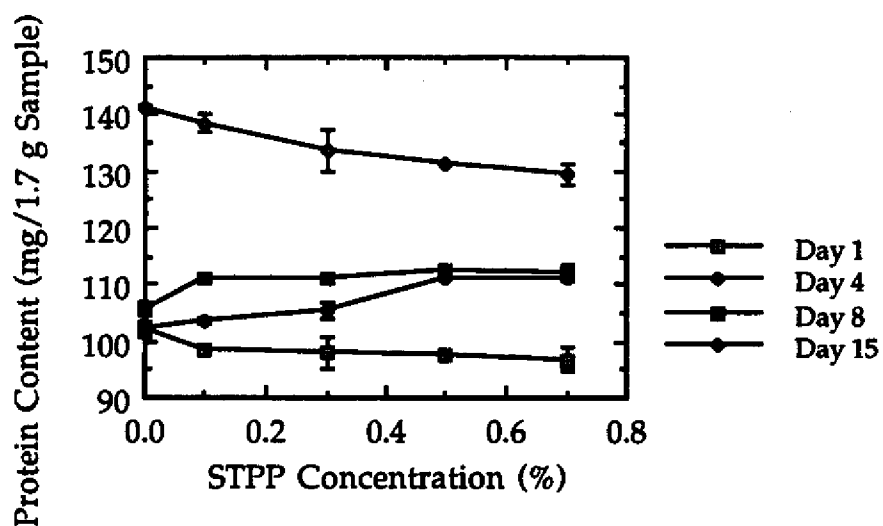


Fig. 15 Effect of storage time on the amount of soluble protein in the supernatant with the addition of STPP

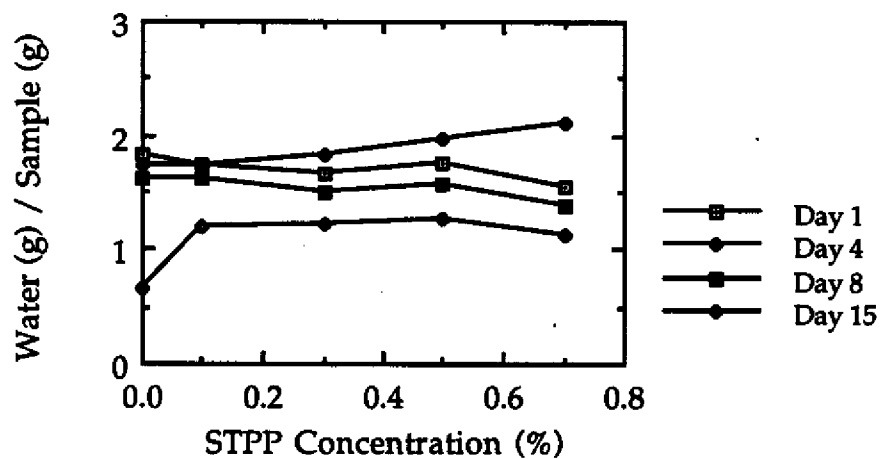


Fig. 16 Effect of storage time on WUA (expressed as water (g)/sample (g)) of minced fresh cod with the addition of STPP

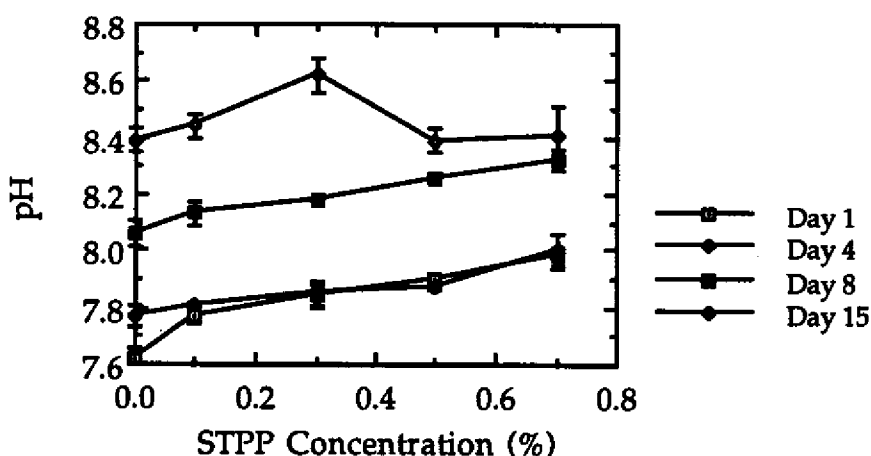


Fig. 17 Effect of storage time on pH of 5% solution of minced fresh sample with the addition of STPP

The effect of storage time on the drip loss of STPP treated fresh minced cod

Fig. 18 shows the drip loss for different storage period. The drip loss increased with storage time. For the samples without STPP treatment, the drip change was large, from 3.5% to 11.9%. STPP prevented the drip loss although the concentration did not affect drip loss. In addition, the STPP had some effect on drip loss with longer storage periods. Day 4 and 8 had similar drip loss, while day 15 showed higher values. This again might be due to spoilage.

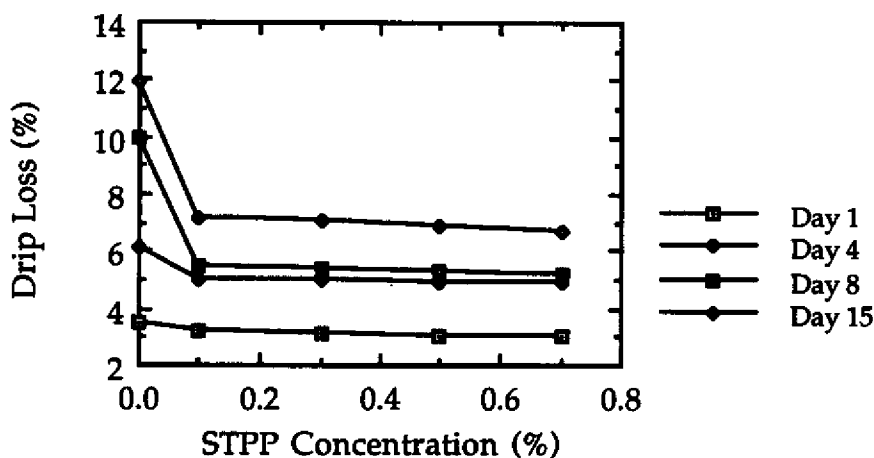


Fig. 18 Effect of storage time on drip loss of minced fresh cod with the addition STPP

The effect of storage time on the cook loss of STPP treated fresh minced cod

Cook loss is shown in Fig. 19. The concentration of STPP did not have a big effect on cook loss compared to the storage time. There was also no big difference between the samples with STPP and the samples without STPP.

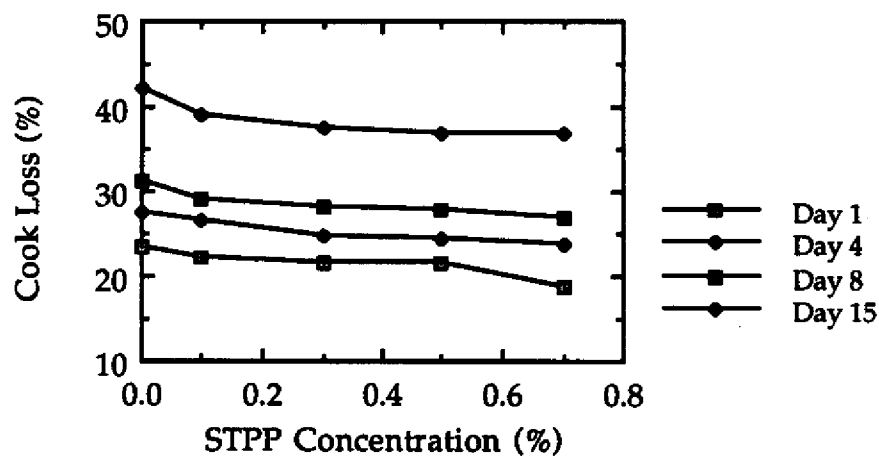


Fig. 19 Effect of storage time on cook loss of minced fresh cod with the addition STPP

Mackerel studies:

Preliminary results on the effect of different concentrations of STPP and dipping time in mackerel samples are shown in figures 20-24. EM decreased with storage time. Both concentration and dipping time (especially the latter) showed an increase in the EM of the samples (see Fig. 20).

As expected then, WUA increased with time, i.e., the older the fish post-mortem, the greater the ability to take up water. The effect of the concentration of STPP was not clearly defined. The highest values for WUA were observed in samples dipped in water (0%) but the lowest were not observed in higher concentrations of the STPP, as we would have expected, but in fact were obtained among the middle concentration levels. The increase in WUA with time, however, seemed to be less in the samples dipped in higher concentrations of the STPP solution. Dipping time appeared to slightly decrease WUA (see Fig. 21).

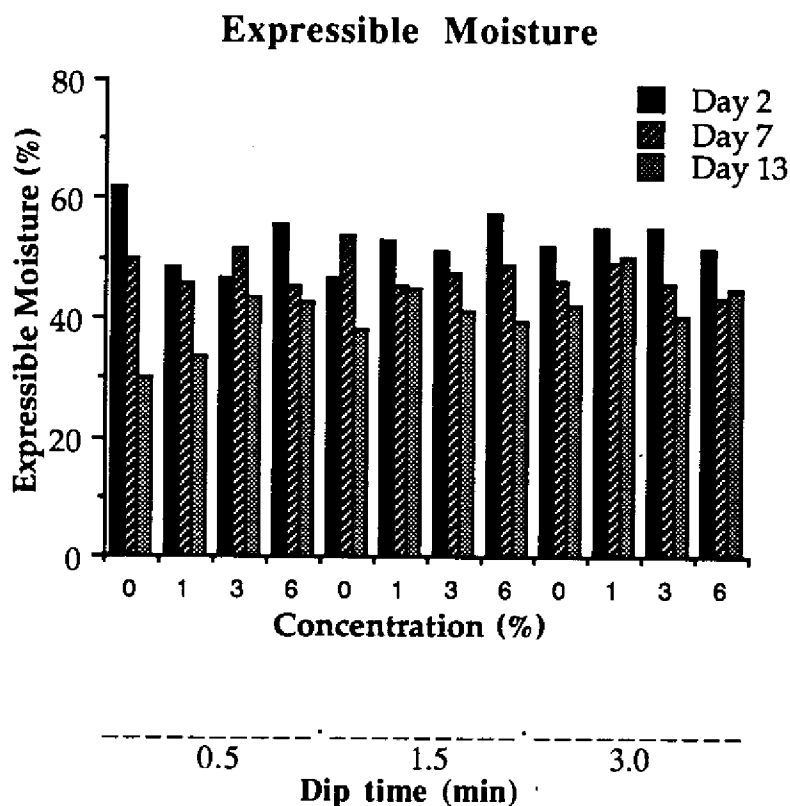


Fig. 20 Effect of STPP concentration and dipping time on EM of mackerel white muscle on days 2, 7 and 13.

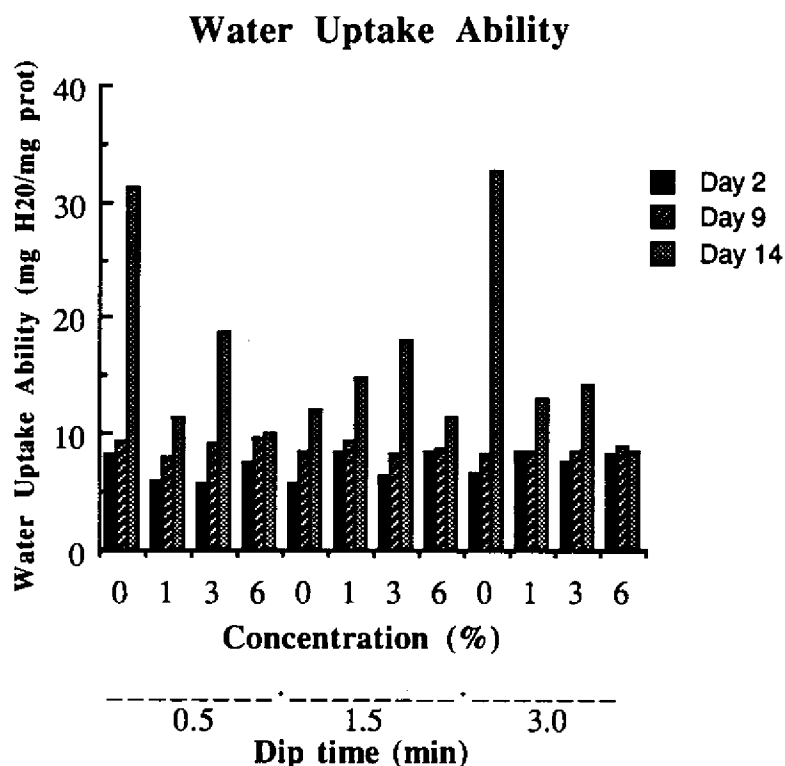


Fig. 21 Effect of STPP concentration and dipping time on WUA of mackerel white muscle on days 2, 9 and 14.

Both the concentration of STPP and the dipping time had the expected effect of increasing the phosphate content of the sample measured as % P₂O₅ (see Fig. 22). These results, however, were not checked against a no-treatment sample which means that the final phosphate uptake of the different samples was not possible to quantify.

Finally, to compare the polyphosphate uptake with the oxidation of fatty acids (i.e., rancidity), a TBA test was performed (see Fig. 23). At higher concentrations of polyphosphate, the TBA values were lower. This effect was also observed with regard to dipping time (i.e., the longer the dipping time, the lower the TBA values).

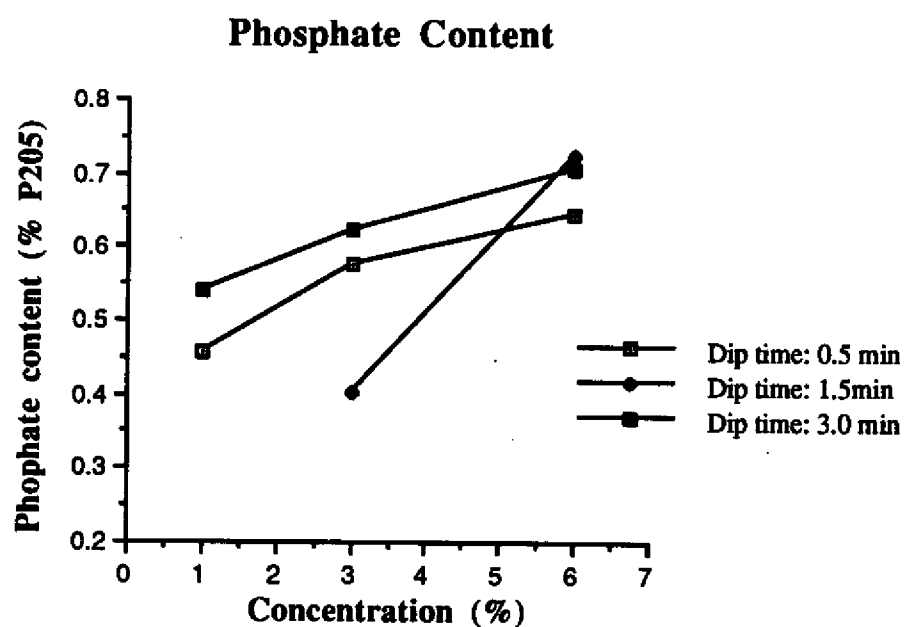


Fig. 22 Effect of STPP concentration and dipping time on the phosphate content of mackerel white muscle.

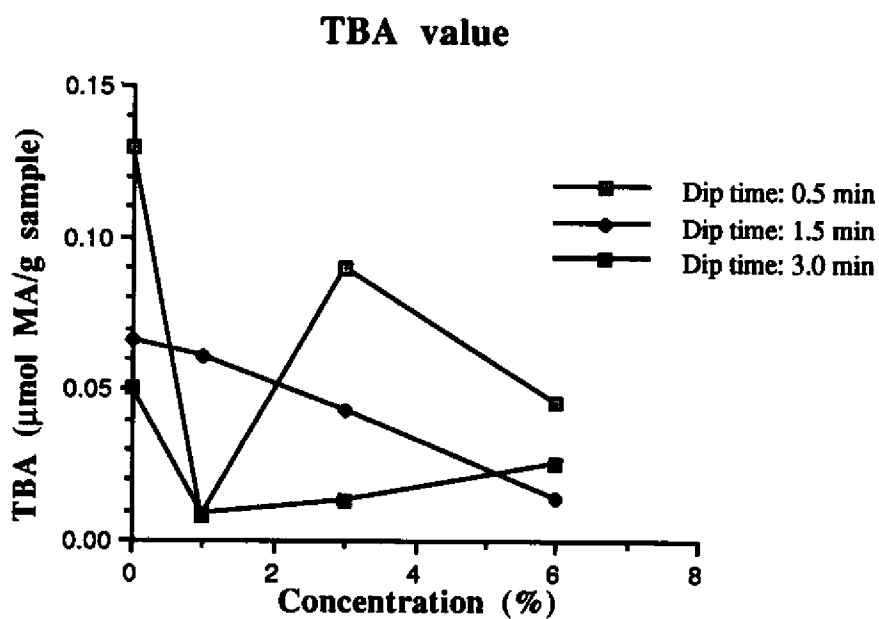


Fig. 25 Effect of STPP concentration and dipping time on the TBA value of mackerel white muscle

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MOISTURE CONTENT OF SCALLOP MEAT: EFFECT OF SPECIES, TIME OF SEASON AND METHOD OF DETERMINING "ADDED WATER"

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INTRODUCTION

Canadian regulations do not allow scallop processors to use phosphate additives. However, depending upon the manner by which scallop meats are handled, water may be added to the meats by their exposure to fresh water. In addition a large portion of scallops, processed within Canada, are exported to the United States and thus will be subjected to the FDA proposed regulation concerning scallop meats that contain greater than 80% moisture content being labelled as containing added water. Therefore the Seafood Quality Investigations Division was asked to investigate moisture content of both Icelandic scallops (*Chlamys islandica*) and sea scallops (*Placopecten magellanicus*), which occur in water adjacent of Newfoundland.

MATERIALS AND METHODS

Sample procurement

During each of April 1991 and September 1992 both wild Iceland scallops and wild sea scallops were caught on the northern half of St. Pierre Bank (NAFO Division 3Ps). In order to minimize both damage and stress, the scallops were caught at the end of a trip, using tows of short duration. All tows were made with an unlined 12ft (3.66m) New Bedford offshore dredge equipped with 3" rings, which were interconnected with three and four links on the top and belly, respectively. Towing speed as approximately three knots with a warp to depth ratio of 3:1. Once each set was completed, live scallops were removed from the catch, sorted according to species, undamaged scallops placed in cabbage bags and stored in sea water. During April scallops were stored in running ambient sea water, whereas during September the increased ambient temperature necessitated that the scallops be stored in chilled (5°C) but aerated sea water.

Upon returning to St. John's, all scallops were immediately transferred to a chilled (5°C) sea water system at the Northwest Atlantic Fisheries Centre. One group of Icelandic scallops and one group of sea scallops were removed from the chilled sea water and processed immediately. Processing consisted of: removing and briefly rinsing the adductor muscle; allowing it to drain to remove excess water; vacuum packaging the larger striated portion of the muscle, and freezing/storing them in a chest freezer at -60°C.

During the Autumn of 1982, sea scallops, of the 1981 year class, were collected as one-year old spat from Garden Cove, Placentia Bay, transported to Spencer's Cove, Placentia Bay, and placed in scallop aquaculture cages, for growth studies. During each of early July and late November, 1985 live cultured sea scallops were collected from these cages. Once collected, live sea scallops were transported to St. John's and stored in an ambient sea water system at the Northwest Atlantic Fisheries Centre. Detailed biological sampling of each sample took place shortly after the samples arrived at the laboratory.

Determination of moisture and protein content

Vacuum packed scallop meats, from each of Iceland scallops and sea scallops procured during each of April and September, were thawed in water kept at 20°C. A Total of 48 Iceland and sea scallops caught during April and September were: finely chopped by hand; placed in preweighed aluminum containers; weighed; dried to constant weight using an air oven operating at 102°C; cooled in a desiccator; weighed; ground and stored in tightly sealed sample jars. The moisture content of a total of 51 giant scallops collected during July and November was determined, immediately after live scallops were shucked, using similar procedures. Crude protein content of the Icelandic scallops caught during April and September was determined, by Kjeldahl, using methods described by AOAC (1).

Assessment of methods used to determine "added water"

The maximum amount of water that could be added to 100g of scallop meat, without the meat containing 80.0% moisture (Proposed U.S. FDA regulation) or having a moisture:protein ratio of 5.0 (2) was calculated. All such calculations were based upon observed protein and/or moisture values, of a variety of scallop meats, harvested during April and September.

RESULTS

Effect of Species

Within each of the two different seasons, the moisture content of meats from sea scallops did not appear to differ from those of meats from Icelandic scallops (Table 1). The frequency distributions of moisture content, of both species, were very similar, even though sea scallops generally spawn during Autumn and Iceland scallops generally spawn during Spring. The moisture content of sea scallops ranged between 74.63% and 80.20% whereas that of Iceland scallops were observed to be between 75.28% and 81.25%. Although there was very little difference between the moisture content of these two species, this does not necessarily mean that the moisture content of either sea scallops or Iceland scallops is similar to that of any of the other eight species harvested throughout the world (Table 2).

The frequency distribution of moisture:protein ratio of meats from sea scallops appeared to be quite similar to the moisture:protein ratio of meats from Iceland scallops (Table 3).

Effect of Season

Within each of the two species, moisture content was dramatically affected by the season during which they were harvested (Table 1).

Table 1. Moisture content of wild Iceland scallop (*Chlamys islandica*) meats and wild sea scallop (*Placopecten magellanicus*) meats harvested during both April and September.

Moisture Content (%)	Observed Frequencies (%)			
	<u>Sea scallops</u>		<u>Iceland scallops</u>	
	April n = 15	September n = 9	April n = 15	September n = 9
≥ 74 < 75	7.1	-	-	-
≥ 75 < 76	28.6	-	13.3	-
≥ 76 < 77	7.1	-	26.7	-
≥ 77 < 78	50.0	-	46.7	-
≥ 78 < 79	7.1	-	13.3	-
≥ 79 < 80	-	77.8	-	-
≥ 80 < 81	-	22.2	-	77.8
≥ 81 < 82	-	-	-	22.2

When harvested during April, 92.8% of the sea scallop meats were observed to contain less than 78.0% moisture with 35.7% of the meats harvested during April containing less than 76.0% moisture (Table 1). However, all of the sea scallops meats harvested during September were observed to contain at least 79.0% moisture with 22.2% of them containing at least 80.0% moisture (Table 1).

Likewise, almost all (86.7%) of Iceland scallop meats harvested during April contained less than 78.0% moisture whereas when harvested during September all of the meats contained at least 80.0% moisture, with 22.2% of these having a moisture content of at least 81.0%.

Although the meats harvested during Spring were obtained during 1991 and those harvested during Autumn were obtained during 1992, it is strongly suspected the large effect of season was primarily a result of high food supply which normally occurs during March to May (4, 5). Observed moisture content of cage cultured sea scallops harvested during both early July and late November substantiated this suspicion. Although all scallops sampled were of the same year class, the time of season during which the scallops were harvested appeared to greatly affect the observed moisture content (Table 4). When harvested during early July 83.3% of the scallop meats contained less than 77.0% moisture, with 63.3% of the meats containing less than 76.0% moisture, but when harvested during late November 95.1% of the meats contained at least 77.0% moisture, with 66.6% of them containing at least 78.0% moisture (Table 4).

Regardless of the reason, the observed moisture content of scallops harvested during early July (1985), late November (1985), late April (1991) and early September (1992) clearly indicated that, moisture content of sea and Iceland scallops was seriously affected by the time of season during which the scallops were harvested.

Effect of Method of Determining "Added Water"

The amount of water that could be added to scallop meat depended upon the natural moisture content and the method of determination, which in turn, was affected by protein content. In general, when scallop meat contained less than 78% moisture the 80% moisture limit allowed more water to be added (Table 5). This degree of difference increased as the natural water content decreased added without the moisture:protein ratio reaching a value of 5.0 (Table 5). In contrast, 20.7g to 26.0g of water could be added to 100g of the same scallop meat without the moisture content reaching the proposed 80.0% limit. This is because, the addition of water (Table 5). For example, with scallop meats containing 74.6% - 75.8% causes both the numerator and the denominator (used to calculate "new" percentage) to change. Alternatively when a moisture:protein ratio is used, the addition of water causes only the numerator, not the denominator, to change. However with scallops that contained 78.6% - 79.7% moisture (16.65 - 17.5% protein), slightly more water (3.6g vs 2.4g, 3.7g vs 1.2g and 8.8g vs 6.8) could be added to 100g of scallop meat if the limit was a moisture:protein ratio of 5.0.

Thus using a moisture:protein ratio of 5.0 rather than a moisture content of 80% to determine the presence of "added water" has some important characteristics. Firstly, it appears to be much more likely to detect the presence of water that had been added to scallops that naturally contained a lower moisture content. Secondly, it appears to be more tolerant of water that had been added to scallops which naturally contain a moisture close to 80%. For example, if a moisture limit of 80% was enforced all of the Iceland scallops harvested during September would have to be classified as having water added, but if the limit was a moisture:protein ratio of 5.0 none of these scallops would have to be classified as having water added.

Table 2. Different scallops that are harvested, commercially, throughout the world.

Name	Location of Catching	Average Count/lb.	Average Yearly Landings (MT)
Bay scallop (<i>Argopecten irradians</i>)	New England to North Carolina	70/90	5,000-8,000
Calico scallop (<i>Argopecten gibbus</i>)	North Carolina to Brazil	150/200	150,000-200,000
Common scallop (<i>Pecten maximus</i>)	United Kingdom, France	40/60	35,000
Iceland scallop (<i>Chlamys islandica</i>)	North Atlantic & Barents Sea	60/100	50,000
Japanese scallop (<i>Pecten yessoensis</i>)	Japan, Korea	10/30	222,000
Panamanian scallop (<i>Argopecten circularis</i>)	Central America	80/120	40,000 (est.)
Peruvian scallop (<i>Argopecten purpuratus</i>)	Peru, northern Chile	80/120	10,000 (est.)
Queen scallop (<i>Chlamys opercularis</i>)	Faeroe Islands, United Kingdom	60/100	12,000
Sea scallop ² (<i>Placopecten magellanicus</i>)	Newfoundland to North Carolina	20/30	130,000
Weathervane/Giant Pacific scallop (<i>Pecten caurinus</i>)	Alaska to Oregon	20/40	4,000

¹From Seafood Leader Buyer's Guide, 1988, p. 266²Also called giant scallop.

Table 3. Moisture:protein ratio¹ of wild Iceland scallop (*Chlamys islandica*) meats and wild sea scallop (*Placopecten magellanicus*) meats harvested during both April and September.

Moisture: protein ratio	Observed Frequencies (%)			
	Sea scallops		Iceland scallops	
	April n = 15	September n = 9	April n = 15	September n = 9
≥ 4.3 - < 4.4	14.3	-	20.0	-
≥ 4.4 - < 4.5	28.6	-	13.3	-
≥ 4.5 - < 4.6	7.1	-	33.3	-
≥ 4.6 - < 4.7	28.6	-	26.7	-
≥ 4.7 - < 4.8	21.4	22.2	6.7	11.1
≥ 4.8 - < 4.9	-	22.2	-	55.5
≥ 4.9 - < 5.0	-	33.3	-	33.3
≥ 5.0 - < 5.1	-	22.2	-	-

¹ Moisture : protein ratio = g moisture/g protein

Table 4. Moisture content of cage cultured sea scallop (*Placopecten magellanicus*) meats harvested during each of early July and late November^a.

Moisture Content (%)	Observed Frequencies (%)	
	Harvested early July n = 30	Harvested late November n = 21
≥ 73 - < 74	3.3	-
≥ 74 - < 75	20.0	-
≥ 75 - < 76	40.0	-
≥ 76 - < 77	20.0	4.7
≥ 77 - < 78	6.6	28.5
≥ 78 - < 79	3.3	38.1
≥ 79 - < 80	3.3	19.0
≥ 80 - < 81	3.3	-
≥ 81 - < 82	-	9.5

^aAll scallops were of the 1981 year class and were harvested during 1985.

(Table 5). For example, with scallop meats containing 74.6% - 75.8% moisture (16.81 - 18.03% protein), 8.8g to 14.3g of water could be

Table 5. Effect of method of determining "added water" upon the maximum amount of water that may be legally added.

	OBSERVED VALUES OF		MAX. G WATER THAT MAY BE ADDED TO A 100 G SAMPLE	
	%M	%P	% M	M/P
Lowest % M.	74.63	17.02	26.0	10.4
Highest % M.	81.25	16.35	N/A	0.5
Lowest % P.	77.72	14.95	11.3	N/A
Highest % P	75.84	18.03	20.7	14.3
Samples Containing 16.51% - 17.02% Protein				
	74.63	17.02	26.0	10.4
	75.21	16.81	23.9	8.8
	77.41	16.57	12.6	5.4
	78.49	16.75	7.5	5.1
	79.51	16.65	2.4	3.6
	79.74	16.70	1.2	3.7
	80.62	16.65	N/A	2.6
	81.25	16.35	N/A	0.5
Samples Containing 17.49% - 18.03% Protein				
	75.28	17.76	23.5	13.5
	75.84	18.03	20.7	14.3
	77.34	17.51	13.2	10.2
	78.63	17.49	6.8	8.8
M = % Moisture P = % Protein M/P = Grams moisture/grams protein				

CONCLUSIONS

Moisture content of sea scallop meats and Iceland scallop meats was not affected by the species being analyzed, but the moisture content of meats from both species was vividly affected by the time of season during which they were harvested. The consistent large effect of season combined with inability of a 80% moisture limit to detect water added to scallops that naturally contain lower levels of moisture greatly reduces the practicality of such a limit.

Although the Inspection Branch, Canada Department of Fisheries and Oceans, agrees with the concept of having a limit concerning the addition of water to scallop meats, it disagrees with the proposal to use a limit of 80% moisture, regardless of species location of catching and season of catching.

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FUNCTIONAL, MICROBIOLOGICAL AND SENSORY CHANGES IN SEA SCALLOPS
(Placopecten magellanicus) TREATED WITH SODIUM TRIPOLYPHOSPHATE
 DURING ICED STORAGE

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Sea scallops are traditionally washed, bagged, ice chilled (in summer) and stowed surrounded with ice in bins onboard harvest boats, then washed or held in chilled sodium tripolyphosphate solutions at processing plants prior to packing. The U.S. Food and Drug Administration identified the practice of soaking scallops for extended periods of time as incompatible with policies prohibiting the uptake of water. To answer questions related to expected weight gains and shelflife properties associated with various processing procedures involving water contact, the International Food Additives Council and the American Scallop Association funded a project which included the work described here. For a copy of the complete project final report entitled "An Evaluation of Processed Atlantic Sea Scallops (*Placopecten magellanicus*)" contact Sea Grant Marine Advisory Program Publications at the Virginia Institute of Marine Science (address above).

The purpose of this study was to evaluate the effect of selected wash and phosphate application treatments on scallop quality parameters and moisture retention during iced storage. Alkaline polyphosphates are most frequently used in seafood and meat systems to improve water binding, texture and other functional properties. However, shelflife extension is a benefit occasionally attributed to their use (14, 16). This effect may be primarily related to metal ion chelation. Molins (9) reported that tripolyphosphates form complexes with alkali and earth metals which are more stable than those formed by pyrophosphates which, in turn, are more stable than those formed by orthophosphates.

In a previous study at Virginia Tech, scallops held in 3 percent and 5 percent solutions of sodium tripolyphosphate for 20 hours maintained higher sensory quality during subsequent iced storage than did either an unprocessed control or scallops held in water prior to storage (T. Rippen, 1990, presented at 50th annual meeting of the Institute of Food Technologists, Anaheim). Results

of this study were also reported at the AFT-TSFT conference in Williamsburg, and a data summary can be obtained from author Rippen. It is not included in this proceedings paper.

MATERIAL AND METHODS

Scallops used in this study were harvested approximately seven days prior to the vessel's return to port; mid-point of an average 14-15 day trip. They were taken from a single resource off Cape Cod, Massachusetts on September 2-3, 1992 and were of a small uniform size (57 meats per pound, average). The shucking, chilling and bagging operations were supervised to assure that handling and temperature control procedures were consistently applied, were representative of industry practice and minimized unnecessary water gains.

At a commercial shore-side facility, six processing treatments were conducted. Five bags (approximately 200 pounds) of scallops were used for each treatment using standard processing equipment. Three 70 pound batches were prepared of each treatment to minimize the effects of batch-wise variability. The six processing methods evaluated in this study were as follows:

FW = scallops washed 20 minutes in fresh water

STP = scallops washed 20 minutes in a solution of 4% sodium tripolyphosphate + 1% NaCl

DIP = FW scallops dipped for 1 minute in a 10% solution of sodium tripolyphosphate + 1% NaCl

S82, S84 and S86 = scallops exposed to a solution of 2.5% sodium tripolyphosphate + 1% NaCl for sufficient time to approximately achieve moisture content targets of 82%, 84% and 86%, respectively.

All solutions were pre-chilled to approximately 48°F in volumes sufficient to produce 2:1 solution:scallop ratios. Scallops treated as described (each treatment variable and washed control) were packed into one pound plastic containers immediately following processing using sanitary procedures. They were labeled, packed in ice and transported the same day to the Virginia Tech Seafood Extension and Research Center in Hampton, Virginia. They were held in ice until evaluated.

Objective Procedures

On days 1, 4, 6, 8, 11, 15, 18 and 20 containers of each treatment (including the wash control) were removed from ice for triplicate analysis (three containers sampled per analysis) for aerobic plate count, moisture and pH. Percents of drip loss and cook loss were determined on days 1, 4, 6, 8, 11 and 15, also in triplicate. An exception was the S86 treatment which was not available for evaluation on day 1.

Aerobic (psychrotrophic) plate counts were determined by standard AOAC methods. Twenty five gram scallop composites were removed aseptically from each container immediately after they were opened. They were diluted 1:10 with 0.1 percent peptone and macerated by stomaching. Subsequent dilutions were made in Butterfield phosphate buffer. One ml portions of appropriate dilutes were plated on Petrifilm APC plates (3M) in duplicate. Plates were incubated at 20°C for 72 hours. Colonies from appropriate plates were enumerated, and results reported as colony forming units per gram (cfu/g).

Moisture was determined by the AOAC oven dry method. Duplicate subsamples (of each replicate) were accurately weighed, dried at 100°C to constant weight (approximately 16 hours) and results reported as percent moisture by weight. pH was measured directly from the opened containers with a standardized Orion pH meter and probe.

Containers of scallops were opened, weighed, spread on a sieve, drained for one minute and reweighed. Results were reported as percent drip loss, by weight. Cook losses were determined by accurately weighing (approximately 300g) scallops, broiling them under an electric coil to an internal temperature of $70 \pm 2^\circ\text{C}$ (approximately 6½ minutes), allowing them to cool for 1 to 2 minutes, then draining and reweighing. Internal scallop temperatures were monitored with a multichannel datalogger (Science Electronics, calibration traceable to a NIST standard) equipped with nude end copper-constantan thermocouples.

Sensory Panel Procedures

On days 4, 6, 8, 11, 15, 18 and 20, each treatment and control were presented to sensory panelists trained in scallop evaluation. Of 15 prospective panelists, 10 were selected based on their commitment and procedural comprehension or acuity. Scallops were evaluated raw for appearance and odor, and cooked for appearance, odor, flavor and texture. Unstructured linear scales, anchored by the terms "fresh" and "not fresh" were used for evaluating each of these parameters (appendix). The purpose here was to simultaneously assess all of the various attributes for each parameter which affect the perceived degree of freshness. This differs from descriptive analysis procedures employing specific category scales, for example, degree of rubberiness as a texture measure. Since any one descriptive term may not fully correlate with freshness or shelf-life, training centered on familiarizing panelists with characteristics indicative of fresh (eg. recently harvested) scallops, those that are not at all fresh (ie. spoiled) and those in between.

Panelists were informed of freshness descriptors commonly used for scallops, such as degree of staleness, bitterness, and off-odors and off-flavors (1). They evaluated scallops of various stages of freshness, compared results and were encouraged to discuss other terms and phrases which describe relative freshness. This process established a common basis for evaluation, however, the descriptors were used only for training. Panelists were instructed that samples should be evaluated independently and that preference or degree of liking (hedonics) was not a test criterion: only degree of freshness.

Recognized sensory panel procedures were followed, including random number generation of sample codes, mixed order of sample presentation, proper lighting and other environmental factors, panel station set-up and care to avoid controllable biases (5, 8). The order of sample parameter evaluation was: raw appearance, raw odor, cooked appearance, cooked odor, cooked flavor, and cooked texture. That is, all samples were evaluated first for raw appearance, secondly for raw odor, and so on. Cooked scallops were held in covered glass containers in a moist heat environment prior to serving to panelists (usually within 10 minutes). They were evaluated warm.

Statistical Analysis

Raw data were analyzed by Analysis of Variance (ANOVA) and, where indicated, means compared by Duncan's multiple range procedure ($\alpha = .05$).

RESULTS AND DISCUSSION

Moisture

Moisture contents on day 4, the first day that all treatments were available, ranged from 80.4 percent for the STP treatment to 85 percent for the S86 treatment. The intended targets of 82, 84 and 86 percent moisture contents for the S82, S84 and S86 treatments were not achieved initially. However, moisture determinations varied during storage and values close to these targets were realized by day 11, Figure 1. Use of sodium tripolyphosphate in STP and DIP treatments did not increase moisture levels compared to FW.

pH

Phosphate treatments generally raised scallop pH as expected (17) compared to FW, with overall reductions in pH during storage, Figure 2. Production of acidic microbial metabolites is likely to be the primary cause of acidification. The release of bases is also expected due to deamination processes. Consequently, the pH values indicate the net release and decomposition of acids and bases in a buffered system; not the specific mechanisms involved. Scallop pH may have some value in quality assessment when processing methods are known.

Aerobic Plate Count

Aerobic plate counts were quite low and generally did not change during storage, Figure 3. Shifts in predominant microflora are possible during this time, however, microbiological populations were not characterized in the study. On days 4, 15 and 18 the DIP treatment APC's were significantly lower than FW APC's.

Maxwell-Miller et al. (7) reported an APC of 7.35 log cfu/g after scallops were held on ice for 14 days. Lower values were reported by Power et al. (11): 5.7 log cfu/g after 18 days on ice beginning with high quality scallops. The microflora was predominately *Pseudomonas* spp. in that study. This compares to APCs of 4.5 (DIP) to 5.5 (FW) log cfu/g for treatments in this study after 18 days of storage. The psychrotrophic incubation procedure used in this study usually produce plate counts higher than those expected from standard APC (35°C) procedures. Mukerji (10) obtained psychrotrophic plate counts of 2.7 to 5.2 log cfu/g for sea scallops cryogenically frozen at sea, and 5 to 6.2 log cfu/g for fresh sea scallops at off-loading.

Antimicrobial properties of polyphosphates have been previously reported, primarily in poultry (3, 4, 12, 15). Vyncke (16) attributed observed shelflife extension of ray meat treated with sodium tripolyphosphate to urease inhibition but not to control of microbial growth.

Drip Loss

The release of free liquid increased during iced storage, with amount of drip and time of release dependant on treatment, Figure 4. The FW scallops exhibited significantly greater drip losses than the phosphate treated scallops, with most of this loss occurring during the first four days of storage. The S86 treatment was significantly less effective at retaining liquid than were the other phosphate treatments. This was probably due to a moisture content in these scallops greater than the water binding capacity of the tripolyphosphate. The least drip loss was achieved by the DIP treatment, with visible weepage occurring only on days 11 and 15. Partial protein denaturation and concomitant loss of water holding capacity during storage was expected, and probably accounts for the overall trends. In a previous study, scallops exposed for two hours to a two percent sodium tripolyphosphate solution experienced no net weight change after subsequent freezing and thawing compared to pretreatment weight (T. Rippen, 1990, presented at 50th annual meeting of the Institute of Food Technologists, Anaheim).

Cook Loss

As with drip loss, cooking losses increased over time, Figure 5. The DIP treatment lost significantly less weight than did FW when cooked during the first six days of iced storage. Most of the apparent differences in cook losses between FW and the other phosphate treatments were not significant, except for S84 on day 4 and S86 on day 11 when less cook loss was recorded for these treatments.

Water may be unbound or bound to various degrees within muscle tissue, and the conditions for its release dependant on changes in protein conformation brought about by chemical, enzymatic and physical effects, including heat induced coagulation. The combined percent weight losses associated with iced storage and cooking are shown in Figure 6. Phosphate treatments generally resulted in less total shrinkage than FW, with DIP producing the least loss of all treatments on days 4 and 6, $p < .05$. Treatments producing significantly less loss than FW were: STP on days 4, 6 and 8; DIP on days 1, 4, 6, 8 and 11; S82 on days 1, 4 and 6; S84 on days 4 and 8; and S86 on days 6, 8 and 11.

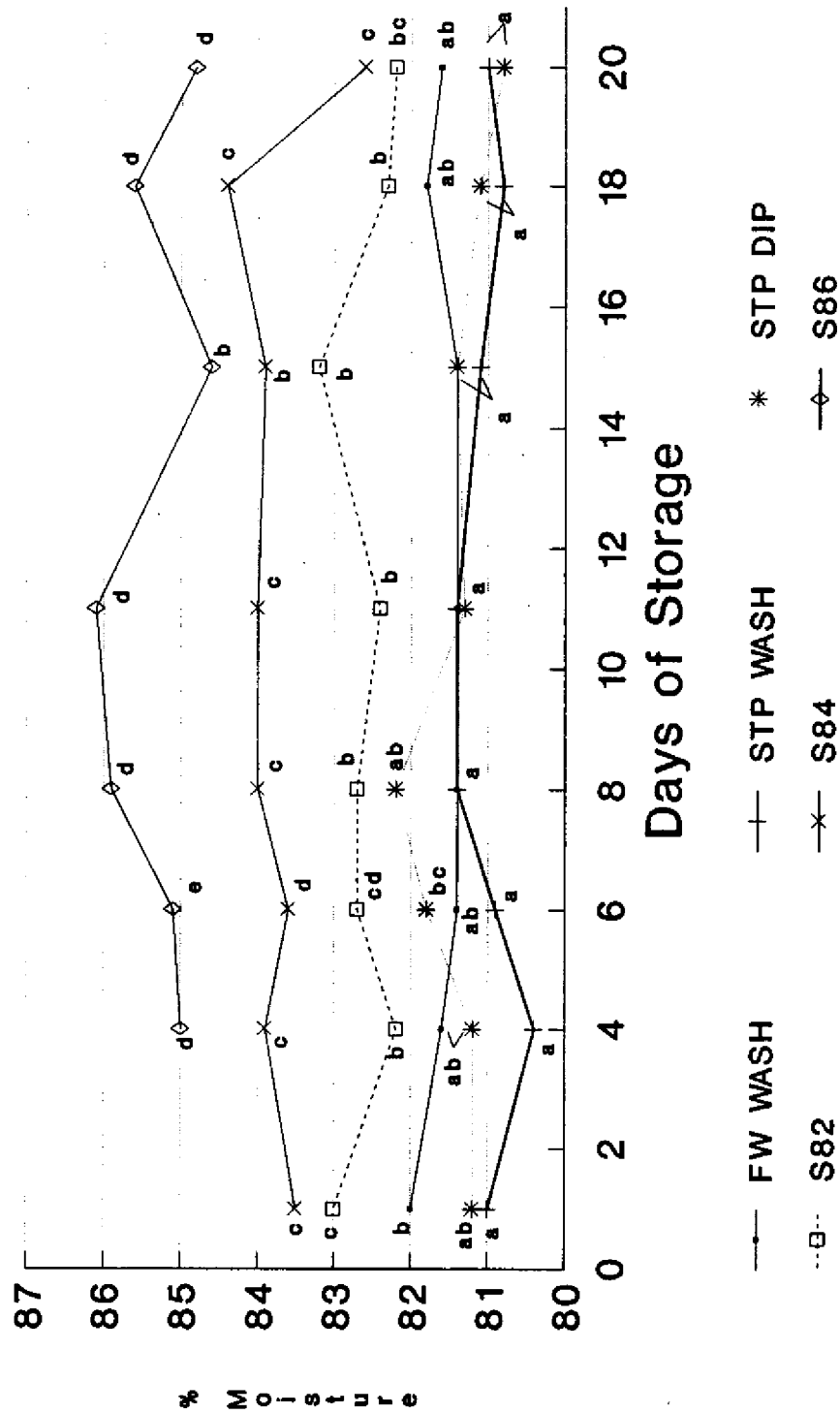
The increased water binding properties of meat systems containing phosphates has been well documented (12, 13, 18, 19).

Sensory Analysis

Appearance and odor scores of raw scallops during iced storage are summarized in Figures 7 and 8, respectively. On day 4, FW was judged to have an appearance that was significantly more fresh than S86 and S84 appearance. Odors of FW, S82 and S84 were judged to be significantly more fresh than STP odor on day 4. No significant differences were identified during the remainder of the storage period.

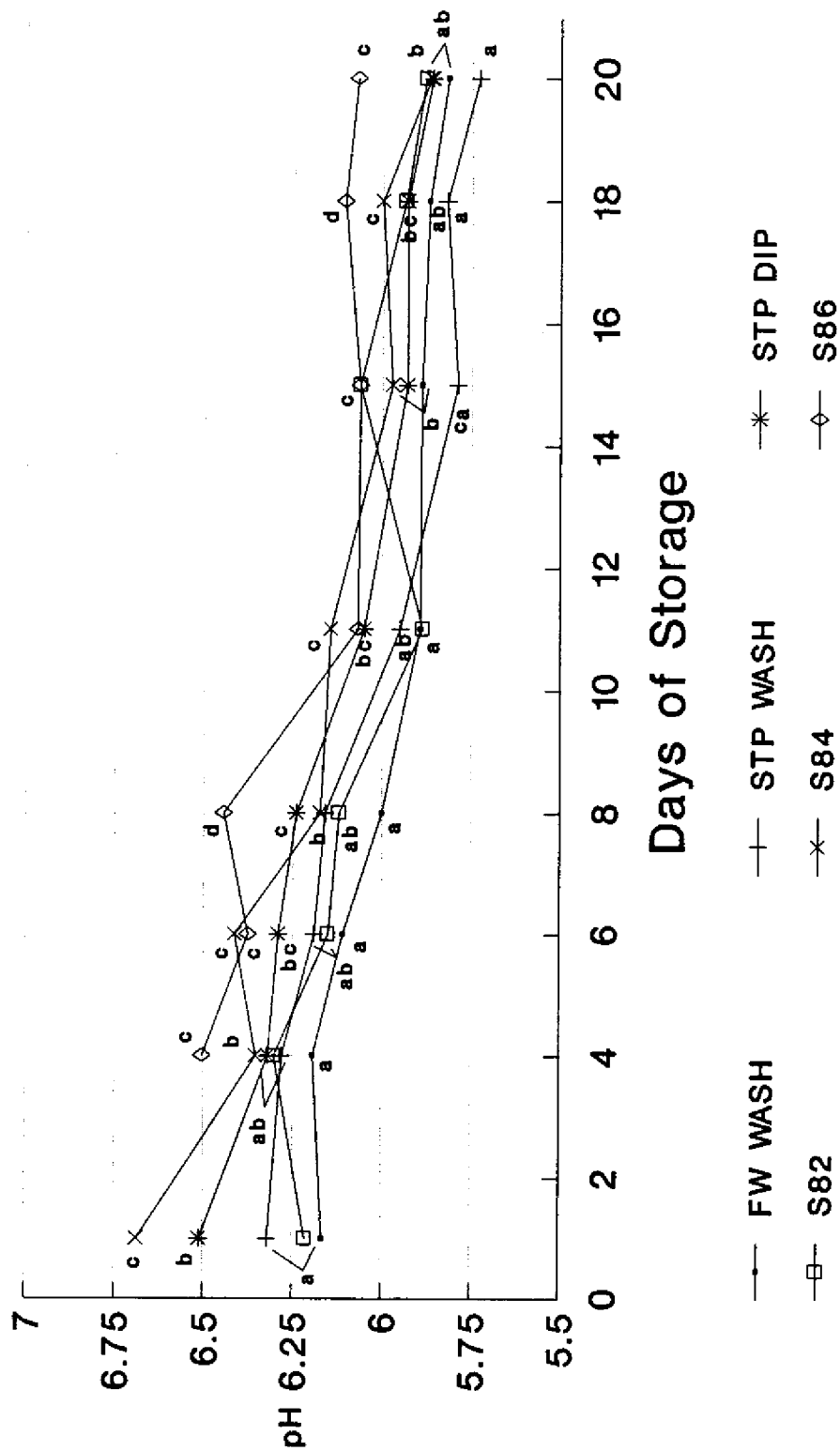
Cooked sensory scores are summarized in Figures 9, 10, 11 and 12. Cooked appearance of FW was judged to be significantly less fresh than S84, S82, DIP and STP on day 6, and less fresh than

Figure 1. Scallop Moisture Content During Iced Storage



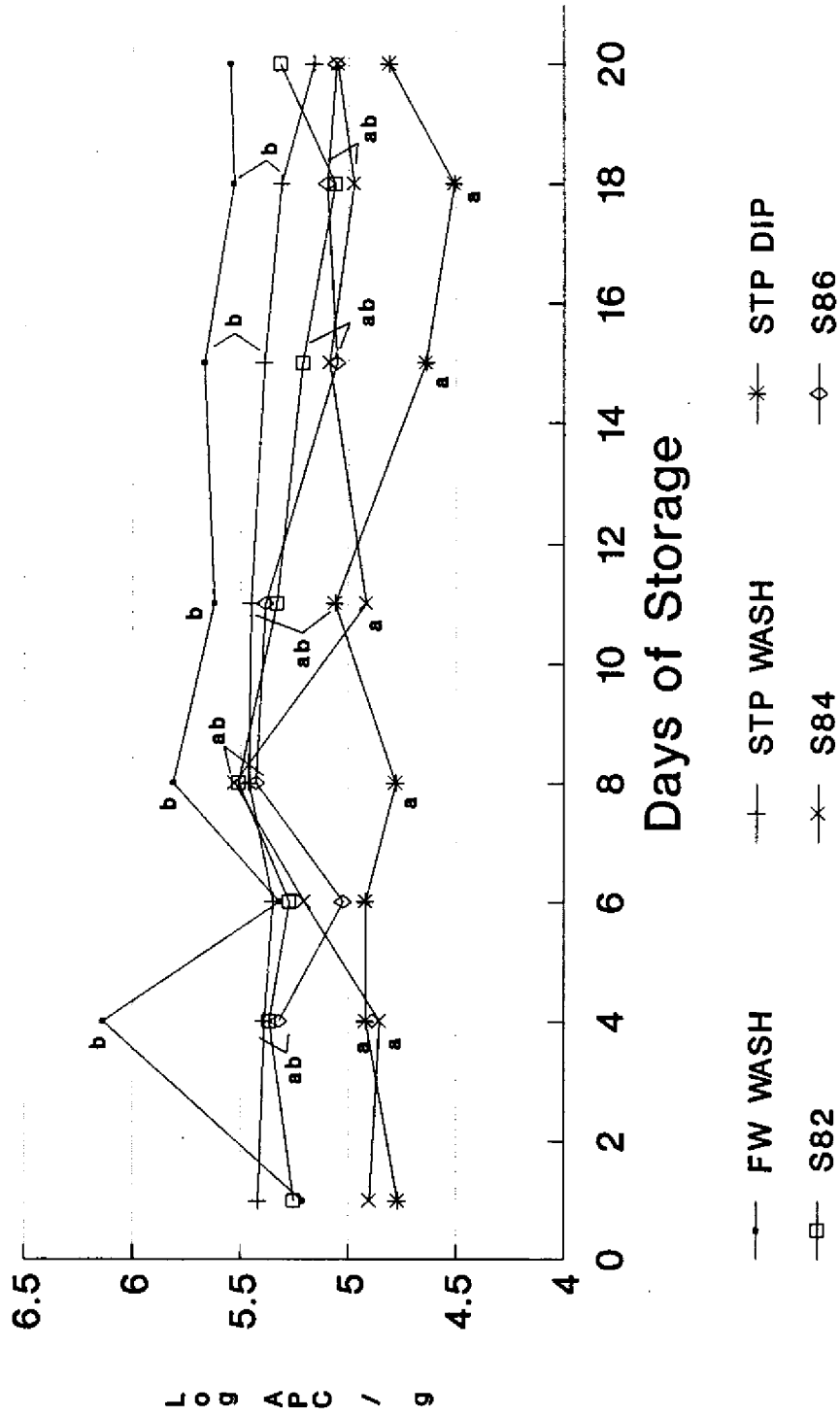
Means each day designated by diff. letters are signif. different, $p < .05$

Figure 2. Scallop pH During Iced Storage



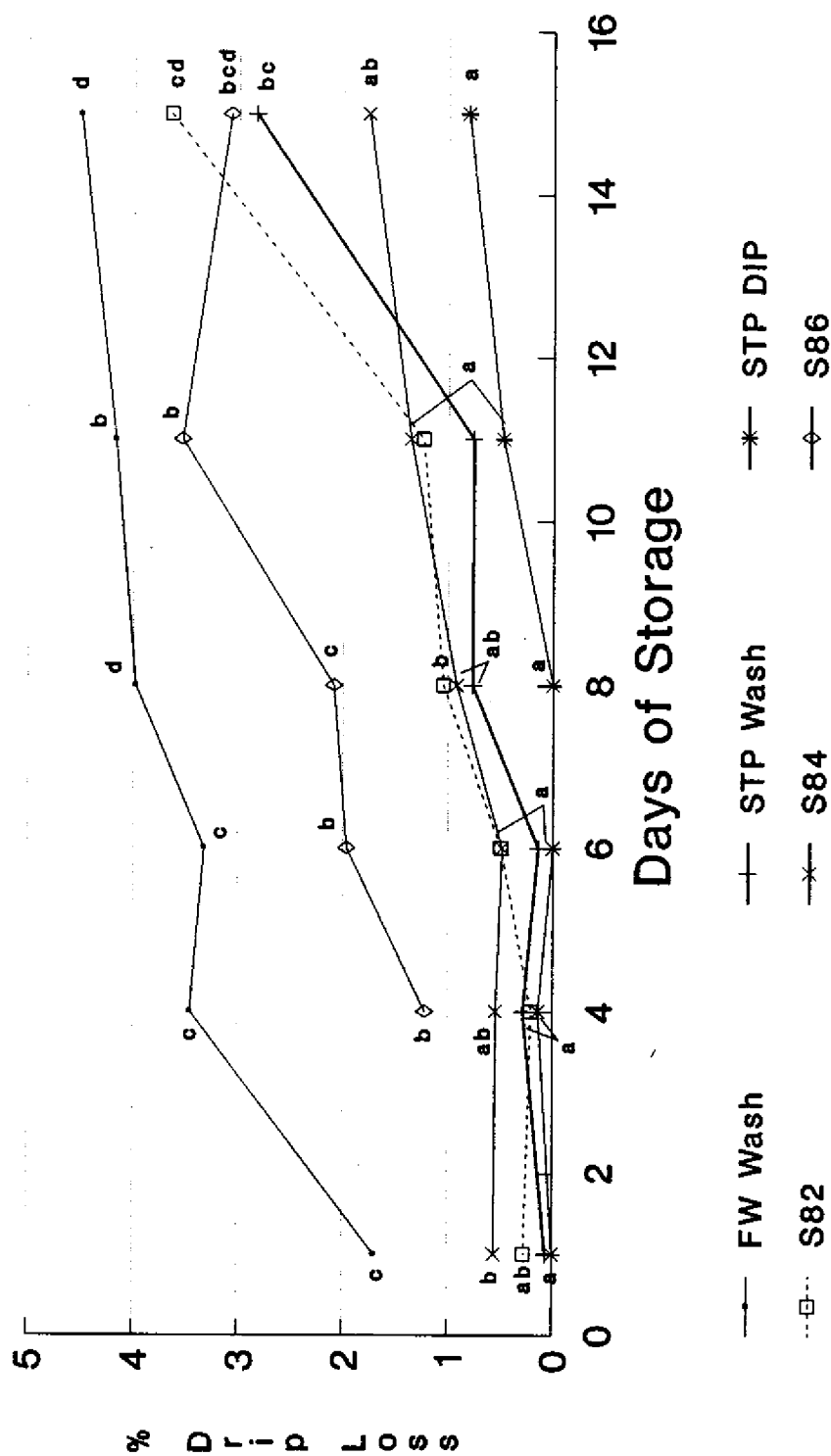
Means each day designated by diff. letters are signif. different, $p < .05$

Figure 3. Scallop Aerobic Plate Counts
(20C) During Iced Storage



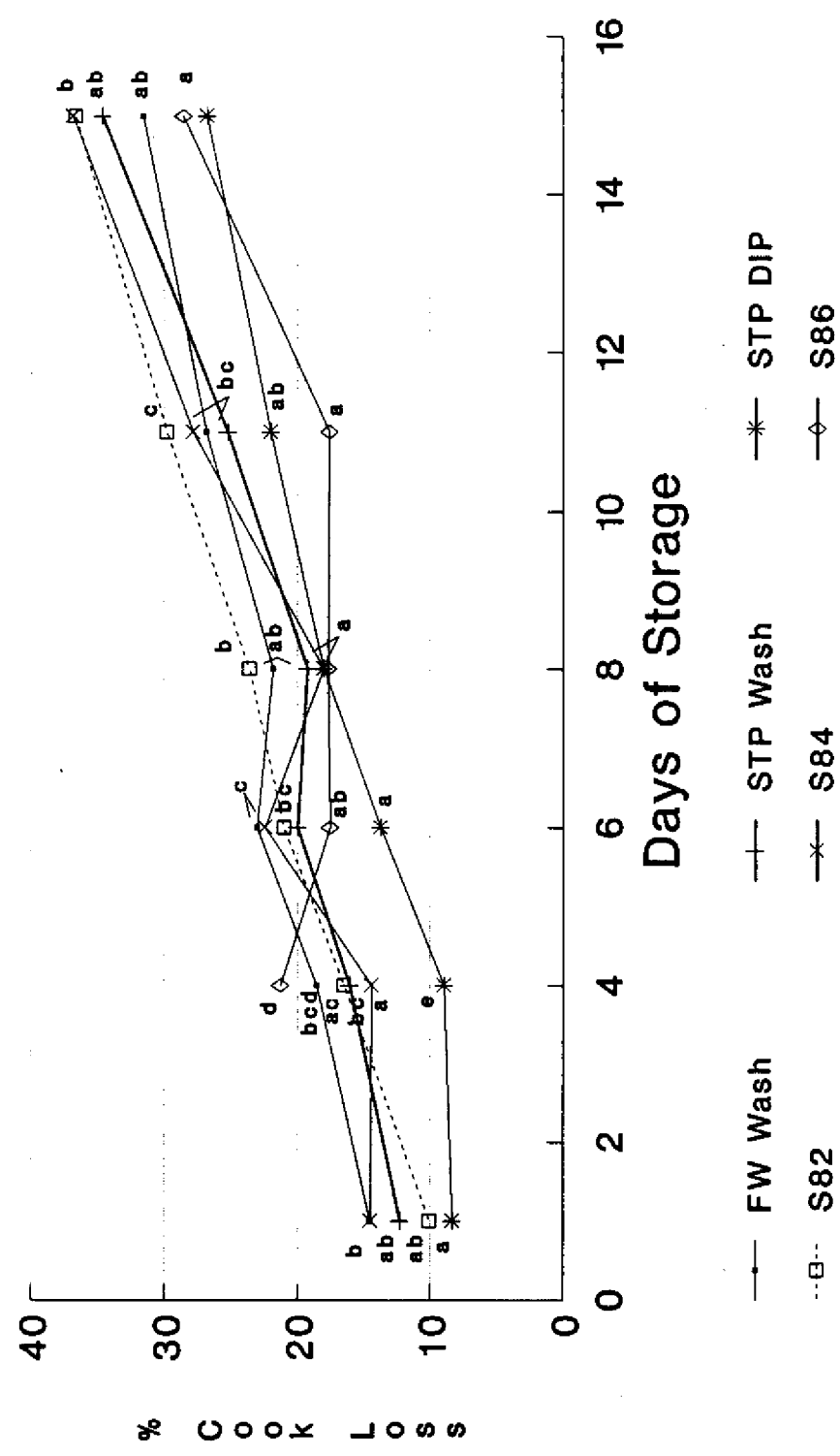
Means each day designated by diff. letters are signif. different, $p < .05$

Figure 4. Scallop Drip Loss During Iced Storage



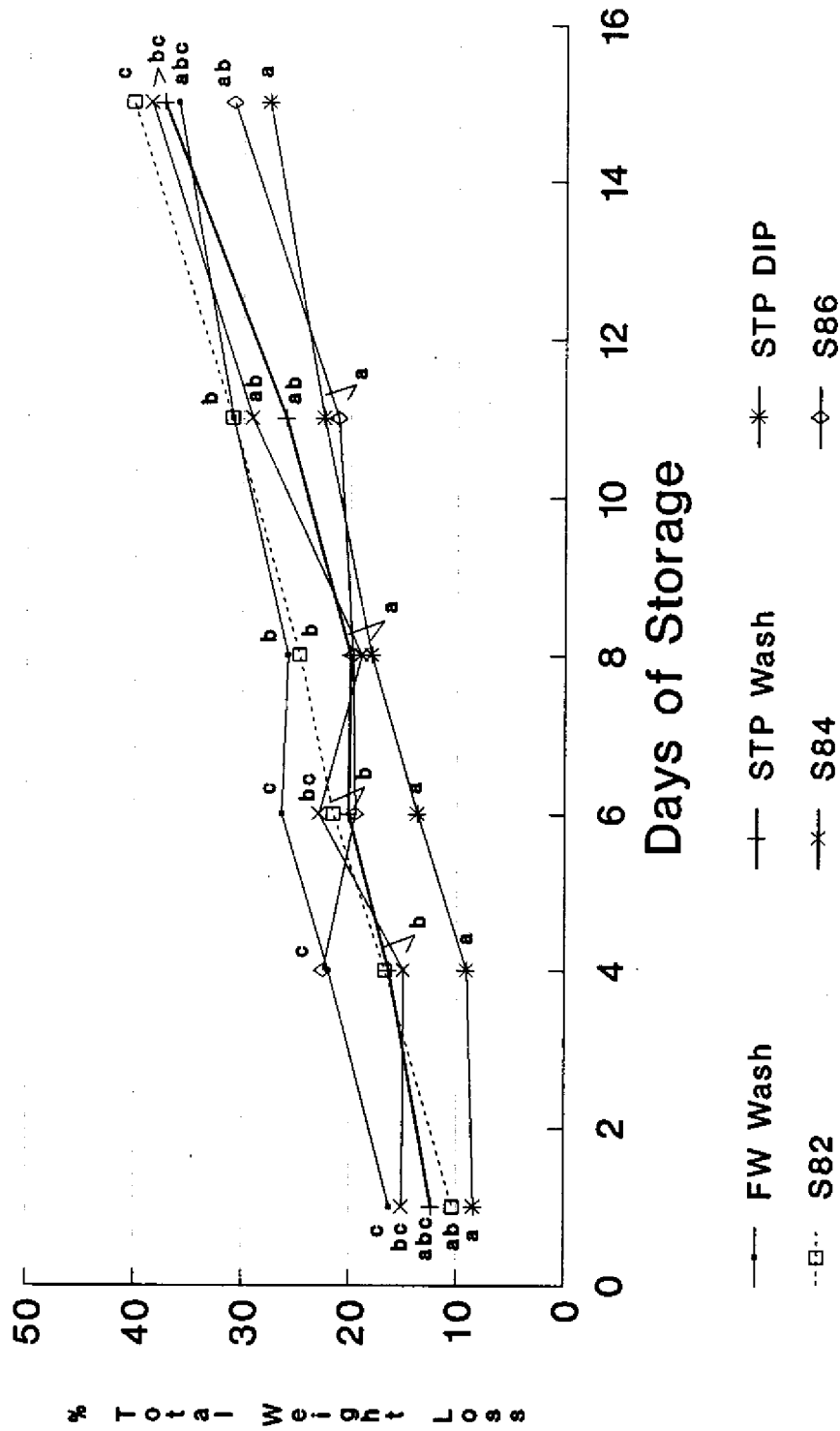
Means each day designated by diff. letters are signif. different, $p < 0.05$

Figure 5. Scallop Cook Losses During Iced Storage



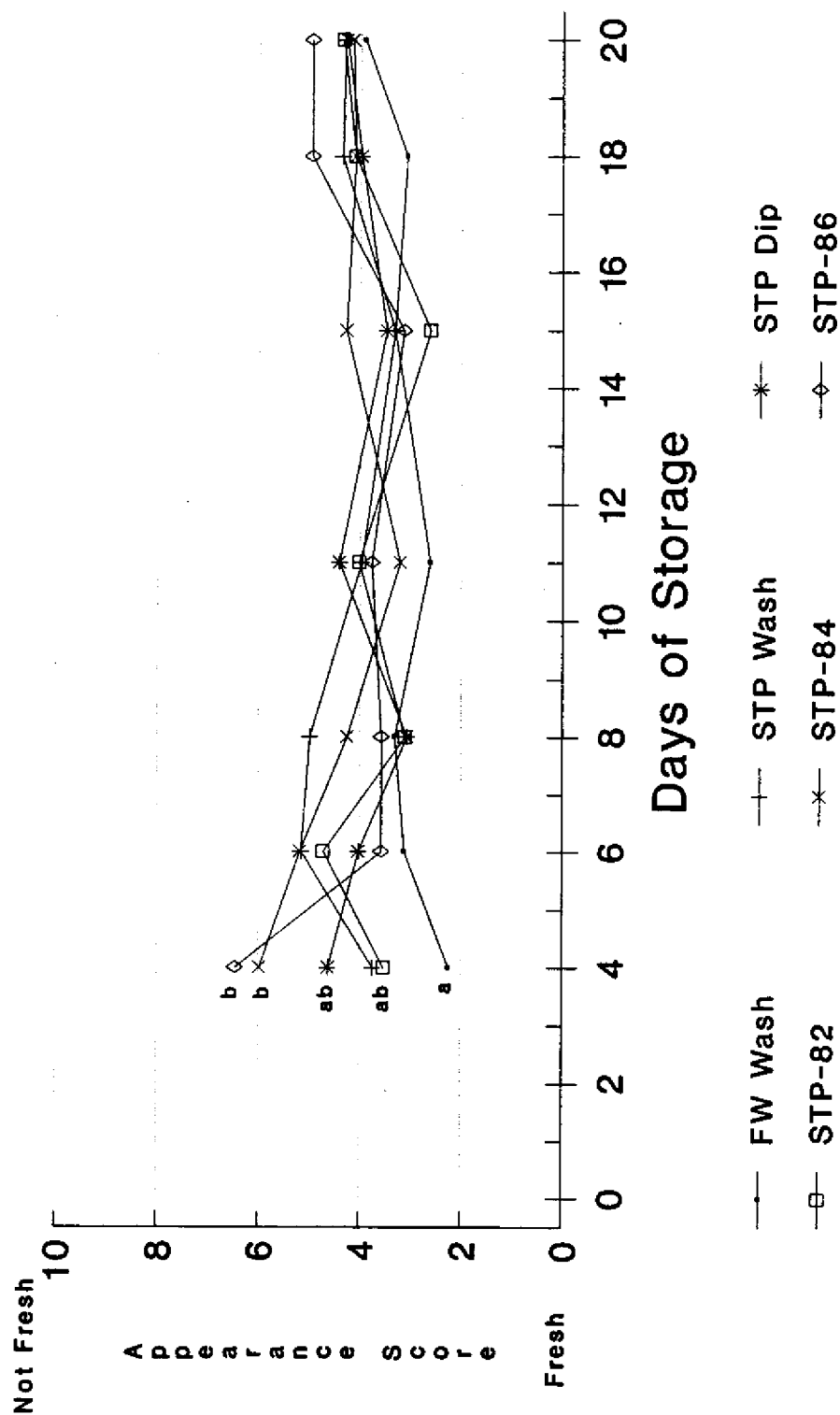
Means on each day designated by diff. letters are signif. different, $p < 0.05$

Figure 6. Combined Scallop Drip and Cook Losses



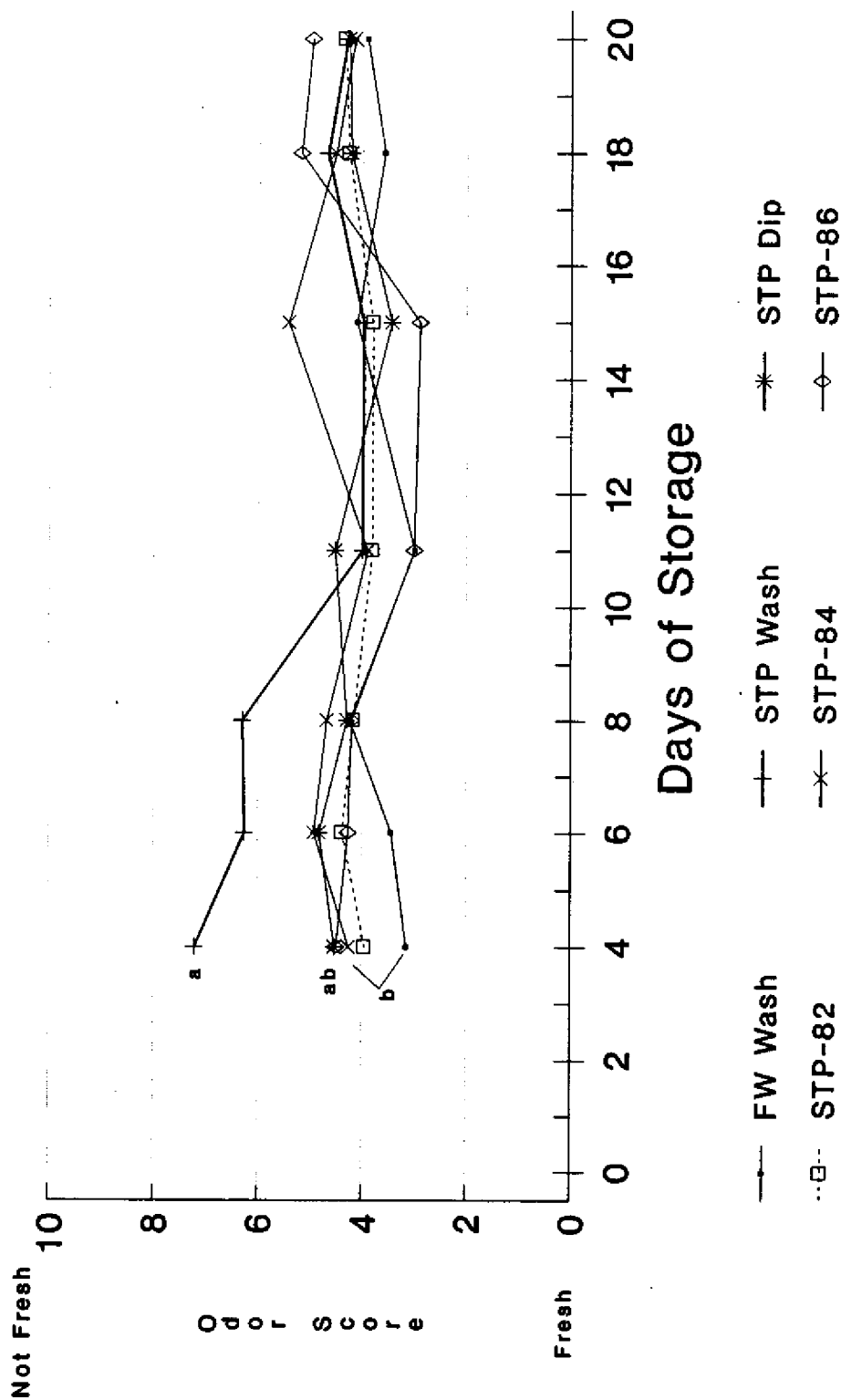
Means each day designated by diff. letters are signif. different, $p < .05$.

Figure 7. Raw Scallop Appearance Score During Iced Storage.



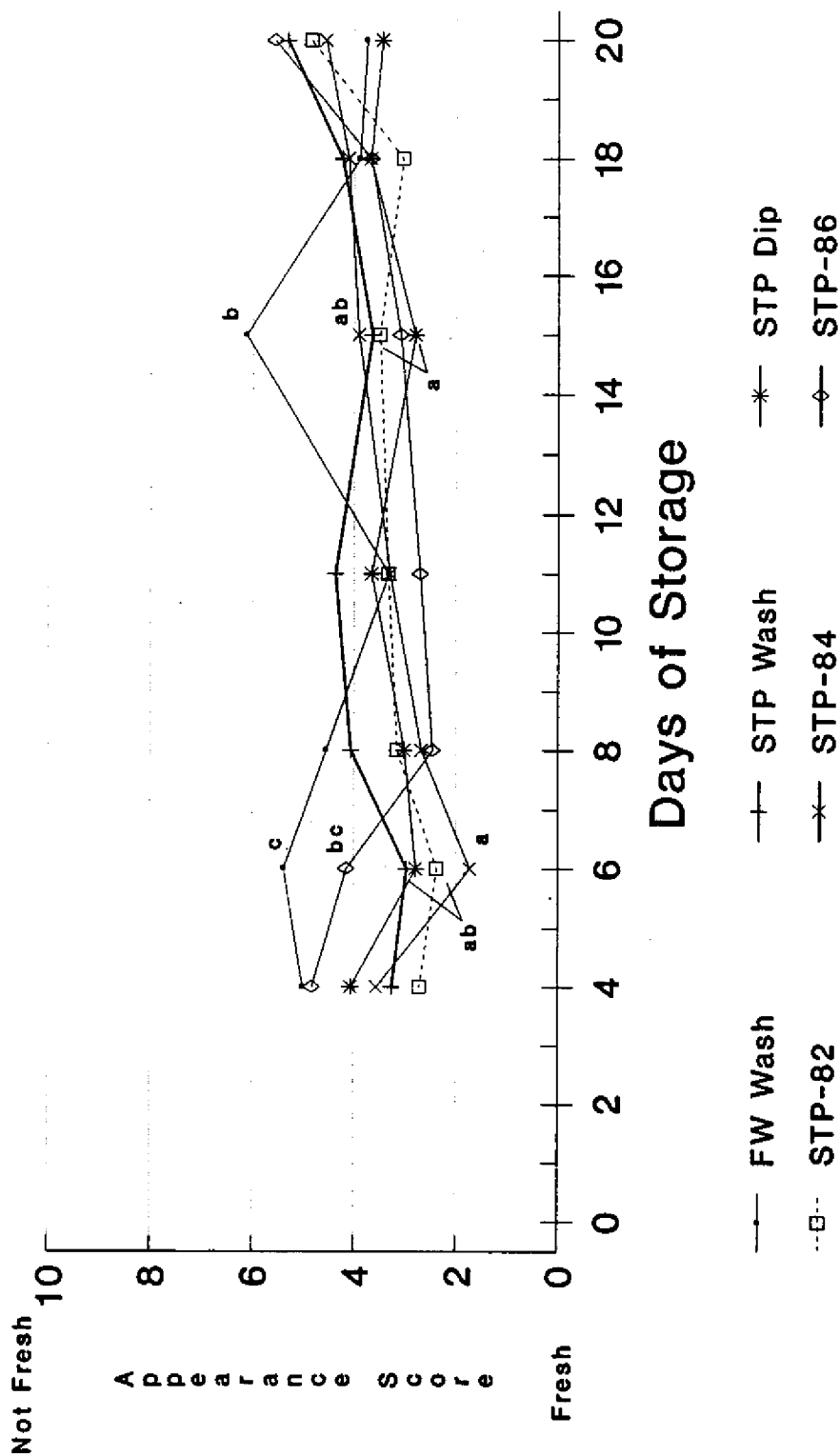
Means on each day designated by diff. letters are signif. different, $p < .05$.

Figure 8. Raw Scallop Odor Scores During Iced Storage



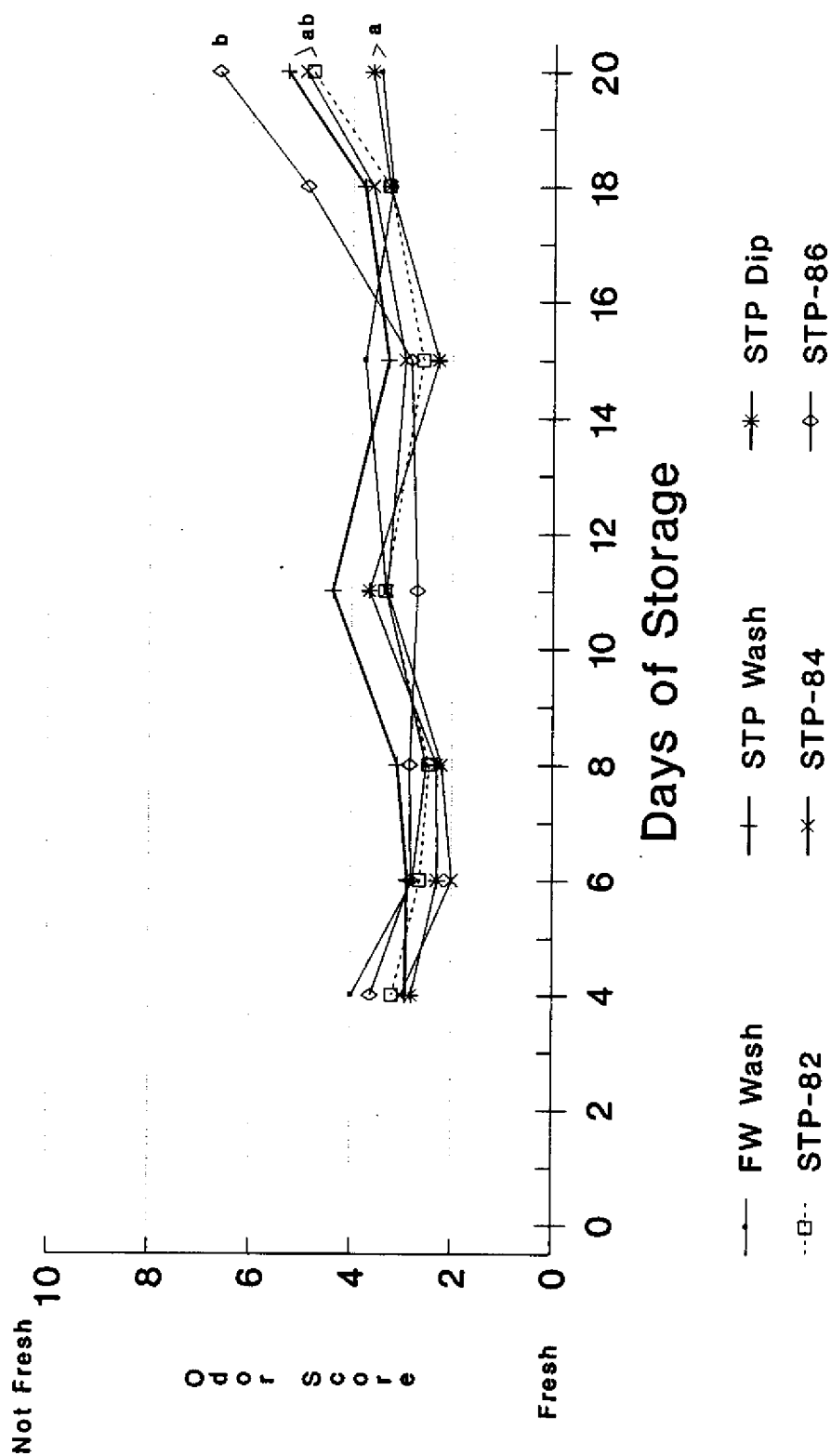
Means each day designated by diff. letters are signif. different, $p < .05$

Figure 9. Cooked Scallop Appearance Scores During Iced Storage



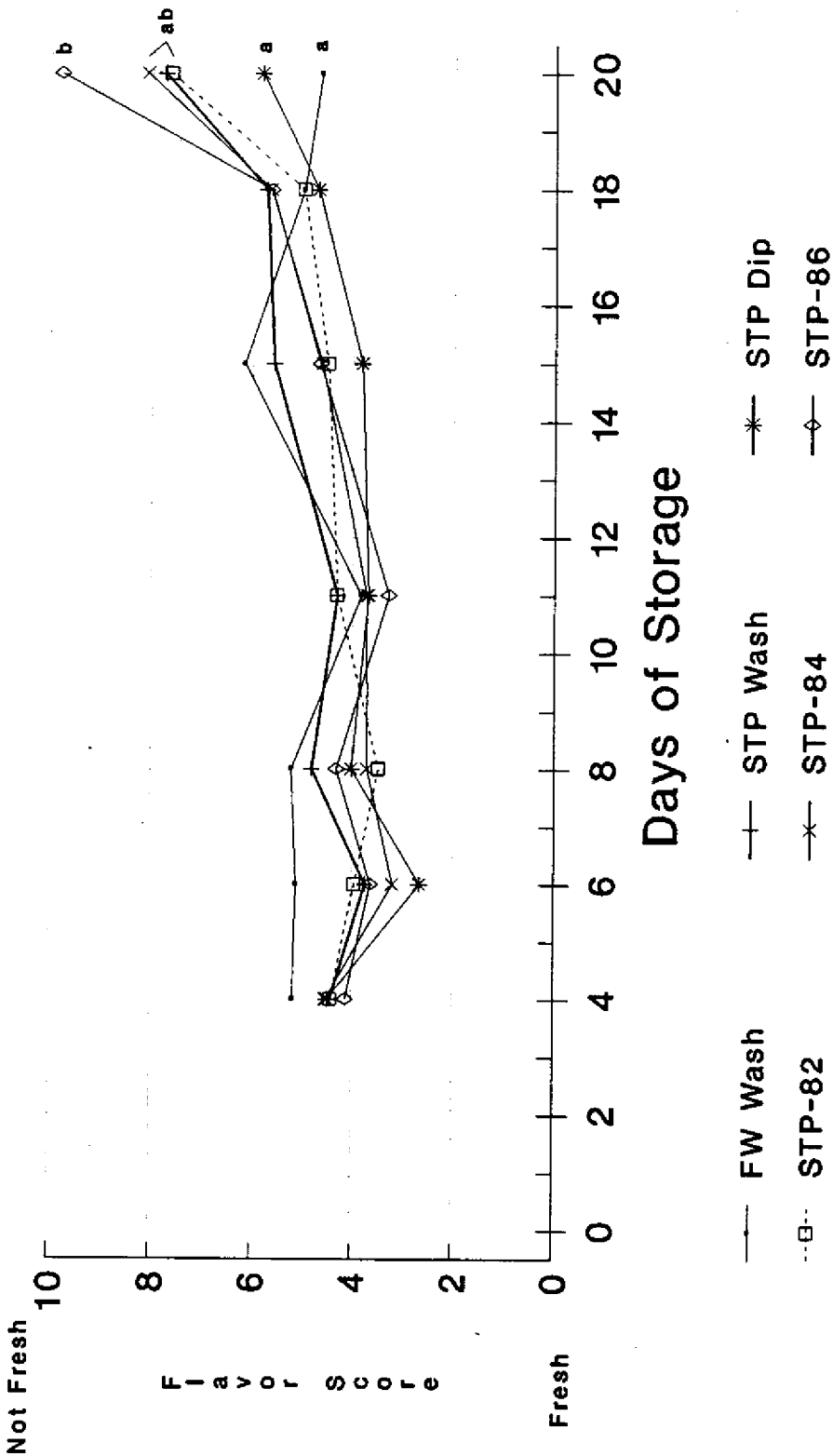
Means each day designated by diff. letters are signif. different, $p < 0.05$

Figure 10. Cooked Scallop Odor Scores
During Iced Storage



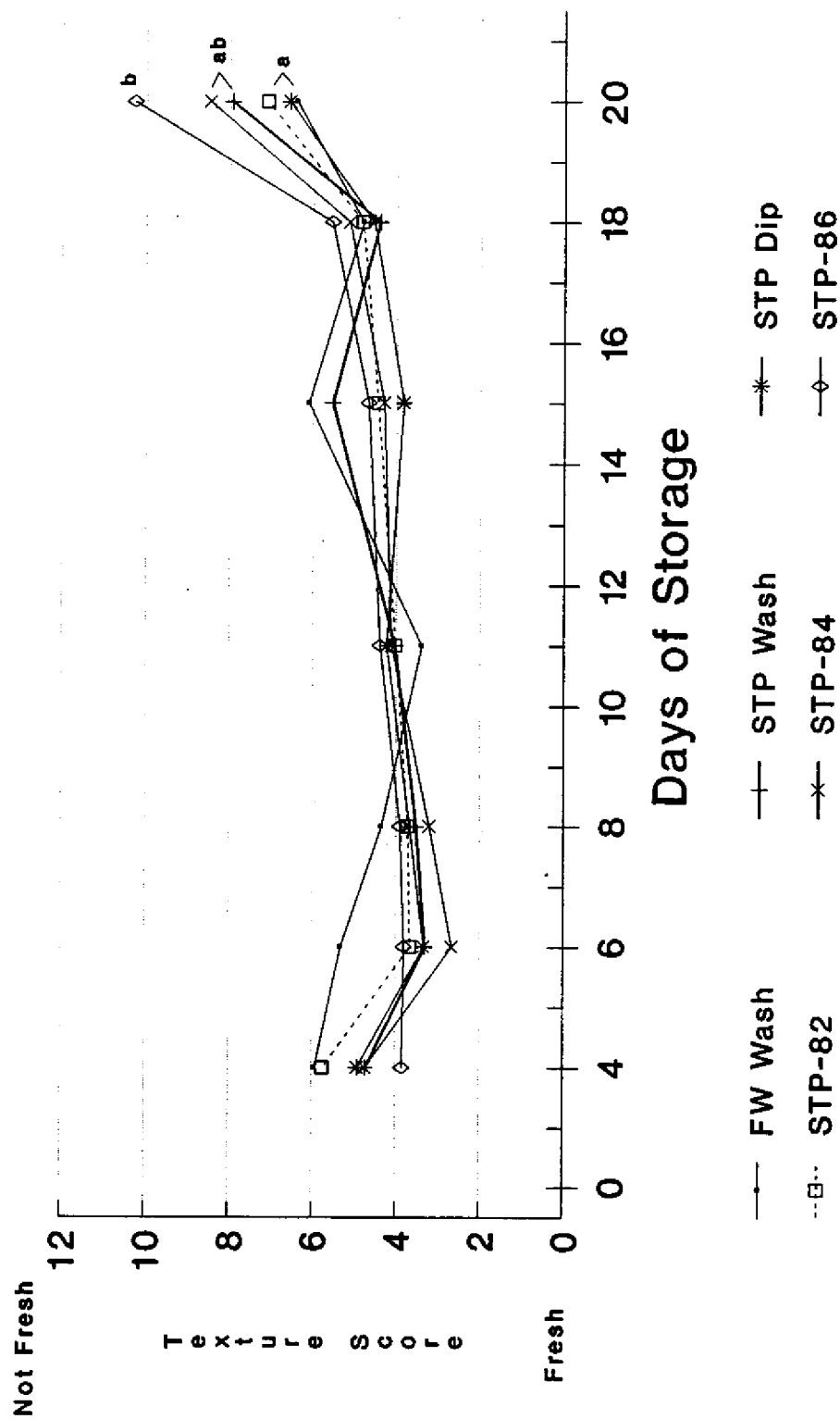
Means each day designated by diff.
letters are signif. different, $p < .05$

Figure 11. Cooked Scallop Flavor Scores During Iced Storage



Means each day designated by diff. letters are signif. different, $p < .05$

Figure 12. Cooked Scallop Texture Scores
During Iced Storage



Means each day designated by diff.
letters are signif. different, $p < .05$

DIP, S86, S82 and STP on day 15, Figure 9. These results are a partial reversal from raw appearance scores. When raw, the appearance of FW scallops is relatively indicative of fresh scallops but, when cooked, they may appear less fresh than phosphate-treated scallops.

Cooked odor, flavor and texture scores were similar over time, with no significant differences until day 20 when FW and DIP scallops rated odor, flavor and texture scores that were significantly more fresh than S86 scallops. The S82 cooked texture was also significantly more fresh than S86 scallop texture on day 20.

In a previous study, scallop sensory quality was maintained longer during iced storage by use of sodium tripolyphosphate (T. Rippen, 1990, presented at 50th annual meeting of the Institute of Food Technologists, Anaheim). In that study, higher APC's were encountered and phosphate extended shelflife up to four days. Lyon and Magee (6) determined that poultry meat held in three percent polyphosphate overnight produced products that were judged to be lighter in color, more tender and less off-flavored than products presoaked in water or two percent sodium chloride only.

Long shelflife is a known characteristic of properly handled sea scallops. As previously noted, APC's did not increase appreciably during iced storage. However, during preparation of sensory panel sessions, researchers observed that scallop odor intensity and, to a lesser extent, flavor intensity diminished rapidly after containers were opened and the scallops prepared. This also is a recognized characteristic of scallops which should be considered when interpreting sensory data. The effect may account, in part, for the very long apparent shelflife of scallops used in this study.

CONCLUSION

The 80 percent moisture content interim regulatory target may not be routinely achievable in properly handled scallops since water contact occurs during chilling, iced storage and washing procedures which are frequently necessary steps. The lowest initial mean moisture value determined in this study was 81.0 percent for the STP wash treatment (STP). The 10 percent STP dip treatment (DIP) generally produced the least drip and cook losses and lowest aerobic plate counts during iced storage compared to freshwater washed scallops (FW).

Sensory shelflife was long for all treatments. Few significant sensory differences were identified among treatments during most of the storage period. The appearance of raw FW scallops was initially perceived to be more like fresh scallops than were the high moisture phosphate-treated scallops (S84 and S86), and the appearance of cooked FW scallops was perceived to be less like fresh scallops than were phosphate-treated scallops, except for the highest moisture phosphate treatment (S86), which was not significantly different.

From this study, the use of sodium tripolyphosphate on fresh scallops appears to have most value in controlling drip losses, cook losses and aerobic plate counts. No additional benefit was apparent by very long exposure to solutions of sodium tripolyphosphate. Shorter exposures to somewhat greater concentrations of STP produced the desired functional effects, generally without exceeding 83 percent moisture.

Appendix. Example Sensory scoresheet used by panel.

NAME:

DATE: September 23, 1992

COOKED SCALLOP EVALUATION

INSTRUCTIONS: Please evaluate the scallop samples with a vertical mark on the horizontal scale based on the characteristic indicated in each block

SAMPLE #:

EVALUATION CHARACTERISTIC:		APPEARANCE
714		
328		
620		
599		
098		
572		
Fresh		Not Fresh

EVALUATION CHARACTERISTIC:		ODOR
714		
328		
620		
599		
098		
572		
Fresh		Not Fresh

EVALUATION CHARACTERISTIC:		FLAVOR
714		
328		
620		
599		
098		
572		
Fresh		Not Fresh

EVALUATION CHARACTERISTIC:		TEXTURE
714		
328		
620		
599		
098		
572		
Fresh		Not Fresh

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THE APPLICATION OF PHOSPHATES IN THE PROCESSING OF PACIFIC SHRIMP,
OR WHAT'S SO DIFFERENT ABOUT THIS USE?

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Simi Valley, California

On October 21, 1992, the Seattle office of the U.S. Food and Drug Administration issued an industry advisory that directed that Pacific shrimp processors would have to begin to label the addition of phosphate to their product. This directive changed a practice that had existed for over ten years, since these processors first used phosphates on their shrimp. The processors, with the guidance of various professionals, both academic and otherwise, believed that this application was classed as a "processing aid".

As most of us know, significant quantities of phosphates are used for shrimp processing. Processors on the East and Gulf Coasts use condensed phosphates to retain moisture in the shrimp. If they did not, there would be a significant loss of moisture (and protein) in the freezer, during thawing, and during heat processing. This would create economic losses and customer dissatisfaction. The direct addition of a phosphate solution by processors in the Southeast adds a specific amount of phosphate to the product, and Food and Drug Administration regulations require labelling that declares the presence of phosphate. This is a proper application of the law.

However, with Pacific shrimp (Pandalus jordani), there is a different reason for phosphate use, and I am here to explain that difference to you. As stated, Pacific Coast shrimp processors, along with numerous knowledgeable people including the authors noted above, believe that their application of phosphates is covered by 21 CFR Ch. 1, Part 101.100, Section ii, paragraphs (b) and (c), part of what is commonly called the "Processing aid regulation". My presentation today will address the reasons that the previously-named authors, and many others, firmly believe that this use of phosphates is definitely a processing aid, rather than a food ingredient.

First, let us look at two of the paragraphs in the processing aid regulation. Paragraph (b) states that processing aids are "Substances that are added to a food during processing, are converted into constituents normally present in the food, and do not significantly increase the amount of the constituents naturally present in the food." Paragraph (c) says that processing aids may also be "Substances that are added to a food for their technical or functional effect in the processing but are

present in the finished food at insignificant levels and do not have any technical or functional effect in that food." Later in this presentation, we will refer back to these two paragraphs. Now, let us look at the processing of Pandalus jordani. Much research has been done on this species of shrimp at the Oregon State University Seafoods Laboratory at Astoria, Oregon.

As many of you already know, this shrimp is much smaller than that which is typically processed by Eastern and Gulf processors, or by those who import green headless shrimp from overseas. Counts may run anywhere from 100 to 350 per pound, in the shell, although harvesting and processing of the smaller sizes, perhaps smaller than 170 per pound, is discouraged, and sometimes even prohibited. This shrimp may be landed at ports from Eureka, California to Westport, Washington, and sometimes even in British Columbia.

Immediately after landing, the shrimp does not process well. Processors determined long ago that allowing P. jordani to age, in ice, for three or four days after catch would improve the ability of the mechanical peelers to remove the shell from the meat. This allows enzymatic action to degrade the connective tissue (collagens) that occur between the musculature and the shell. This action, however, solubilizes some of these tissues, meaning that upon separation, the solubles will then be lost during the intense washing that occurs in a shrimp peeler.

Let's describe the peeling process. Shrimp are delivered to the peeler area in tote bins, with ice still usually present. They are then dumped into the feed hopper on the peeler, where they may remain for several minutes. They then move out of the hopper area on an inclined mesh belt, in a nearly single-thickness layer. The shrimp pass through the cooking area, where they are steamed at 100 degrees centigrade for a period of about 90 to 110 seconds.

After cooking, they immediately fall onto a number of counter-rotating, oscillating rollers that slope down and away from the cooking area. Streams of water under pressure are focused upon these rollers. The loosened shell, along with legs, antennae, and viscera are pinched by the rollers and discharged below. The shrimp flesh, being somewhat soft, slippery and flexible, travels down the rollers and falls into a flume. The shrimp then are flumed into a separator, where any last pieces of shell are removed, then onto an inspection belt prior to freezing.

The industry estimates that up to four gallons of water per pound of shrimp are used after phosphate treatment and cooking, and during the mechanical part of the peeling process.

In the late 1970's Dr. David Crawford, with Dr. Jerry Babbitt, at the Oregon State University Seafoods Laboratory, began to study ways to retain the soluble and insoluble protein lost in the peeling process. The idea was to promote gelatinization of the soluble proteins during cooking. It was noted that considerable amounts of connective tissue were retained in the peeled shell. If the soluble proteins could be gelatinized, this would allow much of the remaining tissue to be pulled from the shell when peeled. Condensed phosphates were selected as the means of performing this action (1).

Initial studies were done with a commercial mixture of mostly sodium tripolyphosphate with some sodium hexa-meta phosphate, called Brifisol[®] D-510, from BK Ladenburg Corporation. Work

began in the lab, and then progressed to plants where phosphate solutions were applied to the shrimp in the peeler's feed hopper. Results were impressive. It required a couple of years for acceptance, but the industry eventually turned 100% to processing with phosphate.

Concurrent further research by the Seafoods Lab found that a solution of up to 6% phosphate could be used, and that very cold treatment temperatures were necessary for best results. The Laitram PC-A peelers at first allowed the hot condensate from the cooking area to flow back into the feed hopper where phosphates were being applied. It was not long until all of the processors had made modifications to the peelers to reduce temperatures in the feed hopper to as close to 0 degrees Centigrade as possible.

A typical operation utilized a recirculating system with a mixing and holding tank for the phosphate solution, a means of refrigeration to maintain cold temperatures, and screens to keep larger particles of shrimp residue out of the system. After these changes were in place, it was not uncommon for processors to increase recovery of meat by as much as ten percentage points, i.e., from perhaps 21% without phosphate to 31% with phosphate. At the price of peeled shrimp, this was a significant savings.

One other change occurred. Due to the softness of the water on the Oregon Coast, and due to the fact that it is very difficult in plant operations to perform precise recovery analysis due to the variability of Pacific shrimp from tote-to-tote, hour-to-hour, week-to-week or whatever time frame is chosen, most processors changed over to straight sodium tripolyphosphate for the cost savings. It was very difficult in this case to achieve truly accurate recovery information that would have shown the difference that could have been achieved with a more sophisticated phosphate, as can be done in a hard water area or with a different product being treated.

At this time, it should be emphasized that phosphate is applied to the shrimp only before cooking, peeling and washing. Contrary to what some believe, there is no phosphate whatsoever applied afterwards, as will be noted later.

Now, let us address the determination that this is a "processing aid". There is a considerable amount of research that justifies this, in the opinion of the referenced authors and myself, and here is some of this information:

In a 1980 paper (1), Dr. Crawford reported that he had determined that phosphorus content of treated shrimp (as P_2O_5) can vary from 537 to 727 mg/100g wet weight, a variation of 190 mg. He also found that pretreatment solutions as high as 6% produced added phosphorus levels of less than 110 mg/100g wet weight over control samples. His conclusion was that the quantity of phosphorus added to cooked shrimp meat...is somewhat less than the range of phosphorus levels naturally occurring in shrimp.

In 1981, Tenhet and others used radiolabelled phosphorus on both fresh and previously frozen raw, peeled shrimp. STP concentration had to be 5% to 10% with a contact time of at least 20 minutes to achieve uniform distribution throughout the muscle. Note that we are discussing the use of phosphate on unpeeled Pacific shrimp, and for a contact time of approximately 5 to 10 minutes,

using 6%—usually less—concentration. It is very likely that phosphate treatment is acting only on the surface of the flesh in our case with Pacific shrimp.

An incorrect idea was recently presented that Pacific shrimp were being treated with phosphate after cooking, causing additional water binding. First, you should know that sodium tripolyphosphate is unstable at elevated temperatures such as in steam cooking. It is quickly and efficiently hydrolyzed to the orthophosphate form, a form naturally occurring in muscle. This molecular form is not active in protein modification, water binding, or sequestration.

A note here, for those who use phosphates, especially those that are more difficult to dissolve, one should never dissolve phosphates in hot water. In fact, you should not even dissolve them in warm water. A good instantized phosphate can be dissolved in cold water.

To point out this hydrolysis effect with scientific studies, Sutton, in 1973 (3), showed that tripolyphosphate was rapidly hydrolyzed to pyrophosphate, then to orthophosphate in cod muscle due to the action of alkaline phosphatases at both 0C and 25C. In 1981, Tenhet and others (4) showed that after two weeks frozen storage, only 12% of total phosphorus in uncooked shrimp muscle corresponded to the originally added STP. By ten weeks, of frozen storage, phosphorus level corresponded to 45% orthophosphate. No heat was applied to facilitate the hydrolysis of the condensed phosphate. It should be very apparent that there is no action whatsoever by the phosphate after cooking, and it is a known fact that there is no phosphate application to the shrimp after cooking. Such application would be a total waste of phosphate and an economic loss.

Another concern has been expressed for the amount of residual phosphate that might remain in cooked shrimp, and the activity of this phosphate. In 1980 Crawford reported (1) that there was an upper limit of 0.258% P_2O_5 uptake in processed shrimp, and as noted above, only 12% of the total phosphorous corresponds to added STP, so it is reasonable to conclude that the amount remaining is insignificant. STP averages 58% P_2O_5 , therefore there would be only 0.018% remaining. This is a level too low to provide any residual humectant or sequestrant effects, even if this was an active form of phosphate.

There have also been concerns expressed that the use of condensed phosphates prior to cooking and peeling results in increased muscle hydration, or a "humectant" effect. Data from Oregon State University shows that there is no significant difference in moisture content between treated and untreated Pacific shrimp. In 1981, Chu (5) compared treated and untreated shrimp, processed at 3, 4, and 7 days post-catch, held in ice before processing. Untreated, the moisture contents were 79.30, 80.39, and 82.04% respectively. Phosphate treated shrimp, however, showed moisture contents of 80.20, 80.88, and 81.30% respectively; not significantly different. It would appear from this data that the treatment resulted in a more consistent final moisture content. It is therefore very apparent that there is no increased muscle hydration nor is there a humectant effect.

In 1979, Nouchpramool (6) showed that the older the Pacific shrimp (time post-harvest and prior to processing), the greater the moisture content, whether phosphate was used or not. With the use of a 6% solution of condensed phosphate on 2-day old shrimp, moisture content increased only 0.30 to 0.58%, with a mean of 0.44%. Two day old untreated shrimp moisture content varied by

1.84%. Any increase in moisture after treatment with condensed phosphate would be within natural variation. Additional work by Nouchpramool showed that use of phosphate increases the meat yield and not the percentage moisture content.

Also, in 1984, Regenstein (7) evaluated the effect of different phosphates on the water binding potential of seafood muscle. At concentrations of up to 6mM, or about 0.6% orthophosphate, there was no net increase in the muscle's water binding potential. Once again, there is no increased muscle hydration nor is there a humectant effect with the presence of orthophosphates. Our conclusion here can only be that, rather than promote water uptake, the use of phosphates results in a more consistent final moisture content and more predictable process yield. Therefore, there is no significant difference in moisture content between treated and untreated cooked Pacific shrimp.

Again, concerning sequestration of ions, sodium tripolyphosphate is considered to be only a very weak sequestrant. Orthophosphate is not a sequestrant and contributes very little, if at all, to water binding. Therefore, it is again very apparent that there is no increased muscle hydration, nor is there any humectant effect. It is also axiomatic that the addition of water to cooked muscle proteins cannot be affected by polyphosphates!

From all of this, we may conclude that phosphates are used in the mechanical peeling of Pacific shrimp to: (i) Effect dissolution of the connective tissue between the muscle tissue and the shell, and, (ii) To facilitate surface protein gelation and increase peeling recovery. This results in efficient separation of the flesh from the shell and has no further effect after deshelling. Neither moisture content nor phosphate content is significantly increased. This application therefore meets the requirements of the F.D.A. "Processing Aid Regulations".

Now, let us look once more at these regulations. I will again show you the two applicable paragraphs, (b) and (c), and embellish them with my comments: Paragraph (b) states that processing aids are: "Substances that are added to a food during processing, are converted into constituents normally present in the food, and do not significantly increase the amount of the constituents naturally present in the food". Please note that I have underlined the word "significantly". It is quite obvious, that if you consider the data that the authors and I have presented, and especially in light of the use of the underlined word, this paragraph has been satisfied.

Furthermore, paragraph (c) says that processing aids may also be "Substances that are added to a food for their technical or functional effect in the processing but are present in the finished food at insignificant levels and do not have any (further) technical or functional effect in that food." Here, we again find that significance is to be considered. I have also added the word "further" that may not be in the regulation, but can be assumed to exist there. Obviously, if a processing aid had no technical effect whatsoever in the food at any time, there would be no reason to use it at all! Common sense tells us that the concern here is for any further effect in the finished product. Therefore, it is also very apparent that paragraph (c) has also been satisfied.

One other note can be added to the above research work: the OSU Seafood Laboratory has just completed another test of treated and untreated shrimp, and I understand that the results confirm all of the previous information. Dr. Morrissey can provide you with more details on this. The final

conclusion is that this application of phosphates can definitely be considered to be a processing aid, and does not require listing in the product's ingredient statement.

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USE OF PHOSPHATES WITH PENAEID SHRIMP

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Water is the largest portion, in both volume and weight, in all edible shrimp products. As the main food component, it has a dominant influence on the sensory attributes, shelf-life and value of the shrimp. Mindful of these concerns, commercial practices have evolved to control, add and retain moisture during shrimp harvest, processing, distribution, storage and preparation. How well these current procedures reflect 'good manufacturing practices,' denoted as federal GMP's (21 CFR Part 110) is in question. Excessive water addition could be deemed adulteration resulting in economic fraud for the buyer, while limited water and water loss can compromise the quality, shelf-life and consumer acceptance of the shrimp products.

COMMERCIAL SITUATION

The current growing concerns for moisture levels in shrimp (Food Chem News, 30 Nov 92) coincides with the most significant period of transition for the nation's penaeid shrimp industry. The domestic fisheries have reached steady-state production in terms of available wild resources and they must compete in a market with an increasing amount of cultured penaeid species from international sources. Since 1980 the total annual U.S. commercial shrimp landings have averaged about 200 million pounds whereas the annual imported poundage has increased from 300 million to over 650 million pounds (Source: Heads-off weight equivalents in USDC/NMFS Fisheries of the U.S., 1991). Domestic processors and food service establishments have grown dependent of foreign aquacultured production. Cultured sources are evolving into foreign processing competition with finished and value-added products. As noted for many domestic industries, competitiveness with an international commodity will require more attention to product quality and good manufacturing practices. Likewise, the new emphasis on seafood safety and nutrition must be incorporated in planning for future shrimp commerce in the U.S. The composition of the shrimp products, particularly moisture content, warrant further consideration relative to GMP's from harvest to consumption.

Use of Phosphates

Use of phosphate agents to influence the moisture content in shrimp was a technology borrowed from the red meat and poultry industries. For penaeid shrimp, the initial intent was to help reduce moisture or drip loss during frozen storage and thawing. This intent, although commonly communicated for well over 25 years, was never formally declared or incorporated by a firm, trade association or regulation. U.S. FDA compliance policy guides (i.e., no. 7303.842), product seizures and letters have referred to sodium tripolyphosphate (STP) to control "drip loss", yet there is no formal regulatory position stated for the use of phosphates with penaeid shrimp. From a commercial perspective, controlling drip loss is a function that is considered necessary for raw or cooked, shell-on, peeled or breaded shrimp. In time additional product benefits were noted through

consumer expectations and retention of cook yield. Similarly, Crawford (1980) introduced phosphate applications for cold water shrimp, *Pandulus jordani*. The work focused on phosphates as processing aids to assist peeling and retain protein. His intent reflected distinct and unique processing conditions and product characteristics for a different shrimp genus. Possibly the intended use for phosphate applications on shrimp would require declared functionality and possible distinction by differing processes and various shrimp genera.

Through commercial practice, shrimp firms have learned of the consequences in product quality and yield due to changes and movements in moisture content. This experience is not well documented in peer reviewed literature or regulatory documents. Noting the simplicity of the moisture analysis, it is surprising that there are not previous studies to document the consequences of handling on the water content in penaeid shrimp production, processing and storage. Most previous studies and reviews have focused on biochemical, microbial and sensory consequences (Green, 1949; Campbell and Williams, 1952; Carroll et al, 1968; Pedraja, 1970; Flick and Lovell, 1972; Cobb et al, 1973; Cobb et al, 1977; Bottino et al, 1979; Chung and Lain, 1979; McCoid et al, 1984; Chamberlain and Lawrence, 1983; Chang et al, 1983; Krzynowek, 1988; and Chen et al, 1990). Limited work has noted the loss of water holding capacity and hydration capacity during temporary refrigeration for shrimp (Shelef and Jay, 1971 and Chen et al, 1990). Patents have introduced the use of phosphates to reduce moisture losses during processes, freezing and cooking of shrimp (Stone, 1981 and Shimp et al, 1983). These former reports are limited to the segments of processing studied and are dated by recent changes in processing.

Handling procedures for many domestic products have changed. There is a growing dependence on cultured penaeid species and some initial processing in international settings. For example, much of the breaded products rely on peeling (shell removal) by separate firms, domestic and international, designed to afford this operation. Multi-firm handling requires additional bulk storage and reprocessing. Traditional 'packing houses' for fresh and frozen shrimp and some domestic breeding operations using more regional wild harvest expect different consequences in product moisture. Their products are still considered 'penaeid shrimp' in U.S. commerce with all other shrimp sources and forms.

Previous work by Gates et al (1985), Williams et al (1981) and Rao et al, (1975) demonstrated moisture migration in breaded shrimp during frozen retail storage. Inability to retain moisture in the edible muscle during the retail case thaw cycles resulted in breaching weight in excess of the allowed 'standards of identity'. Eventual decisions concerning the standards for the U.S. breaded shrimp industry will require additional information on product moisture consequences vs. GMP's. The economic implications of any decisions may represent the most immediate and significant impact on the nation's most valued and established sector of seafood processing.

Moisture Content

The reported moisture contents for penaeid shrimp have varied from 71.8% to 87.0% (Table 1). This range results from natural variations by species, season, product size, molting stage and method of production (wild vs. cultured); differences in handling, processing and product forms; and

Table 1. Reported moisture content in raw penaeid shrimp (chronological order).

Sample Description and Location	Moisture Content (%)	Reference
brown shrimp	76.8	Thompson (1964)
white shrimp	78.2	
1 sample/species Pascagoula, MS		
brown shrimp, Pascagoula, MS 6 bimonthly samples pooled over one year	70.6-78.2	Thompson, et al (1971)
brown shrimp	74.46	Krishnamoorthy, et al (1979)
white shrimp	71.78	
pink shrimp	72.51	
Approx. 11 individual shrimp avg./species Ocean Springs, MS		
<u>P. aztecus, styliiferus, monodon, japonicus,</u> <u>duorarum, indicus, schmitti and setiferus</u>	75.6-81.4 depending on species	Sidwell (1981)
white shrimp, two 5 lbs. boxes TX & MS, winter	82.6	Dudek, et al (1982)
Penaeid and Pandalus	75.9	USDA Handbook No. 8 (1987)
brown shrimp, 5 lbs. retail Louisiana	81.63	Krzynowek and Murphy (1987)
Texas	84.28	
pink shrimp, 5 lbs. retail Florida	82.97	Krzynowek and Murphy (1987)
Honduras	80.10	
white shrimp N. Carolina	84.12	Krzynowek and Murphy (1987)
Georgia	82.03	
Texas	81.90	
<u>P. subtilis</u> , Brazil	80.48	Krzynowek and Murphy (1987)
<u>P. vannamei</u> , Ecuador	79.64	Krzynowek and Murphy (1987)
<u>P. setiferous, notalis, vannamei,</u> <u>aztecus, duorarum and subtilis</u>	80.0 - 84.0 depending on species	Krzynowek and Panunzio (1989)
<u>P. aztecus, indicus, occidentalis,</u> <u>notalis, vannamei, schmitti,</u> <u>setiferus, and duorarum</u> These analysis from 10-16 composite samples per species	78.2 - 82.3 depending on species	Garrido, et al (1992)
<u>Penaeid</u> sp.	78-87% unreported results from processing firms trained in the use of rapid moisture analysis	Personal Communications (1993)

analytical error due to different procedures, misidentification of samples, poor sampling schemes and assumptions. As the data becomes more current the reported moisture contents tend to increase (Table 1). Each report may have satisfied the question of concern for their respective study, but none of these previous studies compared the consequences of moisture relative to GMP's from production through processing and storage. Most studies simply reflect data per one species, most often misidentified or grouped as "penaeid sp.", sampled at one stage of handling. In most instances the product was frozen and held under variable conditions, temperatures and thawing procedures prior to moisture analysis. Simply stated, they are the best data we have to date for penaeid shrimp. Even the most recent data for eight penaeid shrimp species offers limited interpretation relative to GMP's (Garrido et al, 1992). These samples were all obtained prefrozen with 'faith' in the supplier confirmations that they were not processed with any water addition and they were less than 6 months in storage. Any additional history for product handling is unknown. Similarly, the most recent data being reported by current processing firms reflects a very broad range (78 to 87% moisture) that has not been verified in conjunction with current commercial practice.

Also, freezing and cooking can decrease the moisture content in shrimp so as to adversely affect consumer acceptance (Ahmed et al, 1972 and 1973; Webb et al, 1975 and Applewhite et al 1993). Studies have demonstrated that consumers prefer cooked shrimp with a higher moisture content (Webb et al, 1975 and Applewhite et al, 1993). When challenged with boiled pink shrimp (*Penaeus duorarum*) pre-treated with phosphates for water additions vs. untreated samples, a group of prescreened consumer panelists (n=125) significantly preferred all treated products over the untreated controls (Applewhite et al, 1992 and 1993). Their response rated a significantly higher perceived 'value' or 'fair market price' for the treated shrimp. This recent study demonstrates the addition of moisture to shrimp is not necessarily adulteration relative to consumer expectations. This study lacks a comparison of moisture content as a result of GMP's per current commercial practices and products. Cooking consequences per GMP's and related consumer expectations warrants further assessments.

REGULATORY SITUATION

Phosphates are multi-purpose GRAS substances ("generally recognized as safe"; 21 CFR 182.1810), when used in accordance with good manufacturing practices (GMP's). The GRAS status is based on previous commercial practice and these ingredients do not require a formal, food additive status. As 'GRAS' substances their use relative to methods for applications and amounts are based on the functional effects. There are no approved limits for the amount of phosphating agents that can be used in shrimp. A previous proposal to establish a "... 0.5% level ... as served ..." limit on sodium tripolysphosphates (STP) "... in [to be frozen] fishery products ..." was never approved (Federal Register 18 Dec 1979: 74845). This proposed regulation stated limits as established for phosphating agents used with poultry and red meats (9 CFR 381.147 and 9 CFR 318.7). These USDA regulations specifically state the purposes for use of phosphates are to "decrease the amount of cooked out juices" and "help protect flavor". These USDA regulations and current commercial practice attempts to comply with a guideline of 0.5% phosphates as added to the products. This guideline assumes that all of the premeasured phosphate treatment as added or pumped into the product is completely incorporated. A similar assumption is not directly applicable to shrimp and other seafood muscle systems.

Despite these distinctions for seafoods and related phosphate applications, international standards have adopted the 0.5% (5g/kg) guideline for phosphate use (Codex Alimentarius Commission 1976 and 1992). Likewise, the recent unified standards for European Union Council on Foods (1994) specify a 5g/kg maximum added phosphate level for frozen seafoods. These international guidelines do not offer methodology for distinguishing added from indigenous phosphate content.

The actual phosphate constituent for which the 0.5% limited refers to has confused interpretation and analysis. Researchers (Tenhet et al, 1981) and the Codex Alimentarius Commission (1976) imply the specified STP limit is for residual phosphates expressed as P_2O_5 . This constituent is a calculated entity based on the official analytical procedures for phosphorus (AOAC, 1990). A more direct interpretation for the total phosphorus level in untreated or non-phosphated penaeid shrimp (Table 2) nearly equals the proposed 0.5% (500mg/100g) limit when expressed as P_2O_5 . This statement is based on the fact that the phosphate content expressed as P_2O_5 mg/100g edible shrimp is equivalent to 2.286 times the corresponding phosphorus content. Realizing this situation the recent Codex Alimentarius Commission's revision of the International Standard for Quick-Frozen Shrimp and Prawns (1992) 'doubled' their previous 1976 recommended allowance for phosphates. Likewise, for the most probable interpretation that the FDA proposed 0.5% level was strictly for the complete STP compound, this structure is rapidly degraded in all treated seafoods and offers no guideline for assessing the amount of phosphates used (Tenhet et al, 1981; Sturno 1987; Sturno et al 1987; Krynowek and Panunzio, 1993 and Heitkemper et al, 1993).

To further confuse the issue there are no reports published that relate moisture content to the amount or type of phosphates used to treat shrimp. Phosphate suppliers have learned that phosphate blends can be more effective than the traditional use of tripolyphosphates alone. Blend pH (alkaline) is the effective feature in terms of moisture retention. The components in these blends (i.e., hexametaphosphates, pyrophosphate) have not been studied to determine the mode and degree of breakdown in treated products to the pyro- and orthophosphate (phosphorus) forms. This information is necessary to guide commercial and regulatory practice.

The total phosphorus (P) levels in penaeid shrimp offer a simple, direct measure by which to judge previous exposure to a phosphating agent (Table 2). Products with no previous history of phosphate exposure appear to have phosphorus contents less than 250 mg/100g. Higher phosphorus levels suggest previous treatments, but the amount of treatment would still require analysis for moisture content and visual inspections (i.e. glassy appearance, especially when cooked, soapy feel and detectable tastes). Unfortunately, these subjective indicators have not been aligned with phosphorus and moisture contents per phosphate treatments, and they would most likely differ by species.

Confusion for the use of phosphating agents in seafoods has led to the current commercial and regulatory concerns for added water. This issue was initiated by regulatory positions issued to limit the moisture content of sea scallops (FDA, 1991). Realizing attempts to monitor for phosphate residuals offer only evidence for the use rather than the amount used, FDA opted to focus on the primary adulterant of concern, water. Pressured for an interim policy, the FDA and commercial decisions agreed on moisture guidelines that are proving to be inconsistent with natural moisture levels in various scallops and do not reflect current GMP's. This situation has alienated the respective

Table 2. Reported phosphorus levels (mg/100g) in the edible portion of raw penaeid shrimp

Sample Description	¹ Phosphorus Content		References
	Range	Average	
<i>P. brasiliensis</i>		229	² Sidwell (1981)
<i>P. aztecus</i>	235-291	258	
<i>P. indicus</i>	210-397	295	
<i>P. styliferus</i>	169-170	170	
<i>P. monodon</i>	235-345	325	
<i>P. japonicus</i>		340	
<i>P. duorarum</i>		215	
<i>P. schmitti</i>		278	
<i>P. setiferus</i>	187-332	206	
<i>P. aztecus</i> Pascagoula, MS bimonthly samples for one year	207-258	235	Thompson and Farragut (1971)
<i>P. aztecus</i> 5 samples	179-332	249	Tenhet, et al (1981)
Penaeid and Pandalus		205	Exler (1987), USDA Handbook No. 8
<i>P. aztecus, indicus, occidentalis, notalis, vannamei, schmitti, setiferus, and duorarum</i> Three analysis from one 10 lb composite sample per species	149-247 depending on species	217	Garrido, et al (1992)

¹ The phosphate content expressed as P_2O_5 , mg/100g edible shrimp is equivalent to 2.286 times the corresponding phosphorus content. A 218mgP/100g shrimp equals 500mg P_2O_5 , mg/100g shrimp.

² Details for sampling, prior product handling, analytical procedures and proper species identification are lacking in this reference as well as the cited references

parties, complicated commerce, devalued some shipments and compromised consumer confidence (TSFT Symposium, 1993). A similar consequence is possible for penaeid shrimp unless a comprehensive study of moisture content is conducted prior to establishing meaningful and useful guidelines. The moisture content of untreated, raw penaeid shrimp has been reported to exceed the 80% moisture level established to distinguish untreated sea scallops, Placopecten magellanicus.

FDA must also consider the nutritional inferiority of phosphate treated products due to possible water 'dilution' of essential nutrients. As a matter of policy, FDA has adopted an 80% rule for purposes of determining nutrition equivalency (21 CFR 101.9, e, 4). This concern is linked to nutrition labeling and possible determination for imitation foods. It is not clear how nutritional equivalence would apply to raw shrimp when they are not fabricated from new technology. Since raw shrimp is not a 'new food' fabricated from new technology (38 Fed. Register 2138 - 1/19/73 and 20702 - 8/2/73) the primary concern would be total percent indigenous protein. This concern is linked to requirements for nutrition labeling. Data reflecting on the protein consequences due to phosphate treatments for penaeid shrimp are lacking, especially for cooked shrimp.

Another questionable regulation used to control the composition of shrimp products is the standard of identity for breaded shrimp (21 CFR 161.175 and 161.176). The first notice for development of this standard was initiated by the National Shrimp Breeders Association with the National Fisheries Institute in 1961 (Fed. Register 3/31/61, page 2723). These dated regulations differ from similar standards of identity for foods in that they do not declare or emphasize things or ingredients that may be used or included in the shrimp. For example, the standard of identity for canned shrimp (21 CFR 161.173) specifies optional ingredients that may be used with this product form. FDA responses to comments on the standard of identity for canned shrimp specifically stated sodium tripolyphosphate was not a suitable ingredient. In contrast, these breaded shrimp standards are concerned with breading, breading ingredients that are suitable, related product nomenclature and analytical methodology to determine percent breading. This FDA opinion and some industry interpretations have assumed these standards exclude the use of phosphating agents. FDA's opinion was specifically stated in their first draft of the Fish and Fishery Products Hazards and Controls Guide (FDA, 1994) which accompanied their proposed HACCP regulations (Fed. Register 59/19:4142, 1/28/94). These assumptions may lie in previous negotiations and understandings, but it is not evident in the language of the standard of identity.

'Shrimp' as defined in the standards of identity for breaded shrimp, 21 CFR 161.175 and 161.176, is the "tail portion of properly prepared shrimp of commercial species". 'Properly prepared' is not defined except for reference (sections c) to the optional product forms [cuts] and the breading and batter. Section 'd' infers the suitable ingredients for the batter and breading by listing non-suitable ingredients (i.e., artificial flavorings, artificial sweeteners, artificial colors and chemical preservatives). In the same section (d) chemical preservatives that are suitable are: 1) ascorbic acid and antioxidant preservatives. Listing ascorbic acid as a suitable ingredient to prevent 'blackspot' (melanosis) on the shrimp is completely inconsistent with commercial practice for penaeid shrimp about the world. Ascorbic acid, either as a breading ingredient or previous shrimp processing aid, does not provide effective prevention of melanosis on penaeid shrimp. As included on the suitable ingredients list in the standard of identity for canned shrimp (21 CFR 161.173), sodium bisulfite and related sulfiting agents are the primary GRAS ingredients used to retard shrimp melanosis. Sulfites are not included in the standards of identity for breaded shrimp despite their prolific use. Although the standards do

not specifically provide for the use of phosphates, they also do not provide for the use of sulfites. The text in section (d) indicates the listed ingredients all relate to the batter and breading. There is no mention of ingredients that may be used or are non-suitable for use with the 'properly processed' shrimp. There is no mention, inference or exclusion of phosphates either as ingredients in batter, breading or properly processed shrimp. According to current GMP's and GRAS status, phosphates have historically been used in processing of shrimp. Likewise, the non-suitable ingredients listed in section 'd' (i.e., artificial flavorings, artificial sweeteners, artificial colors and chemical preservatives) do not refer to phosphates. FDA's proposal rule on the use of phosphates (FR 44/244:74845, 12/8/79) defined phosphates as antioxidants, flavor enhancers, humectants, pH control agents, sequestrants, stabilizers, thickeners and texturizers. Phosphates are not defined or used as preservatives. Current 21 CFR 182, Subpart D, does not include phosphates in the list of chemical preservatives. Phosphates are not used as artificial flavors, sweeteners or colorants. Simply stated, the dated standard of identity for breaded shrimp does not appear to address the use of phosphates and is inconsistent with current domestic and worldwide practice with penaeid and breaded shrimp.

Mindful of potential product abuse or adulteration due to "excessive" moisture addition through the use of phosphates, FDA has recently attempted to discourage use of any substance "added to, or mixed with the product [seafood] to increase its bulk or weight or to reduce its quality, or make it appear of better or greater value than it is (i.e., through adding water to a product by chemical or other means)." This statement is part of FDA's initial seafood HACCP (hazard analysis and critical control point) inspection proposal (Fed. Register 59/19:4142, 1/28/94). Likewise, this concern was reemphasized in FDA's (1994) proposed HACCP regulations and accompanying Fish and Fishery Products Hazards and Controls Guide. Interestingly, this guide, which was not subject to external review with comments, includes the statement (p. 167) that "Sodium tripolyphosphate is not permitted in breaded shrimp or canned shrimp according to FDA's reference to the respective standards of identity (21 CFR 161.175, 161.176 and 161.173)." Although based on confused regulations, current and future regulatory concerns appear to be for the elimination of indiscriminate and possibly any use of phosphate agents with shrimp.

RECOMMENDATIONS

The continued use of phosphates to treat penaeid shrimp remains in question. Documented abuse has rightfully stirred regulatory and commercial attention. Proper use has not been appropriately defined in commercial practice or regulation. The responsibility to resolve this situation in the best interest of commerce and consumers will rely on industry action. Recommendations:

- 1) **Establish baseline data for the composition of raw penaeid shrimp in attempt to better define the moisture and protein content in raw penaeid shrimp.**

Published data offers a good start (Table 1), but most of the results can be suspect due to previous handling which may have included exposure to phosphates or other agents. Additional work with 'authentic samples' will be required to account for differences by species, harvest conditions, and post-harvest handling. Mindful of the current trends in shrimp production, authenticity of samples would require international liaison. A specific project to address this issue was rejected by the Saltonstall-Kennedy (NMFS) Grant funds in early 1994.

- 2) **Demonstrate the changes in moisture and protein content in penaeid shrimp as a consequence of frozen storage, processing and cooking.**

Published data is limited and often does not account for the influence of phosphating agents. Additional work beginning with authentic, untreated samples must follow through typical processing, storage and cooking regimes. The intent is to demonstrate the need for and influence of water controlling agents in terms of product composition, yield and quality.

- 3) **Declare an intended use and/or function for phosphates in treating penaeid shrimp.**

Based on the results from steps 1 and 2, industry must declare functions and related levels or guidelines for use of the phosphating agents. The functions should include concerns for; a) product protection from frozen storage and thaw drip loss, b) moisture, protein and other nutrient retention during cooking, c) reduced cook loss and product dehydration, and d) maintain product quality attributes (i.e. texture and mouthfeel). All of these functions must be demonstrated with actual product through customary processing and handling.

Likewise, this work should include a demonstration of consumer response to actual treated product. Properly designed consumer evaluation studies offer a real measure of quality consequences and value perception. Since FDA's mission is to protect consumer interest, studies are warranted to assess this interest.

- 4) **Declare appropriate guidelines (i.e. moisture and protein levels) to accompany the intended functions and applications.**

Industry should attempt to control their own situation through reasonable guidelines recommended for commercial standards and regulation. A raw product weight gain between 5 to 10 percent could be reasonable (Tables 3), but it must be justified by steps 1-3. The corresponding protein changes and influence of cooking must be determined. For example, the ham industry bases phosphate water additions based on a protein fat-free (PFF) measure (Table 4).

Table 4. Product labels for ham as determined by PFF standards.

<u>Ham - Cooked and Labeled</u>	<u>Minimum PFF</u>
Ham	20.5
Ham, w/Natural Juices	18.5
Ham, Water Added	17.0
Ham and Water Product, X% of Weight is Added Ingredients	Less than 17.0

Source: USDA, Food Safety Inspection Service, "Introduction to PFF Protein Fat-Free Cured Pork Products" Technical Publication Bulletin, Meat and Poultry Inspection Technical Services, Program Training Division, Washington, DC.

Table 3. Theoretical changes in the percent composition of raw penaeid shrimp due to weight gained (5 to 20%) through to the addition of water as could be influenced by phosphate treatments. Calculations are provided based on five products with initial moisture contents ranging from 77% to 81%.

Percent Weight Gain	Calculated Percent (%) Moisture (M) and Protein (P) Levels.									
	M	P	M	P	M	P	M	P	M	P
0% Initial	77.0	20.0	78.9	19.0	79.0	18.0	80.0	17.0	81.0	16.0
5%	78.1	19	79	18.1	80	17.1	80.9	16.2	81.9	15
10%	79.1	18.2	80	17.3	80.9	16.4	81.8	15.4	82.7	15
15%	80.0	17.4	81	16.5	81.7	15.6	82.6	14.8	83.5	14
20%	80.8	16.7	82	15.8	82.5	15	83.3	14.2	84.2	13

1. Calculations assume an initial 3% ash, fat and carbohydrate content and no loss of protein. All added weight based on added water content.

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DETERMINATION OF TRIPOLYPHOSPHATE AND RELATED HYDROLYSIS PRODUCTS IN PROCESSED SHRIMP

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The Food and Drug Administration is responsible for regulating the use of polyphosphates in seafood. Sodium tripolyphosphate is the most commonly used polyphosphate in the processed shrimp industry; and current regulations limit its use in accordance with Good Manufacturing Practice. The potential exists for economic fraud through mislabeling or excessive use of tripolyphosphate. At this point, the Agency is more concerned with excessive water uptake in treated samples than the amount of phosphate found in the product. However, there is a need for methodology which can 1) provide confirmation of the presence of tripolyphosphate in shrimp and 2) correlate the results obtained for added water with the use and/or abuse of tripolyphosphate.

The determination of total phosphorus by ICP-AES or some other suitable technique will not allow for an accurate and reliable means of regulating polyphosphate use in shrimp because of the widely varying amounts of P found in shrimp naturally [7]. Therefore, it is necessary to analyze for individual polyphosphate species such as tripolyphosphate and pyrophosphate. There are a number of reports dealing with the determination of polyphosphates in detergents by high performance liquid chromatography and ion chromatography [1-4]; however, thin layer paper chromatography often has been used in investigations involving polyphosphates in seafoods [6,8].

The regulation of polyphosphate use is complicated by the fact that polyphosphates are known to undergo hydrolysis. Unfortunately, polyphosphate hydrolysis is catalyzed in shrimp (as well as in other meats) due to the action of tissue phosphatases [5,6,8]. One mole of tripolyphosphate hydrolyzes to one mole of orthophosphate and one mole of pyrophosphate, while one mole of pyrophosphate hydrolyzes to two moles of orthophosphate [5,6,8].

The development of a successful regulatory method for tripolyphosphate in seafood will depend on a number of factors. Of primary concern, is minimizing the amount of hydrolysis which takes place during sample treatment, storage, and analysis. In the case where a significant amount of orthophosphate is formed due to hydrolysis, development of an effective method may be impossible because of high naturally occurring orthophosphate levels. Additionally, it must be shown that higher concentrations of tripolyphosphate and pyrophosphate found in shrimp correlate with greater water retention. Tehnet and co-workers investigated the stability of STP in treated shrimp during frozen storage using P32 labeled tripolyphosphate and thin layer chromatography[8]. This

study showed that some breakdown occurs during treatment; however, the tripolyphosphate concentration remained essentially constant over the period studied (2 - 10 weeks frozen storage). Pyrophosphate and orthophosphate concentrations were not found to stay constant during the same period of frozen storage.

In another study, Reddy found that tripolyphosphate and pyrophosphate concentrations were stable over a 10 month period when stored at -25°C [6]. This study also utilized thin layer chromatography to show that the rate of hydrolysis increased with increasing temperature (5, 10, 25, and 35°C). Additionally, the tissue enzymes responsible for catalyzing hydrolysis in uncooked shrimp were characterized.

Progress towards the development of an ion chromatographic method for the determination of tripolyphosphate and pyrophosphate in processed shrimp will be discussed in this communication.

MATERIAL AND METHODS

Apparatus

The ion chromatograph used in this work consisted of a Dionex Automated Sampler Module, Gradient Pump Module, Liquid Chromatography Module-3, Reagent Delivery Module and Variable Wavelength Detector Module-II. Data collection and reduction were accomplished using a Dionex Advanced Computer Interface and 386/25 microcomputer equipped with AI-450 chromatography software. A Dionex IonPac AS7 anion separator column (4 X 250 mm) and IonPac NG1 guard column (4 X 50 mm) were used.

Operating Conditions

The chromatographic mobile phase was 70 mM nitric acid [HNO_3] at a flow rate of 0.5 mL/min. Detection of the phosphate species utilized post-column reaction with 1 g/L ferric nitrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9 \text{H}_2\text{O}$) in 2 % (V/V) perchloric acid [HClO_4] at a flow rate of 0.5 mL/min. The post-column reagent was mixed with the effluent from the IC column using a mixing tee and a Dionex 500 μL packed reaction coil. Ultraviolet detection of the reaction products was made at 330 nm. The injection volume was 100 μL .

Standards

Food grade sodium tripolyphosphate [$\text{Na}_3\text{P}_3\text{O}_{10}$] was obtained from Monsanto (St. Louis, MO). Sodium pyrophosphate decahydrate [$\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$] was obtained from Aldrich (Milwaukee, WI); and sodium phosphate monobasic [$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$] was obtained from EM Science (Gibbstown, NJ).

Individual stock standards containing either 1,000 or 10,000 $\mu\text{g/g}$ of analyte were prepared in distilled deionized water (DDW) and stored in HDPE bottles. When stored at room temperature, these stock standards were not found to be significantly affected by hydrolysis for at least 30 days. Working standards were prepared daily in the concentration range 5 - 100 $\mu\text{g/g}$.

Sample Preparation

Samples used in this work were various commercially treated products from a single shrimp processor. The samples obtained were packaged and ready for sale.

Frozen samples were placed in zip-lock plastic bags and thawed in cool water. Tails and shells were removed (when necessary). A representative sample (generally 15-20 shrimp) was then composited using a Cuisinart Food Processor (Greenwich, CT). Accurately weighed 0.5 g portions of the composite were then placed in 60 mL HDPE bottles and diluted by a factor of 100 (w/w) with DDW. After thoroughly shaking for approximately 30 minutes on a mechanical shaker, the samples were centrifuged to separate the heavier particulate (the centrifugation step was skipped in later analyses). The analytical sample was then prepared by passing a portion of the extract through a 0.2 or 0.45 μm Nylon 66 syringe filter (Alltech, Waukegan, IL) and an activated 300 mg C-18 sample preparation cartridge (Alltech) in series. The first 2 mL's of sample through the filter series was discarded. The C-18 sample preparation cartridges were activated by passing approximately 10 mL of methanol followed by 10 mL of DDW.

RESULTS AND DISCUSSION

Chromatography and Quantitation

The ion chromatographic method used in this work has been described elsewhere for the analysis of sequestering agents in detergents [3]. The method is well suited for the determination of tripolyphosphate and pyrophosphate; however, orthophosphate elutes very near the void. This limits the linear working range of orthophosphate and makes it susceptible to interferences from unretained sample components. Non-linearity is commonly encountered in ion chromatography when a peak is eluted in the water dip.

It is difficult to obtain sodium tripolyphosphate in a relatively pure form. Generally a small amount of pyrophosphate is present. Because of this, it is best to calibrate the system using single component standards. For quantitative work it is necessary to assay the tripolyphosphate standard. This was done by first determining the concentration of pyrophosphate in a $\sim 500 \mu\text{g/g}$ tripolyphosphate standard. The total amount of phosphorus in the tripolyphosphate standard is then determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Finally, the assay is completed by calculating the amount of P due to tripolyphosphate by subtracting the amount of P coming from pyrophosphate (as determined by IC) from the total amount of P (as determined by ICP-AES). Using this method it was determined that our food grade tripolyphosphate standard contained 92 % (w/w) tripolyphosphate. It should be noted that orthophosphate was not detected above the detection limit in either pyrophosphate or tripolyphosphate 500 $\mu\text{g/g}$ standards.

Peak area responses were used in quantitating the phosphate species. The RSD of peak area response for 12 replicate injections of a mixed standard containing 10 $\mu\text{g/g}$ pyrophosphate and 25 $\mu\text{g/g}$ STP were found to be 0.9 % and 1.2 %, respectively. A linear working range of 0.5 to 50 $\mu\text{g/g}$

pyrophosphate was established. While for tripolyphosphate, the linear range was 10 to 500 $\mu\text{g/g}$ and for orthophosphate a linear range of 10 to 100 $\mu\text{g/g}$ was established. Table 1 summarizes the figures of merit obtained for this separation.

Results for Cooked Product

Figure 1 shows the chromatogram obtained for a commercial sample of cooked shrimp which was treated with tripolyphosphate by the processor. Ortho-, pyro-, and tripolyphosphate were found in this particular sample. In addition, an unidentified peak at a retention time of 6.1 minutes is present in most shrimp samples analyzed. To date, we have not identified this

Table 1. Figures of Merit			
	Ortho-phosphate	Pyro-phosphate	Tripoly-phosphate
Retention Time (min)	4.0	5.0	13.2
Linear Range ($\mu\text{g/g}$)	10-100	1-500	10-500
Correlation Coefficient	0.9994	0.9999	0.9999
Log-Log Slope	1.5	0.98	1.02
Short Term Precision (RSD)	nd	0.9 % n = 12	1.2 % n = 12
nd = not determined			

peak. We have demonstrated that the peak is not sulfate based on a sulfate spike. In addition, preliminary results obtained using an inductively coupled plasma atomic emission spectrometer as a second IC detector (in series with the UV detector), indicate that the unknown peak also does not contain P. No peak was detected at 6.1 minutes when monitoring the 214.9 nm P line.

As a practical note, tripolyphosphate is less strongly retained as the number of shrimp sample injections increases. After the peak moves out of an acceptable window of integration, the column can often be cleaned up by running several column volumes of 200 mM nitric acid. Also, it was found that a 5 mL DDW rinse of the injector after every sample significantly minimized sample carryover problems.

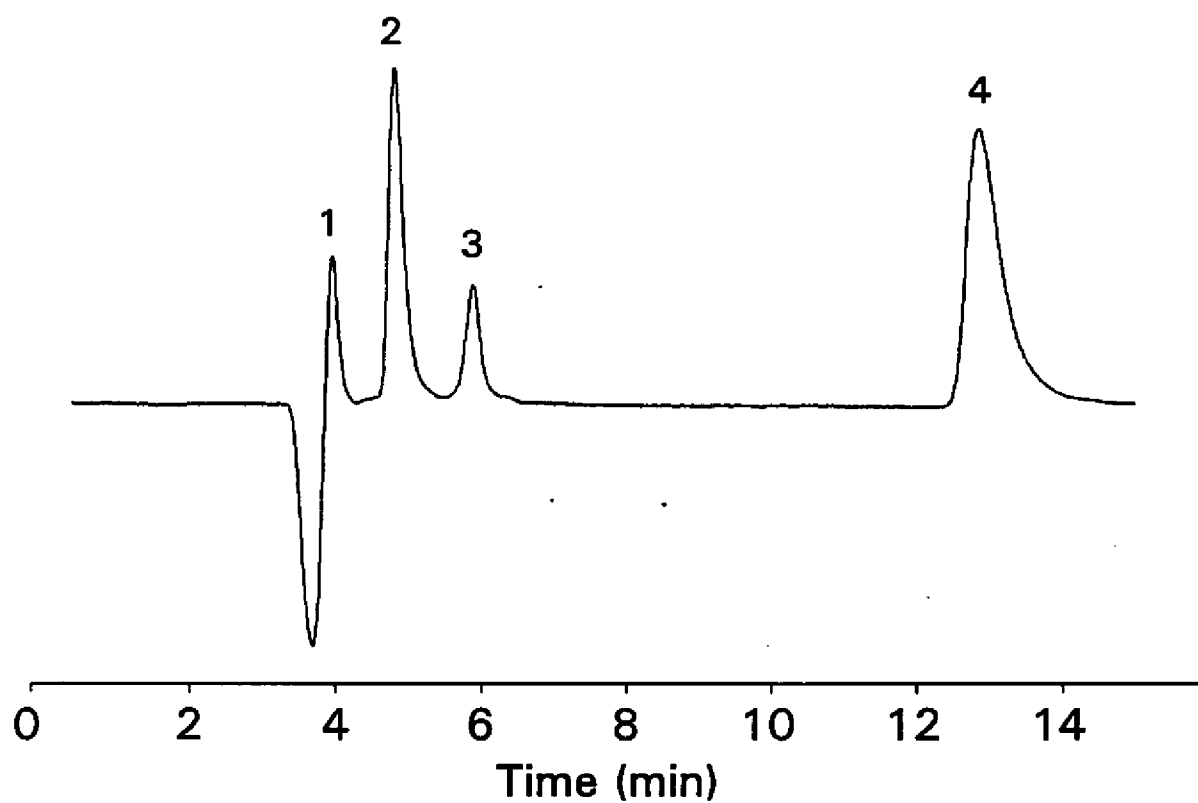


Figure 1. Chromatogram obtained for a cooked processed shrimp sample.
Peaks: (1) orthophosphate; (2) pyrophosphate; (3) unknown; (4) tripolyphosphate.

The method was evaluated for cooked shrimp initially because of fewer problems with hydrolysis. The action of cooking the product apparently slows enzymatic hydrolysis significantly, as will be discussed later. The reproducibility of peak area response for 10 replicate injections of untreated shrimp spiked to contain 25 µg/g pyrophosphate and 50 µg/g tripolyphosphate was found to be 1.0 % and 1.7 % RSD, respectively. Spike recoveries for untreated cooked shrimp were measured by weighing out a sample of homogenized shrimp and spiking with an aliquot of a 10,000 µg/g stock standard. Recoveries for both pyrophosphate and tripolyphosphate were generally acceptable (Range 88 % - 106 %). The data is summarized in Table 2.

Table 2. Spike Recovery Data: Cooked Shrimp		
Spike Level (µg/g), n = 2 unless noted	Pyro- phosphate	Tripoly- phosphate
5	88 %	nd
10	nd	93 %
20	105 %	nd
40 n = 10, (RSD)	100 % (1.2 %)	94 % (1.7 %)
45	106 %	95 %
85	nd	97 %
nd = not determined		

The results obtained for four cooked processed shrimp samples are shown in Table 3. For each sample, both tripolyphosphate and pyrophosphate were detected. The RSD's for 5 replicate weighings were approximately 6 % for each of the composited samples. In addition, a second sample of medium sized shrimp was prepared and the sample extract was analyzed in triplicate for three consecutive days. The extract was refrigerated between analyses. The results of this study showed little day-to-day variation in concentration of pyrophosphate and tripolyphosphate. Averages of 1195 µg/g pyrophosphate and 3195 µg/g tripolyphosphate were obtained over the three day period with RSD's of 9.1 % and 12.1 %, respectively. The same sample was then stored frozen for two weeks and reanalyzed. Seventy-seven percent of the initial pyrophosphate concentration and 92 % of the tripolyphosphate were recovered.

The samples used in Table 3 were reanalyzed approximately 11 months later. The results of this experiment are shown in Table 4. The last column in Table 4 presents the sum of the concentrations of pyrophosphate and tripolyphosphate expressed in terms of % P_2O_5 . For samples A, B, and C the Total % P_2O_5 found after 11 months of frozen storage was 87 %, 89 %, and 103 % of the initial values, respectively. This indicates that very little hydrolysis occurs in frozen storage. Also, it is not known how long these samples were out of frozen storage during the initial analysis.

It is of particular interest that in samples B and C there was very little difference in tripolyphosphate concentration over the 11 month period.

A significant amount of hydrolysis was noted in sample D as indicated by the drop in Total % P_2O_5 from

Table 3. Determination of Tripolyphosphate and Pyrophosphate in Commercially Treated Cooked Shrimp Samples		
Sample Type	Pyrophosphate Conc. ($\mu\text{g/g}$)	Tripolyphosphate Conc. ($\mu\text{g/g}$)
A. Small Salad Size	1410 \pm 84 (4.8 %)	4101 \pm 201 (3.9 %)
B. Medium 55-65/14 oz.	1340 \pm 91 (5.5 %)	3009 \pm 179 (4.8 %)
C. Large 30-40/14 oz.	1306 \pm 90 (5.5 %)	2933 \pm 194 (5.3 %)
D. Peel N' Eat 55-65/14 oz.	817 \pm 45 (2.3 %)	1375 \pm 303 (8.8 %)
Average and 95 % confidence limit reported. Number in parentheses is RSD (n = 5)		

Table 4. Comparison of results after 11 months of frozen storage.			
Sample Type (see Table 3)	Pyro-phosphate Conc. ($\mu\text{g/g}$)	Tripoly-phosphate Conc. ($\mu\text{g/g}$)	Total % P_2O_5
A. 0 mos.	1410	4101	0.46
A. 11 mos.	2090	2740	0.40
B. 0 mos.	1340	3009	0.36
B. 11 mos.	949	2840	0.32
C. 0 mos.	1306	2933	0.35
C. 11 mos.	1218	3024	0.36
D. 0 mos.	817	1375	0.18
D. 11 mos.	231	690	0.09

0.18 % to 0.09 %. However, for this sample the appearance and odor indicated significant decomposition had taken place. It is not known whether this decomposition occurred during frozen storage or if the sample was not promptly frozen after initial analysis. The presence of pyrophosphate in all of the samples treated with tripolyphosphate indicates the need to include pyrophosphate in any quantitative evaluation of tripolyphosphate use. It is not known if the majority of pyrophosphate encountered in these samples originates from the use of tripolyphosphate which contains large amounts of pyrophosphate or if a significant amount of hydrolysis has taken place during treatment and storage. However, the preliminary results obtained for Total % P_2O_5 as described above, warrant further study. It is acknowledged here that any hydrolysis which has occurred produces orthophosphate. The inability to distinguish between the additional orthophosphate and naturally occurring orthophosphate induces a certain amount of error in the determination of how much tripolyphosphate was used.

Results for Uncooked Product

Hydrolysis effects are even more of a concern for uncooked product due to enzymatic hydrolysis. This was especially evident when performing spiked recoveries on untreated uncooked shrimp samples. Recoveries of 2700 $\mu\text{g/g}$ pyrophosphate spikes in two different uncooked samples were 112 % and 84 %. However, recoveries of 5000 $\mu\text{g/g}$ tripolyphosphate spikes resulted in recoveries of 84 % and 46 %. In the latter sample a significant amount of pyrophosphate was found. If both pyrophosphate and tripolyphosphate were determined in the latter sample and the sum of the two species is used to calculate recovery (in terms of % P or % P_2O_5), the calculated recovery increases from 46 % to 96 %.

In a separate experiment the hydrolysis of tripolyphosphate was followed in prepared samples over a period of 3 to 4 days. The same prepared samples were analyzed on successive days and refrigerated between analysis. For two uncooked samples the tripolyphosphate concentration dropped from approximately 2500 $\mu\text{g/g}$ to less than the detection limit in 3 days. While in four cooked samples the tripolyphosphate concentration averaged 94 % of the starting concentration after 4 days.

If it could be shown that tripolyphosphate and pyrophosphate concentrations are stable in frozen storage, it should be possible to evaluate tripolyphosphate use in uncooked product. Obviously the amount of time between sample preparation and analysis would need to be minimized. The major concern then would be how much hydrolysis has taken place during treatment and prior to freezing and how much variation there is in the amount of hydrolysis.

CONCLUSIONS

A method for the determination of pyrophosphate and tripolyphosphate in processed shrimp has been described. The method is generally capable of confirming the use of tripolyphosphate. Recoveries for a 40 $\mu\text{g/g}$ spike of pyrophosphate and tripolyphosphate in cooked shrimp samples were 100 % and 94 %, respectively. An RSD of approximately 6 % was obtained for five replicate weighings of commercially treated cooked shrimp samples.

Quantitative results should preferably be reported in terms of a total concentration of pyrophosphate + tripolyphosphate (expressed as % P_2O_5 or % P). Hydrolysis effects on cooked shrimp during frozen storage were found to be relatively small or non-existent. Whether or not high tripolyphosphate concentrations can be correlated with the amount of added moisture in shrimp needs to be established.

For uncooked processed shrimp the analysis is more difficult because of enzymatic hydrolysis. The same approach as used with cooked shrimp, determining pyrophosphate + tripolyphosphate as % P_2O_5 , may prove useful if the amount of hydrolysis occurring during processing and storage is minimal.

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CONSUMER EVALUATIONS OF PHOSPHATED SHRIMP AND SCALLOPS

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Phosphates are used in the seafood industry primarily to reduce freeze/thaw drip losses that occur during processing and storage. Proper use of phosphates to influence the moisture content in shrimp and scallops is currently in debate relative to regulatory concerns for adulteration vs. commercial concerns for "good manufacturing practices". The objective of the study was to record consumer ratings for various sensory attributes perceived when exposed to phosphated and non-phosphated shrimp and scallops.

Experienced panelist and consumers rated various sensory attributes perceived during encounters with boiled shrimp and broiled scallops previously treated with phosphates. Increasing amounts of water were added to the meats by varying the concentrations of phosphate treatment solutions and exposure times. Initial triangle comparison test for differences in the treatments indicated that over half of the panelist could detect the phosphate treated samples and their ability to distinguish the treated products increased as the moisture content increased. Consumer judgments indicated a preference for the treated product and higher ratings for the phosphated shrimp and scallops were consistent for general appearance, flavor, purchase value and overall quality. The following condensed description of work is based on two papers submitted to Journal of Food Science (Otwell et al. 1993).

METHODS

Pink shrimp (*Peneaus duorarum*) harvested about Key West, Florida, were headed, peeled and deveined without excessive water contact. Sea scallops (*Placopecten magellanicus*) were harvested along the New England coast and processed aboard the vessel by researchers from the Virginia Institute of Marine Sciences. Both products were exposed to various phosphate treatments to obtain increasing amounts of water added. The shrimp were boiled using a standard cook procedure the water was brought to a boil, the shrimp added, the water returned to boil and the shrimp boiled 1 minute. The scallops were placed on broiler pans and the temperature monitored until the internal temperatures reached 160°F.

The product evaluations occurred in two stages. The first stage was a discriminative test involving experienced panelists in a more analytical setting (FSHN labs, Univ. Florida). The panelist were food scientists and students familiar with discriminative product and testing and seafood. This test focused on panelists ability to detect differences between treatment variables. Judgements were based on appearance and taste of cooked samples. The procedure was triangle testing where the panelist were asked to distinguish the odd or different sample amongst three cooked samples.

The second stage in organoleptic testing involved 100-125 consumers randomly recruited by phone and prescreened for age, sex, level of income and familiarity with eating boiled shrimp and broiled scallops. The consumers were assembled and briefed before, during and after the product evaluations to assure their understanding of the questions and rating system. Ratings were based on actual product observations and consumption. The consumers were unaware of the test variables and a 1 to 7 point rating scale was used. The ratings were analyzed for mean differences and variance with significance ($\alpha=0.05$) based on the Walker-Duncan k-ratio test (SAS, 1992).

RESULTS AND DISCUSSION

In general, increasing STP concentration and soak time increased the moisture content in the raw and cooked meats (Tables 1 & 2). In the shrimp, the soaking procedure at 2.0% STP did not impart or retain as much moisture uptake as the tumbling procedures. In tumbling, the higher phosphate treatments increased moisture uptake and retention in the cooked products. In the scallops, the most significant influence was a 6.0 percent change in raw moisture content from the freshwater treated control to the 2.5% STP wash for 24 hours. In both cases, the control samples lost the most water when thawed and cooked while the samples treated with the highest concentrations of phosphates lost the least.

Based on percent correct judgements in the series of triangle comparison tests, the majority of panelist were able to distinguish each phosphated treatment from the controls in both the shrimp and scallops. Product distinction was more obvious for products more heavily phosphated (Tables 3 & 4).

For the consumer evaluations, the participants were asked to rate product appearance and aroma before tasting the product. In all cases, the phosphated product was rated higher than the untreated controls (Tables 5 & 6).

CONCLUSIONS

Consumer evaluations have demonstrated a distinct preference for phosphated shrimp and scallops. The addition of moisture and ability to hold water in cooked product can provide a consumer benefit in terms of flavor and moist mouthfeel. There was no distinct objection to phosphated product appearance, aroma of aftertaste, and additional salt taste was desired. In both cases, the consumers generally felt the phosphated shrimp and scallops meet their expectations and they liked and judged the products to be of high quality and valued more than non-phosphated product.

Weigh changes in pink shrimp, *Penaeus duorarum* as a consequence of the phosphate treatments and the standard cooking (boiling) procedures for the discriminative triangle tests.

Product Treatments	Avg. Percent (%) weight change		
	Raw	%Cook loss ¹ (Boiled)	
	%Uptake	Discrim. test	Consumers
Control	0.0	-28.0	-33.9
<u>Phosphates Treatments²</u>			
2.0% STP, 30 min.S	5.0	-23.5	-33.9
1.5% STP, 15 min.T	10.0	-26.5	-22.5
4.0% STP, 15 min.T	15.0	-11.0	-22.5
6.0% STP, 30 min.T	17.0	- 5.7	-9.3

¹ % uptake and % cook loss are measures in weight change per the original weight of the shrimp before treating with phosphates and before boiling, respectively. The original moisture content in the pink shrimp ranged from 78.5 to 80%. The weight changes primarily reflect changes in the water content.

² All STP (sodium tripolyphosphate) treatments contained 1.0% sodium chloride. The symbols S=soak and T=tumbles

Table 2. Resulting moisture contents in sea scallops, *Placopecten magellanicus* as sampled from selected commercial handling and processing procedures, then frozen (-20°), thawed (raw) and broiled (71° internal).

Product Treatments After Hand-Shucking	Avg. Percent (%) Moisture Content		Percent Change
	Raw, Thawed	Broiled	
Control, freshwater wash	79.5a	75.6	-4.9
Phosphated Dip, 10% STP, 1 min	81.0 bc	78.0	-3.7
<u>Phosphate Washes</u>			
4% STP, 20 min.	80.1a c	76.9	-4.0
2.5% STP, 5 hr.	80.9 bc	77.7	-4.0
2.5% STP, 13 hr.	83.4 d	79.5	-4.6
2.5% STP, 24 hr.	84.3 d	80.6	-4.4

All STP (sodium tripolyphosphate) treatments contained 1.0% sodium chloride.

Average moisture contents (AOAC, 1990) were based on triplicate analyses for subsamples following standard thaw and broil procedures.

Statistical significant differences ($\alpha=0.05$) are denoted by means labeled with different lower case letters according to Bartlett test for homogeneity and Scheffe test for mean comparisons.

Table 3. Discrimination test results for 24 panelists attempting to identify or judge any noticeable difference between cooked samples of control vs. phosphate treated shrimp.

Treatments	% Total Correct Judgements ¹	
	Trial 1	Trial 2
Control	---	---
Phosphate Treatments²		
2.0% STP, 30 min S	58	58
1.5% STP, 15 min T	79	91
4.0% STP, 15 min T	63	83
6.0% STP, 30 min T	100	88

¹ A recorded "correct judgement" required proper product identification in both replicates of the control vs. phosphate samples in triangle paired comparisons per each trial.

² All STP (sodium tripolyphosphate) treatments contained 1.0% sodium chloride. The symbols S=soak and T=tumbles

Source: Otwell et al. 1993

Table 4. Discrimination test results for 24 panelists attempting to identify or judge any noticeable difference between cooked samples of control vs. phosphate treated scallops.

Product Compared to Controls	% Total Correct Judgements		% Moisture Content Cooked
	Trial 1	Trial 2	
Control	—	—	75.6
10% STP/1 min.	58	52	78.0
4% STP/20 min.	69	66	76.9
2.5% STP/5 hr.	73	71	77.7
2.5% STP/13 hr.	66	83	79.5
2.5% STP/24 hr.	77	85	80.6

STP - Sodium tripolyphosphate, plus 1% sodium chloride.

¹ A recorded "correct judgement" required proper product identification in both replicates of the control vs. phosphate samples in triangle paired comparisons per each trial.

Source: Otwell et al. 1993

Table 5. Consumer perception ratings for overall shrimp product 'likableness, quality and value' based on previous evaluations of boiled samples. Value judgements were based on a stated raw product cost of \$4.99 per pound.

Product Treatments	Likableness		Quality		Value	
	Mean	% like	Mean	% like	Mean	%bargain
Control	3.6a	36	3.3a	27a	5	
Phosphate tri ¹						
2.0% STP, 30 min S	4.7b	62	4.2 b	3.4 b	19	
1.5% STP, 15 min T	4.8b	65	4.4 b	3.5 b	14	
4.0% STP, 15 min T	5.0b	71	4.6 b	3.8 c	25	
6.0% STP, 30 min T	4.7b	59	4.4 b	3.5 b	19	

¹ All STP (sodium tripolyphosphate) treatments contained 1.0% sodium chloride. The symbols S=soak and T=tumbles.

Likableness scale: 1-dislike very much; 4-neither like/dislike; 7-like very much; % like is ratings ≥ 5 .

Quality scale: 1-very low quality; 4-neither low/high; 7-very high quality; % high quality is ratings ≥ 5 .

Value scale: 1-paid too much; 4-'fair price'; 7-got a bargain; % bargain is ratings ≥ 5 .

Statistical significant differences ($\alpha = 0.05$) are denoted by any two means labeled with different lower case letters.

Source: Otwell et al. 1993

Table 6. Consumer perception ratings for overall scallop product 'likableness, quality and value' based on previous evaluations of broiled samples. Value judgements were based on a provided raw product cost of \$6.99 per pound.

Product Treatments	Likableness		Quality		Value		
	Mean	% like	Mean	% high qual.	Mean	% bargain	% fair price
Control	4.9a	64	4.4a	47	3.5a	22	31
% STP							
10/1 min.	5.1ab	71	4.7ab	59	3.7ab	20	37
4/20 min.	5.3ab	74	4.8ab	60	3.9ab	27	33
2.5/5 hr.	5.4ab	79	5.0 b	65	3.9ab	30	34
2.5/13 hr.	5.3 b	75	5.0 b	66	4.0 b	29	37
2.5/25 hr.	5.5 b	83	2.2 b	66	4.0 b	30	37

STP - sodium tripolyphosphate, plus 1% sodium chloride.

Likableness scale: 1-dislike very much to 7-like very much; % like is ratings ≥ 5 .

Quality scale: 1-very low quality to 7-very high quality; % high quality is ratings ≥ 5 .

Value scale: 1-paid too much, 4-'fair price' to 7-got a bargain; % bargain is ratings ≥ 5 .

Statistical significant differences ($\alpha = 0.05$) are denoted by any two means labeled with different lower case letters.

Source: Otwell et al. 1993

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COMPUTER-BASED PATHOGEN CONTROL

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The use of computers to monitor and control factors affecting the incidence and proliferation of pathogenic organisms can be an effective tool in safe food production. Pathogen control systems may vary from simple hand held instruments that can periodically monitor storage areas, processes, etc. to state-of-the-art computer designs that provide real-time monitoring of the processing and storage of food products. Factors that affect microbiological growth such as thermal application, pH, salinity, moisture, ionic activity, and employee traffic control can be continuously monitored to ensure that food safety standards are satisfied at all times. As a result, these types of control systems provide the processor with an automated and comprehensive approach to minimizing the occurrence of pathogen producing bacteria.

RESULTS AND DISCUSSION

The proper monitoring of temperature during the process and storage of raw and finished product is perhaps the most critical control parameter for pathogenic bacteria. Proper selection and use of temperature sensors are important elements in this control area. The four types of temperature transducers or sensors used in measuring temperature are thermocouples, resistance temperature detectors (RTDs), thermistors, and integrated circuits (ICs).

Thermocouples are the most common thermal measuring devices used because of their relative simplicity and wide support. Two thermocouple wires of different material when soldered together form a thermocouple junction. The measurable potential across the junction corresponds to a temperature. Because of their wide temperature range and available sizes, they are used extensively in many areas for temperature measurement. Thermocouples are point monitoring devices and are not easily affected by conduction or convection temperature sources other than at the junction point. The accuracy of the thermocouple is limited to plus or minus a degree or two. Of concern is the thermocouple relative low voltage which can be affected by electrical noise at different locations. This problem can be eliminated through isolated circuits and shielding. Thermocouples can be mounted in most operations but are usually limited to stationary processes due to the inability of wires to follow the food product through all processes.

RTD's or Resistance Temperature Detectors are more accurate due to a more linear output signal, but more expensive than thermocouples. Since they are more fragile than thermocouples and

require a voltage source for their operation, they are usually mounted in a permanent and protected location. A variety of sizes are available, however because of the larger mass of RTDs self-heating errors may occur.

Thermistors are used where very accurate temperature measurements are required. The thermistor has a fast response time so it can be used to measure very small increments of temperature change over short time intervals. Thermistors need a power source to establish a temperature reading and are limited to certain temperature ranges. Thermistors are also extremely fragile. A good example of thermistor use would be in a laboratory setting for research purposes.

IC's, or integrated circuits are excellent sensors because they are relatively inexpensive and easy to mass produce. The voltage versus temperature algorithm is linear so temperatures can be calculated with accuracy. Some drawbacks of the IC are slowness to measure change in temperature or poor reaction time, constant need for a power source, and limited temperature ranges.

Remote Sensors are self-contained, power supplied micro circuitry, including memory for storing collected data. They contain one or more of the previous mentioned sensors and can be placed in with the product being measured. The sensor can be used to monitor product or process environment temperature as well as temperature abuse during shipping and handling. With remote sensors the complete picture of time versus temperature can be recorded. The stored data can be down-loaded to a personal computer and translated into a usable form with appropriate software.

Infrared imaging is another method of remote temperature measurement. An infrared monitor measures surface temperatures of various products from a distance. The temperature is measured on the emissivity of product surface and therefore is limited to average temperatures as might be found when scanning food production lines. Changes in moisture on the product surface and air volume movement around the product will make minor changes in the temperature readings found in infrared temperature monitoring.

Traditional methods of measuring temperature in microwave ovens are obsolete due to the heating of metal by microwave excitation. Fiber optic measurement allows for the monitoring of temperature when microwaves are used for the cooking process. The fiber optic cable is expensive and is fragile under shock and shear conditions. The datalogger used to interpret temperature data is the heart of the microwave sensing device and that alone is the leading cost factor when comparing other temperature measurement sensors and systems. However this is the only type of system developed for use in microwaves. Due to consumer demand for convenience in cooking, manufacturers are rapidly developing newer measurement devices for microwave cooking.

Presently there are many systems to choose from when designing a complete pathogen control program to monitor temperature, pH, salinity, moisture, and traffic control. They can range from simple hand-held instruments to state-of-the-art computer systems that continuously monitor all phases of manufacturing. These areas would include process control and monitoring, data logging, quality control, and lab automation. Hand-held instruments and dataloggers provide an economical and efficient method when monitoring food processing and storage areas. They can be easily programmed and moved to various

locations to monitor potential problem areas. Due to the popularity and versatility of personal computers, they have evolved into a cost effective and powerful solution for data acquisition and control on the plant floor. In addition, the evolution of control software has made it easy for the average user to integrate measurement and control instrumentation into a system. Under current technology the user has a choice of four instrument interfacing techniques. They are IEEE 488, also known as the GPIB or HB-1B, data acquisition plug-in boards, RS-232 or serial interface, and the VXI bus. The GPIB bus is available for many sophisticated analytical instruments such as chromatographs and other multiple signal devices. It provides high-speed data transfer (1 Mbytes/sec) and can be used with a variety of computer platforms such as IBM, Apple, and Sun. Plug-in data acquisition boards are rapidly becoming the method of choice due to the low cost and variety of functions. Transducers installed throughout the processing area are connected to the board through signal conditioners. Data collected can be analyzed and displayed as text or graphics, providing the user with a real-time information window of plant floor operations. The RS-232 interface can be found on most simple measurement instruments such as balances and pH meters. It usually is standard on most computer systems and is widely supported by most software programs. VXI was first introduced in 1987 and is regarded as a high performance and rugged interface for industry applications. It combines high speed with a modular design that provides for easy control of advanced measurement systems. All of the above interfaces may be combined on a single system in order to take advantage of instrumentation that will satisfy your cost and performance specifications.

Through the proper selection of sensors and controllers, an automated and comprehensive computer system can be implemented in order to monitor and control those factors that affect the incidence and proliferation of pathogenic organisms in food processing operations.

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RAPID TEST KITS FOR DETECTION OF *LISTERIA MONOCYTOGENES* : A REVIEW

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Listeria monocytogenes is a pathogenic microorganism which can cause serious infections to humans, some of which can be fatal. Estimates suggest that in the United States *L. monocytogenes* is the cause of approximately 1850 cases of serious illness annually, with a fatality rate of 25 % (Pinner, et. al., 1992). This pathogen usually affects consumers with a weak immune system, including children, pregnant women, elderly and immunocompromised individuals, causing spontaneous abortion and death (Farber, et. al., 1991). Due to the severity of these cases, U.S. Food and Drug Administration has established a 'zero tolerance' for *L. monocytogenes* in all cooked ready-to-eat food product. Any product found to be contaminated has to be recalled and destroyed. Several million dollars in product loss has resulted due to destruction of contaminated food products.

Ready-to-eat seafoods (i.e., smoked fish, crab meat, cooked shrimp, lobster meat) have shown a significant degree of contamination incidents, according to State and FDA routine plant inspections. Ideally, before these products are released into commerce they should be tested or monitored for *L. monocytogenes* contamination. Currently the official analytical methodology takes 7 to 14 days to confirm the presence or absence of *L. monocytogenes*. This period makes it impossible for routine monitoring when working with highly perishable goods. Seafood product, with a shelf-life of less than 14 days on the average, can not be held awaiting prolonged analytical results. Faster methodologies are needed to better address this concern. Rapid test kits for the detection of *Listeria* have been developed which offer the industry reliable results in a shorter period of time, 1 to 3 days. Some of these methods do not require extensive training.

There are several test kits commercially available for the detection of *L. monocytogenes* on seafoods. Four of the most popular tests are: VIDAS ImmunoDiagnostic Assay System (bioMérieux Vitex, Inc.), GENE-TRAK *Listeria* DNA Hybridization Test (GENE-TRAK), LISTERIA-TEK ELISA Test System (ORGANON TEKNIKA) and LISTERTEST Immuno-beads Test (VICAM). A comparison of these methodologies is provided to help direct judgements for use. Subsequent trials will be necessary to verify their use in respective commercial settings.

GENE-TRAK *Listeria monocytogenes* is the only test specific for *L. monocytogenes* and is based on a DNA hybridization test followed by a colorimetric detection. The procedure requires sample enrichment and an overall average testing time of 2 1/2 days. Samples are mixed with modified University of Vermont Medium (mUVM-2) for 24 hrs and then spread onto modified Lithium chloride - ceftazidime agar (mLCA) and incubated for another 24 hrs (Appendix 1A). With an sterile swab, growth from mLCA is suspend in 1 mL of Phosphate Buffered Saline (PBS). The suspension is then heated, lysed and washed. The actual GENE-TRAK assay is preformed on a 5 ml aliquot of

APPENDIX 1: Flow Scheme of the different assays mentioned in the paper.

A) GENE-TRAK

Homogenize 25 g of sample and add
225 mL of mUVM-2
(modified University of Vermont medium)

Incubate 24 +/- 2 hrs
@ 35°C

Transfer to mLCA

Incubate 24 +/- 2 hrs
@ 35°C

Resuspend in 1 mL of
Phosphate Buffer Saline (PBS)

DNAH Assay

Confirmation of presumptive
positive colonies
(MOX plates)

B) mini-VIAS

25 g of seafood is homogenized and transferred
to 225 mL of Frazer broth base (Difco)
and supplement

Incubate for 22 +/- 2 hrs
@ 30°C

0.1 mL of Frazer broth
in 10 mL of Frazer broth base

Incubate for 22 +/- 2 hrs
@ 30°C

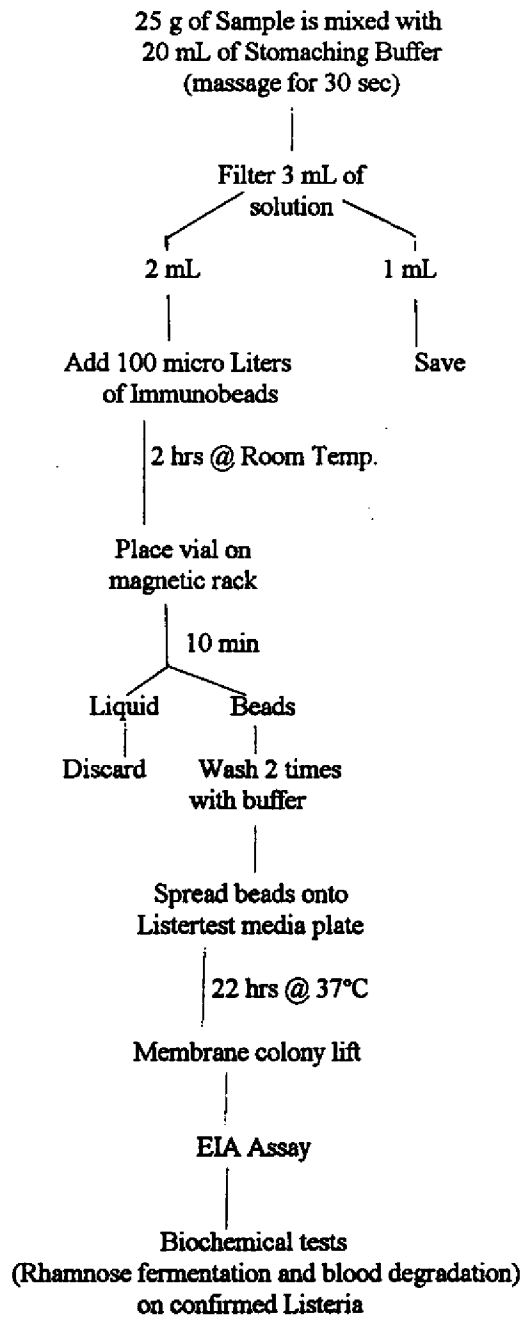
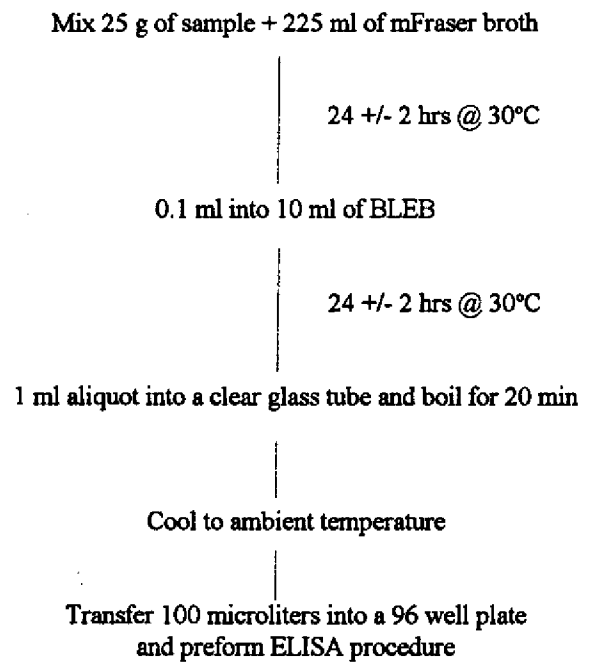
Heat 15 min @ 100°C

Transfer 0.5 mL into the VIDAS
LIS reagent strip and place it
into the automated equipment

Read results

APPENDIX 1 (cont)

C) LISTERTEST

D) *Listeria*-Tek

the treated sample. If L. monocytogenes is present a colored solution will be obtained (GENE-TRAK systems, 1993). This test has the advantage of detecting L. monocytogenes directly, thus reducing the analytical time (Appendix 2) (Rodriguez, 1993). It is labor intensive and requires high level of training for the person performing the test. Strict controls for the lysis and heating steps are necessary.

Mini-VIDAS ImmunoDiagnostic Assay System (BioMerieux Vitex) is an automated system of analysis that will detect *Listeria* spp. from a previously enriched sample. Seafood is enriched for 24 hrs in Frazer broth base plus supplement, then the sample is transferred to frazer broth to be incubated for an additional 24 hrs (Appendix 1B). The enriched sample is heated @ 100 °C for 15 min to lyse the cells, exposing the antigen in the cell for better analytical results and reducing the risk of the technician to infection. An aliquot (0.5 mL) of the enriched sample is placed in a VIDAS LIS reagent strip before positioning in the equipment (Tek Talk, 1993). Results are obtain within an hour. This procedure utilizes Enzyme-Linked Fluorescent Immunoassay (ELFA) as a detection technique and the developed fluorescent product is read by a computer scanner and transformed into positive or negative results (Holloway, J, 1993). This test is not quantitative and takes approximately 2 1/2 days to preform (Appendix 2). With automation one technician could easily preform numerous test simultaneously. The test results are objectives, not depending on worker's interpretation. The only draw-back is that this assay requires high initial investment for necessary equipment and initial reagents.

VICAM Listertest was developed based on the immunomagnetic capture of viable *Listeria* spp. cells. Magnetic beads coated with *Listeria* spp. antibodies are mixed with the sample to be analyze and then separated with the aid of a magnet (Appendix 1C). This mechanical procedure suppresses the need of using an initial selective enrichment. Cells bound to the magnetic beads are washed and plated onto solid selective media for overnight incubation. If growth is present, the colonies are most likely *Listeria*. Further confirmation is based on Enzyme immunolinked assay (EIA) on a membrane imprint of the plate. Colonies can be picked after been confirmed to be *Listeria* and determine if any L. monocytogenes is present.

The advantage of this test is that confirmed, quantitative result (appendix 2) can be obtained within 24 hrs. This test has been found to be equally sensitive to FDA's official method when analyzing *Listeria* in shellfish and environmental samples (Jackson, B. J., et.al., 1993). Due to the specificity of the antibody, this testing procedure performs well for detecting *Listeria* in high bio-burden samples. A disadvantage is labor intensity but this is compensated by the ease of use.

LISTERIA-TEK ELISA Test System (ORGANON TEKNIKA) relays on the specificity of the monoclonal antibodies directed to the *Listeria* spp. antigen, Enzyme-Linked Immunoassay (Appendix 1D). It is labor intensive (Appendix 2), but several samples can be run at the same time by utilizing the 96 well plate format. This format has the disadvantage of requiring a higher level of training by the operator (Appendix 2). Samples are enriched for 48 hrs, then cells are lysed to expose the targetted antigens. EIA procedure is then preformed to determine presence or absence of *Listeria* spp. Biochemical tests must be preformed in order to determine the presence of L. monocytogenes.

Appendix 2: Advantages and disadvantages of rapid test kit assays compared

A. - GENE-TRAK

Advantages

- Sensitivity is 1×10^5 cfu / ml
- Assay will work on high bio-burden
- High specificity
- Results ready in 3 hrs after enrichment
- Low false positive rate

Disadvantages

- non AOAC Approved
- Needs close control on incubation temperatures and procedure
- Positive results will need 48 hrs to be confirmed.

B.- LISTERIA-TEK

Advantages

- USDA Accepted
- Very specific (EIA procedure)
- False positive rate (5.9%)

Disadvantage

- High level of training is required
- Easy cross-contamination during assay
- False negative rate of 8.7%

C.- mini-VIDAS

Advantages

- Specific (EIA procedure)
- USDA accepted
- Low false positive (1.5%)
- Easy to use
- Low level of involvement

Disadvantages

- High initial investment
- Not AOAC approved

D.- LISTERTEST

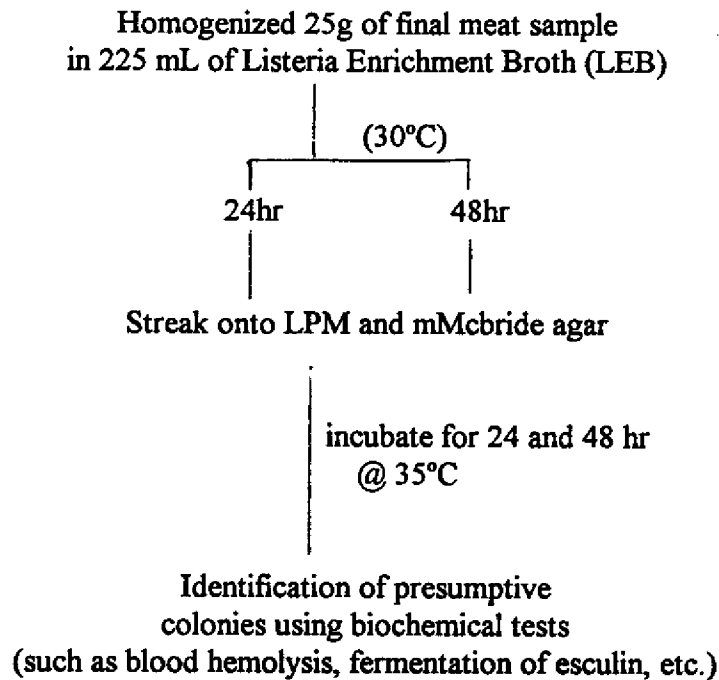
Advantages

- Specific (EIA procedure)
- Results can be obtained within 24 hrs
- Quantitative analysis
- Low false positive

Disadvantages

- High level of involvement
- Not AOAC approved
- Recovery rate of 30 - 85 % depending on the sample

The Food and Drug Administration official procedure (FDA BAM manual, 1984), as can be seen below, will take 4 days to confirm a negative result and 7 to 10 days for a positive.



New and better methods are continuously being develop in order to obtain rapid and accurate results which should be comparable to the official FDA testing procedures. Food manufacturers are constantly seeking for faster and more economical ways of ensuring the required level of safety in their product.

As shown in this paper, rapid test kits are beneficial to the seafood industry, reducing the analysis time, making possible to ensure good quality and product safety at all times. Since seafood is highly perishable processors can not hold the product for more than 2 - 3 days before distribution.

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LISTERIA IN SHELLFISH PLANT ENVIRONMENTS: PREVALENCE AND CONTROL IN VIRGINIA

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Listeria monocytogenes is a pathogen of concern to the crab meat industry. Its persistent presence in plant environments may lead to contamination of finished product thereby becoming a potential health risk. This is particularly significant since crab meat is one of the many products of Virginia's shellfish industry. A preliminary investigation was carried out to determine the presence of Listeria monocytogenes in six crab meat plants from 1989 to 1991. Results obtained with 3057 swabs of different areas showed a 5.1% overall frequency of Listeria monocytogenes in the plants. Incidence during plant operations and after plant cleaning and sanitizing were 6.56% and 4.34% respectively.

A follow up monitoring of 10 plants looking at finished product contact surfaces and non food contact surfaces was conducted during the 1990-91, 1991-92 and 1992-1993 crabbing seasons. Results of 1847 swabs showed overall listeria incidence of 4.0%, 8.5% and 9.0% respectively.

The results obtained from season to season in different plants indicate that control is a function of how keen and persistent particular operators are in setting up a HACCP program in their respective plants. It did not matter that the plant was a small operation or that it is a large operation. The floor, the scrap cans, crab carts, foot stools were the most likely places to recover Listeria monocytogenes.

Listeria monocytogenes is a gram positive, catalase positive short bacillus found in the environment which earned renewed interest as a public health concern after the Jalisco cheese outbreak in 1985. Since then epidemiologic investigations have shown that Listeria is indeed a foodborne pathogen (2, 5, 6, 10, 11, 12).

Listeriosis in humans occurs more often in immunocompromized individuals, in pregnancy causing stillbirths, and a neonatal listeriosis which is often confused with group B streptococcal infection. It is characterized by a central nervous system (CNS) infection with a primary bacteremia and can include endocarditis (4, 5). All 13 serovars of L. monocytogenes can cause listeriosis. However most reported cases are caused by serovars 1/2a, 1/2b, and 4b (3).

In animals it causes abortions, meningoencephalitis and in addition it can be carried in the gastrointestinal tract of animals as well as humans. Listeria monocytogenes has been isolated in almost all livestock species including cattle goats birds and other species (7, 8, 9, 14). In humans L. monocytogenes has been shown to be carried by healthy individuals in the gastrointestinal tract and it is also carried in urogenital tract of healthy females.

Listeria species have also been isolated from the environment and from foods without necessarily a direct relation to human disease. Large scale investigations carried out by different centers have demonstrated that listeriosis is a foodborne disease with the suggestion that an infecting dose and the presence of underlying conditions such as extreme age, pregnancy, immunocompromized status, and other infections could be factors in developing listeriosis (18).

Incidence and growth of L. monocytogenes in seafood has been investigated by Lovett et al.(17) who found the organism surviving and capable of five log growth in fish, shrimp, lobster and cold smoked salmon. Weagant et al.(22) found L. monocytogenes in 15 of 57 samples of frozen seafood which included shrimp, crabmeat, lobster tail, fish and surimi based seafood.

The presence of L. monocytogenes in picked crabmeat thus presents a public health hazard for infants, persons with compromised immune systems, and others with underlying diseases. Because of this FDA has adopted a zero tolerance level for all processed ready to eat seafood items.

In light of the importance of the seafood industry to Virginia's economy and the lack of strict critical controls points in most establishments we investigated the presence of listeria in crabmeat plant environments.

This object of this investigation was: (i) to determine the incidence and foci of L. monocytogenes in crabmeat plant environments, (ii) to determine the colonization process of listeria during the crabbing season, and to study the movement of listeria in the crab plants during operations; (iii) to evaluate standard cleaning procedures and develop a manual for cleaning crab plants.

MATERIALS AND METHODS

The survey protocol was as follows:

In Phase I of the study, six of the sixty plants operating during the crabbing season were selected. The owners and operators of the plants were consulted and participation was voluntary. It was estimated that isolation rates obtained from these plants would then serve as indicators of most likely contaminated areas of the plants. Any plants showing contamination with L. monocytogenes during this preliminary phase would be notified and a regiment of cleaning and sanitizing and adherence to Health Department guidelines would be instituted. The two plants serving as controls were also going to be notified by the third swabbing of any potential listeria contamination.

Swabbing was carried out after the plant had finished a day's operation and cleaned and sanitized. This set of swabs was labelled "Post Op". The process was repeated again the next day early morning before the plant resumed its operation. Swabs were labelled "Pre Op". Finally environmental swabbing of designated sites continued during the day at regular intervals. Swabs were labelled "During".

When a cleaning protocole was instituted, swabbing was performed after proper cleaning and sanitization. If *L. monocytogenes* persisted the process was repeated.

Table A. Plant Areas Selected For Swabbing

A. Receiving Dock	E. Picking Room floor & Contact surfaces
B. Retort Storage	F. Scrap Cans, Chutes etc...
C. Cooling Room	G. Packing Room
D. Picking Room tables,pans,aprons	H. Sinks, Dips etc...
	I. Picking Room Scrap

In the follow up study, Phase II, ten plants were identified. Selection was again on the basis of size, locality and operating procedures. Except for two plants which were selected as control plants during the preliminary study and which continued to serve as controls.

Swabs were taken of food contact surfaces and of non food contact surfaces as outlined in table (A) above. These were kept in 2ml UVM (BBL) and transported to the laboratory where they were then transferred to 18mL UVM broth and incubated 18 to 24 hours at 30°C. After incubation the enrichment was plated on LPM agar (BBL) and modified oxford agar(MOM) (Oxoid). Plates were then incubated at 37°C for 48 hours and observed for growth of typical bluish colonies of *Listeria* on LPM with the oblique light. MOM plates yielded black creamy to grayish colonies with a black halo. Colonies picked were then identified using conventional biochemical and *Listeria* ID kits (Organon Teknica).

Laboratory results were reported to the Division of Shellfish Sanitation which then worked with the plant operator on cleaning procedures and steps to take in order to reduce the risk of contaminating freshly picked crabmeat and thus risk a recall action.

RESULTS

Results of the study are summarized in tables 1 through 7.

During Phase I the prevalence of L. monocytogenes was 6.56%, 4.9% and 3.8% respectively for swabs taken during operations, post and pre operation of the plants (Table 1). L. monocytogenes was isolated in all parts of the plants. An over all incidence of 5.13% was reported.

The receiving docks, floors from the packing room and picking room, scrap cans and scrap chutes, floor drains, green crab containers were the most likely places to recover *Listeria*. An extensive analysis of the isolation sites suggests that *Listeria* is being carried into the plant by workers in the soles of their feet as they enter the plant in the morning. The scrap cans become contaminated and through the hauling and cleaning of these cans other surfaces of the plant become contaminated.

L. innocua was occurring at a higher rate as shown in Tables 1 and 3, however since the study addressed L. monocytogenes specifically as potential pathogen, L. innocua will not be discussed in this report.

Table 1. Recovery of *Listeria* in Six Crab Plants During Phase I

Time	No. Samples	L. mono (%)	L. innocua (%)
Pre Op	1,000	38 (3.8)	151 (15.1)
Post Op	960	47 (4.9)	133 (13.8)
During	1,097	72 (6.56)	138 (12.6)

Incidence in individual plants is recorded in Table 2. It varied from 0.99% to 1.59%. Considering the small number of plants it was estimated that the differences were not significant enough. Incidence rates at the the two control plants however were significantly different from those observed in the four plants. This also indicated what was suspected all along: maily that the institution of a strict quality control program helps reduce the incidence of *L. monocytogenes* in plant environment. This was further demonstrated in the case of Plant I (see Table 3.) which was undergoing a rigorous quality assurance and critical control points hazard analysis (HACCP) at the time of the survey. It was also interesting to note that both control plants were moderate to large processors and that both purchased raw crab from smaller suppliers.

During Phase II, incidence of L. monocytogenes in the ten plants selected varied from zero to 46% as shown in Table 4. below. Some of these plants are the same. Further more environmental incidence did not allow any prediction of the outcome based on redults obtained in Phase I. Plant II is an appropriate example where less than one percent (0.99%) and 22.6% were observed respectively. One can only suggest that the ubiquitous presence of L. monocytogenes coupled with an unstable work force are factors in the recovery rates observed.

Table 2. Prevalence of L. monocytogenes in Crab Plant Environments During Phase I (1989-91)

Plant	No Sites	Pre Op (%)	Post Op (%)	During (%)	Cummulative (%)
I	503	1.25	2.5	1.09	1.59
II	502	1.25	1.25	0.55	0.99
III	497	1.88	2.5	0	1.41
IV	548	1.00	1.88	1.06	1.27
V	504	1.25	0	0.54	0.59
VI	503	16.87	21.25	36.06	25.25
Total	3,057	3.8	4.9	6.56	5.13

The 1993 season's results are summarized in Table 4. Incidence varies from 0% to 46.7%. The important point to note here is that plants I, III and VI have remained free of L. monocytogenes whereas it is being consistently recovered in plant V. Differences in plant practices and work flow may account for the persistence in one and reduction in the other three plants. A summary of incidences is presented in Table 5 to illustrate the point above.

Table 3. Recovery of *Listeria* in Crab Plants Environments During Follow Up Swabbing in 1992

Plant	No. Sites	Pos. Sites	% <i>Listeria</i>	% <i>L. mono</i>
I	30	0	0	0
II	30	9	0.3	22.6
III	30	0	0	0
IV	31	11	35.5	32.25
V	31	7	22.6	6.4
VI	31	5	16.1	0
VII	31	14	45.2	12.9
VIII	30	2	6.7	6.7
IX	30	8	26.7	0
X	31	3	9.7	3.2
Total	305	59	19.3	8.5

Finally we note the prevalence of *L. monocytogenes* among listeria isolates during the study in Table 6. Other species such as *L. seeligeri*, *L. welshmerii* were found but at frequencies less than 1.0%. *L. innocua* was the main competitor of *L. monocytogenes* in isolation rates as shown in Tables 1 and 3. The frequencies of all listeria species in the different plants and the overall rates are shown in Tables 3 and 4. The recovery rates expressed as numbers of *L. monocytogenes* isolates among all *Listeria* isolates is presented in Table 6.

Table 4. Recovery of Listeria in Crab Plants Environments During Follow Up Swabbing in 1993

Plant	No. Sites	Pos. Sites	% Listeria	% <i>L. monocytogenes</i>
I	30	0	0	0
II	44	3	6.8	2.3
III	30	0	0	0
IV	30	15	50	46.7
V	36	6	16.7	11.1
VI	36	3	8.3	0
VII	35	11	31.4	2.8
VIII	30	2	6.7	3.3
IX	31	13	4.2	29.0
X	30	1	3.3	3.3
Total	332	54	16.3	9.0

We also note that *L. monocytogenes* account from one fifth (20.9%) to half (50.8%) of all the isolates.

Table 5. Incidence of *L. monocytogenes* in Crabmeat Plant Environments During Operations

Plant	1992a (%)	1992b (%)	1993 (%)
I	2.3	0	0
II	2.5	22.6	2.3
III	0	0	0
IV	5.0	32.2	46.5
V	5.0	6.4	11.1
VI	2.5	0	0
VII	2.5	12.9	2.8
VIII	5.0	6.7	3.3
IX	12.5	0	29.0
X	2.5	3.2	3.3
Total	4.0	8.5	9.0

a: regular season; b: follow up

Table 6. Incidence of *L. monocytogenes* in Crabmeat Plant Environments During Three Crabbing Seasons

Year	Incidence	Prevalence
1991	5.13	NA
1992a	4.0	20.9
1992b	8.5	43.3
1993	9.0	50.8

DISCUSSION

In this study we have shown that *L. monocytogenes* is present in crabmeat plant environments. Other species isolated included *L. innocua* from all surfaces examined, *L. ivanovii* from scrap cans, chutes, picking room floors, *L. seeligeri* from the receiving docks, cooling rooms, picking room floors, scrap cans and chutes, and *L. welshimerii* from picking room floors, packing room, scrap.

The question of thermal tolerance (5, 10, 11) of listeria does not arise here since what we observed was a pattern of recontamination of the finished product from the soiled waste baskets to the picking tables via the hand trucks and eventually to the picked crabmeat.

The method of isolation used a combination of FDA and USDA in that swabs were kept in cold enrichment for two weeks and replated before final results were reported.

The incidence rates for different plants cannot be used as predictors for incidence from season to season. Rather the institution of a rigorous cleaning and sanitizing program and strict adherence to it are most important factors in controlling the incidence in plant environments thereby reducing the risk of product contamination. *L. monocytogenes* is susceptible to range of disinfectants and sanitizers which are readily available to plant operators. Rossmore and Drenzek (18) and Chermack (6) have shown that efficacy differences existed among biocides approved for use in the food industry and the seafood industry in particular. These products are usually adversely affected by the organic load present at the time of their application.

Farber and Peterkin (7) have summarized a series of listerioses in which listeria was implicated. This table reaffirms the general statement that *Listeria* is a potential food borne pathogen in the elderly, the young, and the immunocompromized hosts. The association of listeria with foodborne outbreaks in which seafood was implicated was described by Lenon et al. (15) in New Zealand.

While a zero tolerance for *Listeria* in shellfish plants is a goal of FDA's Office of Seafood Safety, it might be argued that the frequency rates reported here show the difficulty in eliminating *L. monocytogenes* from seafood plant environments without pushing the cost of the product beyond affordable levels. This was noted in other studies presented at the *Listeria* Conference of Baltimore sponsored by the National Sea Grant, Virginia Sea Grant and the National Blue Crab Industry Association in 1992.

Finally a manual of blue crab operation was developed. It is available through the University Extension Service and the Department of Health's Division of Shellfish. Even in instances where HACCP could be effective the size of the plant did not make a difference in the outcome of seasonal swabbings. What was most important was the commitment of the owners and operators and their perceived risk of economic loss.

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SURVIVAL OF *LISTERIA MONOCYTOGENES* IN LOBSTER MEAT DURING SELECTED HEAT TREATMENT CONDITIONS

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The occurrence of *Listeria monocytogenes* (LM) in crustaceans is a cause of concern for consumers, processors and regulatory agencies. Researchers have reported the presence of LM in lobster (8, 9). The thermal death time for LM in lobster meat has been studied by Budu-Amoako *et al* (2). The Department of Fisheries and Oceans Canada (DFO) has published heat tables to destroy LM in establishments where environment samples have tested positive. In-plant heat treatment of contaminated lobster is often carried out at 80 C or higher. Given that elevated temperatures considerably reduce drained weight and quality of lobster, the present study attempted to determine if LM would survive less harsh heat treatments, yet still be LM-free. The objectives were: (1) To verify if, in the 60 to 80 C range, the partial or full heating times proposed in DFO table A are adequate to completely destroy LM cells inoculated in 320g cans of lobster meat; (2) To evaluate the effect of such heating exposures on processed meat yields.

MATERIALS AND METHODS

Preparation of LM strains

Five strains of LM were provided by Health and Welfare Canada (Sir Frederic G. Banting Research Centre, Tunney's Pasture, Ottawa, Canada): HPB #4 (milk); Scott A (patient); HPB #563 (ground meat); HPB #395 (Ker Noella brie) and HPB #397 (alfalfa). The strains were kept at 4 C on Triticase soy agar (TSA). The preparation of the inocula was done according to the procedure recommended by Brown for thermal studies (1). Prior to each thermal study, the five strains were transferred twice on Triticase soy broth (BBL) with 0.5% yeast extract (BBL) and incubated at 35 C for 24 hours. Two mL of each strain were mixed, centrifuged at 2900 rpm, and the cells were suspended in 10 mL of 0.1% peptone water. The average number of LM cells was $6.4 \times 10^6/\text{mL}$.

Preparation of lobster meat cans

Forty eight hours before each thermal study, live lobster was purchased from a local processor (Paturel's Seafood Ltd, Shediac). It was cooked 15 min in boiling water and immediately cooled in cold water for 5 min. The meat was shucked and kept overnight at 4 C. Each metal can (404 x 206) was fitted with a thermocouple (T-type) placed in the geometric centre. A port of entry for inoculation was also screwed in the can. Each can was filled with 320g of lobster meat, and 140g

of a brine solution containing 1.5% NaCl and 1.5% sodium tripolyphosphate. A lobster tail was fixed on the tip of the thermocouple with fine polyester thread and the can was hermetically sealed (Seamer model HO, American Can Canada Inc). For each trial, two non-inoculated cans were immediately set aside at 4°C to determine initial aerobic count. For each of the experimental cans, the screw of the inoculation port was opened and 1 mL of inoculum was injected in the geometric centre of the can, within the tail fixed on the thermocouple. Nine additional milliliters of brine were added so as to adjust the amount of liquid to 150g. The inoculated cans were kept overnight at 4°C since it has been suggested that LM cells may need time to adjust to their environment in order to multiply (4). Given that freezing at -18°C can inactivate LM cells (3), the lobster cans were not frozen but kept at 4°C.

Heat treatment of lobster cans

Table 1 summarizes the temperatures and time exposures of lobster. Each treatment was repeated six times.

In order to reduce come-up time and better simulate in-plant processing conditions, the experimental lobster cans were submerged in a circulating water bath (Blue M, Electric Co.) which was set at 10°C higher than the projected temperature of exposure. All thermocouples utilized to follow heat penetration were connected to a data logger (Beckman Industrial, model 245). As soon as the central internal temperature of the cans reached 10°C less than the projected study temperature, they were transferred to another circulating water bath (Tecator, model 1024) precisely set at the desired temperature of exposure. When the latter temperature was reached, the cans were removed at the exact exposure time appearing in table 1, and they were cooled in a 7°C water bath until they reached an internal temperature of 30°C. They were kept overnight at 4°C prior to microbiological analyses.

Drained weight

Each can subjected to heat treatment was opened and drained for 90 sec according to the technique used by DFO (A.B. Dewar, 1980, DFO-Halifax, personal communication). The meat was weighed in order to calculate changes in yield attributable to treatment.

Microbiological analyses

Aerobic plate count was determined on two non-inoculated cans for all treatment temperatures studied. The official DFO method (7) was used to enumerate the aerobic organisms present prior to heat treatment. Appropriate dilutions were made and the Petri plates were incubated at $25 \pm 0.5^\circ\text{C}$ for 72 ± 3 hours. All dilutions were done in duplicate.

The LM count in lobster meat was determined after inoculation according to the following method : the lobster can content was macerated in a Waring blender; a 1:10 dilution was made with sterile 0.1% peptone water and subsequent dilutions were made with the same medium; TSA plates containing 5% yeast extract were prepared in duplicate to obtain LM counts. The Petri plates were incubated at 35°C for 72 hours. The microbiological analysis system devised by VITEK (Biomérieux Vitek Inc, Hazelwood Missouri) was used to identify LM. A modified method devised by Health and Welfare Canada which utilizes the Fraser broth (6) was used to confirm the presence of surviving LM cells at the level of less than 1 cell/100g.

Table 1. Temperature and time of heat exposure of lobster samples

Temperature (°C)	Exposure time (min : sec)	Percent of exposure time requested by DFO
60	0:00	0
	5:52	25
	11:44	50
	17:36	75
	23:28	100
64	0:00	0
	1:34	25
	3:09	50
	4:44	75
	6:18	100
68	0:00	0
	0:50	50
	1:15	75
	1:41	100
72	0:27	100
76	0:07	100
80	0:02	100

Statistical analyses

The STATVIEW 4⁺ program (5) was utilized to compute statistical analyses on the raw data. Analyses of variance were done and Fisher's least significant difference was calculated to compare means. The level of statistical significance was set at 0.05%.

RESULTS AND DISCUSSIONMicrobiological counts prior to heat treatment

The number of aerobic organisms present in non-inoculated lobster meat prior to heat treatment is presented in Table 2. The mean number of colony forming units (CFU's) was 3.6×10^6 /g.

Table 2. Microbiological data on non-inoculated and inoculated lobster samples prior to heat treatment

Planned temp. of exposure (°C)	Aerobic count (CFU/g) ¹	<i>Listeria monocytogenes</i> in inoculated lobster (CFU/g) ¹
60	2.7×10^3	6.6×10^6
64	1.3×10^3	5.0×10^6
68	9.3×10^3	8.8×10^6
72	2.9×10^3	3.8×10^6
76	2.9×10^3	3.8×10^6
80	2.9×10^3	3.8×10^6
Mean	3.6×10^3	6.4×10^6

¹ n = 2 for each temperatureCome-up time and heating curve

Figure 1 illustrates the come-up time and heating pattern for each of the six replicates (A to F) at the 60°C trial during 23 min: 28 sec, the time which corresponds to the full heating exposure requested by DFO table A. Initial internal temperature of the cans was $5.9 \pm 0.8^\circ\text{C}$ when they were introduced in the 70°C water bath. When the cans A to F reached an internal temperature of 50°C, they were transferred to the 60°C bath. Total come-up time from 5.9 to 60°C averaged 21.2 ± 1.8 min. For the other trials conducted at 60°C, the cans were removed after the selected heat exposure times presented in Table 1 were reached. A similar procedure was followed for the cans treated at 64, 68, 72, 76 and 80°C.

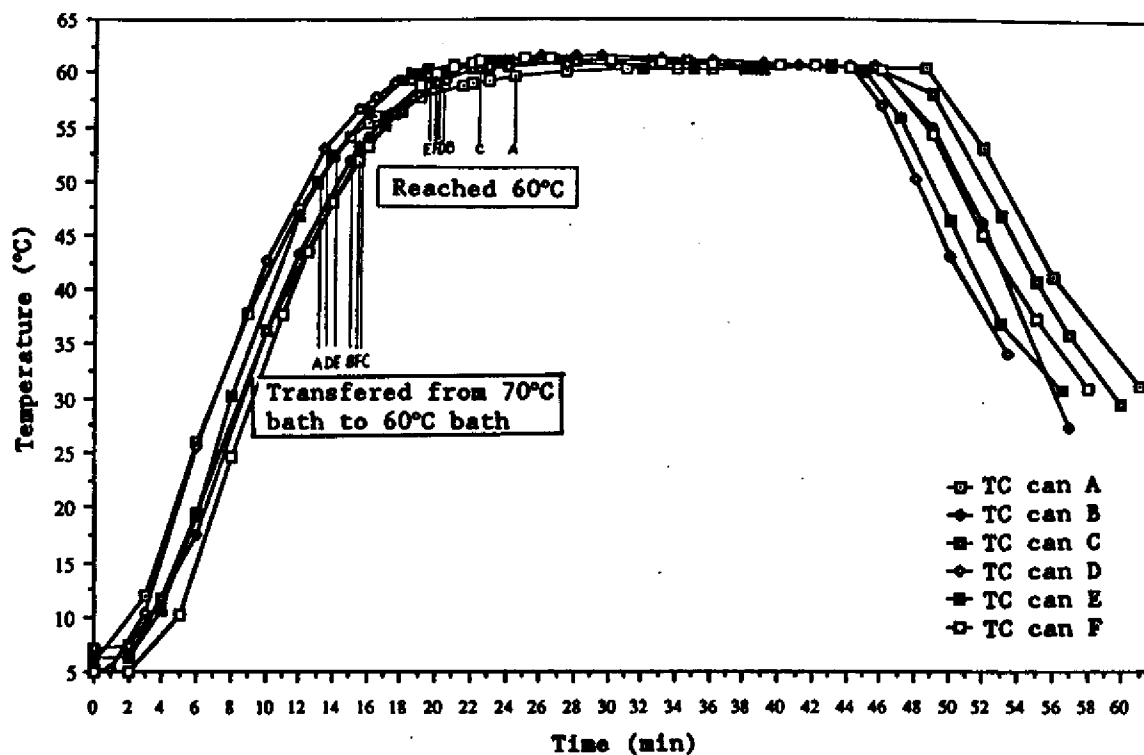


Figure 1. Temperature curves in the center of lobster cans taken from 5.9°C up to 60°C , held there 23 min : 28 sec and cooled.

Mean come-up times to arrive at the targeted temperatures of exposure were between 21 and 25 minutes (Fig. 2). This must be kept in mind when interpreting the data on LM inactivation since partial destruction of LM cells can occur during the come-up time.

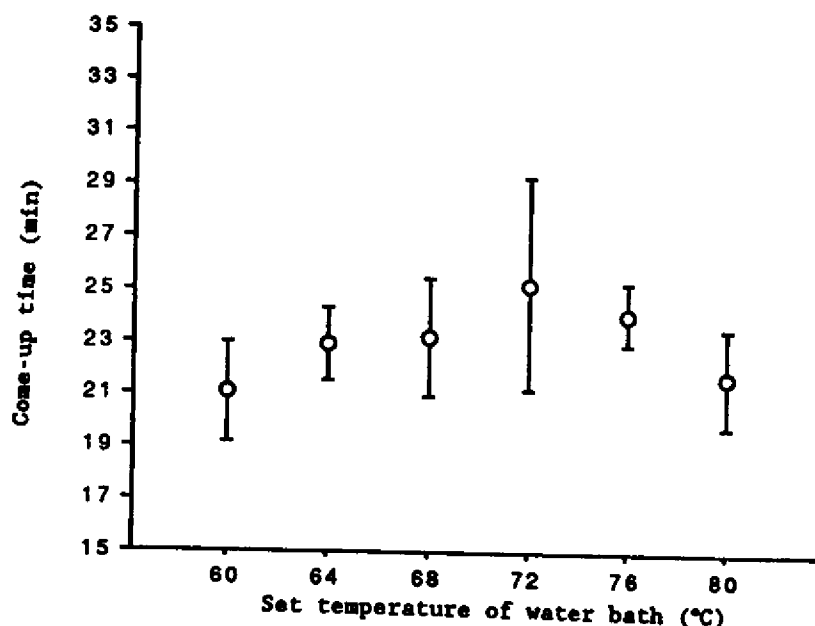


Figure 2. Mean and standard deviation of come-up time for 320g of lobster meat heated at selected temperatures between 60°C and 80°C.

Microbiological data after heat treatment

Table 3 summarizes the microbiological data on the occurrence of LM cells in 320g samples of lobster meat subjected to selected heating treatments.

It was found that at 60°C, unless heat exposure was longer than 11 min : 44 sec, LM colonies were found on TSA plates. However, with a heat treatment of 17 min : 36 sec, LM colonies no longer occurred on TSA plates but, in 5 out of 6 samples, there was a darkening of the Fraser broth and the VITEK system confirmed the presence of LM. A heat treatment of 23 min : 28 sec at 60°C, as requested by DFO, prevented colony growth on TSA but some LM survived in four out of six trials. The four LM positive Fraser broth samples were submitted to the VITEK analytical system and indeed, LM was identified in each case. This finding indicates that a contamination level of 6.6×10^6 with LM requires a longer heat treatment at 60°C than the 23 min : 28 sec requested in Table A issued by DFO.

Table 3. Survival of *Listeria monocytogenes* subjected to various heating processes.

Temperature (°C)	Time of exposure (min : sec)	<i>Listeria monocytogenes</i> (CFU/g) ¹	# of + in Fraser broth ^{1,2}
60	0:00	2.0×10^4	6
	5:52	4.7×10^2	6
	11:44	4.0×10^0	5
	17:36	- ³	5
	23:28	-	4
64	0:00	-	3
	1:34	-	0
	3:09	-	0
	4:44	-	0
	6:18	-	0
68	0:00	-	0
	0:50	-	0
	1:41	-	0
72	0:27	-	0
76	0:07	-	0
80	0:02	-	0

¹N = 6 for each treatment²Darkening of Fraser broth and confirmation of LM³No growth on TSA

At 64°C, no LM growth occurred on TSA when the lobster was held for either 0 sec, 1 min : 34 sec, 3 min : 9 sec, 4 min : 44 sec, and 6 min : 18 sec. However, at zero sec, when the lobster just reached an internal temperature of 64°C, three of the six samples gave a positive response in the Fraser broth and all three were later confirmed positive for LM.

When the lobster samples reached 68°C and beyond, all samples were devoid of LM, even if they were not held at those temperatures for any length of time. This signifies that in-plant heat treatment could safely be carried out at 68°C and beyond, as there is a total inhibition of this pathogenic organism in lobster meat with such heat exposures.

Relationship between lobster meat yield and heat treatment

Table 4 presents the percent weight changes observed in lobster meat after subjecting it to selected heat treatments.

Table 4. Percent weight changes observed in 320g of lobster meat subjected to selected heat treatments

Temperature of treatment (°C)	Exposure time (min : sec)	Weight change (%)
60	0:00	+0.52 ¹ ± 0.99 ^{cd}
	5:52	+0.14 ± 1.31 ^{cd}
	11:44	-0.38 ± 0.97 ^{bcd}
	17:36	-0.28 ± 1.11 ^{bcd}
	23:28	+0.17 ± 0.64 ^{cd}
64	0:00	+0.58 ± 1.03 ^{cd}
	1:34	+0.81 ± 0.90 ^d
	3:09	-0.43 ± 0.43 ^{bc}
	4:44	-0.27 ± 0.84 ^{bcd}
	6:18	+0.14 ± 0.81 ^{cd}
68	0:00	-0.06 ± 0.87 ^{bcd}
	0:50	0.00 ± 1.95 ^{bcd}
	1:41	-0.37 ± 1.58 ^{bcd}
72	0:27	-0.24 ± 0.43 ^{bcd}
76	0:07	-1.13 ± 0.15 ^b
80	0:02	-3.33 ± 1.48 ^a

¹ Means associated with the same letter are not significantly different according to Fisher's least significant difference (p<0.05)

Very little weight changes occurred between 60 and 72°C at the specific heating times tested. Even at the 76°C exposure for 7 sec, the 1.13% weight loss observed did not differ significantly from some of the samples heated between 60 to 72°C. However, at 80°C, there was a 3.33% weight loss and this value was significantly different from all of the other temperatures tested. It must be kept in mind however, that the canned lobster used in this study was not frozen prior to measuring the drained weight, a procedure which departs from current industrial practices. Nevertheless, it can be stated that heat treatment of lobster meat at 80°C is detrimental to yield. Extensive protein denaturation is conducive to a loss in water holding capacity.

CONCLUSION

The results obtained in the course of this study indicate the following:

- At 60°C, heat treatment for 23 min : 28 sec is not sufficient to completely destroy *Listeria monocytogenes*.
- At 64°C, a short heat exposure is required to completely inactivate *Listeria monocytogenes*. With a 1 min : 34 sec exposure, complete destruction has occurred. Given that very little loss in yield is observed at 64°C, contamination risks are reduced by heat treating the lobster for 6 min : 18 sec which is the exposure time recommended in DFO table A.
- At 68°C, *Listeria monocytogenes* is completely destroyed. Treatment at a higher temperature is not necessary for lobster meat.
- Short time heat exposures between 60 and 72°C have minimal effects on the drained weight of lobster meat.
- Optimal heat treatment for 320g of canned lobster lies between 68 and 76°C when come-up time is between 21 and 25 min.

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***LISTERIA MONOCYTOGENES* AND *SALMONELLA* SSP.
IN MODIFIED ATMOSPHERE PACKAGED CHANNEL CATFISH**

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Fish consumption has been on the rise, especially fresh fish products. However, fish are very susceptible to spoilage and losses may be as high as 50% (10). Spoilage of fresh fish is caused mainly by microbial action. Fish are usually in cold waters and thus the predominant gram negative bacteria are able to grow well in refrigeration. One promising technology to extend shelf-life of fresh fish is modified atmosphere packaging, MAP (2). This technology is usually used in combination with refrigeration. This has proven to extend the shelf-life of hybrid bass strips (7) and channel catfish strips (16). Some pathogens may be able to grow in MAP products due to lack of competitive spoilage bacteria and anaerobic conditions created. *Salmonella* spp. (3) and *Listeria monocytogenes* (5) have been found in fish. *Salmonella* spp. have been isolated from channel catfish (CC) fillets (17) and *L. monocytogenes* has been found in CC viscera and fish flesh (9, 17). Some of these pathogens are able to grow at low temperatures and under microenvironments. The objectives of this work were to study the growth of *Salmonella* spp. and *L. monocytogenes* at various temperatures under MAP and look at the effect of a marinade on pathogenic growth inhibition.

MATERIALS AND METHODS

Fresh channel catfish fillets were packaged in 3.0 mil polyethy-lene bags, packed on ice and within 2 h, cut into strips weighing approximately 10 g. Strips were packaged under the corresponding environment after being dipped in 200 ppm chlorine (1:10 fish/chlorine solution v/w) for 2 min, inoculated with 10^4 CFU/mL of the appropriate bacteria (*L. monocytogenes* and *Salmonella* spp.), and stored at 0°C or 10°C. Evaluations were performed for 28 d.

Packaging

Strips were packaged either under air (AIR) or under 90% carbon dioxide balanced with air (MAP). Sixteen strips were placed in 2.7 mil polyethylene bags (Dow Chemical Co., Indianapolis, IN) -- AIR, or in preformed 21.5 x 25 cm² B700 barrier bags (Cryovac Division, W. R. Grace Co., Duncan, SC) -- MAP. The MAP bags were evacuated and backflushed with CO₂ using a Multivac "AGW" (Koch, Kansas City, MO) packaging machine.

CO₂ and O₂ measurements

A Servomex 1450 Oxygen and Carbon Dioxide Analyzer (Norwood, MA) was used to measure the percentage of CO₂ and O₂ of individual packaged samples. The packages were sampled by drawing 30 mL of headspace gas into a 50 mL gas-tight syringe which was then injected into the analyzer.

Preparation of inoculum

One mL inoculum each of 10⁴ CFU/mL of *L. monocytogenes* and *Salmonella spp.* was prepared by incubating a 1 mL frozen cell concentrate in 250 mL of Tryptic Soy Broth and Brain Heart Infusion Broth (Difco, Detroit, MI) respectively, for 24 h at 35°C. Aseptically, 13 mL of each bacterial enriched broth were dispensed in four pre-sterilized centrifuge tubes and centrifuged at 3400 RPM for 5 min using a Centrifric Model 228 Centrifuge (Fisher Scientific). The supernatant from each of four tubes was decanted and the pellets were combined and homogenized to give a final volume of 1 mL.

The cell homogenates were dispensed in 9 mL of 0.1% sterilized peptone solution. A second dilution was made when 3 mL of the previous dilution was suspended in 9 mL of 0.1% sterilized peptone solution. The Optical Density (OD) of the second dilution was measured using a Bausch and Lomb Spectronic 20D spectrophotometer (Milton Roy Co., Rochester, NY). The instrument was calibrated using 0.1% sterilized peptone to measure the absorbance at 540 nm. The OD₅₄₀ of the samples was recorded and labeled as the original dilution. This step was repeated until the samples gave an absorbance of approximately 1.00.

From the original dilution serial dilutions were made with 9 mL of 0.1% sterilized peptone solution. Aseptically, 1 mL from each dilution was pour plated in duplicate, using Standard Plate Count Agar (Difco, Detroit, MI) as the nutrient for the microorganisms. The enumeration of colony forming units per gram of sample (CFU/g) was performed after 48 h incubation at 35°C in an aerobic growth chamber. The corresponding OD values and decimal dilutions were labeled upon each plate. Linear regression was used to correlate OD with values obtained from plate counts.

Microbiological evaluation

Bacterial counts were performed by methods described by Benson (1). After aseptically weighing 25 g, sample was homogenized in 225 mL of 0.1% sterilized peptone solution in a blender for 1 min on low speed. Appropriate decimal dilutions were prepared in 0.1% sterilized peptone solution.

Enumeration of presumptive *L. monocytogenes* and *Salmonella spp.* were done by the pour plate method on selective media using 1 mL of the appropriate dilution. McBride Listeria Agar and MacConkey Agar (Difco, Detroit, MI) were used as the selective media for *L. monocytogenes* and *Salmonella spp.*, respectively. Enumeration of colony forming units per g sample (CFU/g) was performed after 48 h incubation at 35°C.

Addition of marinade

A second experiment was devised to study synergistic effect of MAP and a seasoning mix on fish pathogens. Channel catfish fillets were prepared and inoculated as discussed earlier and treated with 10% tartar mix (TM) (Saffron International, Tupelo, MS) and without tartar mix (CT). They were then packed in 2.7 mil polyethylene bags (Dow Chemical Co.) Air or in B700 (Cryovac) bags and backflushed with 90% CO₂. They were stored at 10°C and analyzed every 4 d for 16 d. Samples were analyzed for CO₂, O₂ and enumeration of *Salmonella* spp. and *L. monocytogenes* as described earlier.

RESULTS AND DISCUSSION

Carbon dioxide concentration in inoculated samples without marinade fluctuated between 84 and 92% while oxygen ranged between 1.5 and 3.5% (data not shown). This range was the result of the lack of tighter control by the packaging machine.

Presumptive *L. monocytogenes* counts on non-marinated CC are shown in Figure 1. Counts for CC packed in AIR at 10°C increased from log 6 CFU/g to over log 8 CFU/g in 4 d while counts for CC stored at 0°C reached over log 8 CFU/g after 6 d. Thus, low temperature had little effect on growth of *L. monocytogenes* in CC strips stored in AIR bags. Harrison et al. (8) reported no growth of *L. monocytogenes* on iced fish and shrimp for as long as 21 d. However, Glass and Doyle (6) observed growth of *L. monocytogenes* at 4°C, in various meat products. *Listeria* are known to grow over a wide temperature range in many fish products (5). Growth of *L. monocytogenes* in MAP at 10°C was similar to that in AIR at 0°C. However, when stored at 0°C, the lag phase was extended for over 8 d and counts did not reach log 7 CFU/g until 28 d.

Salmonella spp. growth was not inhibited in AIR packed samples at 0 or 10°C (Figure 2). However, growth was inhibited at 10°C in MAP products, and at 0°C there was some bactericidal effect. Silliker and Wolfe (15) stated that CO₂ will slow growth of *Salmonella* under mild temperature abuse. Even though it is a facultative anaerobe, it does not grow well at low temperatures and under high CO₂ concentrations.

Addition of a marinade containing mustard, garlic, citric acid, and some spice extracts was used to study its effect on *L. monocytogenes* and *Salmonella* spp. growth in MAP and AIR packed CC fillets held at an abuse temperature, 10°C. *L. monocytogenes* growth was not inhibited in AIR packs treated or not with a fish marinade, tartar mix (TM) (Figure 3). Growth was enhanced in MAP packed products between 6 and 12 d of storage. This might be the result of less competitive bacteria in the MAP product after 6 d. Rorvik et al. (12) reported faster growth of *L. monocytogenes* in vacuum-packed salmon with low initial counts. Addition of tartar mix (marinade) to MAP fillets showed bacteriostatic effect for 4 d but did not lower growth rate after 4 d.

Some of the aromatic compounds in spices are bacteriostatic (13). Shelef et al. (14) reported that rosemary was bacteriostatic at 0.3% and bactericidal at 0.5%. At 2%, rosemary has been shown to be more effective against gram positive bacteria. Mustard contains allyl-isothiocyanate, a compound known to be bacteriostatic to *S. aureus* and other pathogens (11). Garlic contains a diallyl-thiosulfate called allicin which is bacteriostatic to yeasts (4).

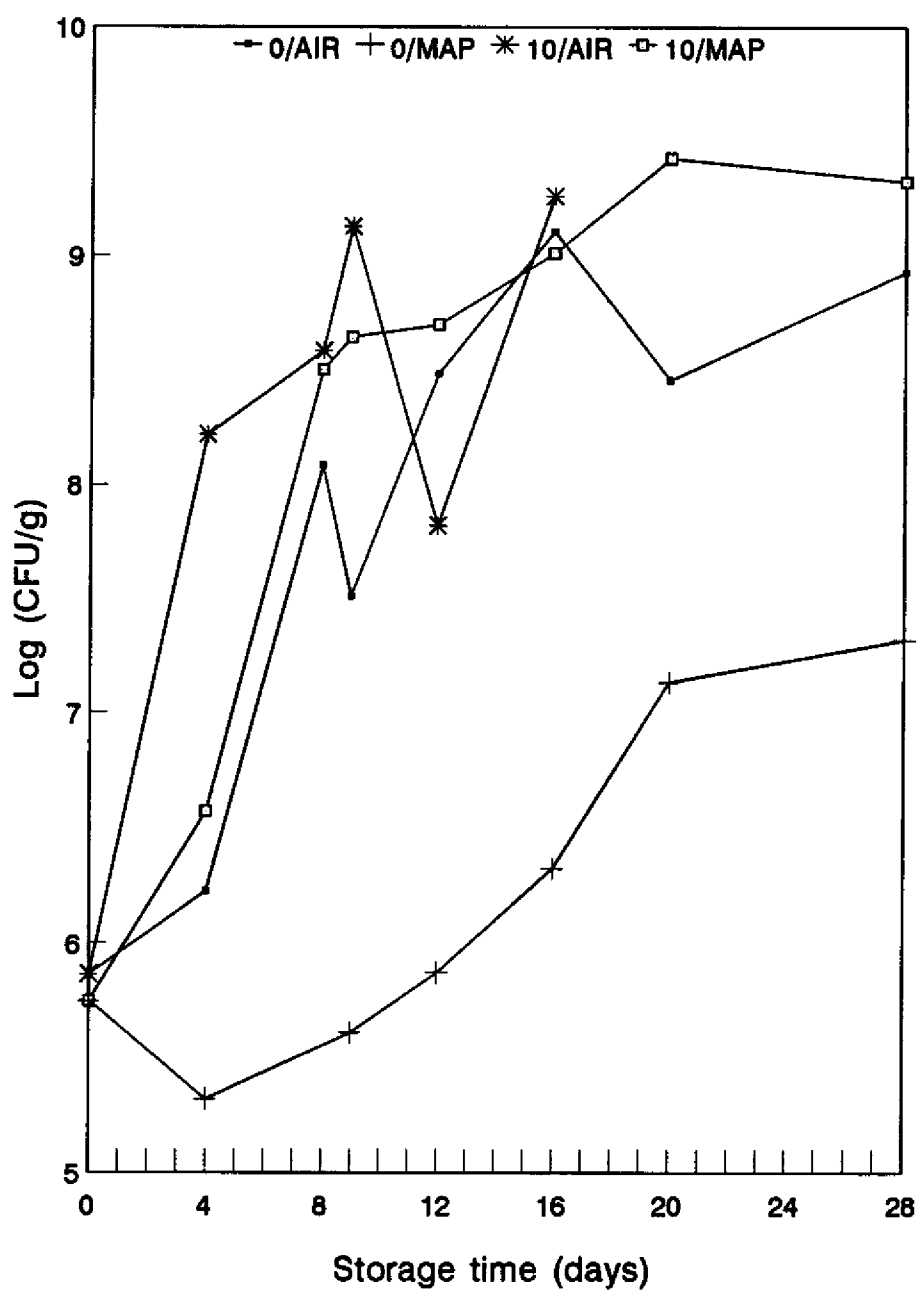


Figure 1. Presumptive *Listeria monocytogenes* in channel catfish fillets stored in air (AIR) and 90% CO₂ (MAP) at 0 and 10°C.

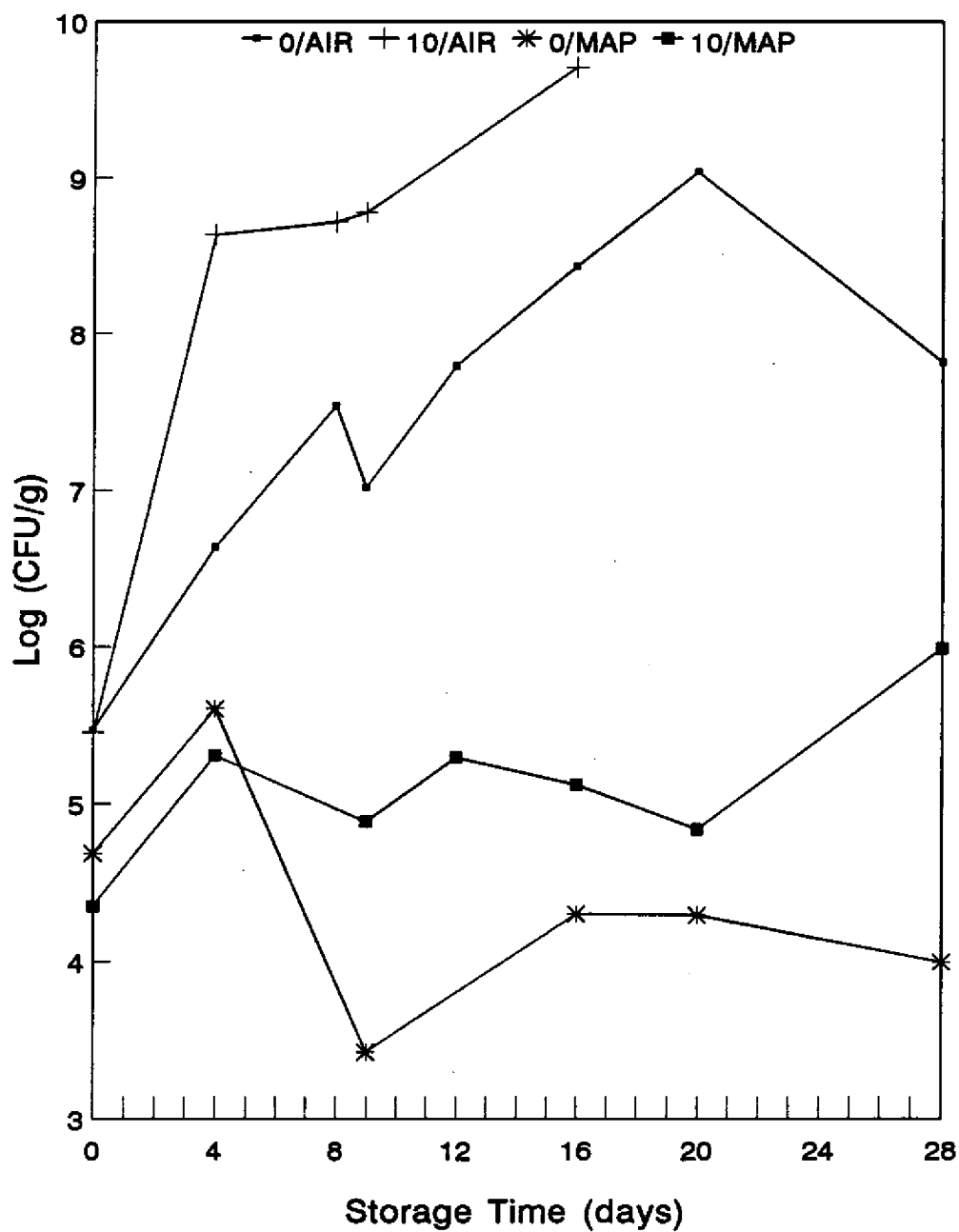


Figure 2. Presumptive *Salmonella* spp. in channel catfish fillets stored in air (AIR) and 90% CO₂ (MAP) at 0 and 10°C.

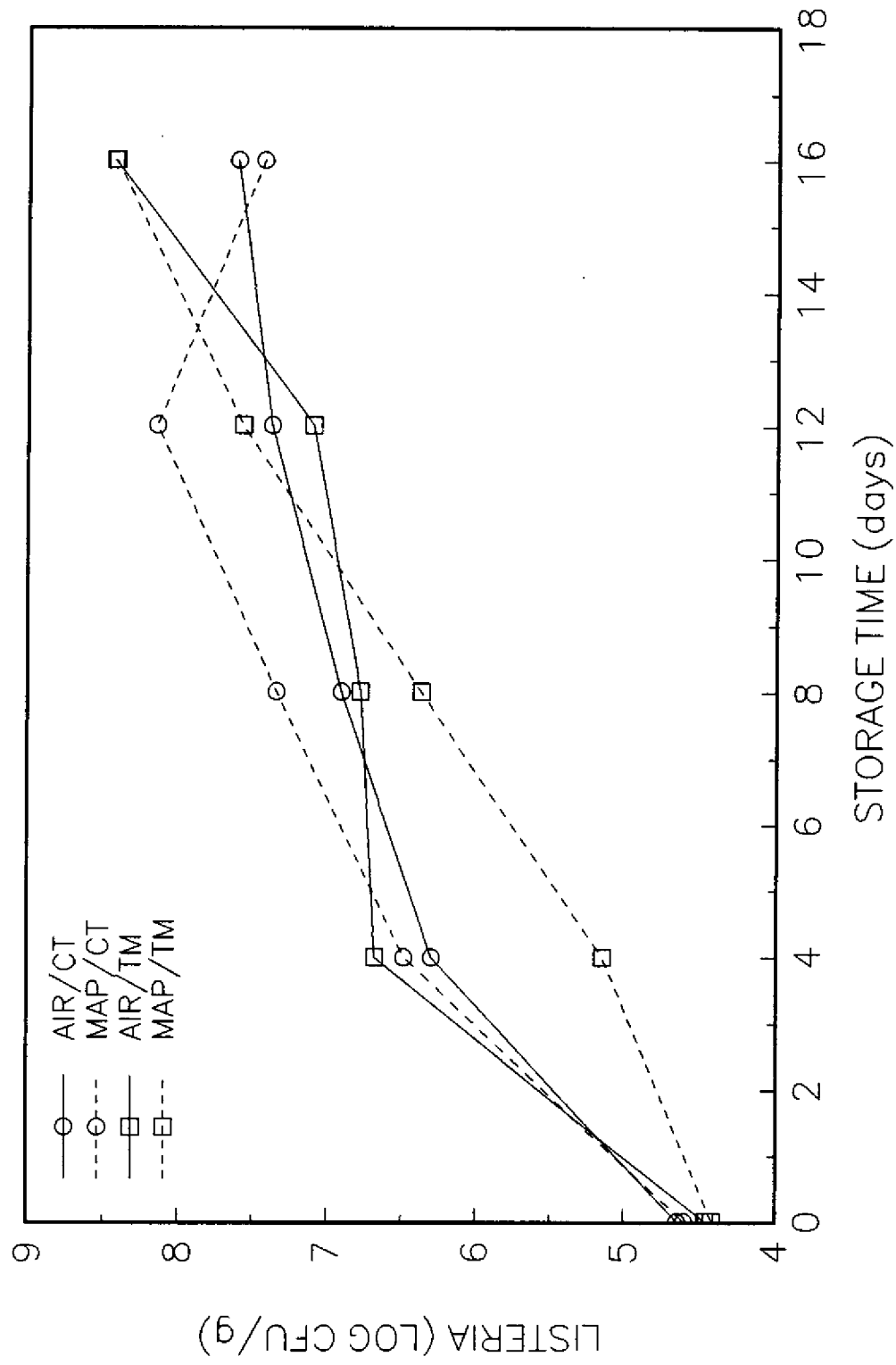


Figure 3. Presumptive *Listeria monocytogenes* in channel catfish filets under air (AIR) and 90% CO₂ (MAP) without (CT) and with tartar mix (TM).

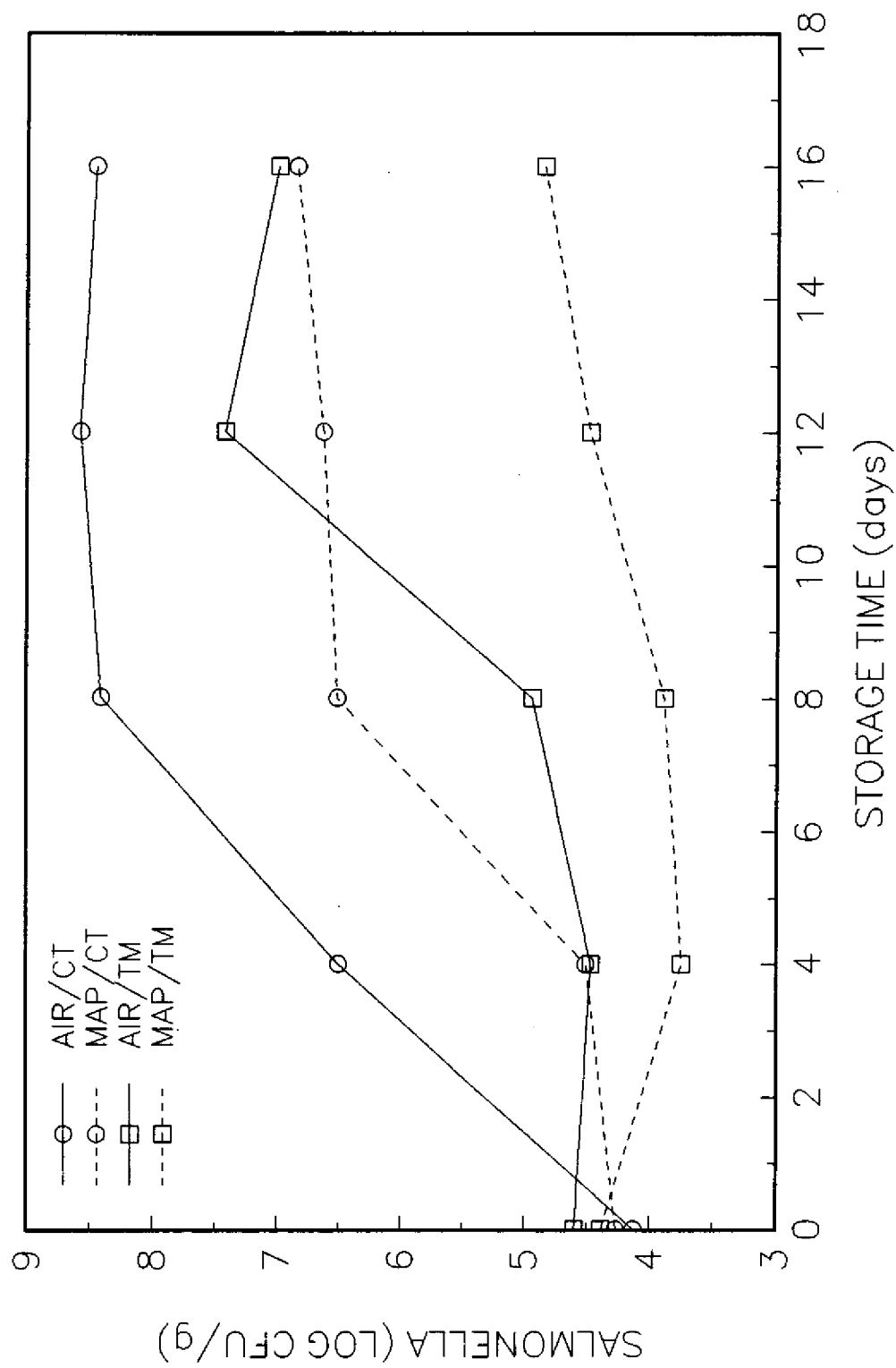


Figure 4. Presumptive *Salmonella* spp. in channel catfish fillets under air (AIR) and 90% CO₂ (MAP) without (CT) and with tartar mix (TM).

Salmonella spp. lag phase was extended in CC fillets with tartar mix packed under AIR (Figure 4). Growth was inhibited for 8 d at 10°C. In combination with MAP, tartar mix was bacteriostatic to *Salmonella* spp. for 16 d in fish held at 10°C.

CONCLUSIONS

It is necessary to combine MAP with very low refrigeration temperatures (~0°C) to be effective in delaying growth of *L. monocytogenes* in CC fillets. Abuse temperatures may increase the rate of growth of *L. monocytogenes* in CC fillets under MAP. *Salmonella* spp. growth is inhibited by MAP even at 10°C but some growth may still occur. Addition of a fish marinade containing spices, essential oils and organic acids will extend lag phase of *L. monocytogenes* in MAP CC fillets at 10°C. Thus, the use of a marinade with antimicrobials may inhibit the growth of *L. monocytogenes* in CC fillets refrigerated at low temperatures.

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NONCULTURABLE *VIBRIO PARAHAEMOLYTICUS*

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Nutrient insufficiency is the most common environmental stress which microorganisms routinely encounter. As nature exerts strong selective pressures in the course of evolution, microorganisms have evolved mechanisms to accommodate those adverse environments (9). Numerous studies have been done on the starvation response of marine microorganisms (1, 7, 11, 15, 18, 20). Many marine bacteria can survive for a long period of time by reorganizing their cellular macromolecules and decreasing the endogenous metabolism and respiration rates (15). Several human pathogens, such as *Escherichia coli*, *Vibrio cholerae*, *Salmonella enteritidis*, *Vibrio vulnificus*, *Shigella sonnei*, *Aeromonas salmonicida* and *Campylobacter jejuni*, have been found to enter into a viable but nonculturable stage rather than to die-off when exposed to low nutrient environment (5, 17, 19, 22, 29). Since there are numerous factors affecting bacterial survival, it is important to understand how these food pathogens survive under the stresses of low nutrient and low temperature.

Vibrio parahaemolyticus is an estuarine bacterium widely distributed in the natural aquatic environment around the world. It has been found that the distribution of this organism is highly seasonable (4, 6). During the summer, the frequency and concentration of the isolates are much higher than these in the winter months. Does the organism enter into the viable but nonculturable state during winter? Over 95% strains of *V. parahaemolyticus* from clinical samples are Kanagawa positive (K^+), producing hemolysis on Wagatsuma blood agar, but the majority of isolates from environmental samples are Kanagawa negative (K^-) (16). This phenomena is not yet understood.

In this study presented here, we examined various factors which could affect the culturability of the organism in low nutrient and low temperature conditions. The difference between K^+ and K^- strains was compared. We also examined the method to resuscitate those nonculturable cells.

MATERIAL AND METHODS

Cultures and media

Two environmental isolates, *Vibrio parahaemolyticus* strains 38C1 and 38C6, were used in this study. Strain 38C1 is K^+ and 38C6 is K^- , both donated by Rita Colwell. Overnight cultures were inoculated 1 to 1000 into fresh Proteose Peptone Beef Extract (PPBE; Difco Laboratories, Detroit, Mich.) containing 1% NaCl (3). The cultures were incubated at 35°C with shaking. The cells were grown to an early log phase (OD_{660} of 0.15-0.25) and harvested by centrifugation at 5000 x g for 15 min. The pellets were washed once with starvation medium. The final pellets were suspended uniformly into an equal volume of starvation medium.

Two starvation media were used in this study. The composition of Morita minimal salt medium (MM) per L of deionized water was: 26.0 g NaCl, 0.8 g KCl, 7.6 g $MgCl_2 \cdot 6H_2O$, 5.6 g $MgSO_4 \cdot 7H_2O$, 5.0 g of Tris buffer (pH 7.8), 0.1 g $CaCl_2$, and 0.1 g Na_2HPO_4 and nine salt solution (NSS) consisted of 17.6 g NaCl; 1.47 g Na_2SO_4 ; 0.08 g $NaHCO_3$; 0.25 g KCl; 0.04 g KBr; 1.87 g $MgCl_2 \cdot 6H_2O$; 0.41 g $CaCl_2 \cdot 2H_2O$; 0.01 g $SrCl_2 \cdot 6H_2O$; 0.01 g H_3BO_3 per L of deionized water.

Acid-washed 1 L screw-capped bottles were used as containers for the experiment. The cultures in starvation media were stored at different temperatures for the temperature experiments. All the cultures were kept in static state.

Cell enumeration

Cell culturability was determined by using nonselective PPBE agar plates. Highly selective medium agar, thiosulfate-citrate-bile-sucrose (TCBS), was used to verify the cultures. The starvation media were used to dilute samples. During sampling, all samples exposed to low temperatures were kept in ice to avoid any temperature abuse. The plates were incubated at 35°C for 2 days.

Total cell counts were determined by the acridine orange staining method under epifluorescence microscope. Direct viable counts were measured according to Kogure et al., (13). Samples were incubated at 35°C with 0.05% filtered sterile yeast extract and 0.002% nalidixic acid for 6 hrs. Only those elongated and swollen cells were counted as viable cells. For the total counts and direct viable counts, a total of at least 10 fields with cells in the range of 30 to 300 were counted for each sample.

Temperature upshift

About 2 ml of cultures were aseptically removed from entire microcosm at low temperature to a 10 ml test tube. The tube was left at room temperature for 3 days without shaking. The resuscitated cultures were spread on PPBE plates.

Most probable number (MPN) determination was performed by the three tube series test using PPBE broth as medium prior to temperature upshift.

Scanning electron microscopy

Both log phase young cells in PPBE broth and the cells exposed for various intervals in starvation medium at 3.5°C were harvested by centrifugation. The cell pellets were washed 3 x with sterile Millonig buffer (pH 7.4, 374 mOSM/kg) (10). Bacteria cells were fixed with 2% (vol/vol) glutaraldehyde in Millonig buffer at room temperature for 1 h. The samples were passed through 13-mm, 0.2- μ m pore size Nuclepore polycarbonate filters and then postfixed in 1% osmium tetroxide at room temperature for 1 h, dehydrated in graded ethanol (75% , 95% and 100%), critical point dried in CO₂ in a DCP1-CPD apparatus, and coated with AuPd in a vacuum evaporator coating apparatus Denton DV503. The coated samples were examined in an Amray 1820 D scanning electron microscope.

RESULTS AND DISCUSSION

The cell morphological change occurred immediately after the cells were suspended in mineral media. Before starvation, log-phase cells of *V. parahaemolyticus* were normally rod-like shape with an average dimension of ca. 0.6-0.9 x 1.6-3.1 μ m. Within a couple of days in starvation medium, rod cells started to demonstrate irregular shapes and finally became coccoid or spheroid. The rate for the cell morphological change was dependent on the strains and the temperatures at which the cells were kept. It was found that at a low temperature, the cells became spheroid quicker and the spheres were bigger than cells at room temperature. In this study, it took about 1 to 2 weeks for strain 38C1 to be sphere at 3.5°C, whereas 38C6 needed at least 3 weeks under the same condition. The spheroid cells had an average diameter of 0.83-1.28 μ m for both strains at 3.5°C as compared with an average diameter of 0.6-0.7 μ m at 25°C. After cells became spheroid, little further size reduction continued at low temperature. The cell morphological change under starvation has been well documented for many marine organisms and pathogenic bacteria (7, 11, 15, 22). It has been proposed that cell size reduction at low temperature is a survival strategy for minimizing cell maintenance requirement and enhancing substrate uptake due to high surface to volume ratio (18, 24)

Scanning electron micrographs (Figs. 1a and 1b) showed that log-phase

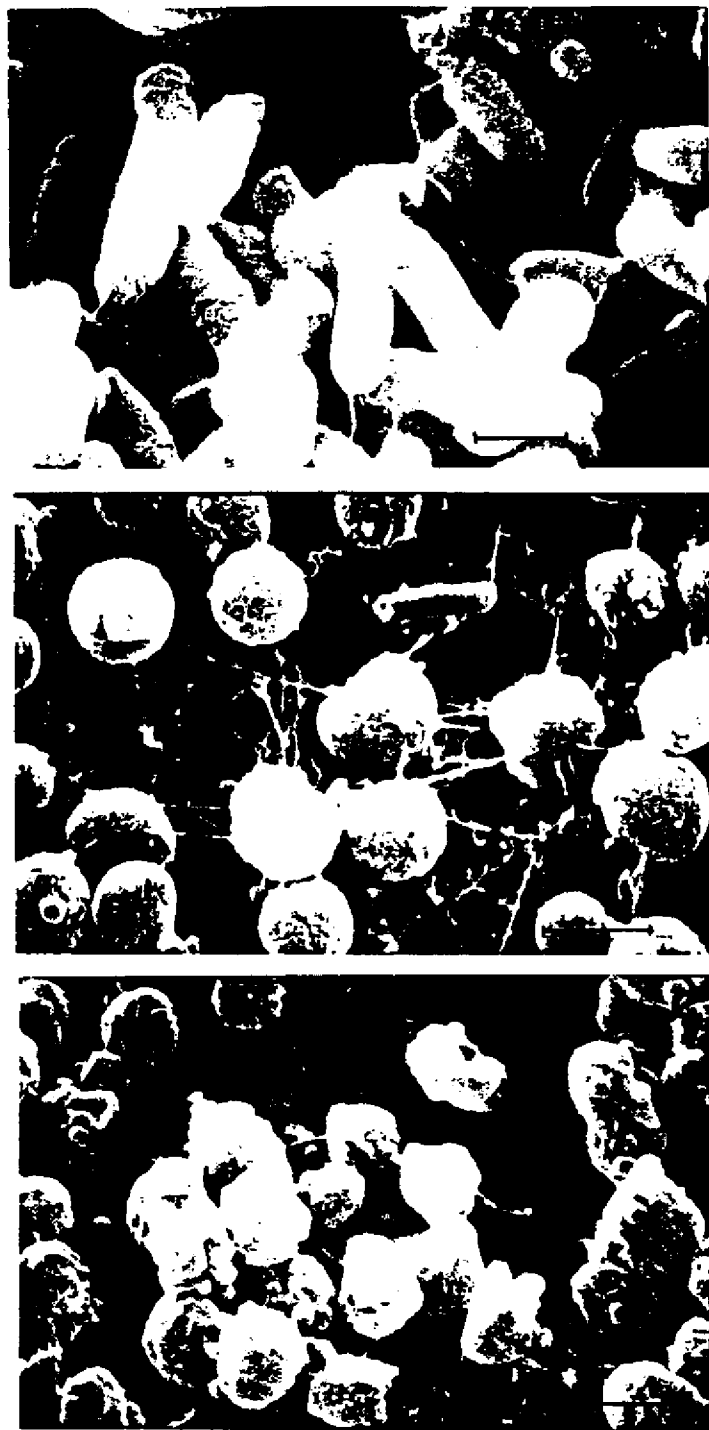


Fig. 1. Scanning electron micrographs of *V. parahaemolyticus* strain 38C1. (a). top, log-phase young cells; (b). middle, 2-wk starved cells at 3.5°C; (c). bottom, 6-wk starved cells at 3.5°C. Bar = 1 μ m.

normal cells were typically rod-like with a single polar flagellum and relatively smooth surface, and 1-wk starved cells were predominately spheroid without flagellum. Interestingly, much polymer-like material formed associated with 1-wk starved cells. It was reported that *Vibrio* DW1 cells formed bridging polymer after 5 h starved in MM medium (7). These polymer-like materials did not look like collapsed flagella since they actually appeared to have some kind of orientation patterns. The functions of these polymers were probably related to cell adhesion. Another important feature of starved cells was the formation of blebs, which appeared in 1-wk starved cells but increased as cells starved at low temperature for longer. The formation of blebs was possibly due to an excess of cell membrane which was too big for shrunken cell contents during starvation. The released blebs from cell surface could possibly supply nutrients for cell regrowth when temperature was upshifted or serve as killing elements for survival cells to maintain viability (27). The 6-wk starved cells did not appear smooth spheroid shapes (Fig. 1c). Despite of completely intact cells, some cells appeared collapsed and parts of the cell envelope adhered together inwardly. *V. cholera* demonstrated dramatic changes in cell shape during long term starvation (11). The thin section micrographs of *V. cholera* showed that cell outer membrane was separated and formed a gap from inner membrane in some part of cell membrane, which agreed with scanning electron micrographs of strain 38C1 of *V. parahaemolyticus* in this study.

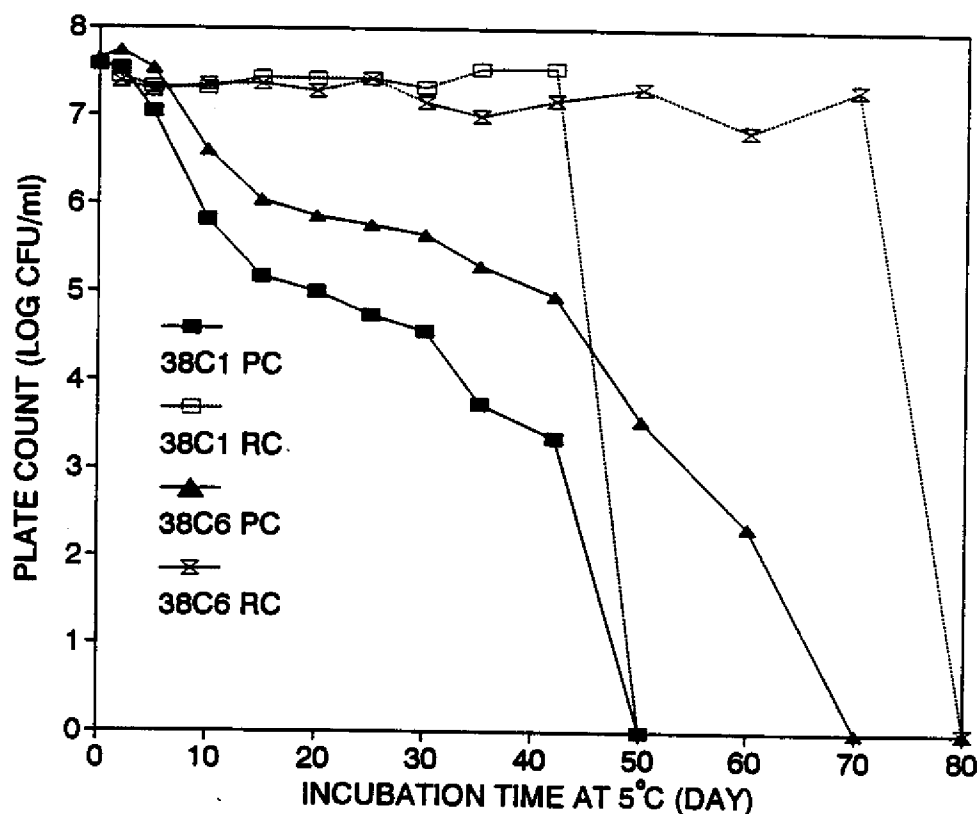


Fig. 2. Survival curves of strains 38C1 and 38C6 during starvation at 5°C.

Fig. 2 showed typical survival curves for both strains 38C1 and 38C6 at 3.5°C in MM medium. The survival curves fell into 3 stages. At beginning of starvation, cells lost culturability rapidly within 2-3 wk, which corresponded to the period of dramatic morphological changes in those cells. Then, a slow decline of culturability persisted for a long time, followed by a quick drop of plate counts to zero.

Many factors could affect the survival of *V. parahaemolyticus* cells. Temperature, medium, physiological age of the cells and the strain difference were among those factors.

Seven temperatures were used to store the starved cultures. They were 0, 2, 3.5, 5, 10, 25 and 35°C. At 10 and 25°C, both 38C1 and 38C6 cells increased their cell numbers at on set of starvation and decreased their cell sizes by more than 50%. Following initial reductive division, the culturability dropped about 1 log cycle quickly within 2 weeks and, then kept constant for a long period of time. Not much difference between survival curves was found for cells stored in 10 and 25°C. Strain 38C1 lost culturability much slower than 38C6 at 25°C (Fig. 3). When temperature was lowered below 5°C, the culturability declined very quickly for both strains. Little difference in culturability was observed at a temperature between 0 and 3.5°C. At 3.5°C, they became nonculturable in 50-80 days. But, at 5°C they survived slightly longer. At an overall low temperature, 38C1 lost culturability much quicker than 38C6. This agreed with the morphological changes.

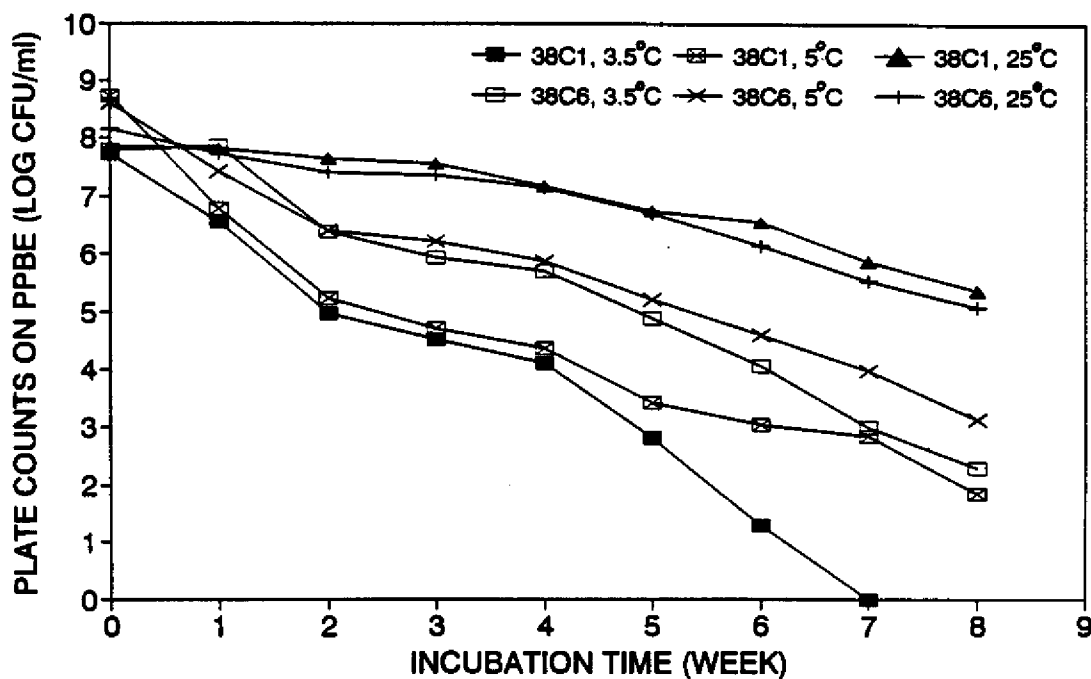


Fig. 3. Effect of temperature on the culturability of *V. parahaemolyticus* strains 38C1 and 38C6 in starvation medium.

In addition to strains 38C1 and 38C6, 8 other strains were tested. All K^+ strains lost culturability quicker than all K^- strains within 50 days in starvation medium at 3.5°C (Figs. 4 and 5). Most strains reached nonculturability within 75 days except P26 and P68. Colwell et al. and other workers reported that *V. parahaemolyticus* occurred in summer months and disappeared in winter. K^+ strains were rarely isolated from aquatic environment (4, 6). The minimal growth temperature for *V. parahaemolyticus* was reported as 5°C (2). The results presented here clearly demonstrate that pathogenic strains are more sensitive to low temperature than nonpathogenic strains of *V. parahaemolyticus*. It has been reported that *V. vulnificus* was temperature dependent, only at 5.0°C could it become viable but nonculturable (14, 28). Unlike *V. parahaemolyticus* and *V. vulnificus*, *C. jejuni* and *Legionella pneumophila* survived longer at low temperature and became viable but non-culturable only at high temperature (37°C) (12, 22). Therefore, different organisms require different temperatures in the survival strategy.

Medium difference had different effect on 2 strains of *V. parahaemolyticus*. At beginning, log-phase normal cells of both strains in the PPBE broth increased their cell numbers when they were exposed to 5°C (Fig. 6). The cell number increase was not due to reductive division since cells were in rich medium. It was possible that cells in divided form prior to exposure to lower temperature produced their daughter cells. Within 2 to 3 weeks, these cells in rich medium lost culturability slower than cells in starvation medium. After that period of time, both strains lost culturability very quickly at a linear rate. Cells suspended in starvation medium survived 2 to 3 times longer than in rich medium. It appeared that cells in starvation medium developed some kinds of resistance after initial quick decline in culturability. It was reported that during starvation cells reorganize their cellular macromolecules and decrease respiration rate and endogenous metabolism, such as degrading some growth relating proteins and synthesizing starvation resistant proteins (15).

The difference between MM and NSS media became clear only after cells were kept in these media for more than one month at 5°C . The NSS medium contains more species of inorganic salts and divalent ions than MM medium but without any phosphate ions. Phosphate ion is a major anion in the bacterial cells participating in many cellular metabolic reactions. It has been reported that phosphate ions enhanced the culturability of *E.coli* cells in seawater (8). The absence of phosphate ions in NSS medium probably resulted in the quick drop of culturability of *V. parahaemolyticus*.

Fig. 7 showed the survival curves of both 38C1 and 38C6 at 5°C . Through out the experiment, total cell counts remained at the initial numbers. The cells stained with acridine orange dye exhibited red-orange fluorescence under epifluorescence microscope. The longer the cells were kept at low

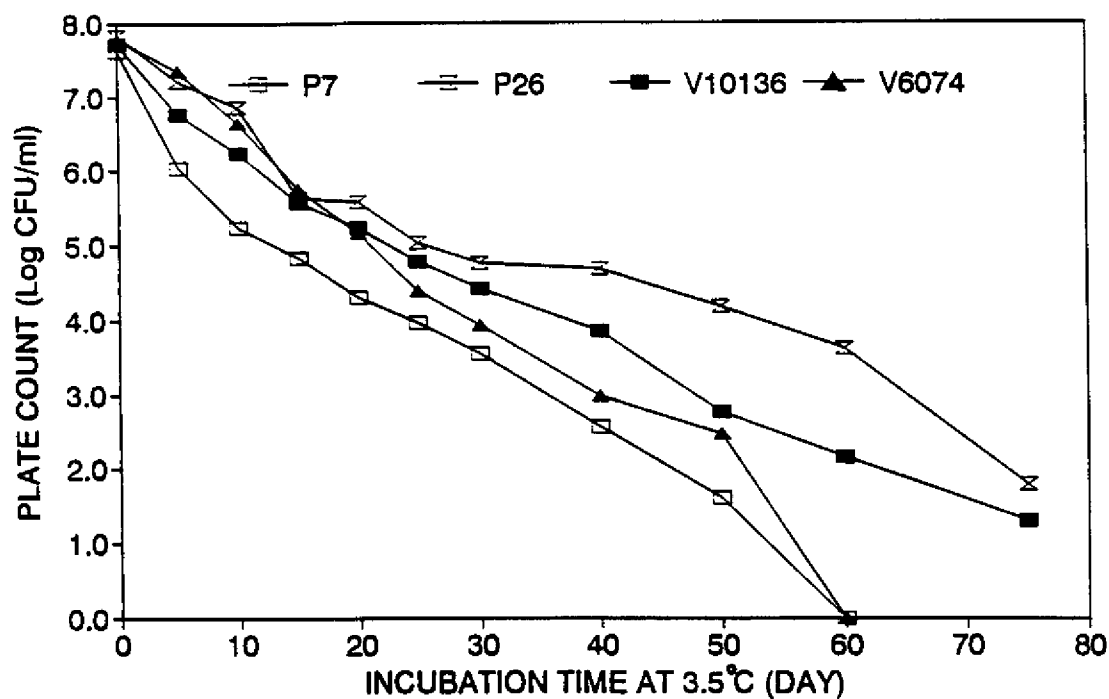


Fig. 4. Survival of 4 Kanagawa positive (K^+) strains of *V. parahaemolyticus* during starvation at 3.5°C.

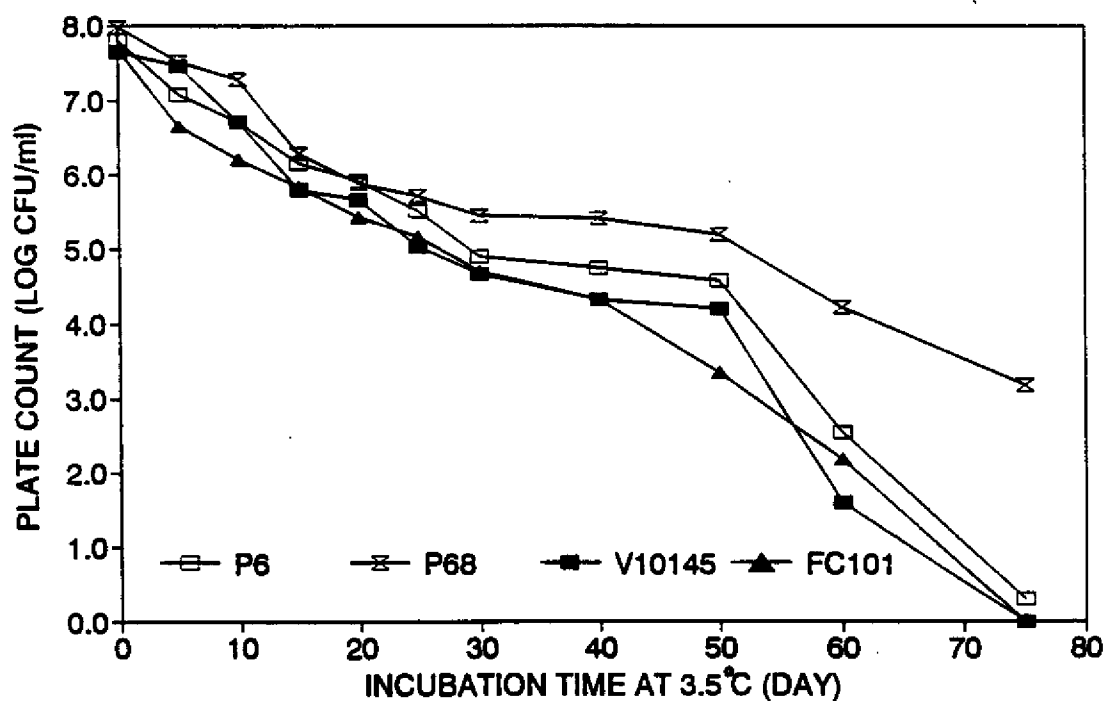


Fig. 5. Survival of 4 Kanagawa negative (K^-) strains of *V. parahaemolyticus* during starvation at 3.5°C.

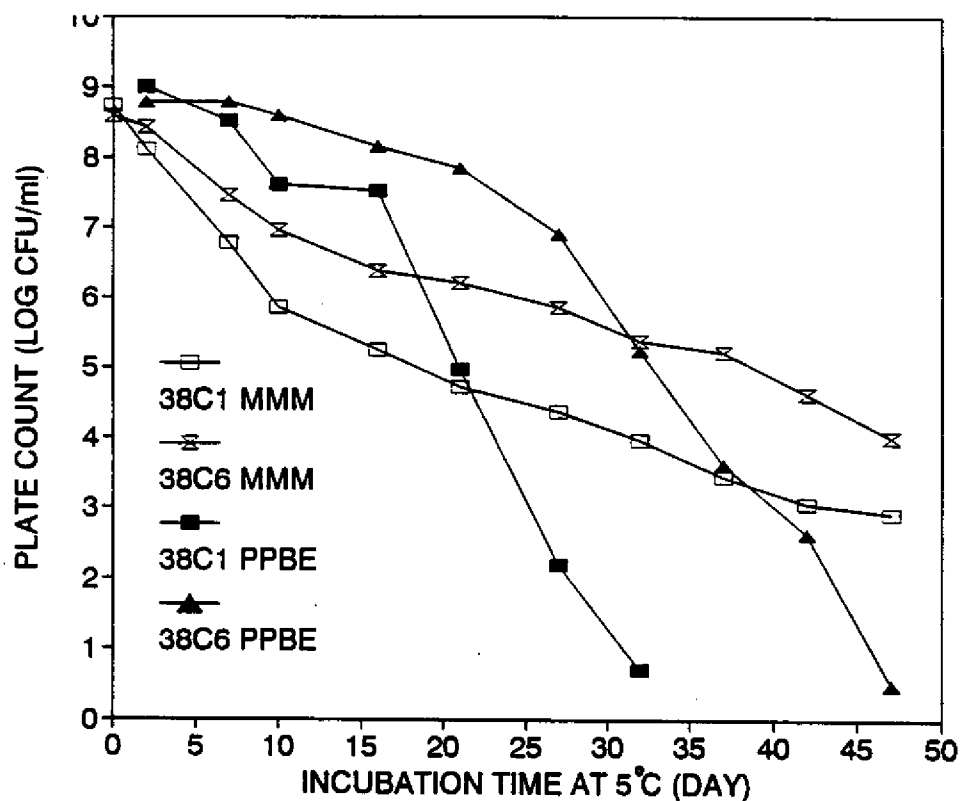


Fig. 6. Survival of strain 38C1 and 38C6 in MM and PPBE media.

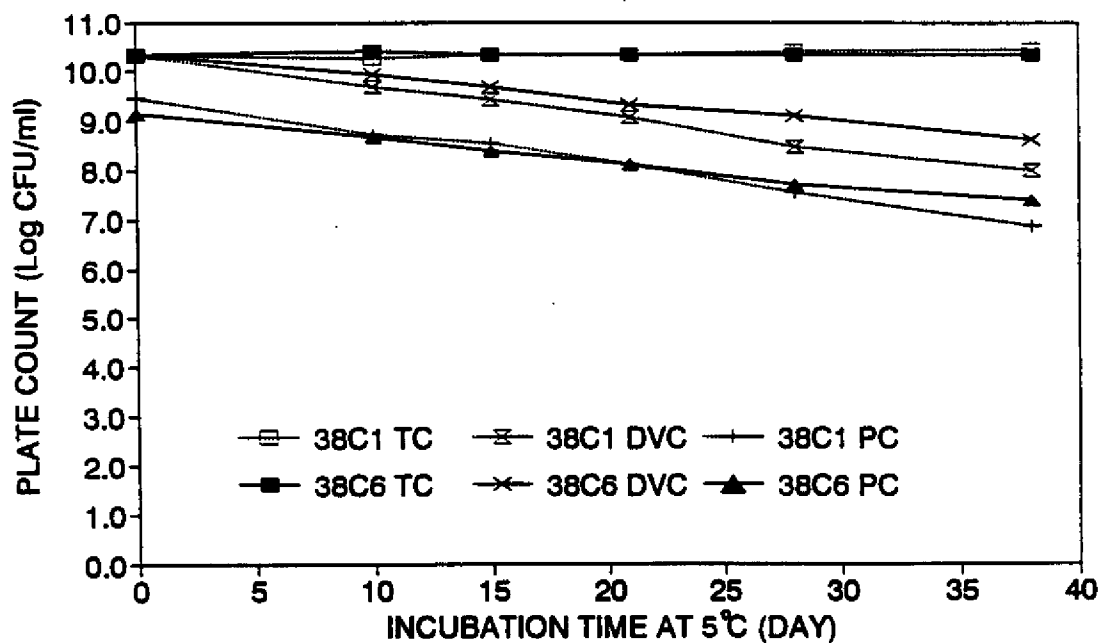


Fig. 7. Survival curves of strain 38C1 and 38C6 of *V. parahaemolyticus* in starvation medium at 5°C.

temperature, the less the density of observed red-orange fluorescence. It was reported that the cells of *Salmonella* in soil labeled with fluorescent antibody (FA) became duller with time during starvation (26). This may indicate that those cells could go further toward dormancy as they were exposed to adverse conditions. The difference between total counts and culturable counts was about 3 log cycles. Direct viable counts (DVC) were always at least 1 log cycle higher than plate counts. Among those elongated cells detected by DVC method, less than 10% were culturable by plate count therefore a significant portion was in the viable but nonculturable stage. *Escherichia coli* demonstrated the same pattern when the culture was tested in salt water (0.5-2.5%) microcosm (29). Unlike *V. parahaemolyticus* and *E. coli*, *V. cholerae* lost the culturability very quickly within 1 day and DVC remained constant (5). Other strains, such as *V. vulnificus*, *S. enteritidis*, and *Campylobacter spp.* reached nonculturability quite rapidly (19, 22, 25). This indicated that there were some differences in response to cold and starvation stresses among different species although they shared almost the same principles for survival.

Several reports have discussed methods for recovery of these viable but nonculturable cells. *L. pneumophila*, *V. cholerae*, *E. coli* and *Campylobacter spp.* (5, 12, 22) have been recovered only through animal passage when they failed to form colonies on agar plates or turbidity in rich or diluted nutrient MPN tubes. For *V. vulnificus*, which became viable but nonculturable cells at low temperature, room temperature resuscitation for 3 days recovered almost all viable but nonculturable cells (19)

Resuscitation process was effective for 38C1, 38C6 and other 8 *V. parahaemolyticus* strains. Following upshift to room temperature for 3 days, both 38C1 and 38C6 cultures kept at 3.5°C could be resuscitated up to 50% of original inocula. Throughout the entire experiment, resuscitated cell numbers remained constant. When direct plate counts dropped to zero, the temperature upshift resuscitation process failed to recover those nonculturable cells (Fig. 2). Under the microscope, after full resuscitation, about 50% of the total cells were rod and the rest were sphere. Within 24 h of resuscitation, it was observed that some rod cells were in divided forms. But after 24 h, no cell division was observed. The total counts were almost constant during resuscitation.

The starvation medium, such as MM medium, is free of nutrients. The cells were washed before resuspension in MM medium. Theoretically, the remaining culturable cells can not multiply during temperature upshift resuscitation. It was originally speculated that all those recovered culturable cells could be resuscitated from those viable but non-culturable cells and few survival cells. In contrast, MPN results demonstrated that cell multiplication could occur during temperature upshift. Cold-stored cultures were diluted into the MPN tubes before temperature upshift. The results of MPN tubes with PPBE medium at room temperature for 3 days were correlated to direct

plate counts before temperature upshift rather than after temperature upshift. The results of MPN tubes with MM medium indicated even fewer bacterial count than direct plate counts (Table 1). Logically, at the highest diluted tube, any growth results from single cell multiplication. If no multiplication occurred during temperature upshift, the MPN results should be closer to the resuscitated plate counts from the original culture than direct plate count before temperature upshift.

Table 1. Comparison of culturable cell numbers between MPN method and plate count method.

STRAIN	BEFORE RESUS.		AFTER RESUS.	
	Storage Time (D)	Plate Count	MPN (PPBE)	Plate Count
38C1	0	1.8×10^9	2.4×10^9	-
38C6	0	2.5×10^9	4.6×10^9	-
38C1	40	2.4×10^2	7.5×10^2	3.8×10^7
38C6	40	2.2×10^4	2.4×10^4	2.5×10^7
38C1	45	1.0×10^1	9.3×10^1	4.9×10^7
38C6	45	6.1×10^3	1.1×10^4	2.5×10^7

"-": not determined.

The effects of cell clumps, cell debris or culturable cell metabolites on the difference between MPN counts and resuscitated plate counts were investigated. Autoclaved cell cultures, ultra-filtered autoclaved cell cultures, and UV-treated cultures were tested but all failed to reach increased MPN counts. Since the nalidixic acid is a DNA synthesis inhibitor, it can inhibit the multiplication of cells without interfering with protein synthesis. Nalidixic acid at 20 $\mu\text{g}/\text{ml}$, 100 $\mu\text{g}/\text{ml}$ or 200 $\mu\text{g}/\text{ml}$ was added to the culture prior to resuscitation and left at room temperature for 3 days. After resuscitation, samples with 20 and 100 $\mu\text{g}/\text{ml}$ nalidixic acid had the same number of rod cells as the control sample, but 200 $\mu\text{g}/\text{ml}$ nalidixic acid totally inhibited cell multiplication (Table 2). From above results, it was certain that the room temperature resuscitation represented regrowth of the few surviving cells in the starved culture. Some reports have demonstrated the possibility of few survival cell regrowth during temperature upshift. (21, 23, 27)

Table 2. Effect of nalidixic acid on the resuscitation of *V. parahaemolyticus* strain 38C6*.

NALIDIXIC ACID ($\mu\text{g/ml}$)	AODC	
	Before Resus. (% sphere cells)	After Resus. (% rod cells)
0	100%	12.6%
20	100%	13.2%
100	100%	12.2%
200	100%	0%

***: Strain 38C6 culture was stored at 3.5°C for 52 days in starvation medium (bay water). The plate count was 4.6×10^3 before resuscitation. After 3-day resuscitation at room temperature, the plate count reached $2.2 \times 10^7/\text{ml}$, which accounted about 11% of original inoculum ($2.1 \times 10^8/\text{ml}$).

Table 3. Effect of initial cell density on the regrowth of strains 38C1 and 38C6 in MMM.

STRAIN	STORAGE TIME (DAYS)				
	0	15	21	26	31
38C1	5.2×10^7	4.0×10^7	5.9×10^7	4.0×10^7	5.2×10^7
38C1 (10x)*	3.8×10^6	4.4×10^6	3.8×10^6	1.2×10^7	0
38C6	6.1×10^7	2.3×10^7	2.2×10^7	3.0×10^7	4.1×10^7
38C6 (10x)*	8.9×10^6	1.2×10^6	2.6×10^6	2.2×10^6	1.9×10^6

***: "10x" means to dilute 10 times of original culture by fresh MM medium at day 0. .

The sources for cell regrowth were investigated in this study. Table 3 revealed that cultures with higher cell densities were always resuscitated to higher cell numbers during room temperature upshift. As seen in Fig. 2, the resuscitated plate counts remained relatively constant throughout the entire experiment. In all experiments, strain 38C1 always reached higher plate count numbers than strain 38C6 during 3-day room temperature upshift. Our results

demonstrated that the numbers of resuscitated plate counts depended on cell densities and strain difference but not numbers of survived plate counts. The amount of released nutrients was determined by initial cell densities and physiological stage of cultures. This conclusion was proved by the observation that the resuscitated cell numbers were always lower than original inoculum. Therefore the amount of nutrients in the medium was a key factor in determining how many cells will be resulted regardless of how many survival cells existed. Nilsson et al reported that the cryptic growth was impossible during temperature upshift because about 50 dead cells need to support the growth of one cell (19). Their calculations were probably based on the growth requirement of log phase young cells rather than starved cells. As for starved cells, they are in stressed conditions and adopt more efficient nutrient uptake metabolism and respiration systems. They can grow and multiply with limited nutrients at an extraordinary rate and cell number.

CONCLUSION

The results reported here have demonstrated that *V. parahaemolyticus* could enter into a nonculturable stage when exposed to low temperature and low nutrient environment. The DVC method showed there was a significant number of viable but nonculturable cells in the culture. The room temperature resuscitation could not recover those viable but nonculturable cells. Therefore, besides temperature upshift and nutrient addition, those dormant cells may need special growth conditions for resuscitation.

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LOW DOSE GAMMA IRRADIATION OF PLESIOMONAS SHIGELLOIDES
IN SHRIMP (PENAEUS SETIFERUS)

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Food irradiation is a process in which food products are subjected to ionizing radiation in order to eliminate spoilage and pathogenic microorganisms and add increased shelf-life. Research involving gamma irradiation of fish and shellfish has focused mainly on low doses to achieve pasteurization of these commodities. Some specific examples are: haddock fillets (1), cod fillets (9), oysters (12), and shrimp (8). Fresh fish treated with gamma irradiation doses up to 3 kGy have resulted in an increase in shelf life of approximately 7 to 15 days when compared with their normal shelf life (11). Gamma radiation is considered a "cold" process versus thermal processing (3), with the advantage of reduction and/or elimination of many pathogenic bacteria as well as spoilage microorganisms resulting in an increase in the shelf-life of seafood with little or no change in their sensory characteristics (11).

The pathogens of main concern in seafood products are bacteria that are naturally occurring in the environment i.e., *Vibrios*, *Aeromonas*, and *Clostridium botulinum* type E. Another microorganism that is widely distributed in the environment, *Plesiomonas shigelloides*, has been recognized as a pathogen (2, 10). *P. shigelloides* has been suspected to be the causative agent in food and waterborne outbreaks of gastroenteritis and many opportunistic infections such as septicemia (13, 7). This bacterium has been linked to

several outbreaks of gastroenteritis due to the consumption of raw oysters and because it has been isolated from other seafoods such as shrimp, clams and fish, its presence in seafood products can be considered a health hazard (6, 7).

The primary objectives of this study were to: (1) determine the effect of low doses of gamma irradiation on Plesiomonas shigelloides in sterile and non sterile shrimp; (2) determine the effect of irradiation on the survival of this bacterium in shrimp samples stored at 0 ± 2 °C (ice) for a period of 21 days and, (3) to evaluate the recovery of this microorganism in both selective (Plesiomonas agar) and nutrient-rich, non-selective (Brain Heart Infusion agar) media.

MATERIALS AND METHODS

Organism:

Plesiomonas shigelloides strain 7-1 isolated from Blue crab (Callinectes sapidus) was obtained from the culture collection of the Department of Food Science, Louisiana State University. The bacterium was stored at refrigeration temperature (5 °C) in Brain Heart Infusion Broth (BHIB), (Difco Laboratories, Detroit, MI), and was transferred once a week to fresh media (BHIB). To prepare the inoculum, the bacterium was transferred to fresh BHIB and incubated at 35 °C for 14 hours to obtain an approximate concentration of 1×10^9 cfu/ml of cells in the stationary phase. A 0.1 ml/g of the working culture was added to the shrimp samples in order to achieve an approximate concentration of 10^8 p. shigelloides per gram of sample. Inoculated samples were then transferred to the Nuclear Science Center at Louisiana State University in a cooler containing ice for treatment with low dose Cobalt-60 gamma irradiation (0-4 kGy).

Preparation of the Samples:

Fresh iced shrimp were purchased whole from a local supermarket. The whole shrimp were de-headed, packed in polyethylene plastic bags and were frozen at -20 °C until needed. Samples were thawed at room temperature (25 °C) prior their use in the experiments. After thawing, shrimp samples were prepared as follows. Shrimp were peeled and the tail meat was ground in a Kitchen Aid^R mixer with a food grinder attachment (model FG-A) (Hobart Corporation, Troy, OH) to provide a homogeneous shrimp paste sample.

Two treatments were performed using Cobalt-60 gamma irradiation. One set of samples was sterilized by autoclaving at 121 °C for 15 minutes prior to inoculation and irradiation; the second set consisted of

non-sterile (raw) samples which were inoculated and then irradiated. Control samples (non-irradiated/inoculated), were also prepared for both treatments. Samples of 10 g were placed into 60 ml nalgene containers. Triplicate samples were prepared for each radiation dose, treatments, and controls.

Irradiation Procedure:

Samples packed in ice were placed into a water secure diving bell and lowered into a source pit for irradiation from a Cobalt-60 source emitting 0.02 kGy per minute. Samples were exposed to doses of 0, 1, 2, 3 and 4 kGy. Samples were then removed and placed on ice for transfer and storage in the Food Science Department for microbiological enumeration over a period of 21 days.

Microbiological Enumeration:

Shrimp samples were examined for the presence of P. shigelloides at 0, 7, 14, and 21 days of storage at 0 ± 2 °C. Two different media were used for detection of the bacterium in the inoculated samples. Brain Heart Infusion agar (BHI) (Difco Laboratories, Detroit, MI) was used for plate counts, and a selective medium, Plesiomonas agar (PL), developed by Miller and Koburger, 1985, was also used for the selective isolation and counting of this bacterium. Samples were diluted in a 1:10 dilution in a Stomacher sample bag (Seward Medical Stomacher^R '400' bags) using sterile alkaline peptone water and blended for 1 minute using a Stomacher Lab-Blender 400 (Tekmar Company, Cincinnati, OH). Appropriate serial dilutions (10^{-2} to 10^{-8}) of samples in alkaline peptone water were prepared with duplicate aliquots of 0.1 ml being plated, by surface plating, on both media (BHI agar and PL agar) and incubated at 35 °C for 48 hours. The plates were counted in accordance to the Bacteriological Analytical Manual (4). A Darkfield Quebec^R Colony Counter (American Optical, Buffalo, NY) was used as a background light to obtain the colony count. The lowest limit of detection of this methodology was 10^2 cfu/g. The number of survivors 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 at each radiation dose and time of storage respectively.

Statistical Analyses:

To study the effects of low gamma irradiation on the survival of P. shigelloides, an analysis of variance for repeated measures was applied using a SAS-GLM program (14). Pair-wise comparisons to determine significant differences among the different mean radiation levels was performed using the Tukey test (5).

Significant differences were found when the p value was less than 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

In the non sterile shrimp samples inoculated with an approximate concentration of 10^7 cfu/g of P. shigelloides there was an initial aerobic plate count of 10^7 total microorganisms/g. After irradiation the total number of microorganisms was reduced with doses of 1 to 4 kGy (Figure 1). Results show that there was a 3-5 log reduction of the total number of microorganisms on day 0 on BHI agar. However, beginning with the first week of storage there was an increase in the number of colonies recovered throughout the 21 days of storage for all treatments. When comparing gamma irradiation doses, the Tukey test showed that inoculated non-irradiated samples (control) and those treated with irradiation were significantly different ($p < 0.05$). The effects of low dose gamma irradiation specifically on P. shigelloides are shown in Figure 2. With an irradiation dose of 4 kGy, counts were below detectable levels throughout the entire 21 days of storage period. A reduction of at least 5 log cycles was achieved using a 3 kGy dose, but on day 21 a 2 log cycle increase in numbers was seen. Thus, it appears that some Plesiomonas colonies were able to survive. Counts were below the limit of detection for both 1 and 2 kGy on days 0 and 7; however, the number of P. shigelloides recovered after seven days of storage increased with both 1 and 2 kGy. The Tukey test showed that non-irradiated inoculated (control) samples were significantly different ($p < 0.05$) from the treated samples. A 4 kGy dose was also significantly different ($p < 0.05$) when compared to any of the lower irradiation doses. No significant differences ($p > 0.05$) were found for 1 and 3 kGy; however, there was a significant difference ($p < 0.05$) between 1 and 2 kGy.

Sterile shrimp samples were inoculated with an approximate concentration of 10^8 cfu/g P. shigelloides. Figures 3 and 4 show the effects of low dose gamma irradiation on the recovery of this bacterium on BHI agar and PL agar, respectively. In Figure 3 doses of 3 and 4 kGy on day 0 reduced the original population of P. shigelloides by at least 6 log cycles. On day 21, counts were still at the limit of detection. On day 0 a dose of 2 kGy reduced the original population of this organism by approximately 6 log cycles, while 1 kGy reduced it by 4 log cycles. However, the number of colonies recovered for these two doses gradually increased after 7 and 14 days of storage. This increase

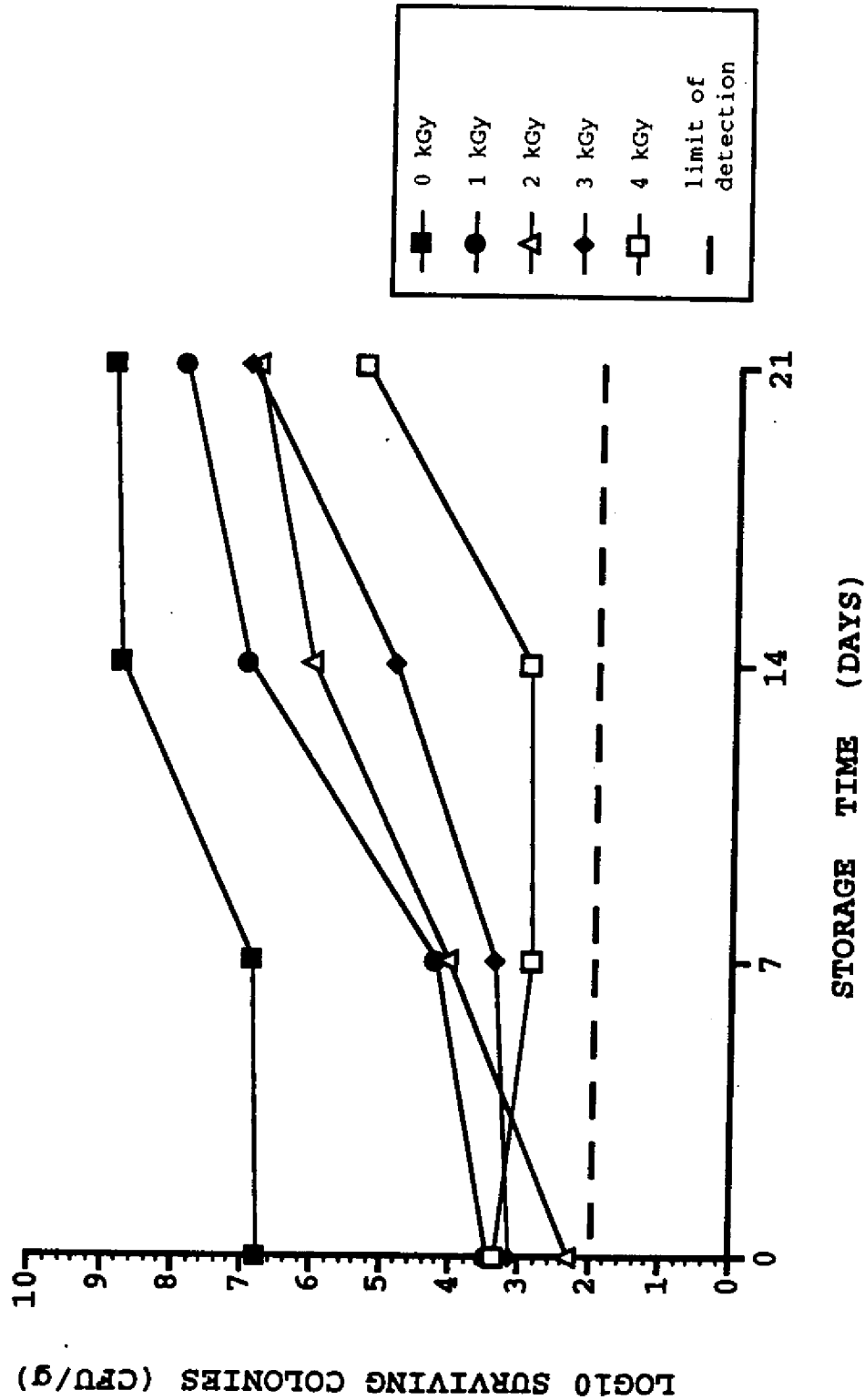


Figure 1 Effect of gamma irradiation on the recovery of normal shrimp microflora (plated on Brain Heart Infusion agar) with an additional 10^7 inoculation of *P. shigelloides* in shrimp stored at $0 \pm 2^\circ \text{C}$.

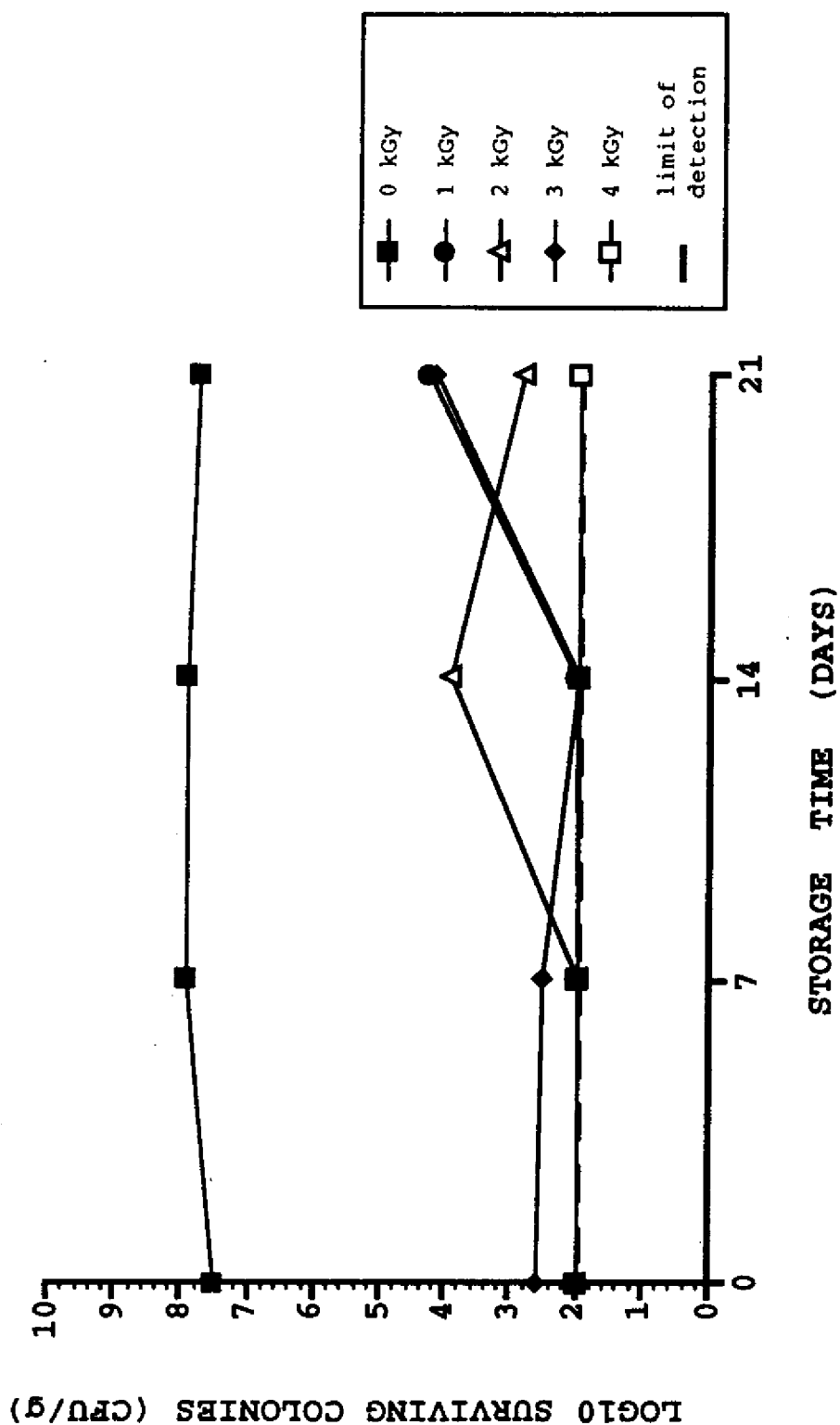


Figure 2 Effect of gamma irradiation on the recovery of *P. shigelloides* (plated on *Plesiomonas* agar) in non sterile shrimp stored at 0 ± 2 °C

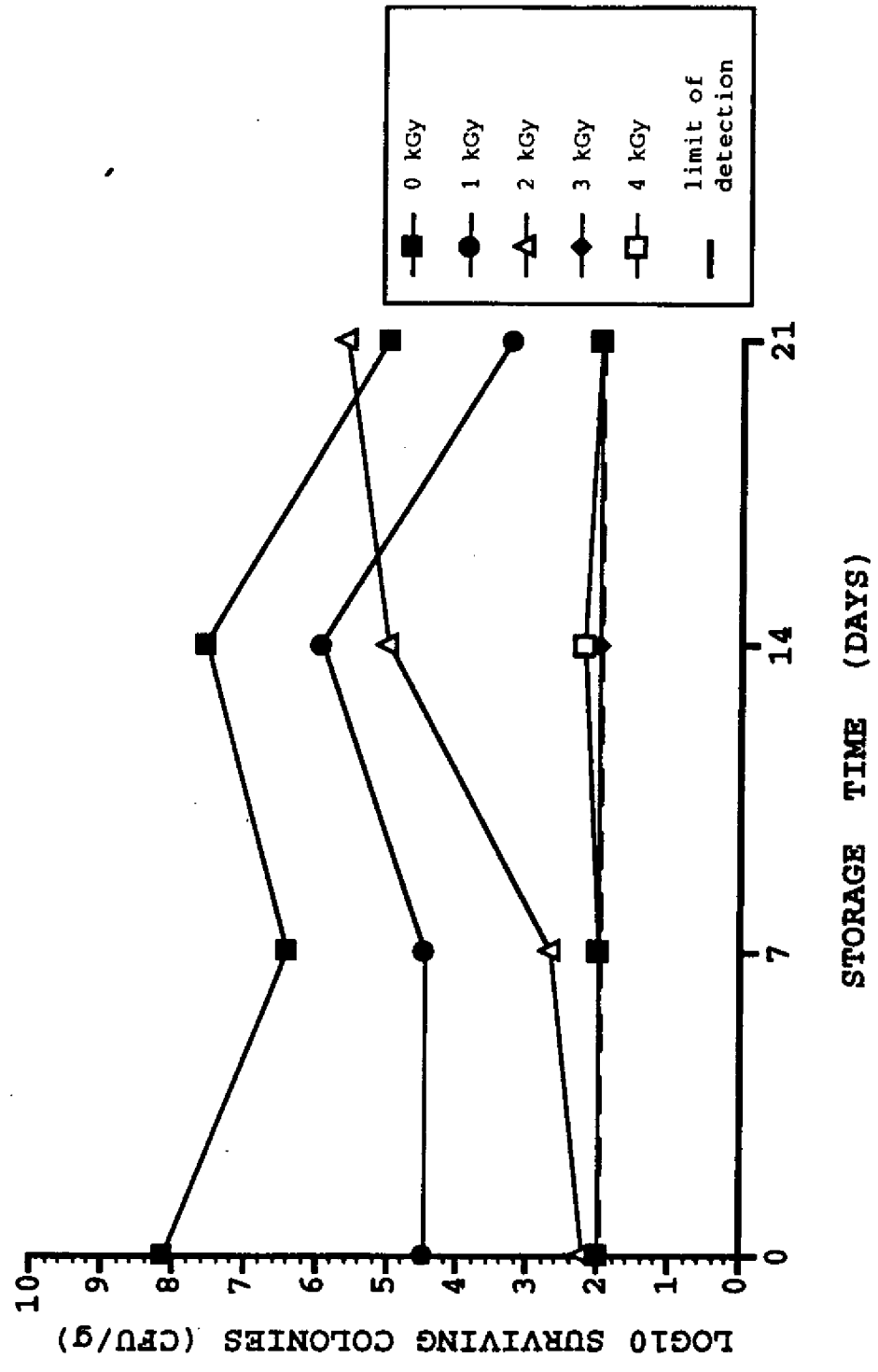


Figure 3 Effect of gamma irradiation on the recovery of *P. shigelloides* in sterile shrimp (plated on Brain Heart Infusion agar) stored at 0±2 °C

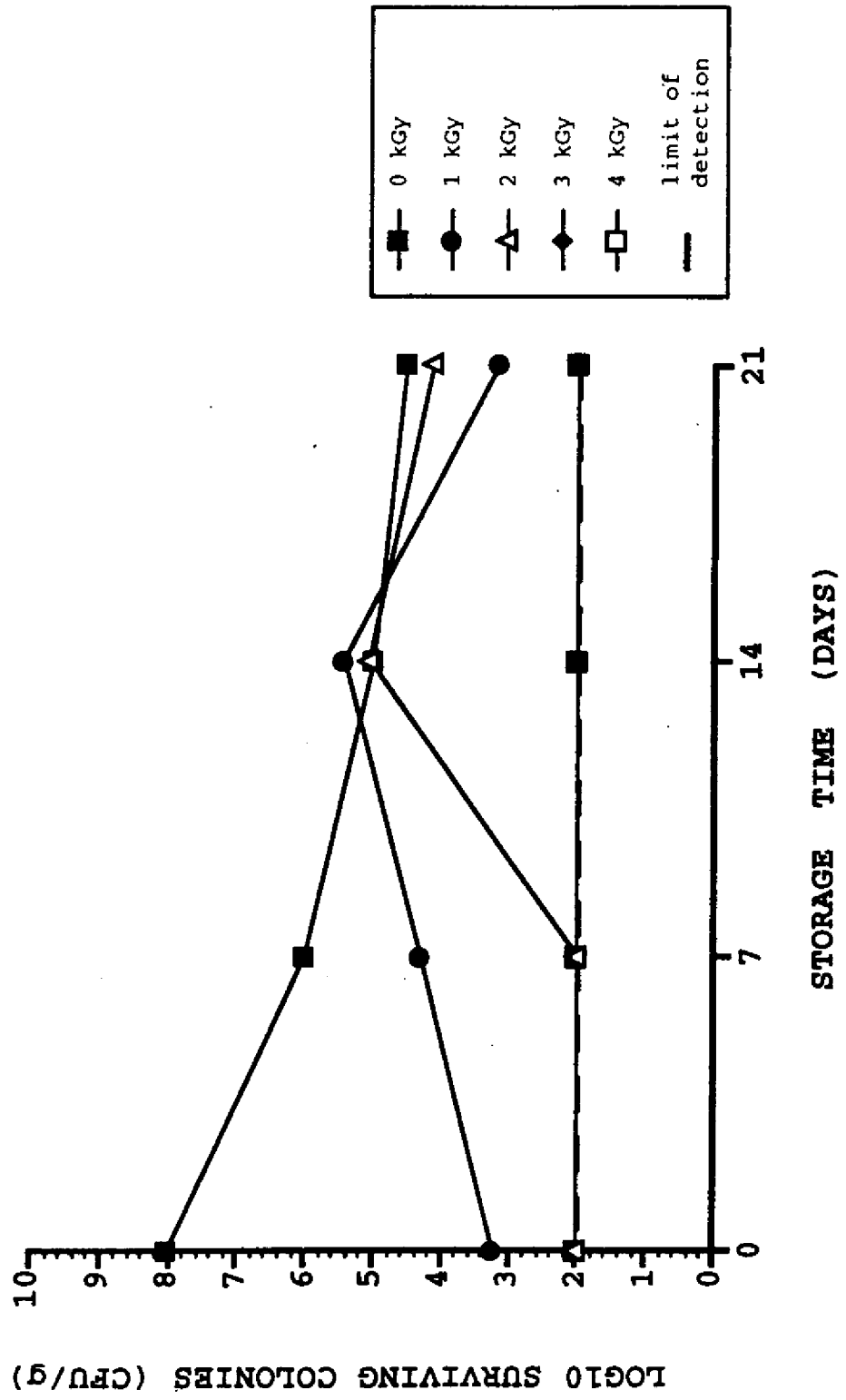


Figure 4 Effect of gamma irradiation on the recovery of *P. shigelloides* (plated on *Plesiomonas* agar) in sterile shrimp stored at 0±2 °C

may have been due to recovery from the effects of radiation. On day 21 the numbers recovered for 1 kGy were lower than for 2 kGy. This may have been due to an increase in the rate of mortality of the cells or to a slower growth rate. The Tukey test applied to these data showed that non-irradiated inoculated (control) samples were significantly different ($p < 0.05$) from the samples treated with irradiation. Applied doses of 1 and 2 kGy and 3 and 4 kGy were not significantly different ($p > 0.05$) between themselves, but they were different ($p < 0.05$) when 1 and 2 kGy were compared with 3 and 4 kGy. Samples plated on the selective Plesiomonas agar are shown in Figure 4. For samples treated with 3 and 4 kGy no observable counts were found at the limit of detection for the entire storage period of 21 days. P. shigelloides numbers in sterile shrimp samples treated with 1 and 2 kGy were reduced by 5 and at least 6 log cycles respectively, on day 0, but the numbers increased in the first 14 days of storage and then decreased on day 21. The Tukey test showed the same significant differences for the different irradiation doses as the samples plated on the nutrient-rich non-selective agar.

Statistical analysis of the data for both sterile and non sterile samples plated on selective and non-selective media showed that there was an interaction ($p < 0.05$) between radiation levels and storage times. The results indicated that the gamma irradiation treatment had an effect on the survival of P. shigelloides in shrimp stored at 0 ± 2 °C since it reduced the numbers of this bacterium in the treated samples.

From the results of the present study, it was apparent that low dose gamma irradiation had an effect on the survival of P. shigelloides. The lower numbers of colony forming units recovered after irradiation indicated that there was some inactivation of the original cells. Cells that survived were those presumably only injured or unaffected by gamma irradiation. Surviving injured colonies were found to be much smaller in size than colonies that were not subjected to the irradiation treatment, due probably to a slower growth rate. When comparing the number of colonies recovered from sterile and non-sterile shrimp samples, it appeared that recovery was greater for sterile samples. This suggests a possible competition of the normal shrimp microflora with P. shigelloides in non-sterile samples making it more difficult for the Plesiomonas to survive.

When comparing the number of colonies recovered in the two media, it appears that the number of colonies recovered was lower for the selective medium than for the nutrient-rich non-selective medium. Thus, this reduction in colonies recovered can be related to the inhibitory properties of the selective agar. The same

reduction in recovery was found by other researchers in experiments involving other microorganisms treated with irradiation (15). The growth of injured cells was much slower in the selective agar. The greatest recovery of injured cells was in the non-selective agar (BHI). This was to be expected since the non-selective agar does not contain inhibitory compounds thus allowing for a better recovery.

Another interesting result from the present study was that the Plesiomonas strain used was capable to maintain growth at a temperature of 0 ± 2 °C. This contradicts findings from other researchers who found that the minimum growth temperature for other strains of Plesiomonas is 5 to 8 °C (7, 10). The capability of P. shigelloides of maintaining growth at low temperature could pose a health hazard in refrigerated foods since this bacterium has been classified as a food and water borne pathogen.

CONCLUSION

Food irradiation has been useful for reducing microbial spoilage and as a means to reduce the threat of food borne illnesses. Use of gamma irradiation doses from 1 to 3 kGy have been useful in the reduction of several spoilage organisms in seafood, i.e. Pseudomonas and Achromobacter, resulting in enhanced refrigerated shelf-life (11). However, depending on the type of seafood, gamma irradiation doses above 2 kGy have resulted in adverse sensory effects (12).

The results of the present study have shown that irradiation dosages of 1-4 kGy were effective against the food and water borne pathogen P. shigelloides at an proximate concentration of 10^8 cfu/g, which represents a "worst case scenario". Although lower gamma irradiation dosages reduced the number of P. shigelloides initially, the bacterium was capable of recovering and multiplying at 0 ± 2 °C. In conclusion, the present study has provided information on the effects of low dose gamma irradiation on P. shigelloides in shrimp.

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CONTROL OF *LISTERIA MONOCYTOGENES* IN BLUE CRAB MEAT USING LACTIC ACID BACTERIA FERMENTATES

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Listeria monocytogenes is frequently associated with fresh, frozen, and ready-to-eat seafood products and epidemiologically implicated in a limited number of seafood-related listeriosis episodes (5, 8). The association of L. monocytogenes with seafood (incidence from 12 to 26%) has also resulted in several product recalls (12). Moreover, the organism is difficult to control in seafood, because this pathogen can grow at refrigeration temperatures, survive in brine solutions, and tolerate extremes in heat and pH (12).

The frequency at which L. monocytogenes is isolated from seafoods and its growth and survival properties warrant the investigation of additional barriers to supplement traditional control practices. The purpose of the present study was to evaluate lactic acid bacteria (LAB) fermentates to control L. monocytogenes in crab meat. Blue crab meat was selected as the test substrate because the prevalence of L. monocytogenes is higher in this product compared to other seafoods (6), and the organism grows better on crab meat than on certain other seafoods (7).

MATERIALS & METHODS

Listeriae

L. monocytogenes strains 101M (serotype 4b; meat isolate), NCTC 5105-3 (serotype 3a; human isolate; hypo-hemolytic variant of NCTC 5105), and V7 (serotype 1/2a; raw milk isolate) were grown at 37°C in Tryptose Phosphate (Difco Laboratories, Detroit, MI) broth and maintained as previously described (11).

Microbiological sampling of crab meat

Fresh blue crab (*Callinectes sapidus*) meat was obtained from retail markets in Florida and sampled immediately upon arrival in Madison, WI for total bacterial load by direct-plating on Plate Count Agar (Difco) and for *L. monocytogenes* by direct-plating on Listeria Enrichment (LE; Difco) agar and by enrichment using the FDA procedure (10).

Inoculating crab meat

Fresh blue crab meat (ca. 2 kg) was sterilized in a 4 L glass beaker by autoclaving. The meat was cooled to 4°C in an ice bath and then inoculated with 1 L of the listeriae cocktail to levels of 5×10^5 CFU/g. The beaker was inverted 3 times to distribute the inoculum throughout the meat and then held at 4°C for 5 min. The crab:listeriae slurry was then poured (aseptically) into a large funnel containing a sterile stainless steel sieve that drained into a 1 L graduated cylinder. Crab meat was pressed using a large sterile pestle until 1 L of fluid (original volume of inoculum) was recovered. *L. monocytogenes* levels in the inoculated crab meat were determined by homogenizing 20-g samples of meat with 180 ml of 0.1% peptone, spread-plating dilutions onto LE agar, and incubating plates 24–48 h at 37°C. Numbers of *L. monocytogenes* in the original inoculum and the fluid collected from the crab meat were also determined by spread-plating appropriate dilutions on LE-agar plates as above.

Washing crab meat with antimicrobials

The LAB fermentates tested included Alta 2341 (Quest International, Sarasota, FL), Enterocin 1083 (1), and Nisin (Sigma Chemical Co., St. Louis, MO). Each of the fermentates were tested at levels of 0 (control; deionized water), 2,000, 10,000, and 20,000 arbitrary units (AU)/ml. Antilisterial agents were adjusted to equivalent arbitrary units using the spot-on-lawn assay (4).

Crab meat inoculated with *L. monocytogenes* was divided into several 200-g lots for "washing" with antilisterial agents. Each wash solution (200 ml) was separately added to individual lots of crab meat in a 600 ml beaker. The beakers were inverted 3 times to distribute the antimicrobials before the meat was drained using a sterile funnel and sieve as described above. The entire washing procedure required 30–60 s for each antimicrobial. Treated crab meat was divided into 20-g aliquots and placed into sterile petri dishes. Duplicate samples were monitored for viable listeriae during storage at 4°C as described above.

Crab meat pH

The pH of crab meat was monitored both before and after washing with antimicrobials using a sanitized electrode. In a second set of experiments, the pH of inoculated crab meat (in the absence of antimicrobials) was adjusted with 1 N HCl or 5 N NaOH to pH values ranging from 2–12. A 100-g sample of inoculated crab meat (ca. 10^6 CFU/g) plus 200 ml dH₂O were combined in a 500 ml beaker. The crab:listeriae slurry was then adjusted to a target pH while gently stirring with the aid of a magnetic stir bar. Excess fluid (200 ml) was drained from the crab meat as described above and the pH of the crab meat was recorded. Counts of the pathogen in the crab meat were determined just prior to adjusting the pH of the meat and 60 min after pH adjustment.

Statistical analyses

The data reported are average values from duplicate samples and two separate trials. Data were analyzed statistically with the SAS program (SAS Institute, Inc., Cary, NC), wherein analysis of variance and separation tests of the least significant difference of the means were used to compare antimicrobials based upon \log_{10} CFU unit counts of *Listeria*. All analyses were done at the 95% confidence level.

RESULTS

Occurrence of *L. monocytogenes* in crab meat

L. monocytogenes was found in 3 of 4 lots tested by enrichment and in one lot by direct-plating at levels of 75 CFU/g (Table 1). In comparison, the total bacterial load of fresh crab meat ranged from 4.8 to 5.3 \log_{10} CFU/g (mean of 5.1; 4 samples).

Table 1. Incidence of *L. monocytogenes* on fresh crab meat.

Trial	Direct Plating	Enrichment
1	75 CFU/g meat	+
2	^a --	+
3	--	+
4	--	--

^a-- *Listeria* spp. not detected

LAB fermentates as antilisterial agents in crab meat

The behavior of *L. monocytogenes* was examined in crab meat in the presence of three levels of LAB fermentates (Figure 1). Counts of the pathogen remained relatively constant and were not significantly different ($p < 0.05$) from the control in samples washed with each of the fermentates used at 2,000 AU/ml. In contrast, counts of *L. monocytogenes* decreased 1.2 (Alta), 2.1 (Enterocin), and 2.6 (Nisin) \log_{10} units/g within 0.04 d in crab meat washed with 10,000 AU/ml. Thereafter, counts of the pathogen increased somewhat over 6 d, but did not return to initial inoculum levels. An initial 2-3 \log_{10} unit decrease was also observed with wash solutions containing levels of 20,000 AU/ml. More important, the recovery/resurgence of *Listeria* was lessened over the 6 d sampling period when treated with 20,000 AU/ml (ca. 0.5-1.1 \log_{10} units) compared to 10,000 AU/ml (ca. 0.8-1.6 \log_{10} units).

Effect of pH on *L. monocytogenes* in crab meat

Experiments were also conducted to delineate between the antilisterial activity of the antimicrobials used to wash crab meat and any pH effects contributed by these same antimicrobials. The pH of the crab meat as received was pH 8.1 (average of 12 samples), whereas the pH of the individual wash solutions at 20,000 AU/ml was 5.1 (Alta), 5.8 (Enterocin), and 3.8 (Nisin). The pH of crab meat immediately after washing with antimicrobials was 7.4 (Alta), 7.5 (Enterocin), and 5.2 (Nisin). In general, the pH of crab meat (pH 8.1) decreased somewhat after washing with LAB fermentates.

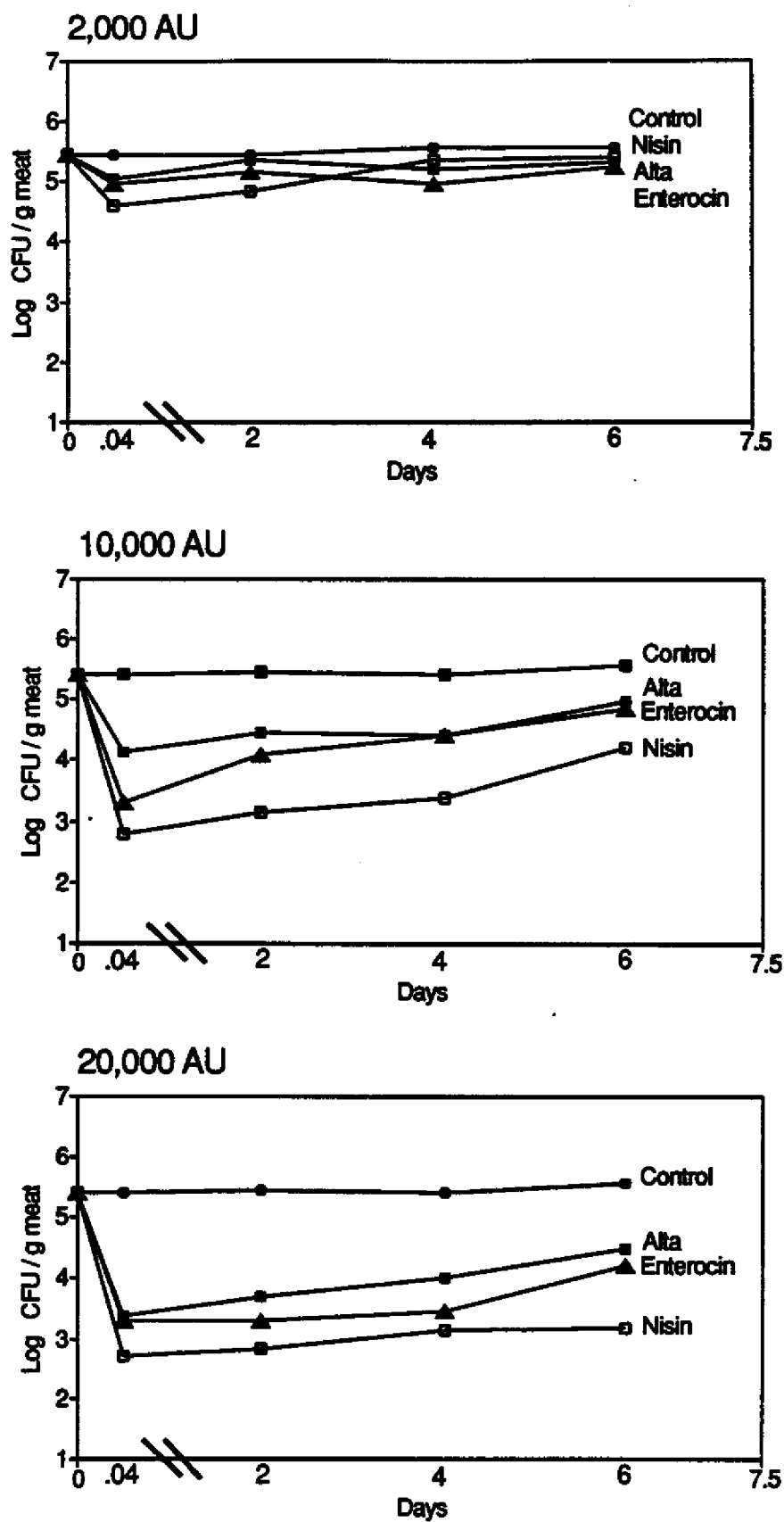


Figure 1. Behavior of *L. monocytogenes* in crab meat washed with fermentates and stored at 4°C for 6 days. Data shown are an average of 2 trials.

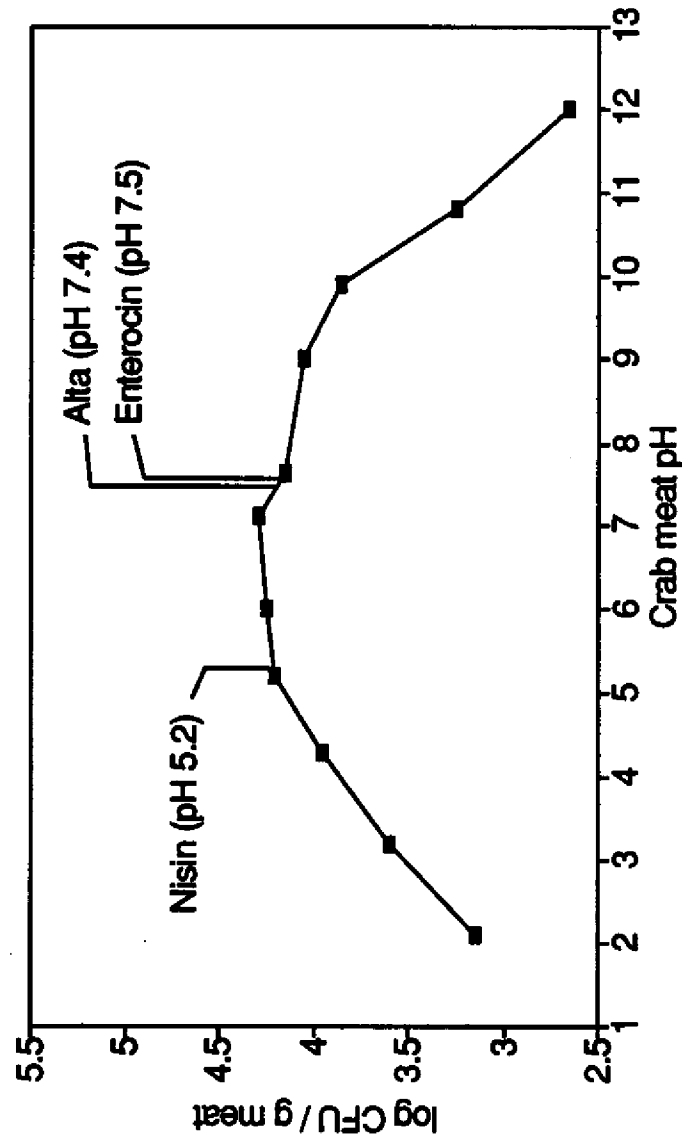


Figure 2. Behavior of *L. monocytogenes* in pH-adjusted crab meat. Superimposed on the curve are pH-values of crab meat after treatment with Alta, Enterocin, or Nisin (20,000 AU/ml each). Data shown are an average of 2 trials.

After establishing the baseline pH data reported above, the survival of L. monocytogenes was examined in crab meat within 60 min of adjusting the pH to values ranging from pH 2-12 to ascertain the effect of pH on pathogen survival in the absence of any antimicrobials (Figure 2). Pathogen numbers remained relatively constant ($\pm 0.2 \log_{10}$ units/g) compared to the control over the range of pH 4.4-10. As depicted in Figure 2, Alta, Enterocin, and Nisin did not alter the pH of crab meat to inhibitory levels.

DISCUSSION

Although L. monocytogenes is inactivated by proper thermal processing of crab meat (9), the results herein and those reported by other investigators (3) revealed the pathogen is present in retail product. With the exceptions of listeriosis episodes in New Zealand in 1980 and 1992 and one in Connecticut in 1989, as well as a few sporadic cases, seafoods are not a common vehicle of listeriosis compared to other food vectors (see references 2, 8, 12). However, the link of listeriosis with the consumption of seafood and the association and ability of L. monocytogenes to grow/survive in crab meat represents a potential threat to the consumer and, as such, prompted us to evaluate LAB fermentates as interventions.

To our knowledge, there have been few, if any, reports detailing the use of LAB fermentates to control L. monocytogenes in crab meat. As described herein, a significant initial decrease in pathogen numbers was observed after washing with Alta, Enterocin, or Nisin at levels of 10,000 or 20,000 AU/ml, and counts of L. monocytogenes increased only slightly after 6 d at 4°C. Moreover, listericidal activity was not due solely to pH. Additional studies are warranted to characterize the mechanism(s) by which the aforementioned antimicrobials inhibit L. monocytogenes on crab meat. Regardless, the results of the present study agree with and expand upon our previous efforts, and those of others, establishing the efficacy of LAB bacteriocins for controlling L. monocytogenes in foods.

CONCLUSION

Washing crab meat with LAB fermentates may serve as an additional hurdle to decrease the likelihood of listeric illness.

ACKNOWLEDGMENTS

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RELATIONSHIP BETWEEN PARALYTIC SHELLFISH TOXIN
PRODUCING DINOFLAGELLATE ALGAE AND ASSOCIATED BACTERIA

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In 1988, Kodama et al. (1) published the first of a series of five papers (1-5) documenting observations that paralytic shellfish toxins (PSTs) i.e. derivatives of saxitoxin (STX) were produced by intracellular bacteria harbored by toxic dinoflagellate algal members of the genus *Gonyaulax*, later ammended to the genus *Protogonyaulax*, and more recently to the genus *Alexandrium*. Kodama et al. (1) purified the algal cultures to an axenic state i.e. free of external bacteria by culturing them in the presence of 1 ppm gentamycin sulfate, 15 ppm ampicillin, and 15 ppm cloxacillin. Following purification, they then disrupted the algal cells, plated the resulting preparations onto Marine Agar and isolated bacteria from resulting colonies that were allocated to the genera *Moraxella* (2,4) and *Bacillus* (5). Mouse lethality data and high performance liquid chromatograms were presented, documenting that these pure cultures of bacteria produced toxic derivatives of STX. We undertook to repeat these studies with toxic strains of the genus *Alexandrium* and *Gymnodinium* obtained from Bigelow Laboratories, representative of North American isolates.

We found initially that the mixture of antibiotics used by Kodama et al. (1) were lethal to our dinoflagellate cultures. This approach was therefore abandoned. Dinoflagellate cells are about 30m in diameter and appear as small dots to the naked eye unless in chains. With the use of an inverted microscope we employed the single cell isolation technique to dinoflagellate culture *A. tamarense* 1312. This involved capturing single cells with glass micro

pipets drawn out to an inside diameter of about 50 μ and then releasing the single cell into a drop of sea water and repeating this process six times to thoroughly wash the cell free of extracellular microbial contaminants. We were able to purify cells of culture 1312 in this way so that they were completely free of extracellular bacteria. However, a pink yeast always accompanied these individually manipulated dinoflagellate cells. The peptide yeast inhibitor Delvocid (Sigma) was then incorporated into several agar media to document its ability to inhibit colony formation by this pink yeast. When agar plates were incubated at 32°C, 50 ppm Delvocid was found to be completely inhibitory to the pink yeast when incorporated into Marine Agar and Tryptone Yeast Extract Agar (Table 1). However, Brain Heart Infusion Agar was significantly less inhibitory with this concentration of Delvocid. When parallel plates were incubated at 20°C, Delvocid at a concentration of 10 ppm in all three plating media completely inhibited this yeast (Table 2). Delvocid at a concentration of 50 ppm was found to be non inhibitory to *Moraxella* and *Bacillus* (Table 3) obtained from Kodama (2, 4, & 5). The incorporation of Delvocid at 50 ppm into these agar media therefore allowed us to plate disrupted cell preparations of culture 1312 in attempting to isolate intracellular bacteria from dinoflagellate cells. We were eventually able to purify additional strains of toxic dinoflagellae cultures by adding 1,000 ppm penicillin G directly to algal cultures followed by incubation for 3 - 4 weeks. The dinoflagellate cultures purified to an axenic state are given in Table 4. Toxicity levels as determined with the mouse bioassay were similar for non-axenic and axenic cultures (Tables 5, 6, 7, and 8). When the cells of all four toxic axenic cultures were disrupted in a micro blender with glass beads so as to physically rupture 50% to 90% of the dinoflagellate cells (Figs. 1, 2, 3, and 4) no colonies were ever found to develop on the above plating media, indicating the complete absence of intracellular bacteria in toxic cells. The similar blending of presumably toxic bacterial cells from cultures received from Kodama (2, 4, & 5) was found not to reduce the number of colony forming units in the cell suspensions (Fig. 5). In 1992, Sako et. al. (6) published on the Mendelian inheritance of PSTs in both *A. catanella* and *A. tamarensis*. In this work, the authors specifically cited the 1988 paper of Kodama and et al. (1). They genetically crossed two opposite mating type strains. One strain produced gonyautoxins 4 and C4 and the other strain produced neosaxitoxin and saxitoxin. The F1 progeny were found to produce only one parental toxin profile which segregated independently from the mating type. The authors concluded that PST production is

Table 1. Delvocid inhibition of pink yeast isolated from *A. tamarense* strain CCMP-1312.^a

Delvocid (ppm)	LOG CFU/ML		
	Marine Agar	Brain Heart Infusion Agar	Tryptone Y. Ex. Agar
0	7.6	7.7	7.7
1	8.0	7.6	7.6
10	6.5	7.1	1.3
50	<1	3.3	<1
100	<1	2.7	<1
500	<1	<1	<1

^a Plates were incubated for 14 days at 32°C.

Table 2. Delvocid inhibition of pink yeast isolated from *A. tamarense* strain CCMP-1312.^a

Delvocid (ppm)	LOG CFU/ML		
	Marine Agar	Brain Heart Infusion Agar	Tryptone Y. Ex. Agar
0	7.3	7.6	7.8
1	7.0	7.8	7.3
10	<1	<1	<1
50	<1	<1	<1

^aPlates were incubated for 14 days at 20°C.

Table 3. Delvocid resistance of *Moraxella* A and *Bacillus* intracellular isolates of Kodama et al. (2.4) and Ogata et al. (5).^a

LOG CFU/ML					
Isolate		Delvocid (ppm)	Marine Agar	BHI Agar	T. Y. Ex. Agar
<i>Moraxella</i>	A	0	8.92	8.91	8.98
"	"	50	9.18	9.04	9.15
<i>Bacillus</i>	spp.	0	8.75	8.80	8.82
"	"	50	8.64	8.85	8.76

^aPlates were incubated for 14 days at 20°C.

Table 4. Toxic dinoflagellate algal cultures purified of Extracellular Microorganisms.

CULTURE	STRAIN
<i>Alexandrium tamarense</i>	CCMP-1312
" "	CCMP-117
" <i>catanella</i>	CCMP-1493
<i>Gymnodinium catenatum</i>	CCMP-412

Table 5. Toxicity levels of axenic and non-axenic dinoflagellate *A. tamarense* strain CCMP-1312.

PARAMETER	NON-AXENIC	AXENIC
Algal culture age (days)	9.0	12
Algal cell count/ml ($\times 10^3$)	11.9	2.4
CFU/ml ($\times 10^5$)	1.6	None
MU/750 ml	114	60
MU/ 10^4 algal cells	0.10	0.25

Table 6. Toxicity levels of axenic and non-axenic dinoflagellate *A. tamarense* strain CCMP-117.

PARAMETER	NON-AXENIC	AXENIC
Algal culture age (days)	17	18
Algal cell count/ml ($\times 10^3$)	10.8	4.5
CFU/ml ($\times 10^6$)	5.1	None
MU/750 ml	142	88
MU/ 10^4 algal cells	0.13	0.19

Table 7. Toxicity levels of axenic and non-axenic dinoflagellate *G. catanella* strain CCMP-1493.

PARAMETER	NON-AXENIC	AXENIC
Algal culture age (days)	17	15
Algal cell count/ml ($\times 10^3$)	3.0	9.8
CFU/ml ($\times 10^5$)	1.0	None
MU/750 ml	6.8	17
MU/ 10^4 algal cells	0.02	0.02

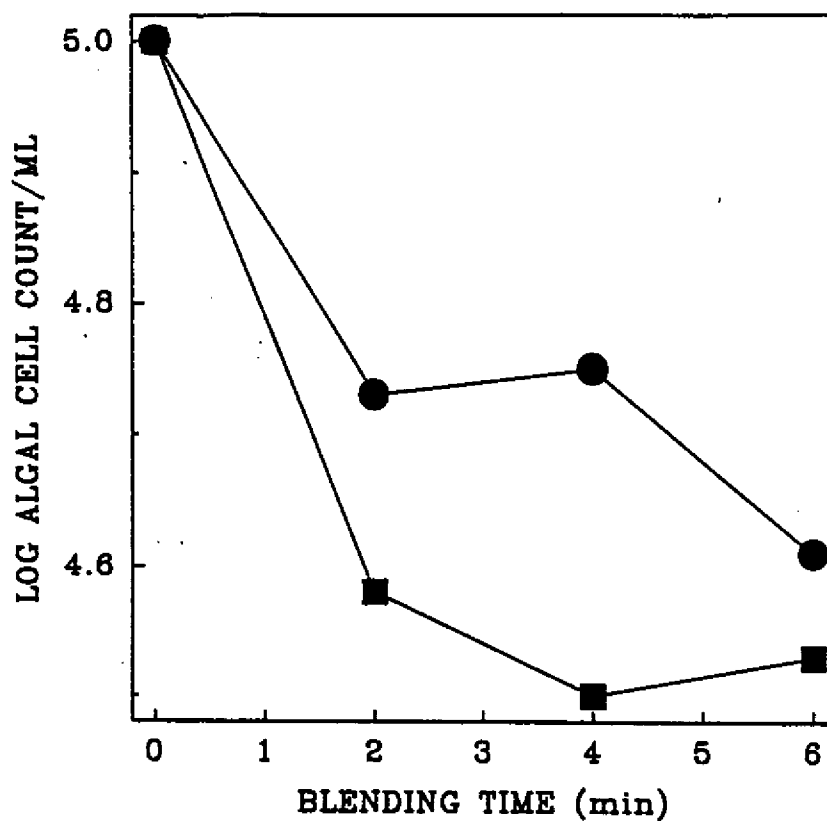


Fig. 1. Resistance of *Moraxella* A and B and *Bacillus* isolates to disruption by blending with glass beads.

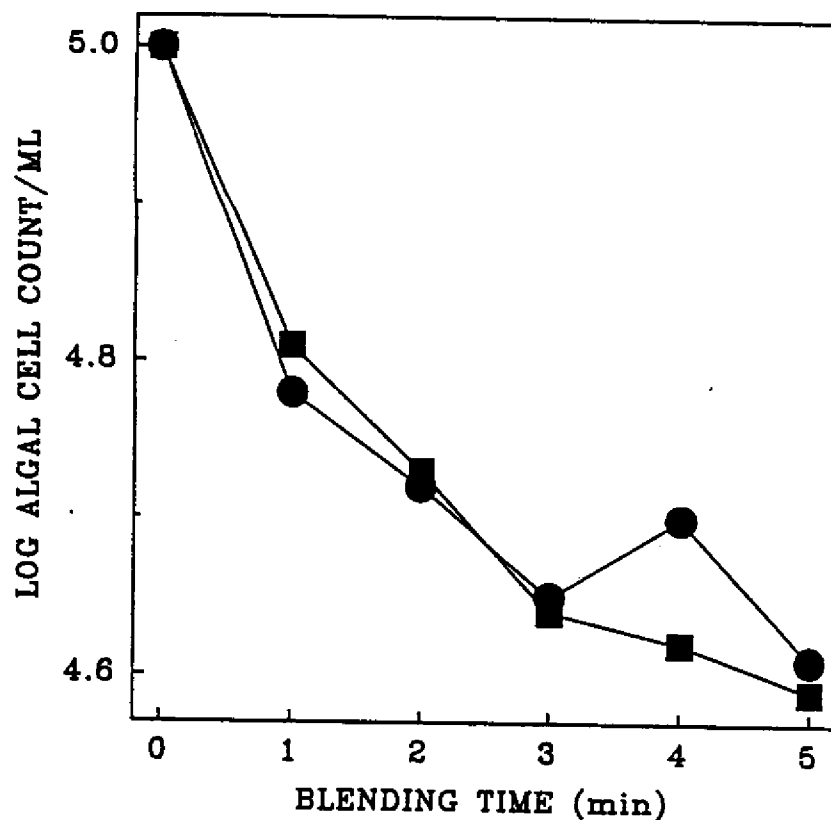


Fig. 2. Rates of cellular disruption of *A. tamarensis* strain CCMP-1312 by blending with glass beads. Duplicate destruction plots presented.

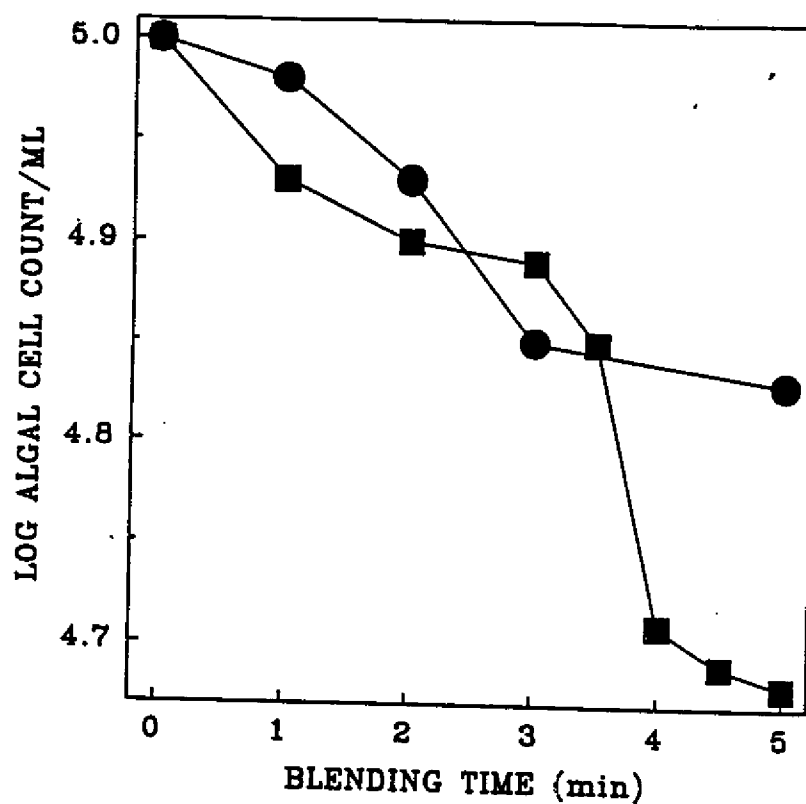


Fig. 3. Rates of cellular disruption of *A. tamarense* strain CCMP-117 by blending with glass beads. Duplicate destruction plots presented.

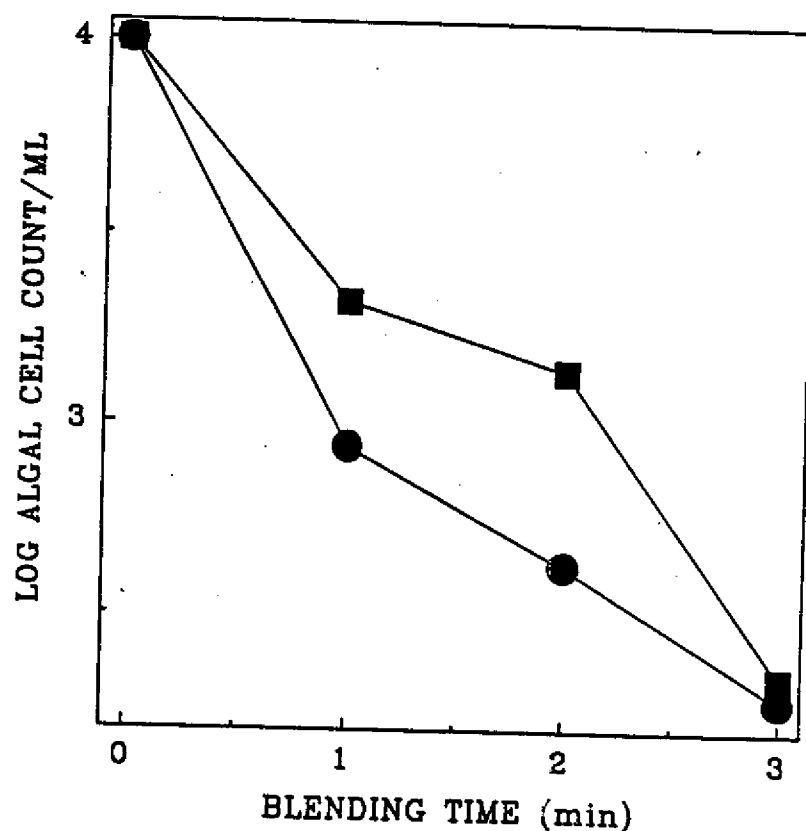


Fig. 4. Rates of cellular disruption of *A. catanella* strain CCMP-1493 by blending with glass beads. Duplicate destruction plots presented.

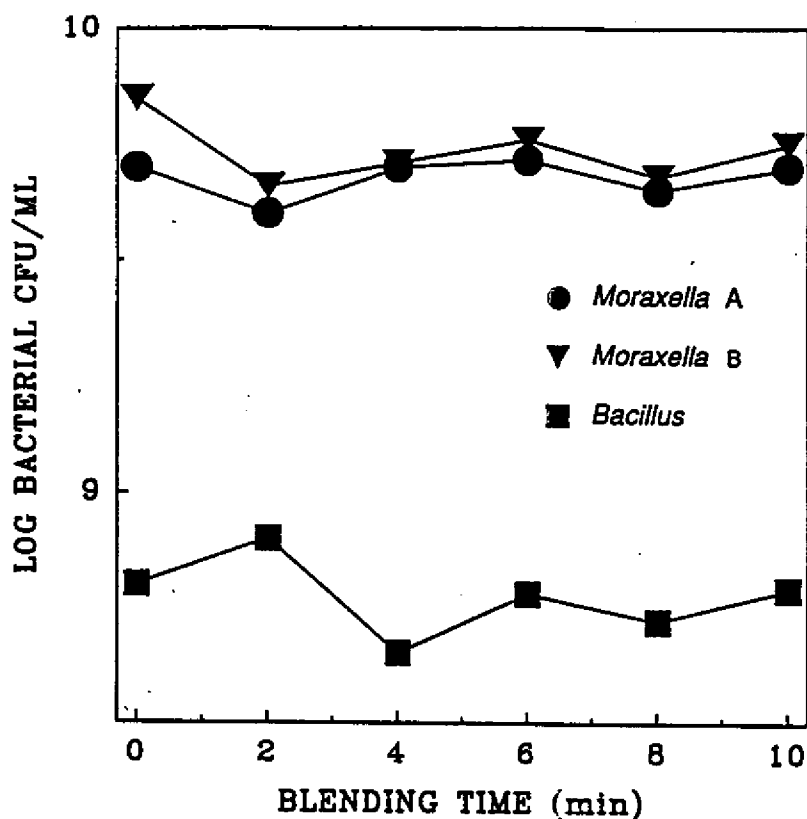


Fig. 5. Rates of cellular disruption of *G. catenatum* strain CCMP-412 by blending with glass beads. Duplicate destruction plots presented.

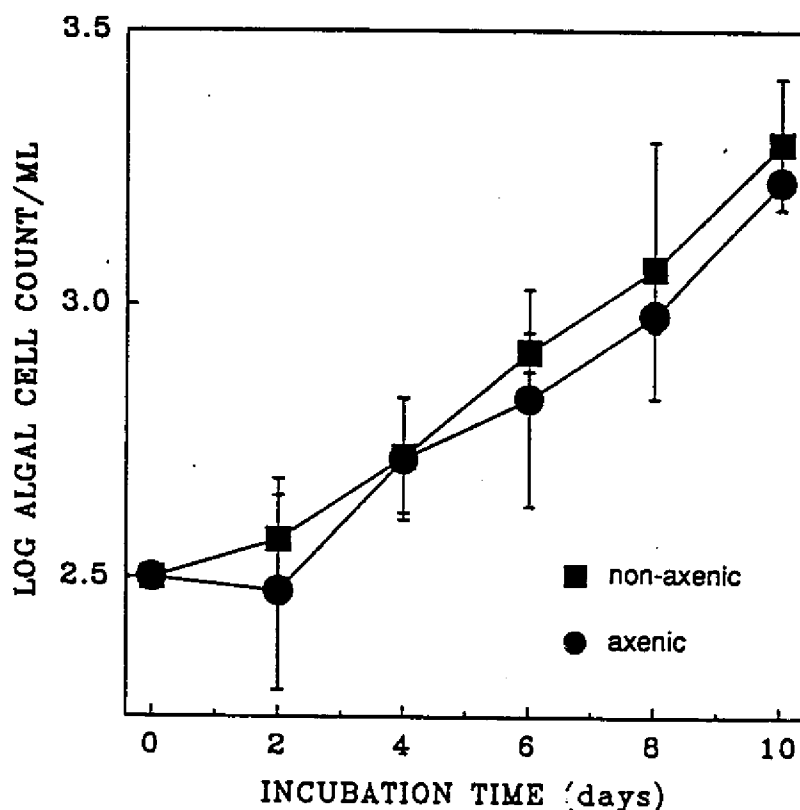


Fig. 6. Increase in microscopic cell counts of *G. catenatum* strain CCMP-412 in axenic and non-axenic cultures. Axenic and non-axenic algal cultures were grown at 20°C in f/2 (24 ppt NaCl) algal medium supplemented with soil extract. Error bars represent standard deviations of duplicate studies.

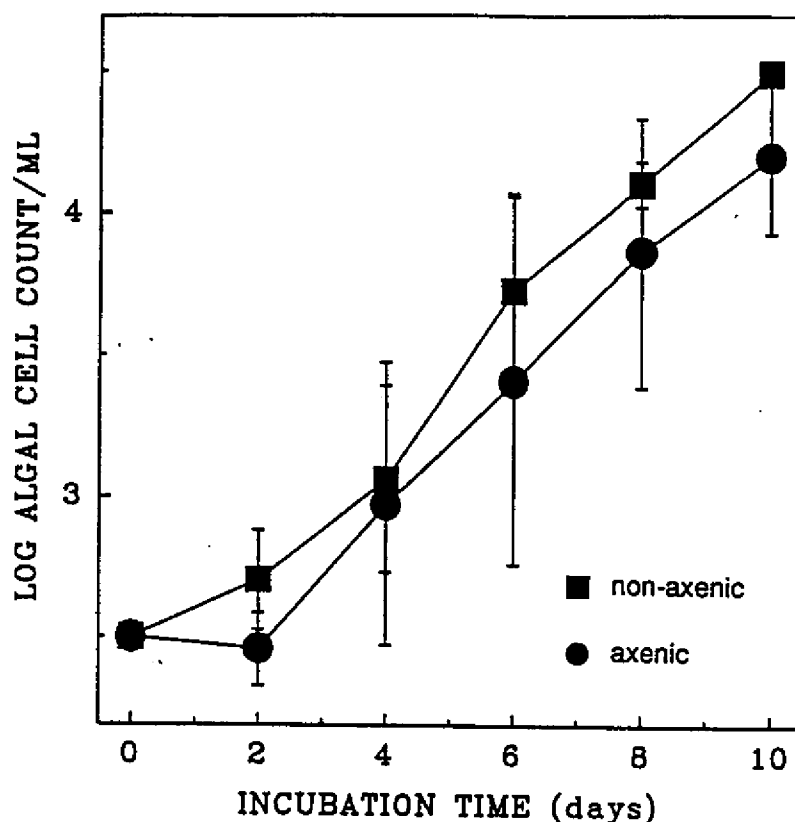


Fig. 7. Increase in microscopic cell counts of *A. catenella* strain CCMP-1493 in axenic and non-axenic cultures. Axenic and non-axenic algal cultures were grown at 20°C in f/2 (32 ppt NaCl) algal medium. Error bars represent standard deviations of duplicate studies.

Table 8. Toxicity levels of axenic and non-axenic dinoflagellate culture *G. catenatum* CCMP- 412.

PARAMETER	NON-AXENIC	AXENIC
Algal culture age (days)	9.0	12
Algal cell count/ml ($\times 10^3$)	11.9	2.4
CFU/ml ($\times 10^5$)	1.6	None
MU/750 ml	114	60
MU/ 10^4 algal cells	0.10	0.25

inherent in nuclear genes of the dinoflagellate.

If intracellular bacteria were involved in toxin production, one would expect a mixing of bacteria in the zygote and the F1 progeny resulting in hybrid profiles occurring in the F1 generation cells. No hybrid profiles occurred. In addition, no studies have come forth on the nutritional and environmental factors that influence toxin production by such *Moraxella* and *Bacillus* isolates nor have confirming studies emerged from other laboratories.

In addition to the studies above, we followed the rates of dinoflagellate cell development in non-axenic and axenic cultures. No significant difference was observed in the rate at which cell densities increased (Figs. 5 & 7).

We have therefore concluded that: (1) extracellular contaminating microorganisms exert no influence either on toxin production or on the rate at which the density of dinoflagellate cells increase and (2) that the cultures we examined were devoid of intracellular bacteria.

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VIBRIO VULNIFICUS IN SHELLFISH:
AN EXAMINATION OF PUBLIC HEALTH RISKS
AND PUBLIC HEALTH POLICY RECOMMENDATIONS

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INTRODUCTION

The purpose of the paper is to examine the characteristics and significance of disease associated with the bacteria *Vibrio vulnificus* in oysters and how this affects public health policy. It is necessary that we determine the nature of the organism, describe the disease with which it is associated, determine its environmental distribution, determine the public health risk, discuss the state's legal liability as well as moral and ethical obligations, and outline the proposed responses and their potential impacts upon the public and the industry.

DISCUSSION

Nature of organism

By understanding the nature of the organism we can understand the implications of causing disease via oysters. Oysters filter the water in their environment to extract their food. *Vibrio vulnificus* (*V. vulnificus*) is a marine bacterium which is found throughout the marine environment within U. S. coastal waters (9,10,14,19,21,22). It is also found in brackish water environments (13). The bacteria are highly motile, free living, gram negative, curved, rod-shaped, autochthonous inhabitants of the waters from which molluscan shellfish are harvested (5,16). *V. vulnificus* is a halophilic, (salt-loving) non cholera, marine vibrio which is most frequently isolated from marine environments with a temperature greater than 20°C (68°F) and a salinity of 7‰ to 16‰ and is only rarely found in environments cooler than 17°C (62.6°F) (20). Oysters harvested from waters which exhibit these salinity and temperature regimes have the potential to cause *V. vulnificus* infections. Since *V. vulnificus* occurs in Virginia waters which exhibit these specific characteristics, we must address the disease caused by this infection.

Description of the organism

In order to perceive the public health implications of the presence of V. vulnificus in oysters, we must understand how the infection can occur, what are its manifestations and what is the prevalence rate of the disease. V. vulnificus is a highly virulent and invasive pathogen which is identified with three different disease syndromes (9,23). The first syndrome is a progressive infection characterized by a few diarrheal symptoms, primary septicemia and cutaneous lesions, it carries a 50% case fatality rate (7). The second is a wound infection which results in skin lesions and occasionally septicemia, with a 13% case fatality rate (4,13,23). The third is acute diarrhea which is limited and rarely fatal (12). Infection occurs most often in immunocompromised hosts which include patients who have neoplasia of the immune system, a hematopoietic disorder, liver disease or alcoholism, chronic renal failure, or acquired immune-deficiency syndrome, or patients who are receiving pharmacological immunosuppression for neoplasia or transplantation of an organ (8). Additionally, patients with diabetes mellitus, individuals with naturally low levels of gastric acid or those taking prescription or over-the-counter medication to reduce stomach acidity are at increased risk of infection (12). Those persons with hepatic disorders and iron metabolism dysfunction are at much higher risk of progressive infection which often results in death (14,18). Infection with V. vulnificus comes from exposure of lesions to the marine environment, injuries from marine animals, or the ingestion of raw or partially cooked shellfish (13,14,18).

Epidemiology

In Virginia the incidence of V. vulnificus infections has been low in comparison to the Gulf states. From 1974 to 1992 there were 29 cases of V. vulnificus infections identified by the Virginia Division of Consolidated Laboratories and reported to the Office of Epidemiology; 3 resulted in death (VDH Epidemiology Program 1992). The average annual incidence for this period in Virginia is 0.53 cases per 100,000 population with a mortality rate of 0.05 deaths per 100,000.

In the state of Florida from 1981 through 1992 there were 124 confirmed cases of V. vulnificus infections; 43 resulted in death (Florida Department of Health and Rehabilitative Services Epidemiology Program 1992). The average annual incidence for this period in Florida is 1.09 cases per 100,000 population, with a mortality rate of 0.37 deaths per 100,000.

In the state of Louisiana there were 85 confirmed cases of V. vulnificus infections from 1981 through 1992, of which 25 resulted in death (Louisiana Department of Health and Hospitals, Epidemiology Program 1993). The average annual incidence in Louisiana is 2.01 cases per 100,000 population, with a mortality rate of 0.59 deaths per 100,000.

The demonstrated incidence of V. vulnificus infections in a Maryland Chesapeake Bay community was 1.6 per 100,000 per year from 1974 through 1988 (6). These numbers indicate a significantly higher incidence rate demonstrated in this clinical study than the reported Virginia annual incidence rate. These clinical studies tend to provide more comprehensive data than the state reports. The state statistics may not be accurate due to underreporting, improper diagnosis and other confounding factors. Therefore, the average annual incidence rate in Virginia may be higher than 0.53 cases per 100,000 population.

Environmental Distribution

The next step is to uncover why the incidence rates are higher in the Gulf than in Virginia. The key to answering this question is found in determining how the organism is distributed in the environment. Concentrations of *V. vulnificus* vary widely in the water column, sediment and oysters. The recent cooperative work between the Virginia Department of Health, Division of Shellfish Sanitation, and the University of Florida has shown that there is a statistically significant difference between Gulf of Mexico waters and Chesapeake Bay waters. The concentrations of *V. vulnificus* are, on average, somewhat greater in the Gulf waters and persist for longer periods presumably due to higher temperatures, a more temperate yearly climate and higher salinities.

A number of studies indicate that there is a strong correlation between temperature, salinity, and the presence of *V. vulnificus* in seawater and oysters (9,10,11,14,15,17). As stated earlier, *V. vulnificus* has been isolated from waters with a wide range of temperature and salinity readings, but the highest concentrations of the organism have been found in waters with temperatures of 17°C to 31°C and salinities of 15‰ to 25‰ (17).

Most oysters harvested in Virginia come from waters that fall outside the temperature and salinity ranges preferred by *V. vulnificus*. The majority of oysters presently harvested in Virginia are from waters with salinities less than 15‰. Harvesting on public oyster grounds is limited to the months from October to May. The oyster harvest statistics from the Virginia Marine Resources Commission indicate that the months, in order of number of bushels of oysters caught, for the years 1988 to 1992 are January, October, November, December, February, March respectively (Virginia Marine Resources Commission, Fisheries Plans and Statistics, 1993). The average monthly water temperatures (calculated from the daily mean) for the months October to May, from 1947 to 1992, for the Chesapeake Bay watershed (as measured at the Virginia Institute of Marine Science pier) range from a low of 4.12°C in January to a high of 26.61°C in August with the months of November through April having average temperatures below 17°C (Virginia Institute of Marine Science, 1993). Therefore, it is less likely that oysters from Virginia will have concentrations of *V. vulnificus* as great as those from the Gulf states. Additionally, the majority of oyster landings in Virginia occur in months when the water salinities are outside of the optimum range for the growth of *V. vulnificus*. Consequently, the likelihood of oysters harvested from Virginia having significant numbers of *V. vulnificus* is low whereas for the Gulf oyster it is high. We must address the risks associated with oysters caught from Virginia and those caught from the Gulf.

Public Health Risks

What are the public health risks posed by *V. vulnificus* in oysters that are harvested, distributed and processed in Virginia? In order to adequately address this question we must outline how the oyster industry in Virginia currently operates. The season of oyster harvesting from areas open to harvest by anyone who purchases the appropriate licenses is October through May. Harvest from private leases from the state occurs year round. The major portion of the processing takes place from October through March. Due to the shortage of Chesapeake Bay oysters, their prices are high. In contrast, due to their abundance, Gulf oysters are, at times, only half the price of Virginia oysters. So, based on price differential and availability of the resource, oysters from other states, particularly

Gulf states, are imported into Virginia to be processed. The oysters that come to Virginia from Florida, Louisiana and Texas have a warning label. After these oysters are processed, they go out under the label of a Virginia facility. Since Virginia does not require a warning label, the information concerning the risks associated with these oysters is lost.

Risk is a quantitative assessment of the degree of harm to health that may be anticipated. Safety is a value judgement of how much risk an individual or a community is willing to accept (2). These concepts are crucial in our examination of this issue.

As noted earlier, the disease and death rates from V. vulnificus infections are much lower in Virginia than in Florida and Louisiana. The results of the 1993 Commonwealth Poll estimate that there are 847,554 raw shellfish consumers in the state of Virginia (18.1% of the population 18 or older). Of this group, 92,882 (10.9%) have at least one high risk characteristic. These high risk characteristics are liver disease, stomach disorders, diabetes, immune system disorder or immune suppressant drug therapy. The 1988 Florida Behavioral Risk Factor Survey estimated the prevalence of raw oyster consumption and host factors that may facilitate V. vulnificus infections (4). Approximately 2,950,818 adults in Florida are raw oyster eaters (23% of the population 18 or older) and 70,820 (0.5%) of these raw oyster eaters have liver disease (one of the high risk characteristic) (3).

There appears to be a lower risk of disease and death from eating oysters caught in Virginia. Some of the possible explanations are: 1) The concentration of V. vulnificus in Virginia oysters is lower. 2) The majority of oysters are harvested in the months where the water temperature is lower and, therefore, there are fewer organisms in the water and in the oyster. 3) The salinities in the areas where oysters are harvested do not provide optimum conditions for the proliferation of the organism. 4) Consumers are educated and ask for oysters caught in Virginia. 5) The serotype of V. vulnificus found in the Gulf is more virulent than that of the serotype found in Virginia waters.

Estimates indicate that approximately 90% of the oysters processed in Virginia are from the Gulf. The greater risk of disease and death from eating oysters caught in Gulf waters may be explained as follows: 1) The concentrations of V. vulnificus in Gulf oysters is higher than Virginia oysters. 2) During the months in which the public oyster harvest season in Virginia is closed, processors may purchase oysters from the Gulf. It is during these summer months that the water temperatures are highest and, therefore, the concentrations of V. vulnificus would be highest. 3) Salinities in the areas of harvest in the Gulf provide the optimum conditions for the proliferation of the organism. 4) Shucked oysters are not identified as to the place of harvest, so consumers are not aware that they may be buying Gulf oysters.

Florida has shown through their work that there is a substantial difference in the risk of disease and death between nonraw oyster eaters, raw oyster eaters and raw oyster eaters with liver disease. Of the oysters implicated in V. vulnificus infections in Florida all were harvested from the waters of the Gulf or southern Florida. (Fla. HRS Epidemiology Program)

RELATIVE RISK RATIOS FROM FLORIDA DATA

NONRAW OYSTER EATERS

.02 illnesses/1000 adults/35 years; .003 deaths/1000 adults/35 years

RAW OYSTER EATERS

.03 illnesses/1000 adults/35 years; .008 deaths/1000 adults/35 years

RAW OYSTER EATERS WITH LIVER DISEASE

2.43 illnesses/1000 adults/35 years; 1.53 deaths/1000 adults/35 years

There is also a risk of V. vulnificus infection via a wound exposed to the marine environment. This risk exists obviously for anyone exposed and increases if they have a liver disorder, are immunocompromised or exhibit any other factor which may increase their susceptibility. The mortality rate for a wound infection is significantly smaller than for primary septicemia.

Legal Liability

If someone is infected with V. vulnificus from oyster ingestion and dies, the state's legal liability will be questioned. This issue has arisen in California and Louisiana.

In a recent California case, an individual became sick from ingesting raw oysters from the Holiday Inn at Fisherman's Wharf in San Francisco (Kilpatrick v. Holiday Inns Inc.). The attending physician concluded that the symptoms resulted from the consumption of raw oysters tainted with Vibrio cholera. The man sued the Holiday Inn and the companies (United Shellfish, Inc., Fisherman's Wharf Seafoods, Inc. and Pearson's Seafood) that supplied and transported the shellfish. The suit was based upon causes of action for negligence, negligence per se, strict liability and breach of warranty. This case was settled out of court with the trucking companies that shipped the shellfish paying damages to the plaintiff.

In Louisiana a Mississippi man became infected with V. vulnificus from consumption of Louisiana oysters (William Winstead v. Ed's Live Catfish & Seafood Inc., et al. Parish of E. Baton Rouge, LA)(17). The original verdict found that the state was liable for not having adequately warned the public of the risks associated with consuming raw oysters. The case was appealed and the lower court's ruling was overturned. The most important findings are as follows:

1. The failure of the Department of Health and Human Resources to warn the consumer that a person with certain underlying risk factors should not consume raw oysters was not a cause, in fact, of consumer's illness. The Department was not liable since the consumer was not aware that he had any of the underlying risk factors.
2. Since the Department of Health and Human Resources had undertaken studies of Vibrio vulnificus bacteria and knew that it posed risk to a small segment of the population, it had a duty, once it had sufficient information, to issue a warning to the public concerning the health hazards possibly associated with Vibrio vulnificus

bacteria in raw oysters. However, the Department fulfilled its duty by publishing a report summarizing what the Department knew about bacteria at the time and by stating that physicians should warn patients with any of the underlying risk factors not to eat raw oysters.

3. It was an error for the trial court to create a duty on the DHHR to warn when no such duty exists in Louisiana statutory or case law.
4. It was an error for the trial court to conclude any act or omission of DHHR was a cause, in fact, of the plaintiff's injury.
5. It was an error for the trial court to conclude that the DHHR did not warn the public about the Vibrio vulnificus bacteria and its relationship to raw oyster consumption.

A similar case was also brought to court in Louisiana (Theresa Simeon, et al. v. Big Easy, Inc., M.J. Billick Oyster Co., Dept. of Wildlife and Fisheries, La. Dept of Health Civil District Court New Orleans, LA)(18). In this case Floyd Simeon Sr. and his son ate raw oysters at the Sweet Pepper grill in New Orleans. Thereafter, Simeon was hospitalized for pain associated with severe blisters on his legs. After attempts to stop the spreading of the blisters failed, Simeon's legs were amputated. Despite these efforts, Simeon subsequently died. Death resulted from septicemic infection caused by V. vulnificus. Mr. Simeon's daughter later brought suit against the restaurant, suppliers and the Louisiana state agencies that hold responsibility for monitoring the sanitation of oysters. As with the previous case, the original verdict found that the state was liable for not having adequately warned the public of the risks associated with consuming raw oysters. The case was appealed and the lower court's ruling was overturned. The most important findings are as follows:

1. The Department of Health and Human Resources (DHHR) fulfilled its duty to warn concerning danger of bacteria in raw oysters by publishing monthly morbidity reports targeting the warning to health care providers. Since the bacteria most often attack a small percentage of the population, ie. those with liver disease, kidney disease, or other immune-suppressive condition, this method of warning was determined to be adequate.
2. A newspaper article quoting Department of Health and Human Resources (DHHR) officials concerning dangerous bacteria present in raw oysters was offered to show that the public was warned that the dangers in eating raw oysters are relevant, not hearsay.
3. Statistics from United States Department of Commerce, National Marine and Fisheries Service, offered to prove extremely low incidence of bacteria infection in oysters and to show it was more prudent to target warnings to health care providers, who would inform those very few who were at risk, were determined to be admissible in action arising from the death of restaurant patron.

4. The fact that the DHHR chose not to aggressively disseminate the information contained in the Monthly Morbidity Report to the general public does not constitute a breach of the duty to warn. Since the bacteria only attacks a small percentage of the population, i.e., those with liver disease, kidney disease or other immune-suppressive condition, the DHHR was reasonable in its conclusion that the best way to warn these people was through the medical community. The Monthly Morbidity Report was sent to every physician in Louisiana and the health departments of several states, including Mississippi. The court held that this satisfied the duty to warn which was incumbent on the DHHR due to its specialized knowledge and position of public trust.

In this case one judge on the Fourth Circuit Court of Appeals of Louisiana held a dissenting opinion. In this minority opinion he writes: "Considering the DHHR's knowledge about the extreme danger of this bacteria and its position of public trust, I find that DHHR owed a duty to the general public to adequately warn of the possibility of contracting a deadly disease by eating raw oysters. A warning to the medical community absent a concurrent warning to suppliers and retailers of oysters as well as the general public was not sufficient."

The liability of the state as determined by the court indicates that, if the effort is made to inform and educate about the risks associated with raw oyster consumption, then state liability is minimized. This effort is considered valid if it is targeted toward the medical community and the general public via the media. Warning labels on the product are not deemed to be necessary in order to fulfill the agencies' responsibility to warn.

Proposed Response To The Issue

There is an increased risk of disease from eating raw oysters. Due to the ubiquitous nature of many bacteria and their opportunistic abilities, they will always pose a risk to those who eat food which has not been subjected to a bactericidal process. There currently is no feasible method available in harvesting, processing or distribution to render the oyster free of harmful bacteria without cooking it. Therefore, disease prevention must focus on educating those at the greatest risk (ie. raw oyster eaters with liver disease, stomach disorders, and immune deficiency disorders) about the magnitude of risk associated with this behavior.

The Interstate Shellfish Sanitation Conference (ISSC) is a consortium of state agencies, federal agencies and industry that formulates policy for adoption by the United States Food and Drug Administration (FDA) into the National Shellfish Sanitation Program (NSSP). It is this program that provides the specific guidelines of sanitation which must be followed by the industry and the operational rules under which the states must implement their programs. The purpose of the NSSP is to provide uniformity and to assure that shellfish shipped interstate are safe. The ISSC has addressed the V. vulnificus concerns as follows:

1. In Issue 92-311, the ISSC adopted: "The Interstate Shellfish Sanitation Conference should formally address the public health significance of naturally occurring pathogens and biotoxins associated with the consumption of raw molluscan shellfish. Specifically, the ISSC should continue to investigate and address harvest, handling,

storage, process, distribution, education, and public information activities particularly those activities targeted toward those at-risk consumers."

2. In Issue 92-117, the ISSC adopted: "ISSC should seek technical assistance from federal agencies regarding water temperature and naturally occurring pathogen levels in water and shellstock at different storage temperatures and its public health significance. In addition, an evaluation of commercial feasibility should be conducted."
3. In Issue 92-304, the ISSC adopted: "The Interstate Shellfish Sanitation Conference should work with FDA and industry through the Education Committee to develop educational programs to reduce health risks associated with consuming raw shellfish. Such programs should be geared to both the general public and at-risk groups. The Conference should develop consumer education brochures targeted to at-risk groups and the medical/health community, promote 'Dear Doctor' letters to physicians, and develop other methods of educating consumers."
4. In Issue 92-305 the ISSC adopted: "...as part of the ISSC on-going educational efforts, if individual States require a point-of-sale message, the following language is recommended:

CONSUMER INFORMATION

As is the case with consuming other raw protein products, there is a risk associated with consuming raw shellfish. If you suffer from chronic illness of the liver, stomach or blood or have immune disorders, do not eat these products raw."

The position of the ISSC is that: "Certain individuals with underlying medical problems (cirrhotics, diabetics, persons with renal failure, persons with hemochromatosis, persons who are immunosuppressed) are at high risk for infection by Vibrio vulnificus. The Conference has stated and continues to emphasize that such individuals must not consume raw molluscan shellfish. The Conference believes that aggressively targeted educational efforts are appropriate means of reducing illnesses from Vibrio vulnificus. Such targeted education will be accomplished by reaching the high risk population through their various support groups (i.e. professional, medical, social, voluntary, and governmental organizations). Further aggressive Conference actions include recommendations to states and the shellfish industry to take immediate steps to institute harvesting, processing, and handling procedures which will eliminate illnesses attributable to Vibrio vulnificus. These procedures should include appropriate rapid cool-down and continued chilling of shellfish to control growth of potentially pathogenic Vibrio species. In addition, the ISSC will promote and encourage research to develop control mechanisms which will effectively reduce these illnesses." (ISSC News Release, March 21, 1991).

The position of the FDA is that: "Reports of illness and death attributable to Vibrio vulnificus infection following the ingestion of raw oysters emphasize the importance of warning certain high-risk patients against eating raw or lightly (partially) cooked seafood. These patients include those with: -liver disease and other diseases with possible or definite hepatic involvement, such as alcoholism, malignancy, hemochromatosis, thalassemia major, -therapeutically induced or naturally low gastric acid, -compromised, immune systems (including AIDS and AIDS-related complex). There are presently no known sanitation or other public health controls that can limit the harvesting of shellfish to those free of Vibrio vulnificus or control the presence of the organism in raw shellfish in the marketplace. Vibrios are not expected to withstand normal cooking procedures. Shellfish-borne Vibrio infections can be prevented by cooking seafoods thoroughly and keeping them from cross-contamination after cooking. Cooked seafood is best stored at 60 degrees C (140 degrees F) or greater, or 4 degrees C (39.2 degrees F) or below. Vibrio vulnificus is naturally occurring in the marine environment, and it is considered to be an opportunistic pathogen in individuals having compromised immune systems or medical disorders. The control measure of choice at this time is adherence to Good Manufacturing Practices associated with shellstock handling and transportation." (FDA Drug Bulletin, April 1988, Volume 18, Number 1).

The FDA has agreed with the ISSC "Issues" 92-311, 92-304, and 92-305 (October 9, 1993 letter from FDA to the ISSC).

Both FDA and the ISSC recommend that this issue be investigated and addressed by analyzing and examining harvest, handling, storage, process, and distribution of the product. This recommendation is currently being pursued by the ISSC and the VDH. Additionally education and public information activities, particularly those activities targeted toward at-risk consumers, should be initiated. The FDA and ISSC feel that consumer education brochures should be targeted toward at-risk groups and the medical/health community by promoting "Dear Doctor" letters to physicians, and by developing other methods of educating consumers.

CONCLUSION

Educating persons who have liver disease, stomach disorders, or immune deficiency disorders about the risk in eating raw oysters is most effectively accomplished via the physician because 1) the physician has the most complete knowledge of the patient's state of health and therefore, can assess individual risk; 2) the patient is more likely to heed a physician's advice as opposed to a general warning via a label, notice in eating establishments, news releases etc.; and 3) one-on-one communications are more effective than mass communications.

An informed physician can be the most effective communicator of the risks associated with raw oyster consumption to high risk patients. If we target specific physicians, we have a smaller, more informed audience to reach with the information and can more easily repeat the message so that it is reinforced. If we wish follow up to assure that the message is getting to the physicians and that they are relaying it to their high risk patients, we can measure the effectiveness of our effort.

Optimally, the information should be available in a pamphlet form in the physician's waiting room as a mechanism to reach more individuals. The groups that are at highest risk of infection from eating raw oysters are more likely to be visiting a physician. By placing this information in the waiting room area, the individual may be stimulated to question the physician concerning consumption of raw oysters. In addition to educating the physician, this would increase the likelihood that the consumer at greatest risk would be informed and that the physician would provide advice on this matter.

The appropriate agency should also distribute a news release concerning the risks involved with the consumption of raw oysters. This release could be provided on a yearly basis to reinforce the message. It would also be sent to all other state public health agencies so that they may pass this information on to their citizens as they see fit.

Warning labels on the product are another alternative but warning labels tend not to elicit the positive response that physician's advice would. According to Dr. Louise McFarland, the director of the Epidemiology program in Louisiana, warning labels on the product are not entirely effective. That state has not seen a significant decrease in cases of disease or death as a result of its warning label program. She said that a well directed public education campaign which included health professionals was a much more effective method of reducing risk than warning labels. Warning labels may not be taken seriously. In most instances, warning labels rarely reach the intended audience because, if they are not included at the point of sale (ie. usually at the restaurant or retail outlet), the information does not get to the consumer. Indications from states that have labeling requirements are that they actually have little effect on reducing the number of cases of *V. vulnificus* infections via oyster consumption. The majority of health professionals that we have surveyed indicate that, in their opinion, warning labels are the least effective means by which to educate the public concerning the risk of eating raw oysters and that information disseminated to health professionals and the mass media was most effective. We share this opinion and recommend a comprehensive public education/information campaign be implemented in Virginia.

In order to implement the physician and general public education campaign, it will be necessary to communicate the message via mailings, distribution of printed information at physicians' organization meetings, speaking at physician meetings, information printed in professional journals, provision of printed materials to be placed in physicians' office waiting rooms, and through news releases to both the print and electronic media. It will be necessary to follow up with a mail or telephone survey to estimate the effectiveness of this campaign.

There are certainly some other more controversial measures that can be taken to minimize the potential risk of disease. These include restricting harvesting from Gulf waters to months which exhibit lower concentrations of *V. vulnificus*, require an approved irradiation regimen for all shellfish, require thermal processing of all shellfish prior to marketing and restrict the sale of raw shellfish. Are these measures reasonable in light of the potential risk? Probably not, but they will certainly continue to be proposed as long humans continue to contract vibriosis from ingesting raw shellfish. An educated public is the best line of defense against this disease.

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PROPERTIES OF FISH GELATIN

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During the manufacture of gelatin, collagen from connective tissue (skin, bone, sinew) is converted into a soluble, gel forming material which can be used as a food component or as a glue. Gelatin, commonly processed from pork and beef, is widely used as a commodity because of its gel forming and surface active properties. However, the Kosher/Halal community does not accept most currently available gelatin because the processing does not meet their religious requirements. Although a few low Bloom (less than 100 Bloom) fish gelatins have been available to this community, the gelatins were not comparable to commercial high Bloom gelatins. In order to fill this need, a few companies, including Food Industry Testing, Inc. (Miami Beach, FL), have developed a process (currently being patented) to obtain a high Bloom fish gelatin from warm-water fish skins. This product, in addition to the great benefit it represents to the Kosher/Halal community, has a high value to the fish industry's waste management effort by yielding an additional income for a by-product that is commonly wasted. The two main purposes of this study were to study the properties of these high Bloom fish gelatins derived from fish skin and to compare them to the ones from a well-known commercial gelatin.

MATERIALS AND METHODS

Seven fish gelatin samples: 300 Bloom fish gelatin (lots T5001, T5401, TA30104, and T5104), 250 Bloom fish gelatin (lot T25104), 210 Bloom fish gelatin (lot T5113), and 200 Bloom fish gelatin (lot T5502), were investigated. In addition, a commercial product, Knox® gelatin (Englewood Cliffs, NJ, lot 5-35001-08) was studied alone, and mixed 25%,

50% and 75% by weight with 300 Bloom fish gelatin (T5001) or with 200 Bloom fish gelatin (T5502). All these fish gelatin samples were prepared into 5% (w/w) solutions for the study of the effect of dissolving time on solubility, pH, melting point, and minimum solubility. For the determination of the minimum solubility point and the effect of concentration on pH, melting point and gel strength, the 300 Bloom fish gelatin (T5104) was used.

Effect of dissolving time on fish gelatin solubility

Fish gelatins (300 Bloom (T5104) and 200 Bloom (T5502)) solutions were prepared with deionized distilled water and kept at 40°C for 0, 1, 2, 3, 4, 5, and 6 hr. Each sample was centrifuged at 16,000 rpm for 15 min at approximately 40°C and the protein content of the supernatant was determined by Lowry (1951). The solubility was expressed as the percent of total protein (measured prior to centrifugation).

pH in solution

pH was measured using a glass electrode pH meter (pH-103 Metrohm/Brinkmann, Brinkmann Instruments Inc., Westbury, NY). All solutions were prepared with deionized distilled water and equilibrated to 40°C in a water bath for at least one hour. The calibration buffer (Certified Buffer Solutions, pH 4.00±0.01 @ 25°C, Fisher Scientific Co., Fair Lawn, NJ, lot 87623-24) was also kept in the water bath.

Minimum solubility

Eight 30 mL fractions of the 5% solution were prepared and kept at 40°C for at least one hr. The pH was adjusted with 0.1 N HCl or 0.1N NaOH to pH 3.8 to 5.2 (0.2 pH increments). These samples were centrifuged at 16,000 rpm for 15 min at approximately 40°C. A Lowry protein determination of the supernatant gave the total soluble protein (Lowry, 1951). The pH of the fraction with the lowest protein content in the supernatant (i.e., less soluble protein) was defined as the "minimum solubility point." After the approximate minimum solubility point was determined, the pH range was reexamined using 0.1 pH increments. The minimum solubility was calculated as a percent of the total sample weight and as a percent of the total protein (measured prior to centrifugation).

Melting point

The melting point measurement method described by Wainwright (1977) was modified. The test determines the temperature at which gels soften sufficiently to allow drops of chloroform to sink through them. Solutions were prepared with 0.1N NaCl, equilibrated at 40°C, and the pH adjusted to 7.0 with 0.1N NaOH. A 5 mL aliquot of each sample was transferred to a small glass tube (Fisherbrand® borosilicate disposable culture tubes, 12 x 75 mm, Fisher Scientific Co., Pittsburgh, PA) previously coated with Sigmacote® (Sigma Chemicals Co., St. Louis, MO, lot 23H6097) to prevent sticking. The samples were degassed in a vacuum desiccator for 5 min. The tubes were then covered with Parafilm® (Laboratory Film, Greenwich, CT). After heating in a

water bath at 90°C for 15 min, the gelatin solutions in the glass tubes were cooled immediately in ice-chilled water to 10°C and matured for 4 hr. Five drops of a mixture of chloroform and a reddish brown dye (Food color AFO OWS 550, Miles Inc., Elkhart, IN, lot 5-057039) were placed on the surface of the gel. The gels were put in a water bath at 10°C and the bath was heated at 0.2-0.4°C/min. The temperature of the bath was read from an electronic digital thermometer ($\pm 0.2^\circ\text{C}$, NBS (NIST), Serial No. 457261). The temperature at which the chloroform drops began to freely move down through the gel was taken as the melting point.

Gel strength

This method was modified from the NE-123 (Northeast Regional Hatch Protein Functionality Group) gelation method (Chong Lee, personal communication). Solutions from 2% to 12% (w/w) were prepared with 0.1N NaCl and equilibrated to 40°C. The pH was adjusted to 7.0 with 1N NaOH and the solutions stirred for 30 min. Following hydration, the samples were transferred to glass tubes (19 x 175 mm) which were previously coated with Sigmacote® and closed on one end with rubber stoppers (Fisherbrand® No. 2, Fisher Scientific Co., Pittsburgh, PA). The samples were degassed in a vacuum desiccator for 10 min. The other end of each glass tube was closed with a second rubber stopper, to prevent moisture loss and cook swelling, and the glass tubes were placed vertically in a 90°C water bath for 15 min. The glass tubes were then cooled to 10°C immediately in ice-chilled water for 20 min and kept refrigerated (4-10°C) overnight. The gels were removed from the glass tubes and cut into about 8 cylinders 19 mm long. Each cylinder was then compressed on an Instron Universal Testing Machine (Canton, MA, Model No. 1101) to determine the failure force. The cross-head (CCT load cell, 200 lb) speed of the Instron, interfaced with an IBM PC-XT, was set at 12.7 cm/min. The gel strength of each sample was calculated from the average failure force of several cylinders.

Sensory analysis

Cubes with 2.75% gelatin (w/w) were made from 300 Bloom (T5104 and TA30104) or 250 Bloom fish gelatin and Knox® gelatin, and cranberry juice (Ocean Spray Cranberries, Inc., Lakeville-Middleboro, MA) or grape juice (Sunny Square grape juice, Commander Foods, Inc., Syracuse, NY). One third of the juice was added cold to the fish gelatin and allowed to sit for 1 min. The rest of the juice was heated (high) in a microwave (Amana Touchmatic II Radarange® microwave oven, Amana Refrigeration, Inc., Amana, IW) for 1 minute, added to the cold mixture, and stirred until the gelatin was completely dissolved (about 5 min). Because the color of the fish gelatin was different from the Knox®, a few drops of red food color (McCormick, Hunt Valley, MD) was added. The mixture was poured into a 9" square pan and kept overnight in a refrigerator, and then cut into 1" cubes. A triangle sensory test (Larmond, 1987) was used to determine if the panelist could tell the difference

between the two products made from fish gelatin and Knox®. The panelists were also asked to record how great the difference was and which sample(s) (odd or pair) was preferred. Only correct answers on the triangle test were kept for further evaluation.

RESULTS AND DISCUSSION

Fig. 1 shows the effect of dissolving time (i.e., the time between gel solution preparation and the solubility test) on the protein solubility (%). The major change (i.e., a difference in solubility of about 12%) seemed to occur during the first hr. After 1 hr, the solubility changed slightly (i.e., an increase in solubility of about 3%) for both samples. Based on these results, all the samples that were prepared for the rest of the tests were allowed to stand for at least 1 hr.

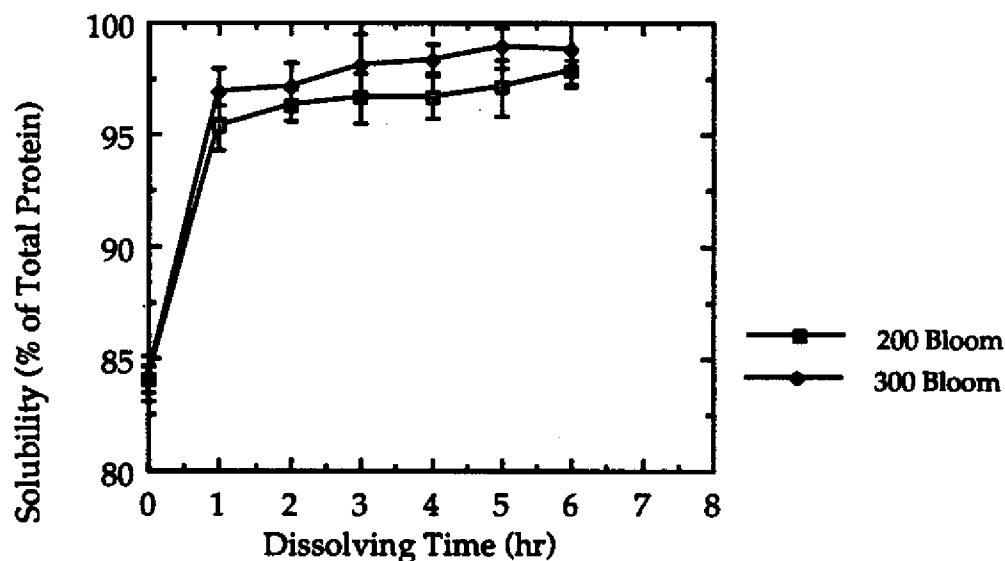


Fig. 1 Effect of dissolving time on fish gelatin solubility (% of Total Protein)

Table 1 shows pH, melting point, the minimum solubility point, and the minimum solubility of all samples. The pH ranged from 4.98 to 5.27. The lowest pHs were observed in 210 Bloom (T5113) and 300 Bloom (T5104) gelatin. The rest ranged from 5.17 to 5.27. The pH values were found to be significantly different by one way ANOVA statistical analysis from Bloom to Bloom and even within a single Bloom (300) ($p < 0.05$). However, the pH range was only of 0.29 pH units. The pH of the mixtures of 300 Bloom and Knox® were not significantly different from either 300 Bloom or Knox®. On the other hand, the pH of the mixtures of

200 Bloom and Knox® were significantly different from Knox® ($p < 0.05$) but not from 200 Bloom fish gelatin.

Table 1. pH, melting point, minimum solubility point (MSP) and minimum solubility (MS) of gelatins as 5% (w/w) solutions

Samples	pH	Melting Point (°C)	MSP (pH)	MS (% of Total Protein)	MS (% of Total Weight)
300 Bloom fish gel (T5001)	5.21	24.3	4.34	97.29	83.03
300 Bloom fish gel (T5401)	5.22	24.7	4.35	96.89	82.13
300 Bloom fish gel (TA30104)	5.27	24.7	4.38	96.33	84.19
300 Bloom fish gel (T5104)	5.05	24.6	4.38	96.66	84.08
250 Bloom fish gel (T25104)	5.27	24.0	4.38	95.95	86.09
210 Bloom fish gel (T5113)	4.98	23.7	4.38	96.24	84.93
200 Bloom fish gel (T5502)	5.20	23.5	4.38	95.42	85.55
Knox® Gelatin	5.17	29.6	4.34	96.34	83.41
25% 300 Bloom fish gel (T5001) + 75% Knox®	5.20	28.6	4.35	96.71	83.07
50% 300 Bloom fish gel (T5001) + 50% Knox®	5.18	27.3	4.35	96.19	82.74
75% 300 Bloom fish gel (T5001) + 25% Knox®	5.19	25.9	4.36	96.32	82.89
25% 200 Bloom fish gel (T5502) + 75% Knox®	5.21	26.8	4.35	95.47	83.74
50% 200 Bloom fish gel (T5502) + 50% Knox®	5.24	26.3	4.35	95.55	84.23
75% 200 Bloom fish gel (T5502) + 25% Knox®	5.23	25.2	4.36	95.16	84.72

The concentration of the solution seemed to affect the pH slightly (Fig. 2), although from 2% to 20% gelatin the shift was only about 0.10 units.

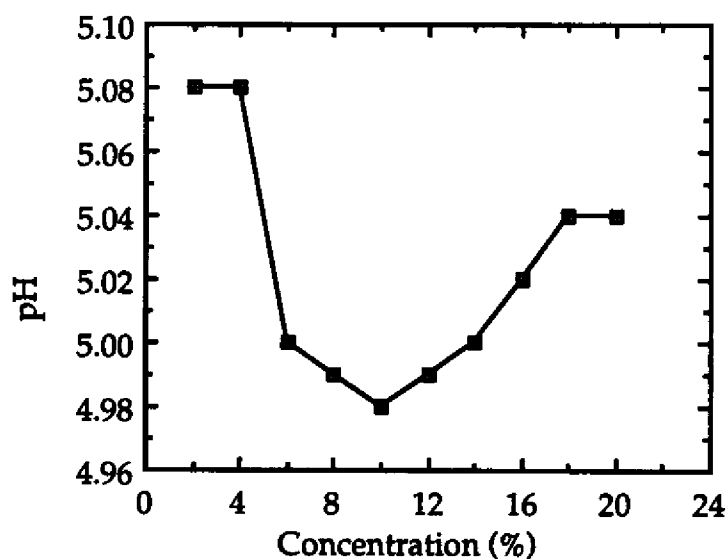


Fig. 2 pH at different concentrations of 300 Bloom fish gelatin (T5104)

Two tests were performed to measure the minimum solubility of 5% 300 Bloom (T5104) (Fig. 3). Since the lowest solubility was observed at pH 4.38 in both cases, this value was considered the minimum solubility point of this fish gelatin sample. The minimum solubility point of all samples (see Table 1) showed a mean of 4.36 ± 0.02 pH units. The minimum solubility was $96.18 \pm 0.02\%$ of the total protein content or $83.91 \pm 0.13\%$ of the total weight. Furthermore, all 200 Bloom to 300 Bloom samples had a minimum solubility of about $96.14 \pm 1.39\%$ of total protein content which was very close to the minimum solubility of Knox® of about 96.34% of total protein content. This was also true when compared as percent of total weight. The minimum solubility points were also very close between the fish gelatins (4.37 ± 0.02) and the Knox® gelatin (4.34).

Two of the 300 Bloom fish gelatin and Knox® mixtures (25% and 50% fish gelatin) had higher minimum solubilities (expressed as percent of total protein content) than either the Knox® or the 300 Bloom fish gelatin whereas the sample of 75% fish gelatin had the lowest minimum solubility (see Fig. 4). All the mixtures, however, had a higher protein solubility than either the Knox® or the 300 Bloom fish gelatin at other pHs. In the case of the 200 Bloom fish gelatin which had a lower solubility (at all pH values) than Knox® gelatin, the protein solubility of the mixtures were between the two (see Fig. 5).

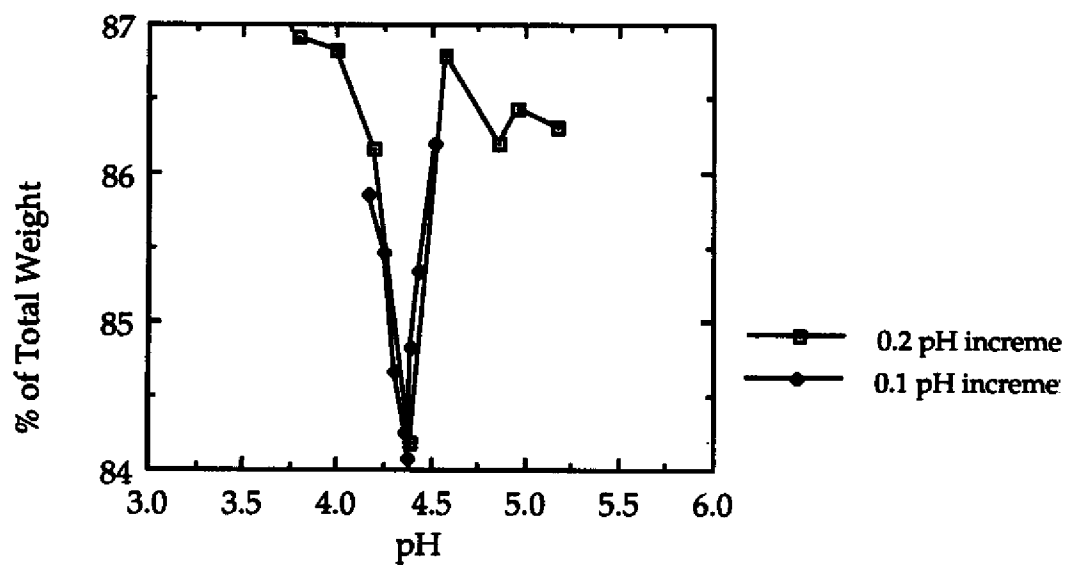


Fig. 3 Solubility of 5% 300 Bloom Fish Gelatin (T5104) at different pHs

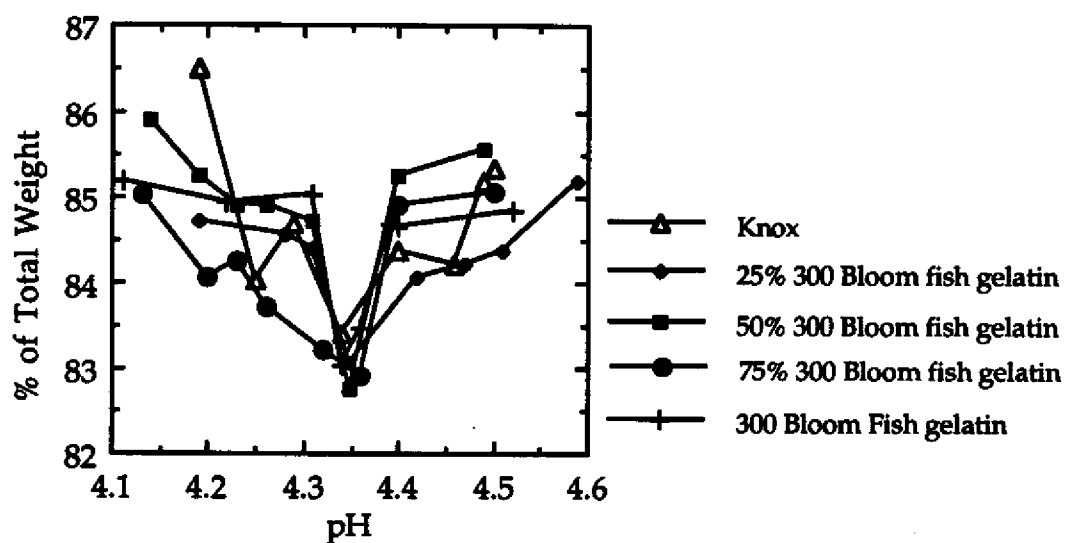


Fig. 4 Solubility of the mixtures of 300 Bloom fish gelatin (T5104) and Knox® gelatin

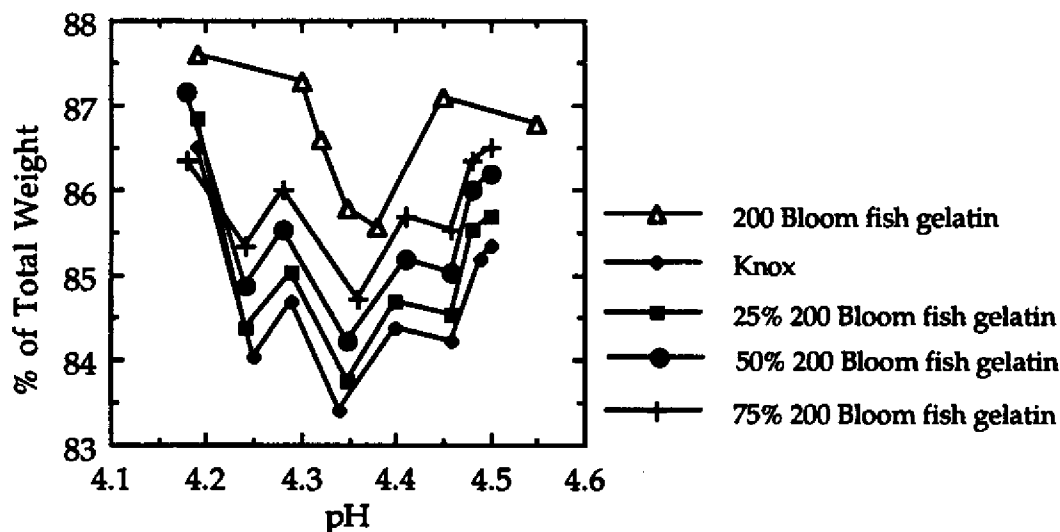


Fig. 5 Solubility of the mixtures of 200 Bloom fish gelatin (T5502) and Knox® gelatin

The gel melting point seems to be concentration dependent (Fig. 6). An increase in the concentration of the gel solution, from 2% to 12%, resulted in an increase in the melting point from 21.4 to 24°C. The mean melting point of all Bloom fish gelatin samples was around $24.2 \pm 0.5^\circ\text{C}$ which is 5°C lower than that of Knox® gelatin (29.6°C) (see Table 1). As with pH, melting point was significantly different, measured by one way ANOVA, from Bloom to Bloom and even within one single Bloom (300) but different lots ($p < 0.05$). The melting point increased with the Bloom in fish gelatin samples, and although significant, the difference from the lowest Bloom (200) to the highest Bloom (300) was only 1°C (see Fig. 7).

The effect of mixing Knox® gelatin with either 200 Bloom or 300 Bloom fish gelatin is seemed in Fig. 8. Since the fish gelatins have lower melting point than Knox® gelatin, it was expected that the melting point would decrease with the addition of the fish gelatin. It is interesting though that the pattern observed was not a straight line (i.e., an additive effect) but instead suggested a discontinuity, especially with 0-25% 200 Bloom fish gelatin. In addition, a second-order polynomial curve was fitted to the 300 Bloom data which coincided exactly with the experimental curve ($y = 29.614 - 3.8343 \times 10^{-2}x - 1.4857 \times 10^{-4}x^2$, $R^2 = 1.000$).

A linear relationship between gel strength (lb) and concentration (%) can be seen in Fig. 9 ($R^2 = 0.991$).

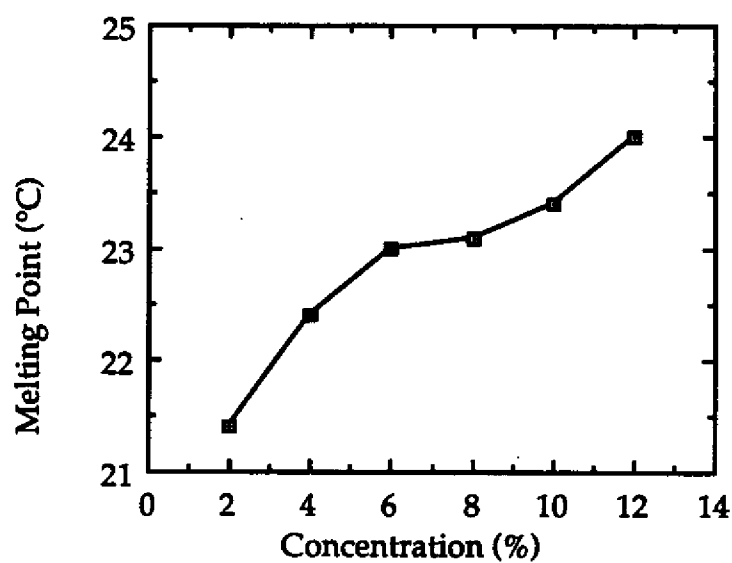


Fig. 6 Melting point as a function of concentration using 300 Bloom fish gelatin (T5104)

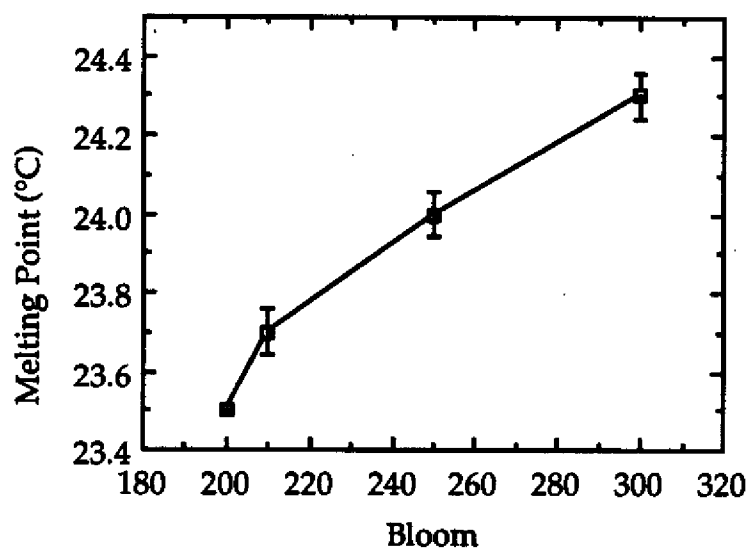


Fig. 7 The relationship of fish gelatin Bloom and melting point

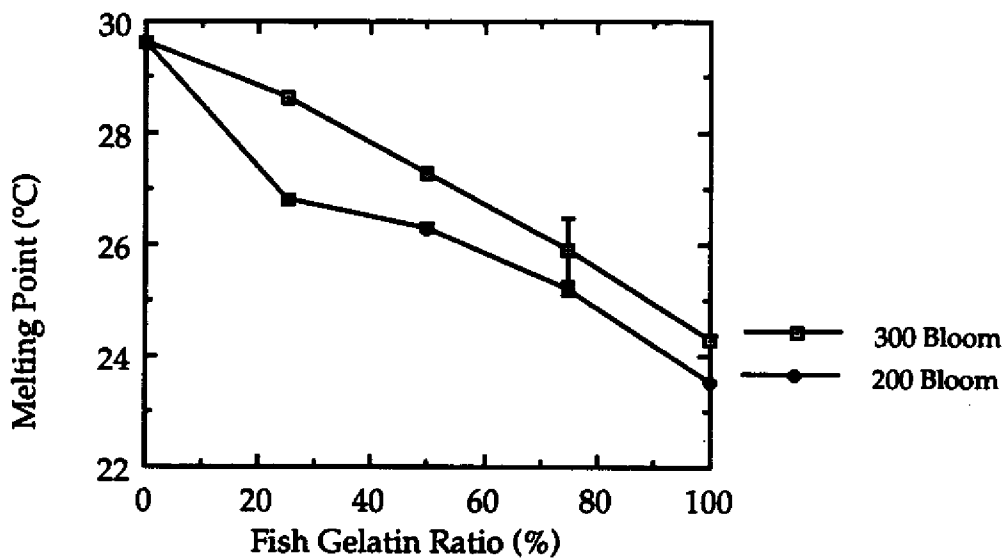


Fig. 8 The relationship between melting point and fish gelatin ratio in mixtures of 200 Bloom (T5113) and 300 Bloom (T5001) and Knox® gelatin

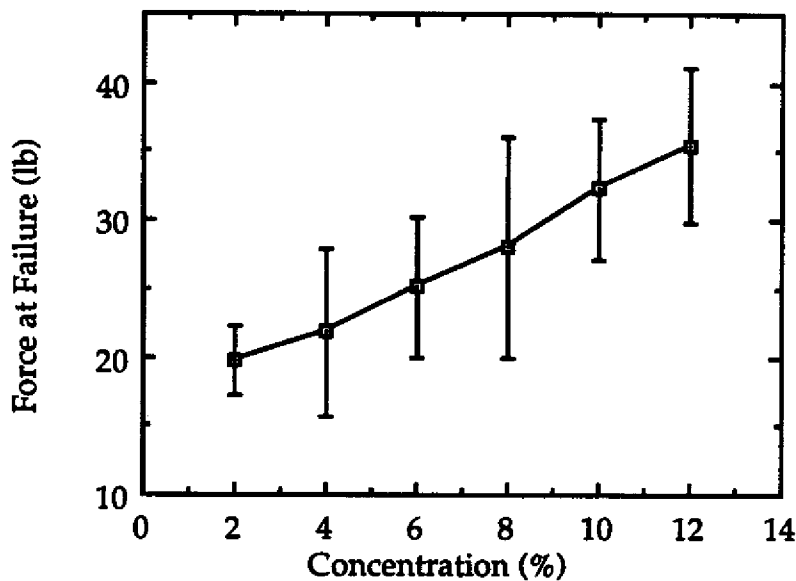


Fig. 9 Gel Strength (lb) as a Function of Fish Gelatin Concentration (300 Bloom fish gelatin (T5104))

The results of the sensory triangle tests are shown in Table 2. In all cases, panelists were able to distinguish between the Knox® and the fish

Table 2. Sensory analysis of Knox® and fish gelatin cubes by triangle test

Samples	Panelist Numbers	Correct Response	Degree of Difference				Preference	
			(Of Correct Responders)					
		N	Slight	Moderate	Much	Extreme	Knox®	Fish Gelatin
Knox® vs 300 Bloom fish gelatin (T5104) [†]	25	18	3	8	4	3	17	1
Knox® vs 250 Bloom fish gelatin (T25104) [†]	17	13	3	6	4	0	8	5
Knox® vs 300 Bloom fish gelatin (TA30104) [†]	17	14	3	4	7	0	7	6
Knox® vs 250 Bloom fish gelatin (T25104) ^{††}	24	19	7	8	3	1	11	8

[†] 2.75% gelatin cubes made with cranberry juice

^{††} 2.75% gelatin cubes made with grape juice

gelatin samples ($p < 0.05$). For the 300 Bloom fish gelatin (T5104), of the panelists, who correctly identified the odd sample, all but one liked the Knox® gelatin better than the fish gelatin. Among the panelists that liked the Knox® product better, 9 panelists said that its flavor was better, 6 panelists said that both its flavor and texture were better, and 1 said that its color was better. With the 250 Bloom fish gelatin (T25104) sensory test, 8 preferred the Knox® gelatin, 5 preferred the fish gelatin, and 1 thought that neither was more acceptable, even though the fish gelatin was "softer and sweeter." Another panelist agreed that the fish gelatin was softer, however, the panelist observed that although the flavor was the same, the texture was better in the Knox® gelatin.

In a second 300 Bloom fish gelatin (TA30104) sensory test, 7 preferred the Knox®, 6 preferred the fish gelatin and one did not have a preference but declared that the texture of fish gelatin was better, while the flavor of Knox® gelatin was better. Finally, for the 250 Bloom fish gelatin (T25104) cubes mixed with grape juice, instead of cranberry, 11 preferred the Knox® gelatin while 8 preferred the fish gelatin. Among the comments made about the fish gelatin samples were that it was sweeter and had a stronger flavor. A different aftertaste was also noted by some panelists. In summary, although Knox® gelatin was preferred among the panelists, more than 1/3 of the panelists preferred the fish gelatin cubes. The most common objection to the fish gelatin was the aftertaste which suggests that some process improvement is needed.

CONCLUSION

There is a clear effect of concentration, at least from 2% to 20% solutions of 300 Bloom fish gelatin, in pH, melting point and gel strength. Both melting point and gel strength increase with an increase in the concentration. The lowest pH was obtained in 10% solutions and the highest pH was obtained at the two ends of the concentration range that was used. Increasing the Bloom resulted in a small increase in the melting point, but all Bloom fish gelatin samples had a lower melting point than Knox® (about 5°C lower). The pH of Knox® (5.17) was found to be in between the pH range of the fish gelatin samples (4.98 to 5.27). There was clearly an effect of the ratio of fish gelatin in the mixtures of Knox® with 300 and 200 Bloom fish gelatin on all the properties studied (i.e., pH, melting point, and minimum solubility). The effect, however, was not additive and therefore could not be easily predicted. The minimum solubilities of the fish gelatin samples were similar to the ones obtained for Knox® expressed either as % of total protein or % of total weight. Sensory analysis showed that most panelists distinguished fish gelatin from Knox® and although there was a clear preference for the latter, more than one-third of them preferred the fish gelatin. The most

common objection to the fish gelatin cubes was the aftertaste which suggests that there is still area for improvement.

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Storage Stability of Hydrogenated Menhaden Oil
Shortening In Cookies, Crackers, and Snacks

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Fish oil has been a significant source of fat for many people for centuries. It is widely used in Europe, the Far East and South America in such products as margarine, shortening, and cooking and salad oils. The U.S. actually consumed hydrogenated fish oil from 1921 - 1951 but this practice ceased when the California sardine fishery failed. In 1988, menhaden oil accounted for 98% of the total U.S. production of marine oils (3).

Fish oil contains the same fatty acids as vegetable oil and animal fats, plus additional long chain polyunsaturated fatty acids (C₂₀ and C₂₂). When hydrogenating this wide range of fatty acids, the oil crystalizes into the beta-prime form. The hydrogenation process alters the physical characteristics of the oil and gives unique functional properties. For instance, it has high crystal stability, unlike most animal and many vegetable oils. Since there is no crystal growth, it retains its smoothness and plasticity. It also has better creaming properties than vegetable oil shortening and it is resistant to "work softening" during pastry manufacturing (4).

Despite these advantages, the seafood industry has been reluctant to manufacture fish oil shortening for

two reasons: 1. the shortening might have reversion ("fishy") flavors that might be imparted to the baked product (16), and 2. the shortening might be prone to develop autoxidative rancidity

because of the high polyunsaturated fatty acid content of fish oil (18, 7, 10). However, a specially processed deodorized fish oil mayonnaise, when properly packaged and stored at 2C for 14 weeks was organoleptically acceptable and was not significantly different from soy oil mayonnaise. Moreover from chemical analyses, there were few signs of oxidation (12).

Hydrogenation decreases the tendency to oxidise and increases flavor stability (5). In an unpublished study by Stauffer et al. (unpublished study, JAOCS), refined, bleached, and deodorized partially hydrogenated menhaden oil (PHMO) was used to make cookies, crackers, and deep fried extruded snacks. The PHMO items were as organoleptically acceptable as those made with partially hydrogenated vegetable oil (PHVO).

Li Hsieh and Regenstein (13) found that the best analytical tests for early oxidation of fish oil mayonnaise are peroxide value (PV) and thiobarbituric acid (TBA), while for later oxidation total carbonyl compound (TOC) and anisidine value (AV) were better.

This research is a companion work to that of Stauffer et al. (unpublished study, JAOCS) shelf-life study of cookies, crackers, and deep fried extruded snacks using sensory analysis. The objective of our work was to determine the oxidative stability of PHMO shortening in the same cookies, crackers, and deep- fried extruded snacks using PV, AV, and Totox.

MATERIALS & METHODS

Raw Materials

Menhaden oil was produced by Zapata Protein Inc. It was extracted from the fish, then refined, bleached, and deodorized by standard methods. Hydrogenation, analysis, and blending of basestocks, and preparation of shortenings was carried out in the facilities of POS Pilot Plant Corporation (Saskatoon, Saskatchewan). Table 1 shows the preparation of PHMO basestocks and hardstock. Table 2 shows the production of the PHMO all-purpose shortening that was used in the cookie and cracker basecakes, PHMO filler fat that was used in the cream filling, and PHMO frying fat that was used in deep-fried extruded snacks.

The baking, deep frying, and sensory evaluation tests were done at the American Institute of Baking (AIB, Manhattan, KS). The formulas were standard ones, as used by the AIB for ingredient and process testing.

Two sets of each type of cookies, crackers, and snacks were supplied. One set was formulated with PHMO as the fat component (shortening, filler fat, spray oil, or frying fat). The other set served as the control and was formulated with a partially hydrogenated vegetable oil (PHVO) shortening.

Table 1 - Preparation of PHMO basestocks and hardstock

	Basestock #1	Basestock #2	Hardstock
Temperature, °C	175 - 200	160	175 - 200
H ₂ pressure, psi	20	40	20
Ni concentration	0.1%	0.1%	0.1%
Time, min.	103	99	150
<u>Solid Fat Index</u>			
10.0°C	33.1	39.5	78.2
21.1°C	20.3	27.0	80.3
26.7°C	13.5	21.0	82.0
33.3°C	1.1	9.6	85.8
40.0°C	0	0	88.5
Mettler m.p., °C	35.8	38.9	58.2
Iodine value	76	71	4.6

Table 2 - Production of PHMO shortenings

	All-Purpose	Filler Fat, Frying Fat
Basestock #1	60%	-
Basestock #2	-	100%
Hardstock	10%	-
RBD canola oil	30%	-
<u>Solid Fat Index</u>		
10.0°C	25.4	39.5
21.1°C	19.2	27.0
26.7°C	16.5	21.0
33.3°C	11.1	9.6
40.0°C	3.6	0.0
Mettler m.p., °C	43.6	38.9
Smoke point, °C	248	240
ACM, hrs.	162	273

Two types of cookies were tested: a rotary sandwich cookie with a cream filling, and a wire cut butter crunch cookie. The experimental sandwich cookie and butter crunch cookie basecake were made with all-purpose PHMO shortening (see Table 2); the control cookie basecake used partially hydrogenated soybean/cottonseed oil shortening (BBS-C, Capital City Co., Karlshamns, Columbus, OH). The experimental sandwich cookie cream filling was made with PHMO filler fat and the control was made with partially hydrogenated coconut oil filler fat (PUR-CO 92, Capital City Co.).

Snack crackers used all-purpose PHMO shortening in the experimental cracker and were sprayed with all-purpose PHMO. Control crackers contained partially hydrogenated soybean/cottonseed oil shortening and sprayed with hydrogenated canola oil (Lobra 70, Capital City Co.). The spray oil, 12-15%, was applied to the hot crackers as they exited the oven.

Experimental extruded snack pellets were deep fried in PHMO frying fat shortening, while the control snacks were deep fried in all vegetable frying fat (XXX Vcream, Bunge Corp.) Extruded snack pellets were fried in a floor model fryer (Filter Magic, Fry Master Corp., Shreveport, LA) at 204C for 10 sec.

Storage

All test material were stored at room temperature. Since the cookies and crackers were packed (by the supplier) in clear plastic bags, they were exposed to fluorescent light and some sun light.

Sensory evaluation

Sensory evaluation of the product was conducted using 20 untrained panelists. Products were tested using a triangle test at 0, 1, 2, 4, and 6 months after baking. Panelists were asked to taste the samples in the order indicated and identify the odd sample. They were also asked to indicate the degree of difference between the samples and the acceptability of each.

Peroxide value (PV)

This is the most widely used method to determine the degree of oxidation (17). The primary oxidation products of oils and fats are hydroperoxides, the quantity of which can be quantitatively measured by determining the amount of iodine liberated by its reaction with potassium iodide. The peroxide content is expressed in terms of milliequivalents of iodine per kilogram of oil or fat.

Fat extraction for the determination of PV was done by the procedure of Ke and Woyewoda (11). The PV for the extracted fat was determined by A.O.C.S. official methods (1, 2).

Anisidine value (AV)

Anisidine value measures the later oxidation of fat by measuring alpha-beta unsaturated aldehydes of fats and oils. This method of determining the degree of oxidation is extensively used in European countries (17).

To measure anisidine values, the method for fish oil as published in the International Association of Fish Meal Manufacturers (IAFMM) Fish Oil Bulletin (8) was used.

Totox

An extension of these methods is the Totox value, which is a measure of total oxidative deterioration, with an emphasis on primary oxidation. Totox is the sum AV and twice the PV (6, 9, 17).

Statistical analysis

The general linear model of Minitab 7.2 (Minitab, Inc., State College, PA) was used to do the analysis of variance, analysis of covariance, and regression, since it can be used with unbalanced designs and missing data.

For each test, analysis of variance with respect to time, treatment and the interaction of time and treatment was determined ($p < 0.05$). In order to determine significance ($p < 0.05$) between overall means of treatments, a one-way analysis of variance was done with time averaged out.

RESULTS & DISCUSSION

Sensory evaluation

Sandwich cookies. At 0 and 1 month, panelists could detect a slight difference, although not at the 5% significance level. By the 6th month, 0 panelists picked the correct odd sample. With regard to preference, the panelists seemed to slightly prefer the control sample, but the split was so small as to be considered random. Again, results of the triangle test indicated that there was no significant difference between sandwich cookies made with PHMO as both the basecake and the filling and the control cookie.

Butter crunch cookies. For the triangle test, with 20 panelists, 11 correct responses corresponds to a 5% significance level. This was reached only when the cookies were evaluated after 2 months of storage Table (Table 3). On the other test times, the selections appeared to be random. Of the panelists who correctly identified the odd sample, the panelists' preference, also appear to be random, except for perhaps the 2nd month, where 7 out of 12 preferred the PHMO cookies. Results of the taste panel tests indicated that there was no significant difference between the experimental butter crunch cookie and the control cookie

Table 3 - Sensory analysis^a of sandwich cookies overtime by triangle test

Months	Correct Response	Preference (Of Correct Responses)		
		Fish Oil	Control	Neither
0	10	3	5	2
1	10	4	5	1
2	3	-	3	-
4	7	3	4	-
6	0	-	-	-

^a20 untrained panelists: 5% significance requires 11 correct responses.

Table 4 - Sensory analysis^a of butter crunch cookies over time by triangle test

Months	Correct Response	Preference (Of Correct Responses)		
		Fish Oil	Control	Neither
0	6	1	4	1
1	5	4	1	-
2	12	7	3	2
4	5	3	2	-
6	0	-	-	-

^a20 untrained panelists: 5% significance requires 11 correct responses.

Table 5 - Sensory analysis^a of snack crackers over time by triangle test

Months	Correct Response	Preference (Of Correct Responses)		
		Fish Oil	Control	Neither
0	12	2	8	2
1	14	2	12	-
2	10	-	9	1
4	7	2	6	-
6	4	-	4	-

^a20 untrained panelists: 5% significance requires 11 correct responses.

Table 6 - Sensory analysis^a of deep-fried extruded snacks over time by triangle test

Months	Correct Response	Preference (Of Correct Responses)		
		Fish Oil	Control	Neither
0	9	2	7	-
2	9	1	8	-
4	4	2	2	-
6	6	2	4	-

^a20 untrained panelists: 5% significance requires 11 correct responses.

Snack crackers. Only at 0 and 1 month, could the panelists detect a significant difference between the experimental and test crackers with a preference toward the control crackers (Table 5). Comments indicated that some of the panelists could pick up a slight fishy taste. As the crackers "aged", the differences between the crackers seemed to decrease.

Deep-fried extruded snacks. If an oil is prone to autoxidative rancidity and development of reversion flavors, using it to make a deep-fried extruded snack item is a stringent test. The snacks were not coated with any flavoring materials, so the panelists were experiencing the crunchy texture of the bland substrate with the frying oils as the only source of flavor. As shown in Table 6, the ability of the panelists to detect the correct odd sample did not reach significance during the entire period of the study. At the beginning of the study, there was a slight preference for the control samples. However, this does not seem to be significant in view of the undetectable difference between the PHMO and control samples.

Biochemical evaluation

Since Totox includes contributions from both AV and PV, only the Totox data will be presented.

Sandwich cookies. The Totox of the sandwich cookie basecake and the cream filling made with PHMO shortening was slightly higher than the control throughout most of the study (Fig. 1). However, except for the initial period, both fluctuated at fairly low levels (as compared to high-quality crude soybean oils, which will generally have a Totox value of less than 3; for crude soybean sunflower oil, the value is generally less than 5 (15)). The differences in Totox between the experimental and control sandwich cookies were nonsignificant for both the cookie and cream layers.

Wire cut butter crunch cookies. Totox values for the control cookies were higher than the experimental cookies at 2 and 8 months; and lower at 4 and 6 months (Fig. 2). The differences in Totox for butter crunch cookies were nonsignificant. Totox values were acceptable, since for the most part, the values were under 5.

Snack crackers. Totox in PHMO snack crackers were generally higher than the controls with a fair bit of scatter, but the differences generally decreased with time (Fig. 3). By the end of the study the differences were slight. Also, except for 0 and 6.5 months, the Totox values for PHMO snack crackers showed good stability, since the values were less than 5. The differences in Totox for snack crackers were significant. However, by the end of the study (8.5 months) the Totox values were essentially the same. Also, with the PV test, there was no significant difference.

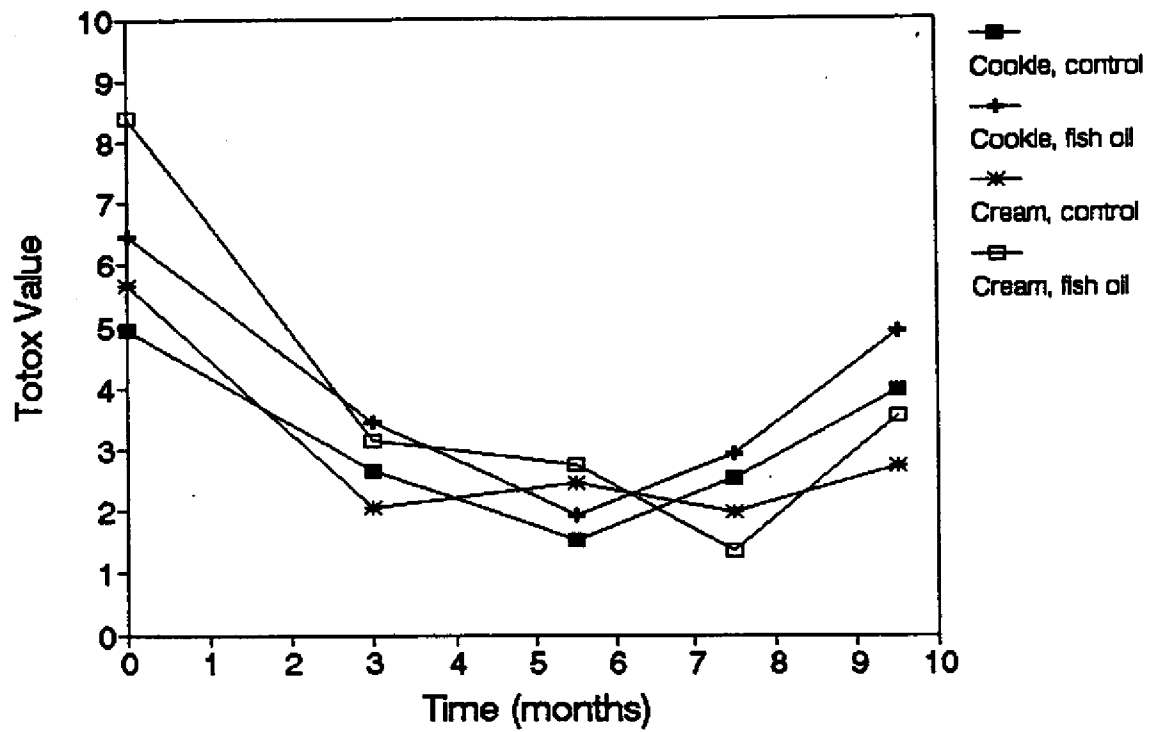


Fig. 1 - The effect of time on rancidity of sandwich cookies

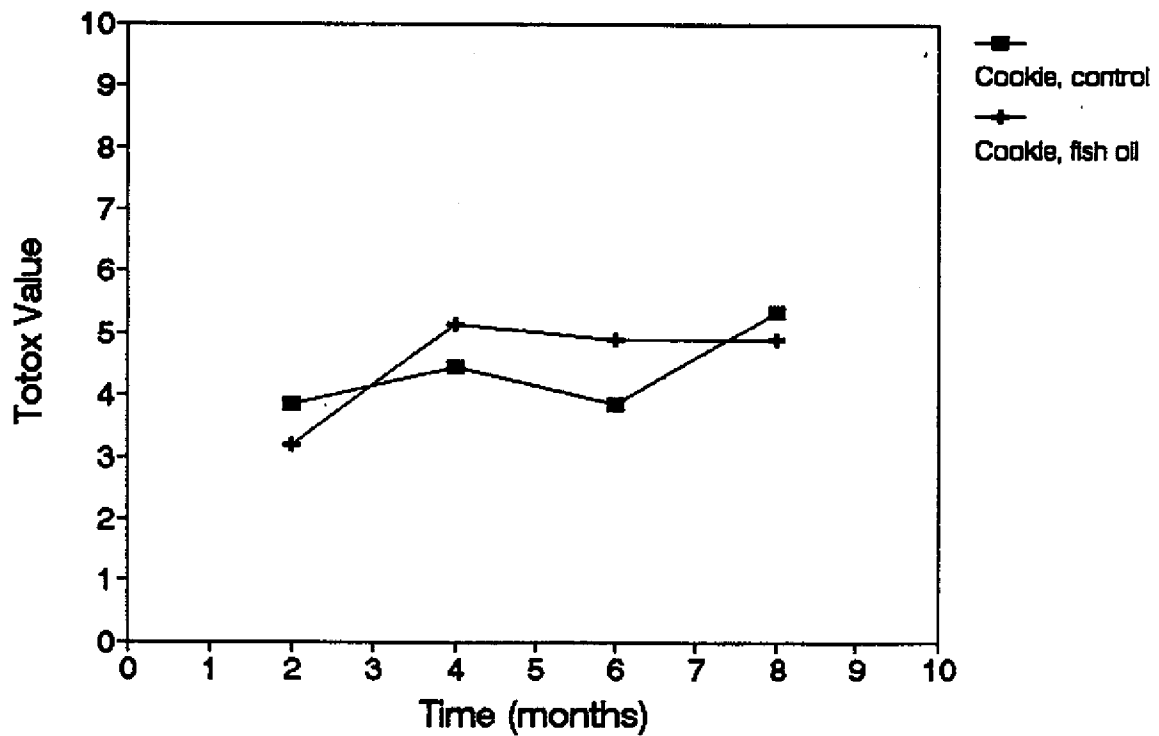


Fig. 2 - The effect of time on rancidity of butter crunch cookies

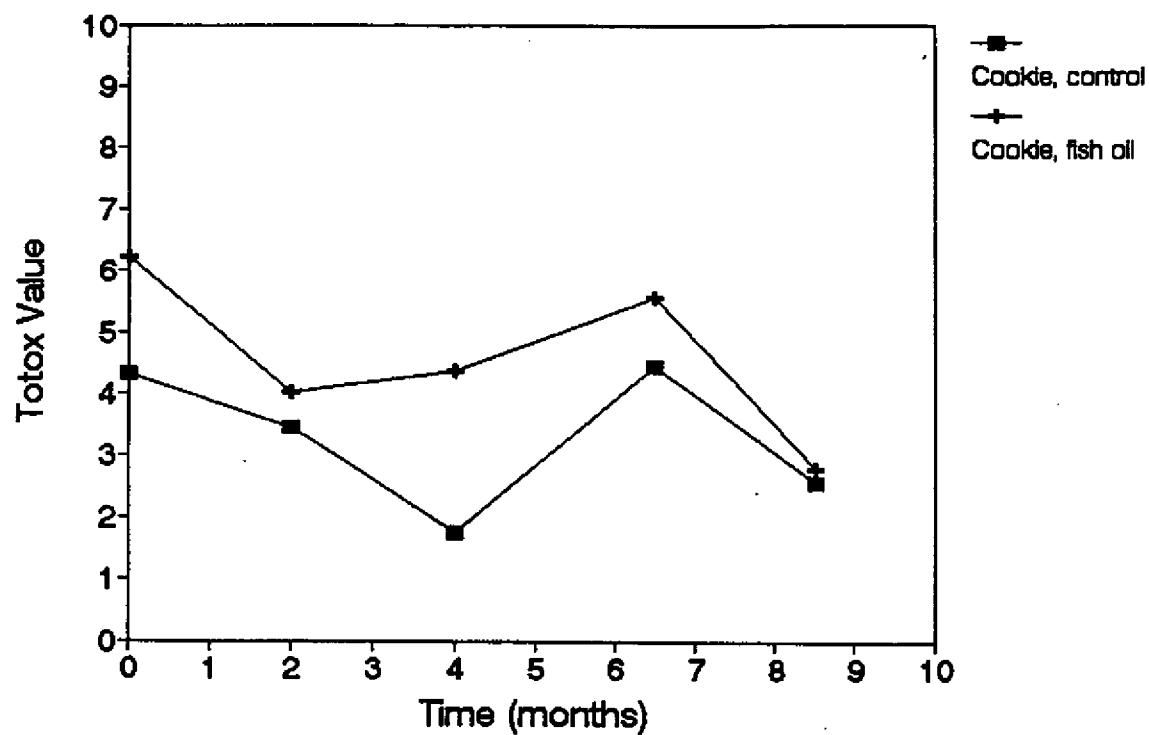


Fig. 3 - The effect of time on rancidity of snack crackers

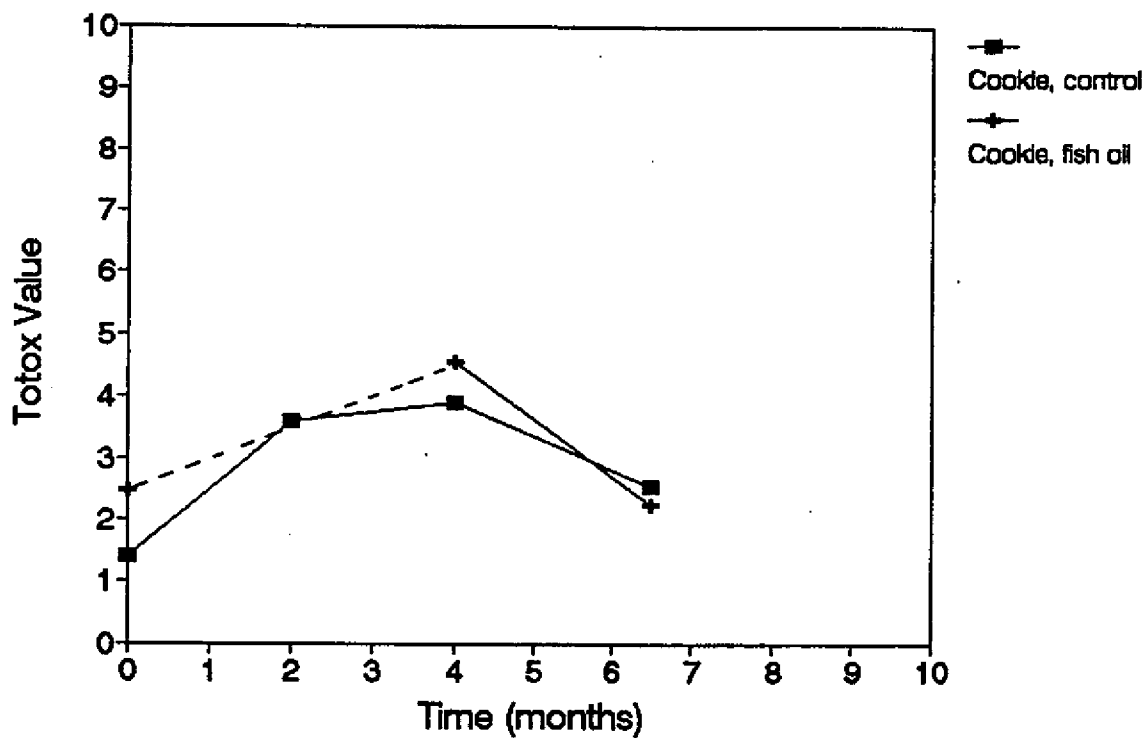


Fig. 4 - The effect of time on rancidity of extruded snacks

Deep-fried extruded snacks. PHMO deep-fried extruded snacks had higher initial Totox, but by the 6.5 month, the Totox was lower than the control (Fig. 4). The data for the 2nd month was missing, therefore a dashed line was used to connect the 0 month and the 4th month. Both fluctuated at fairly low levels. With Totox, there was no significant difference between PHMO and the control extruded snacks.

CONCLUSION

Data from the taste panels indicate that there are no significant differences between PHMO shortening cookies, snacks and deep fried extruded snacks; and those made with control PHVO shortening (except for the butter crunch cookies at the 2nd month, and snack crackers at 0 and the 1st month.) For the most part, differences between the products decreased with increasing shelf-life.

No significant difference for Totox was found when PHMO shortening cookies, crackers, and deep-fried extruded snacks were compared to similar items made with PHVO shortening (except the AV and Totox of snack crackers, and Totox of sandwich cookies). Since there was little difference between the oxidative stability of the PHMO cookies, crackers, and deep-fried extruded snacks and the controls, PHMO shortening may be used in cookie, cracker, and deep-fried extruded snack formulations without concerns about rancidity.

These results showed that there was little difference between the oxidative stability of the fish oil cookies and crackers and controls, therefore hydrogenated menhaden fish oil may be used in cookie, cracker, and deep-fried extruded snack formulations without concerns about rancidity.

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EFFECTS OF SODIUM LACTATE AND POTASSIUM LACTATE ON COLOR, PH, AND
BACTERIA COUNTS OF TILAPIA HELD IN OVERWRAPPING, VACUUM SKIN
PACKAGING, AND MODIFIED ATMOSPHERES

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Sodium or potassium lactate, a salt of lactic acid, is a Generally Regarded As Safe (GRAS) chemical. It is often used in bakery products including biscuits, cakes and confectionery to keep them moist and give them a soft, crumbly texture, and in cheese to enhance the action of antioxidants. It is also used in jam and marmalade, and margarine to regulate the acidity. In recent years, sodium lactate has been used in meat products as a flavor enhancer and an antimicrobial agent (7). However, due to the fact that consumers have a trend to reduce the sodium intake, potassium lactate has been used to partially substitute sodium salt.

Previous studies indicated that sodium lactate inhibited microbial growth and prolong the shelf life of meat products. Duxbury (4) reported that beef roast injected with sodium lactate resulted in increased shelf life of the roasts. Bacterial growth was inhibited and the microflora came to be dominated by Lactobacillus species. Chirife and Fontan (2) stated that sodium lactate has been shown to decrease the water activity sufficiently to inhibit bacterial growth. de Witt and Rombouts (3) reported that the antimicrobial effects of sodium lactate may due to the increased permeability of cellular membranes for lactate ion at a high pH. However, feedback inhibition by lactate was proposed as the mechanism of action in the delay of toxin production by Streptococcus faecalis (8) and by Clostridium botulinum (5).

Little information regarding the effect of sodium lactate or potassium lactate on fishery products was available. Williams and Rodrick (10) reported that sodium lactate functioned to retard microbial growth, and stabilize color and odor characteristics in the skinned catfish fillets. Total aerobic plate counts of cod fillets were also big reduced when 12% of sodium lactate solution was used to immerse the fillets for 2 min (1). However, no study of sodium lactate or a mixture of sodium and potassium lactates on tilapia has been made. In order to extending the shelf life of fresh product at the retail level, the objectives of this study were to determine the reduction of microbial populations on tilapia fillet treated with sodium lactate or a mixture of sodium and potassium lactates

held in different packaging system, and to evaluate the effect of treatments on pH, Torrymeter reading and color changes during refrigerated storage.

MATERIALS AND METHODS

Tilapia

Tilapia (*Oreochromis* spp.), purchased from the Delkab Farmers Market (Decatur, GA). The fish were headed, gutted and filleted and then transported on ice to the University of Georgia, Athens, GA. The tilapia fillets were treated with 8% sodium lactate or a mixture of 4% sodium lactate and 4% potassium lactate solutions for 2 min.

Treatments

All treated fillets were packaged by either overwrapping with polyvinylidene films (Saran wrap, oxygen transmission rate: 5 cc/m²/24 hr/atm at 20°C and 43% RH), or vacuum-skin packaging with low density polyethylene films (Surlyn, oxygen transmission rate: 930 cc/m²/24 hr/atm at 23°C and 75% RH) using a Trigon Intact RM 331 Mark III Mini Intact machine (Trigon Packaging Corp., Redmond, WA), or modified atmosphere packaging with 70% CO₂ and 30% N₂. Packaged fish were stored at a 4°C walk-in cooler for 16 days. Three packages of each treatment were randomly removed from cooler at a 4-day interval for determination of pH, color values (L, "a", "b"), Torrymeter readings and microbial counts.

Microbiological Evaluation

A 5x5 cm section of fillet was collected by using a sterilized blade. The sections were then placed in stomacher bags with 100 ml of 0.1% peptone buffer solution (Bacto) and stomached for 2 min by a Stomacher (Seward model 400, London, England). The aliquots of proper dilution were plated onto plate count agar and incubated at 20°C for 4 days to enumerate a total psychrotrophic bacterial count (9). The aliquots were also plated onto trypticase peptone glucose yeast extract agar (TPGY) and incubated at 20°C for 4 days to enumerate total anaerobic plate counts (9). The BBL GasPak^R anaerobic chamber with BBL GasPak^R Co₂ gas packs (Becton Dickinson Microbiology Systems, Boston, MA) were used to create an anaerobic environment for incubation.

Surface pH Measurement

Triplicate pH readings were taken from the surface of each sample fish by using a Fisher Scientific probe (No. 13-620-286) and a Corning pH meter (model Y140, Corning Inc., NY, NY).

Color Analysis

The color of fish skin surface was measured prior to removal of the core sample by using a Colormeter (Minolta CR-200, Osaka, Japan). Hunter color values of L (lightness), "a" ("+":redness; "-":greenness) and "b" ("+":yellowness; "-":blueness) were recorded. Triplicate measurements were taken from each fish.

Torryster readings

Six spots of fillet were measured using a Torryster (gr Torry Station, Scotland, UK). The reading ranges from 1 to 16.

Data Analysis

Statistical analyses (6) were performed on pH, color values, Torryster readings and microbiological data by means of PC SAS. The Duncan's multiple range test was used to determine any significant differences among samples from fish with different treatments.

RESULTS AND DISCUSSION

Effects of lactates treatment and packaging system on psychrotrophic bacterial growth on tilapia

On day 8, control samples and overwrapped samples treated with either 8% sodium lactate or the mixture of 4% sodium lactate and 4% potassium lactate exhibited obvious spoilage, while modified atmospheres packaged ones had significantly lower bacterial counts (Table 1). Vacuum skin packaged fillets treated either sodium lactate or the mixture exhibited an onset of spoilage. No significant differences between vacuum skin packaged samples or between MAP samples were found regardless of chemical treatment. Results indicated that 8% sodium lactate had the same effect as the mixture of sodium lactate and potassium lactate. Modified atmosphere packaging significantly retarded microbial growth on tilapia and prolong the shelf life for two times.

The same growth pattern of anaerobic bacteria as psychrotrophic bacteria was found in tilapia (Table 2). Modified atmosphere packaging did not promote the growth of anaerobic bacteria on lactates treated tilapia.

Effects of lactates treatment on surface pH of tilapia as affected by packaging system

Both lactates treatment significantly reduced the initial pH on tilapia skin surface and this effect last till day 8 (Table 3). On day 12, no significant difference on pH among samples was found except the MAP samples. This was because that all the samples were spoiled except the MAP ones. The absorption of carbon dioxide by the fish was another factor to cause the lower pH value.

Effects of lactates treatment and packaging system on color of tilapia fillet

No clear difference on Hunter color values of all samples was found regardless of sodium lactate treatment and packaging system (Tables 4, 5, and 6). Results implied that neither lactates treatment nor packaging system had no effect on Hunter color values of tilapia fillets.

Table 1. Psychrotrophic bacteria count (logCFU/cm²) of tilapia as affected by lactates treatment and packaging system at 4°C

Day of storage	control	8% sodium lactate			mixture		
		OW	VSP	MAP	OW	VSP	MAP
0	2.69a	2.69a	2.69a	2.69a	2.69a	2.69a	2.69a
4	5.55a	4.98ab	4.60bc	2.86d	4.39bc	3.86c	2.59d
8	8.82a	8.13b	7.20c	3.18d	8.00b	6.95c	2.46e
12	9.15a	9.09a	8.53b	4.63c	9.26a	8.12b	5.23c
16	-	-	-	6.24a	-	-	5.93a

a,b,c Means in a column followed by the same letter are not significantly different at level of 0.05

Table 2. Total anaerobic bacteria count (logCFU/cm²) of tilapia as affected by lactates treatment and packaging system at 4°C

Day of storage	control	8% sodium lactate			mixture		
		OW	VSP	MAP	OW	VSP	MAP
0	2.64a	2.64a	2.64a	2.64a	2.64a	2.64a	2.64a
4	5.18a	4.91ab	4.02c	2.71d	4.25bc	3.80c	2.59d
8	8.82a	8.13b	7.64c	3.25e	8.10b	7.17d	2.73f
12	10.26a	9.85ab	9.68ab	4.56c	9.37ab	8.81b	4.92c
16	-	-	-	6.06a	-	-	6.64a

a,b,c,d,e,f Means in a column followed by the same letter are not significantly different at level of 0.05

Table 3. Surface pH of tilapia on skin side as affected by lactates treatment and packaging system at 4°C

Day of storage	control	8% sodium lactate			mixture		
		OW	VSP	MAP	OW	VSP	MAP
0	6.78a	6.43b	6.43b	6.43b	6.46b	6.46b	6.46b
4	6.60a	6.46ab	6.47ab	6.29b	6.39b	6.49ab	6.34b
8	6.75a	6.44b	6.33b	6.33b	6.51b	6.42b	6.42b
12	6.95ab	7.11a	6.78abcd	6.50cd	6.84abc	6.60bcd	6.36d
16	-	-	-	6.27a	-	-	6.34a

a,b,c,d Means in a column followed by the same letter are not significantly different at level of 0.05

Table 4. Hunter L values of tilapia on skin side as affected by lactates treatment and packaging system at 4°C

Day of storage	control	8% sodium lactate			mixture		
		OW	VSP	MAP	OW	VSP	MAP
0	52.72a	53.09a	53.09a	53.09a	50.96b	50.96b	50.95b
4	54.03ab	53.30ab	52.98ab	56.63a	52.74b	52.36b	54.45ab
8	52.98a	55.35a	53.77a	53.14a	52.81a	54.10a	54.54a
12	55.34abc	58.36a	53.31bc	55.28abc	56.99ab	52.27c	54.98abc
16	57.74a	57.73a	49.95b	55.40a	58.27a	51.87b	56.91a

a,b,c Means in a column followed by the same letter are not significantly different at level of 0.05

Table 5. Hunter "a" values of tilapia on skin side as affected by lactates treatment and packaging system at 4°C

Day of storage	control	8% sodium lactate			mixture		
		OW	VSP	MAP	OW	VSP	MAP
0	-0.86a	-1.29a	-1.29a	-1.29a	-1.04a	-1.04a	-1.04a
4	-0.89a	-1.38abc	-1.23abc	-1.53bc	-1.25abc	-1.00ab	-1.67c
8	-1.75b	-1.86b	-1.58ab	-1.24a	-1.67ab	-1.62ab	-1.54ab
12	-2.26b	-1.56bc	-1.41ab	-1.33ab	-1.71ab	-1.17a	-1.52ab
16	-1.67abc	-0.97a	-1.09a	-1.90c	-1.51abc	-1.10ab	-1.81bc

a,b,c Means in a column followed by the same letter are not significantly different at level of 0.05

Table 6. Hunter "b" values of tilapia on skin side as affected by lactates treatment and packaging system at 4°C

Day of storage	control	8% sodium lactate			mixture		
		OW	VSP	MAP	OW	VSP	MAP
0	-4.95a	-3.97a	-3.97a	-3.97a	-3.97a	-3.97a	-3.97a
4	-2.20ab	-3.57b	-3.67b	-1.74a	-3.53b	-2.83ab	-3.35ab
8	-0.87a	-2.03ab	-3.34bc	-2.49abc	-2.25abc	-3.81c	-1.80ab
12	-0.90a	-0.70a	-2.58a	-1.91a	-0.98a	-1.82a	-1.53a
16	-1.29a	-1.69a	-2.72a	-2.08a	-1.40a	-2.29a	-1.42a

a,b,c Means in a column followed by the same letter are not significantly different at level of 0.05

In summary, at 4°C the overwrapped tilapia fillet had shelf life of 6 days with any lactate treatment. Significant effects on microbial growth were found for fish treated with the combination of modified atmosphere packaging and dipping in the 8% sodium lactate solution or in the mixture of 4% sodium lactate and 4% potassium lactate. Vacuum skin packaging had also shown a reduction for psychrotrophic bacteria growth, while the overwrapping had only little effect.

ACKNOWLEDGMENTS

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EFFECTS OF TANNIC ACID, GALLIC ACID, AND PROPYL GALLATE ON STORAGE LIFE OF CATFISH

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Tannins are present in many plant products including strawberries, grapes, apples and tea. The substances are categorized as phenolic compounds and can be divided into two categories: hydrolyzable and condensed tannins. The hydrolyzable tannins can be further divided into two classes: gallotannins and ellagitannins. Upon hydrolysis, the gallotannins yield gallic acid and glucose while ellagitannin yield ellagic acid and sucrose.

Tannic acid has been used as a clarifier for many years. It is also used to refine fats. Tannic acid is of amorphous powder, glistening scales, spongy masses. It exhibits yellow, white, and brown colors. It is usually odorless but occasionally with an faint odor, and with an astringent taste.

On the antimicrobial activity, tannic acid is inhibitory to many foodborne bacteria including Aeromonas hydrophila, Escherichia coli, Listeria monocytogenes, Salmonella enteritidis, Staphylococcus aureus, and Streptococcus faecalis while gallic acid and ellagic acid are not. When tannic acid is hydrolyzed, the ester bond is broken to release gallic acid and/or ellagic acid and the antimicrobial activity is lost (1).

The antimicrobial activities of tannin include inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation (4).

Propyl gallate is the Common name for n-propyl 3,4,5 trihydroxybenzoate. Blending BHA with propyl gallate shows particularly efficacious in preserving flavor of oils and fats. Propyl gallate discolors when in the presence of iron or copper, unless it is treated with citric acid to form citric acid metal ion compounds to reduce discoloration.

Propyl gallate is a GRAS antioxidant, white or off white powder. It is odorless and has slightly bitter taste. Legal requirements for antioxidants and oxygen interceptors permitted in meat and poultry processing : to retard rancidity rendered animal fat or a combination of such fat and regular fat: 0.01% ; 0.02% in combination. For dried Meats, 0.01% based on total weight are suggested (2).

The objectives of this study were to determine the effects of tannic acid, gallic acid, and propyl gallate on psychrotrophic plate count of overwrapped catfish fillets, and to evaluate the color and surface pH changes during refrigerated storage for 12 days.

MATERIALS AND METHODS

Catfish

Fresh farmed catfish (*Ictalurus punctatus*) were purchased from the Dekalb Farmers Market, Decatur, Georgia. Fish were headed, eviscerated, skinned, and filleted at the market's facilities. The fillets were transported on ice to the Food Science Department, University of Georgia in Athens and then the ice chests were placed in the 4°C walk-in cooler.

Treatment

Tannic acid, gallic acid and propyl gallate were purchased from the Sigma Co. (St. Louis, MO). One percent tannic acid (TA), 1% gallic acid, and 1% propyl gallate were used to treat fillets. One hundred pieces of fillets were randomly assigned to the treatments. These treatments had used ice to cool the temperature down close to 0°C to minimize fish spoilage. Fillets were dipped in the appropriate treatment solutions for 30 minutes and drained before packaging procedures.

Packaging and Storage

The treated fillets were placed on polystyrene trays (1 fillet per tray) and overwrapped with Saran^R (PVDC, polyvinylidene chloride, oxygen transmission rate = 5 cc/m²/24hr/atm, at 20°C, 43% R.H.; Dow Chemical Co., Indianapolis, IN). All packaged fillets were stored in a 4°C walk-in cooler until sampling days on day 0, 4, 8, and 12. Three packages of each sample were used for all the tests.

Microbiological Analysis

A size of 25 cm², from the central portion of fillets, was obtained and individually homogenized for 30 seconds with 100 ml sterile 0.1% peptone solution in Fisher polyethylene sample bags using Seward (England) Laboratory Blender Stomacher 400. Serial dilutions were further made in 0.1% peptone, and suitable dilutions were plated by spreading 0.1 ml onto the surface of Plate Count Agar (Difco, Detroit, MI). Psychrotrophic plate counts were made in duplicate. Plates were incubated for 48 hours at 20°C. The results were expressed as the log₁₀ of cfu/cm².

Color Measurements

The Hunter L, "a", "b" were measured by using a Chroma Meter CR200 (Minolta Camera Ltd., Osaka, Japan). The value L measures lightness which range from 0 to 100; "a" measures chromaticity where positive value indicating redness and negative value indicating greenness; while

positive value of "b" indicating yellowness and negative value indicating blueness. Color measurements were made with the packaging unremoved to avoid the effect of packaging material. Three random spots were measured and the average data were recorded.

pH Measurement

Surface pH was measured using a Corning M140 pH meter (Corning Medical & Scientific Instruments, Halstead Essex, England). pH was measured on three random spots on each fillet and the average reading was recorded.

Statistical Analysis

Analysis of variance was performed on the data by means of PC SAS. The Duncan's multiple range test was used to determine if any significant difference among samples as affected by packaging method and different additive treatment.

RESULTS AND DISCUSSION

The only significant difference in psychrotrophic plate count on Day 4 was displayed by the sample treated with propyl gallate (Table 1). However, no significant different difference among samples were found at the end of 12 days storage. Looking at the pH changes, samples treated with gallic acid and tannic acid were found to have significant lower pH than control and propyl gallate treated samples (Table 2). There were no significant difference between propyl gallate treated samples and control.

Table 1. Psychrotrophic plate counts (log CFU/cm²) of overwrapped catfish fillets as affected by different additives during refrigerated storage

Day of storage	Control	Tannic acid	Gallic acid	Propyl gallate
0	4.45(0.10)a	4.23(0.24)a	4.10(0.71)a	3.74(0.24)a
4	6.94(0.14)a	6.95(0.57)a	6.32(0.57)a	5.49(0.12)b
8	8.41(0.13)a	8.51(0.30)a	8.14(0.09)a	7.54(0.33)b
12	8.79(0.21)a	8.65(0.37)a	8.70(0.11)a	8.56(0.15)1a

a,b

Means in a row with the same letter are not significantly different at $p=0.05$ ($n=3$)

Table 2. Surface pH of overwrapped catfish as affected by different additives during refrigerated storage

Day of storage	Control	Tannic acid	Gallic acid	Propyl gallate
0	6.56(0.16)a	5.66(0.25)b	5.05(0.20)c	6.70(0.25)a
4	6.83(0.08)a	6.39(0.14)b	6.02(0.12)c	6.45(0.12)b
8	6.82(0.08)a	6.89(0.77)a	6.41(0.11)a	6.60(0.16)a
12	6.97(0.14)b	6.79(0.04)b	6.70(0.26)b	7.75(0.14)a

a,b

Means in a row with the same letter are not significantly different at $p=0.05$ ($n=3$)

The color of the catfish fillets turned lighter (as observed with higher L values) with chemical additives treatment (Table 3). The fillets treated with tannic acid exhibited significant lower "a" (Table 4) and higher "b" (Table 5) values in overwrapped samples, indicating that the treated samples were of green and yellow tinge as compared to the control samples. On the other hand, samples treated with propyl gallate showed no significant difference in "a" and "b" values as compared to the controls.

Table 3. Hunter L value of overwrapped catfish as affected by different additives during refrigerated storage

Day of storage	Control	Tannic acid	Gallic acid	Propyl gallate
0	55.12(3.84)c	56.38(2.12)c	69.46(0.89)a	62.20(1.63)b
4	56.38(2.12)c	65.43(1.46)a	61.68(2.27)b	59.87(1.24)bc
8	55.42(2.56)b	60.38(1.51)a	61.27(1.47)a	59.62(1.00)a
12	58.45(1.89)a	59.85(2.49)a	57.80(6.73)a	57.27(1.20)a

a,b,c

Means in a row with the same letter are not significantly different at $p=0.05$ ($n=3$)

Table 4. Hunter "a" value of overwrapped catfish as affected by different additives during refrigerated storage

Day of storage	Control	Tannic acid	Gallic acid	Propyl gallate
0	-1.43(0.48)a	-2.60(0.37)b	-1.88(0.24)a	-1.48(0.18)a
4	-1.51(0.24)a	-2.20(0.03)b	-1.92(0.20)a	-1.68(0.08)a
8	-1.49(0.49)a	-1.99(0.57)a	-2.16(0.18)a	-1.75(0.18)a
12	-2.51(0.17)a	-2.14(0.32)a	-2.70(0.34)a	-2.35(0.34)a

a,b Means in a row with the same letter are not significantly different at $p=0.05$ ($n=3$)

Table 5. Hunter "b" value of overwrapped catfish as affected by different additives during refrigerated storage

Day of storage	Control	Tannic acid	Gallic acid	Propyl gallate
0	-2.56(0.62)b	0.15(4.55)a	-2.37(0.97)b	-3.24(0.40)b
4	-2.09(0.61)b	4.35(0.35)a	-1.49(0.73)b	-1.74(0.41)b
8	-0.48(1.40)b	3.85(1.40)a	-0.02(1.04)b	-1.44(0.46)b
12	-0.13(2.41)bc	4.17(0.74)a	1.60(0.57)b	-0.71(1.57)c

a,b,c

Means in a row with the same letter are not significantly different at $p=0.05$ ($n=3$)

The psychrotrophic plate count of the water of the fish tank (in Dekalb's Farmer Market) was found to be log 5 cfu/g which is higher than normal. Leung et al (3) reported an initial psychrotrophic plate count of log 2.45 cfu/cm² on channel catfish harvested from the Fisheries Research Unit, Auburn University, Auburn, Alabama. Therefore, the initial psychrotrophic plate counts of catfish fillets for this study was higher, contributing to a shorter shelf-life, that is about 4 days. Color changes of fillets as affected by chemical is undesirable for consumers' acceptance of the product. Therefore, propyl gallate could be used without affecting the color changes of the fillets.

In summary, fillets treated with propyl gallate had significantly lower psychrotrophic plate counts on day 4 and 8 as compared to the control. pH of fillets treated with tannic acid and gallic acid was significantly lower than that of control and fillet treated with propyl gallate. All treated samples had significantly lighter than control, while samples treated with tannic acid had significantly higher "b" value (yellowness) and higher "-a" value (greenness) than other samples. In general, the propyl gallate showed the potential to reduce the psychrotrophic bacteria counts on catfish fillet. However, further studies on sensory evaluations have to be done in the future.

ACKNOWLEDGMENTS

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EXTENDING SHELF LIFE OF SEAFOOD WITH ACETIC ACID

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The seafood producing and exporting nations of the world are continuing to experience the need to ship by air fresh fish stored in ice. It is well known that fish stored in that manner have a rather short shelf life depending on a number of factors that ultimately determine the quality and safety of the product. One definition of shelflife is the length of time from the day of capture that fresh seafood can safely be in the marketplace (Regenstein and Regenstein, 1991). There are many ways of preserving seafood, such as drying, salting, smoking, and freezing. However, most of those method alter the original fresh seafood characteristics.

Acetic acid has been used for centuries as an acidulant in foods. Therefore, acetic acid is not subjected to many food law restrictions. In the U.S., acetic acid is considered GRAS. It has a more powerful degenerative effect on living cells than mineral acids with the same hydrogen ion concentration because of its ability to dissolve lipids. The undissociated form of acetic acid is able to penetrate through the cell membrane and enter the interior of the cell where it acts to denature proteins. Between pH 6 and 5, the action of the acetic acid only doubles (Woolford, 1975), whereas, the undissociated portion increases about seven fold in this range. A study conducted by Adams and Hall (1988) provides convincing support for the view that the undissociated part of acetic acid is the active factor. A concentration of 1% and 3% acetic acid can provide a basic protection against pathogenic microorganisms, especially Clostridia (Anderson, 1984). Acetic acid is approved for use as a sanitizer on red meat carcasses (Federal Register, 1982). One \log_{10} reduction in count can be obtained from proper sanitizing with acetic acid (Anderson, 1984). The effect of acetic acid was studied on vacuum-packaged pork (Mendonca, 1989). Treatments containing 3% acetic acid lowered the aerobic microbial counts and effectively inhibited Enterobacteriaceae. Dickson (1992) has shown that acetic acid as a sanitizing agent on beef tissue was independent of the level of initial contamination with a consistent reduction in populations, irrespective of cell numbers.

The object of this study focused on studying the effect of acetic acid on both filleted and dressed fish stored at refrigeration temperature for different periods of time.

MATERIALS AND METHODS

Preparation of fish

All seafood were obtained from local seafood processing plants, Seafresh U.S.A. Inc., Narragansett, RI and Town Dock, Galilee, RI. The fish were brought in as whole fish except for dogfish which were brought in as fillets or backs. After the fish had been headed, bled, and gutted, they were washed and drained and then dipped in various concentrations of acetic acid for a different periods of time. The fish were then stored in open plastic bags at refrigeration temperature. The following analyses were conducted periodically for up to 3 weeks.

Microbiology analysis

a) Sample preparation : 11 g of fish were aseptically transferred to a sterile blender jar with 99 ml of 0.1% sterile peptone water and homogenized at a high speed for 1 minute. This gave a 10^{-1} dilution. Serial dilutions of 10^{-2} , 10^{-3} , and 10^{-4} were prepared by using 0.1% peptone water.

b) Total Bacterial Count (TBC) : TBC was conducted aerobically by using plate count agar method according to Post (1988). Plate count agar(Difco) was prepared by homogenizing 23.5 g agar in 1000 ml of deionized water.

Duplicate samples of 1 ml and 0.1 ml of each dilution were pipetted into sterilized and appropriately labeled petri dish. Twelve to 15 ml of cooled (44-46°C) plate count agar were poured into each plate. The sample and agar medium were immediately mixed and then allowed to solidify. The petri dishes were inverted and incubated aerobically at 37°C for 48 hours. The total colony counts were recorded as total bacterial counts or colony forming units per gram (cfu/g).

Biochemical analysis

a) Trimethylamine(TMA) was determined according to Woyewoda et al(1986). Twenty five g of comminuted fish and 50 ml of 7.5% Trichloroacetic acid (TCA) were added to a small blender jar and blended well. The homogenate was filtered through Whatman #2 filter paper and was kept in the freezer (-20°C) before further analysis.

Aliquots (1 or 2 ml) of samples and water (2-3 ml) was added to a clean screw-capped tube to bring the total volume to 4.0 ml for both standard and blank. 1.0, 2.0, and 3.0 ml of working standard solutions containing 10.0 ug TMA/ml were used. The blank contained 4.0 ml of water. One ml of 10% formaldehyde, 10 ml of dried toluene and 3 ml of 25% KOH were added to the tube respectively and the mixture was blended in a vortex mixer for 15 seconds. About 7 ml of toluene (upper layer) was transferred to another tube containing 0.3-0.4 g of anhydrous Na_2SO_4 and mixed with a vortex mixer again. Four ml of this toluene solution was transferred into a test tube containing 4 ml of 0.2% picric acid in toluene. The absorbance was measured in a Perkin Elmer Lambda 4B Spectrophotometer at 410 nm. The result was expressed in mg TMA/100 g.

b) Total acidity was done in accordance to AOAC (1990). The fish sample was comminuted and 5 g were weighed into a screwed-capped bottle and 100 ml of distilled water was added. The sample was blended well in a rotator for 30 minutes and filtered through Whatman paper #4. Ten ml of the mixture was put into a 250 ml Erlenmeyer flask and 100 ml of distilled water was added. The mixture was then swirled to mix. A few drops of phenolphthalein indicator was added, and the mixture was then titrated with 0.1 N NaOH to a persistent pink color. The titer was recorded and calculated as %acidity.

c) pH determination : Twenty g of comminuted fish were measured directly without any addition of water according to the procedure of Woyewoda et al (1986) by using the Fisher Accumet Model 910 pH meter.

Sensory evaluation

Sensory evaluation were conducted after verifying the safety of the product by microbial counts and TMA. The nine points hedonic test according to Meilgaard et al (1991) was used for treated dogfish that had been stored for up to 9 days at 3-5°C compared to the control (fresh fish). All samples were deep fried in hot (185°C) corn oil for up to 4 minutes before they were served to the panel. Samples were evaluated for taste, texture, odor, appearance and general acceptance and statistically analyzed.

RESULTS AND DISCUSSION

The effect of acetic acid was tested on dogfish (*Squalus acanthias*) fillets, dressed whiting (*Merlangius merlangus*), dressed butterfish (*Peprilus triancathus*) and squid (*Loligo vulgaris*) mantles.

Table 1. Total bacterial count (cfu/g) for acetic acid treated Dogfish fillets dipped for 2 minutes in different concentrations of acetic acid and stored at 3-5 °C

<u>STORAGE TIME</u> Days	<u>TOTAL BACTERIAL COUNT (CFU/G) IN SAMPLES</u>				
	Control	1%	2.5%	5%	10%
0	2.5x10 ²	1.1x10 ²	2.0x10 ¹	<10	<10
2	1.7x10 ³	4.9x10 ²	3.0x10 ²	4.0x10 ¹	1.4x10 ²
6	1.0x10 ⁴	8.5x10 ²	3.0x10 ¹	<10	6.0x10 ¹
13	2.2x10 ⁶ *	2.5x10 ²	1.4x10 ²	4.0x10 ¹	9.0x10 ¹
20	3.0x10 ⁸ *	1.2x10 ⁴	1.0x10 ²	6.0x10 ²	4.0x10 ¹
36	NA	NA	2.3x10 ³	5.5x10 ²	4.0x10 ¹

NA : Not analyzed

* : Deteriorated, very high count

Table 1 showed the total bacterial count (cfu/g) for acetic acid treated dogfish fillets dipped for 2 minutes in different concentrations of acetic acid and stored at 3-5°C for different periods of time. The untreated samples(control) showed the expected increased microbial population with time. By dipping the fillets in the different concentrations of acetic acid, a significant decrease in the bacterial count was brought about with increasing concentration of acetic acid. A 10% solution of acetic acid arrested significantly the growth of the initial bacterial population.

Table 2. TMA(mg TMA/100 g.) in acetic acid treated dogfish fillets, dipped for 2 minutes in different concentrations of acetic acid and stored at 3-5 °C

STORAGE TIME Days	AMOUNT OF TMA (mg/100g) IN SAMPLES			
	Control	2.5%	5%	10%
0	0.6	0.8	0.7	0.4
2	1.1	0.9	0.7	0.8
6	1.9	0.9	0.7	0.8
13	3.1	1.1	0.6	0.8
20	30.4	1.5	0.9	1.4
36	45.5	2.1	0.9	1.7

Table 2 shows the corresponding effect of acetic acid on the Trimethylamine (TMA) production on the same samples. Although the TMA level of the control sample rose to about 45 mg per 100 g sample, the treated samples developed a very insignificant level of TMA.

In Table 3 the pH and the % acetic acid levels of the different treatments are shown. Again there was a rise in the pH for the control group, from pH 6.3 to pH 8.2 over a period of 20 days at refrigeration temperature. On the other hand, the treated samples either maintained the pH level at about pH 6.0 or lowered it to about pH 5.0 for the 10% acetic acid treatment. This pH drop may not be substantial enough to produce dramatic microbial inhibition, yet in conjunction with the undissociated portion of acetic acid apparently does induce some inhibition as seen in the previous tables. The absorbed acetic acid was about 0.7% in the fillets and did not vary with time.

Table 3. pH and acetic acid content (%) in treated dogfish fillets, dipped for 2 minutes in different concentrations of acetic acid and stored at 3-5°C

STORAGE TIME			pH AND %ACIDITY IN SAMPLES					
Days	Control		2.5%		5%		10%	
	pH	%AA	pH	%AA	pH	%AA	pH	%AA
0	6.3	0.0	6.0	0.3	5.7	0.6	5.0	0.8
2	6.4	0.0	6.0	0.3	5.8	0.6	5.0	0.8
6	6.5	0.0	6.0	0.2	5.9	0.6	5.1	0.7
13	6.8	0.0	6.1	0.2	6.0	0.6	5.1	0.7
20	8.2	0.0	6.1	0.2	6.0	0.6	5.2	0.8
36	NA	NA	6.2	0.2	6.0	0.6	5.4	0.7

NA : Not analyzed

It was of interest to examine the treated products organoleptically by a taste panel. Table 4 shows that for the treated products at the 2 % acetic acid concentration, there was little difference in the general acceptability score, however, that difference was significant if the 5% acetic acid treatment was compared to the control (fresh sample). The general negative remark was the slightly sour taste of the heavily treated samples.

Table 4. Sensory evaluation of fresh dogfish fillets and fillets that were dipped in different concentrations of acetic acid and stored for 9 days at 3-5°C

CHARACTERISTICS	TASTE PANEL SCORE		
	Control(0 day)	2% 2 min. dip	5% 7 min. dip
Taste	7.56 ^a ±1.18	5.93 ^{ab} ±2.15	5.63 ^{ab} ±2.39
Texture	8.13 ^a ±0.83	7.50 ^{ab} ±1.20	7.38 ^{ab} ±1.85
Odor	7.13±0.64	6.31±1.49	5.88±1.46
Appearance	6.88±2.17	6.88±1.64	7.13±1.89
Gen Accept.	7.69 ^a ±1.03	6.94 ^a ±1.32	6.38 ^{ab} ±2.07

Average values from 8 judges ± standard deviations. Different upper case letters represent statistical difference between means within the same row (p<0.05).

Value 1 - Dislike extremely

Value 9 - Like extremely

Dressed whiting was treated with acetic acid and both the bacterial population and the TMA were determined. Table 5 shows the delay of bacterial growth as the concentration of acetic acid is increased. The 5% and 10% acetic acid gave the best results, limiting the bacterial population to about 10^4 and 10^2 respectively in a period of 24 days at refrigeration temperature. The control group deteriorated after six days of the storage. The TMA test (Table 6) confirmed the above results.

Table 5. Total bacterial count (cfu/g) for acetic acid treated whiting (dressed fish) dipped for 3 minutes in different concentrations of acetic acid and stored at 3-5 °C

STORAGE TIME Days	TOTAL BACTERIAL COUNT (CFU/G) IN SAMPLES			
	Control	3%	5%	10%
0	3.5×10^4	3.0×10^3	2.6×10^3	3.8×10^2
2	8.6×10^4	9.5×10^3	9.3×10^3	1.07×10^2
4	8.0×10^5	1.1×10^4	6.7×10^2	<10
6	$>6.5 \times 10^6$ *	1.1×10^5	6.3×10^3	<10
9	$>6.5 \times 10^6$ *	8.0×10^3	1.0×10^3	<10
11	NA	4.5×10^4	2.1×10^3	<10
15	NA	7.5×10^5	1.7×10^3	<10
19	NA	7.2×10^6	8.0×10^3	3.5×10^2
24	NA	NA	7.5×10^4	7.0×10^2

NA : Not analyzed

* : Deteriorated, very high count

Table 6. TMA (mg TMA/100 g.) in acetic acid treated dressed whiting, dipped for 3 minutes in different concentrations of acetic acid and stored at 3-5 °C

STORAGE TIME Days	AMOUNT OF TMA(mg/100g) IN SAMPLES			
	Control	3%	5%	10%
0	0.4	0.4	0.3	0.3
4	1.3	0.4	1.4	0.2
9	38.9	5.6	0.2	0.2
15	NA	10.1	0.3	0.2
19	NA	14.0	0.5	0.4
24	NA	NA	0.2	0.3

NA : Not analyzed

Another species tested was dressed butterfish (Table 7). Table 8 also shows the inhibitory effect of acetic acid on the bacterial growth and demonstrates the dramatic effect of the acid on squid mantles.

Table 7. Total bacterial count (cfu/g) for acetic acid treated dressed butterfish dipped for 3 minutes in 5% of acetic acid and stored at 3-5 °C

<u>STORAGE TIME</u> Days	<u>TOTAL BACTERIAL COUNT (CFU/G) IN SAMPLES</u>	
	Control	5%
0	6.9×10^3	3.0×10^2
8	1.6×10^6 *	4.4×10^2
14	1.3×10^7 *	1.2×10^4
18	NA	5.2×10^4

NA : Not analyzed

* : Deteriorated, very high count

Table 8. Total bacterial count (cfu/g) for acetic acid treated on squid mantles dipped for 3 minutes in 2% and 5% of acetic acid and stored at 3-5 °C

<u>STORAGE TIME</u> Days	<u>TOTAL BACTERIAL COUNT (CFU/G) IN SAMPLES</u>		
	Control	2%	5%
0	7.0×10^1	<10	<10
6	2.6×10^2	<10	<10
14	NA	<10	<10
19	NA	<10	<10
26	NA	1.6×10^3	<10

NA : Not analyzed

It is important to note that besides the inhibitory effect produced on the bacterial growth, the treated samples appeared clean, had less of a fishy odor, were free of blemishes, and no objectionable odor could be detected. However, a faint sour odor could be identified in the samples treated with higher concentration of acetic acid.

CONCLUSIONS

Low concentrations (3-5%) acetic acid appeared to retard microbial growth in fish fillets and dressed fish when dipped in the acetic acid solution for a few minutes. The TMA values remained very low throughout the storage period at refrigeration temperature.

The process which can extend shelf life of fish for up to three weeks produces a clean looking product, free of mucous, blood, etc. Using this treatment on scombroids might be able to decrease the onset of histamine production in scombroid poisoning. The mild tartness which is often experienced can be removed by dipping the fish in water before meal preparations. Such an inexpensive process should allow temporary storage at refrigeration temperature before transportation by air or other means.

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PRESERVATION OF GURRY HYDROLYSATES WITH THE PROPIONIC ACID FERMENTATION

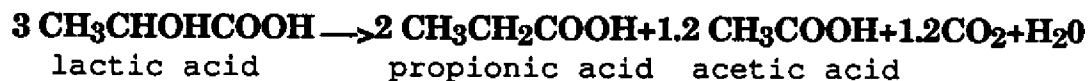
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When gurry hydrolysates are used as fertilizers, the reduction of their pH with a mineral acid such as phosphoric acid or sulfuric acid to facilitate preservation significantly reduces the pH of the soil and therefore greatly restricts repeated application for many crops. Alternatively the use of an organic acid to reduce the pH has the advantage that lactic acid or propionic acid are completely utilized by soil microorganisms with repeated application of the fertilizer to soils. The pH of the soil is therefore not with the use of organic acids for the reduction of pH of gurry hydrolysates. Propionic acid is a well recognized mycostatic agent at acidic pH values and if produced by fermentation entails only the cost of glucose and inoculum preparation, and eliminates the handling of concentrated acids. Propionic acid bacteria convert glucose to propionic acid along with a smaller quantity of acetic acid according to the following equation (1)



Alternatively, the propionic acid bacteria will convert lactic acid as an initial substrate to propionic acid and acetic acid according to the following equation (2):



MATERIALS AND METHODS

Preparation of cod gurry hydrolysate

Cod gurry consisting of heads, tails, and frames was ground and hydrolyzed with 0.05% papain at 71°C for 15 min. with constant agitation. The temperature was raised to 90°C for 5 min., the bone chips screened out and the temperature rapidly reduced to 30°C. In all

studies where absorbance at 600 nm was taken as a determination of relative cell density, hydrolysate was centrifuged at 10,000 g for 10 min, the pH reduced to 4.0 with phosphoric acid, and then filter clarified and sterilized through 0.2m porosity membranes after the addition of glucose and preservatives unless otherwise stated.

Fermentation studies

Unclarified cod hydrolysate was steam sterilized in screw capped tubes (20 x 200 mm). Appropriate volumes of a sterile glucose stock solution (80g/120 ml, prepared with distilled water) were then added so as to yield glucose concentrations of 2.5%, 3.0%, 3.5%, and 4.0% (w/v) in a final volume of 20 ml. Cells of *Propionibacterium acidipropionici* ATCC 4875 were inoculated into tubes containing 10 ml of MRS broth which were then incubated for 96 hr. The cell density of ATCC culture 4875 was then determined with dark-phase microscopy with the use of a Petroff-Hausser bacteria counting chamber. The cell density was then adjusted so as to yield 5×10^6 cells/ml when 0.1 ml of culture was added to duplicate tubes containing 20 ml of hydrolysate. The concentrations of glucose, propionic acid, and acetic acid were determined by high performance liquid chromatography as previously described (3). All incubation was at 32°C unless otherwise stated.

RESULTS AND DISCUSSION

We first screened 15 cultures of *Propionibacterium* for their ability to grow in Lactobacilli MRS broth (MRS) and in filter clarified cod gurry hydrolysate containing 3.0% glucose. Representative cultures and data are given in Table 1. Cultures ATCC 4875 and CCM 1860 yielded the lowest pH value in MRS broth (pH 4.5) after 96 hr of incubation at 32°C (Table 1). With cod hydrolysate, a pH of 4.0 was achieved only with culture ATCC 4875 after 96 hr of incubation. Culture ATCC 4875 was therefore used in all further studies.

When 2.5% glucose was added to cod hydrolysate, 96 hr were required to reduce the pH to 4.5 (Fig. 1). A final pH of 4.2 was achieved after 192 hr of incubation with a propionic acid yield of 1.3%. Glucose concentrations of 3.0%, 3.5%, and 4.0% yielded similar fermentation profiles. With 4.0% glucose an incubation time of 96 hr was again required to reduce the pH to 4.5 (Fig. 2). The final pH after 192 hr of incubation was 4.0 with a yield of 1.75% propionic acid. Although a propionic acid concentration over 1.0% at a pH of 4.0 can be considered to result in a high level of

Table 1. pH reduction by strains of *Propionibacterium* ^a.

CULTURE	FINAL pH ^b	FINAL pH ^c
CCM 1857 <i>P. freudenreichii</i> subsp. <i>freudenreichii</i>	5.4	4.8
P95 <i>P. freudenreichii</i> subsp. <i>shermanii</i>	5.0	5.3
P26 <i>P. jensenii</i>	4.7	4.3
P42 <i>P. acidipropionici</i>	4.6	4.5
ATCC 4875 <i>P. acidipropionici</i>	4.5	4.0
CCM 1860 <i>P. jensenii</i>	4.5	4.3

^aIncubation was for 96 hr at 32°C.

^bMRS broth containing 3.0% glucose (initial pH 5.9).

^cCod hydrolysate containing 3.0% glucose (initial pH 6.8).

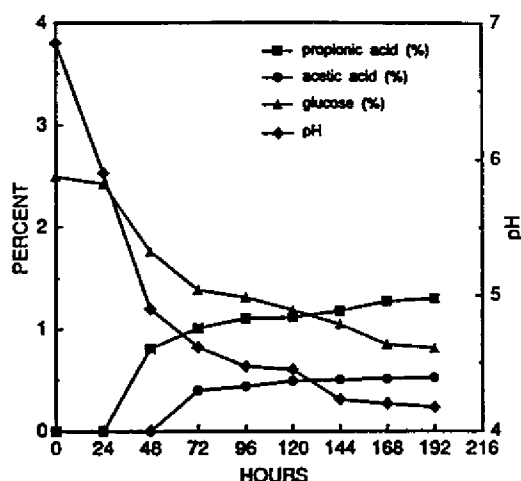


Fig. 1. Rates of glucose utilization, propionic acid formation, acetic acid formation, and pH reduction in hydrolyzed cod gurry containing 2.5% glucose

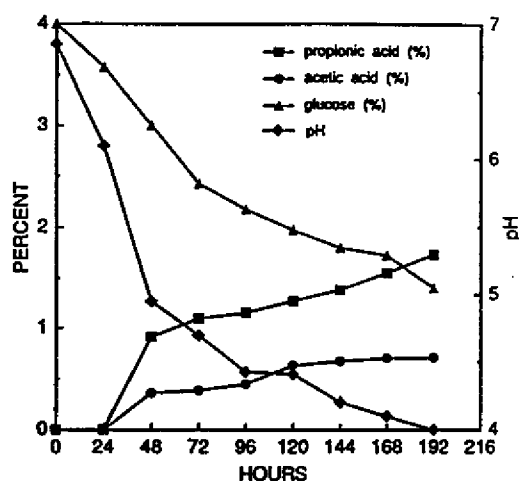


Fig. 2. Rates of glucose utilization, propionic acid formation, acetic acid formation, and pH reduction in hydrolyzed cod gurry containing 4.0% glucose.

preservation, the prolonged period of incubation required to reduce the pH to 4.5 cannot be considered acceptable in terms of allowing the development of contaminants during the first 48 hr of incubation in non sterile

hydrolysate as would be used by industry. This notably slow fermentation is due to the well known slow growth of propionic acid bacteria and to the fact that the majority of propionic acid is produced after cultures reach a maximum cell population. The development of contaminants under similar conditions of fermentation in non sterile hydrolysate containing 4.0% glucose is shown in Figure 3. When a 20% inoculum (v/v) was used so as to result initially in 1×10^9 colony forming units per ml of hydrolysate containing 4% glucose the ratio of propionibacteria to contaminants increased over 100-fold (Fig. 4). However, the time required to reduce the pH

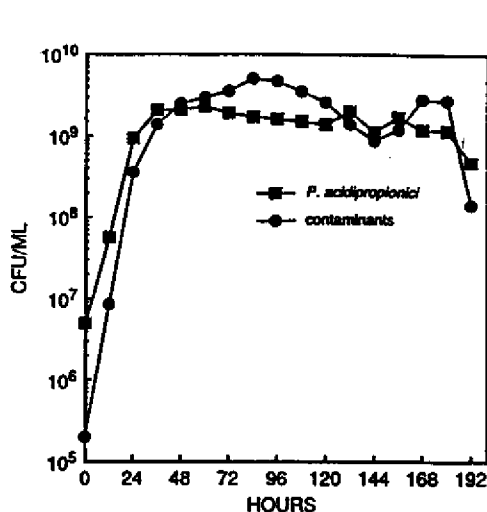


Fig. 3. Development of CFU of *P. acidipropionici* & contaminants in cod hydrolysate. Hydrolysate contained 4.0% glucose and was not sterilized. Initial CFU/ml of *P. propionici* were 5×10^6 .

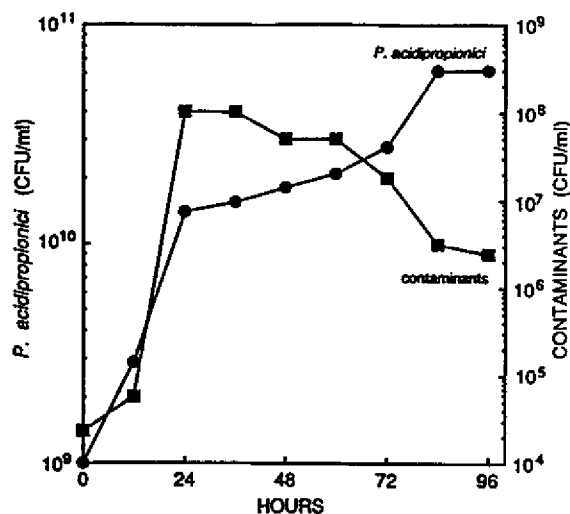


Fig. 4. Development of CFU of *P. acidipropionici* as 20% inoculum and contaminants in cod gurry hydrolysate. Hydrolysate contained 4.0% glucose.

to 4.5 was lowered only to 72 hr (Fig. 5). In contrast, the time required for *Lactobacillus plantarum* to reduce the pH to 4.5 with cod gurry hydrolysate containing 4.5% corn syrup is only 7 hr (Fig. 6).

Our major concern therefore, with respect to the propionic acid fermentation of hydrolyzed gurry is the selective development of propionic acid resistant microorganisms. Recently, a commercial producer of hydrolyzed gurry for use as fertilizer forwarded a sample to us that contained an actively metabolizing yeast that resulted in the development of significant gas pressure inside the plastic container although

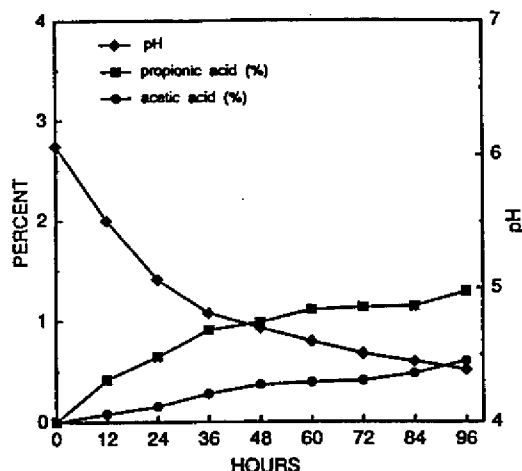


Fig. 5. Rates of propionic acid and acetic acid formation and pH reduction with the use of a 20% inoculum of *P. acidilactici*. Hydrolysate contained 4.0% glucose and was not sterilized. An inoculum volume of 20% (v/v) was used.

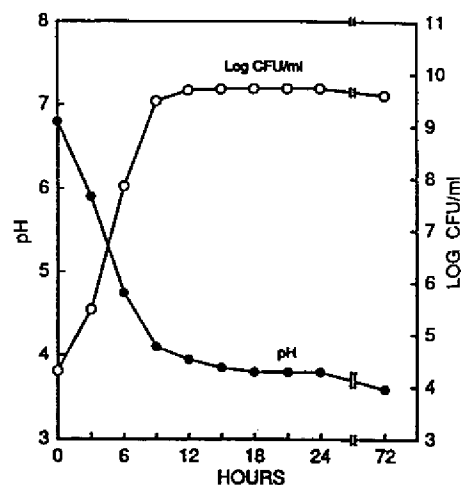


Fig. 6. Lactic acid fermentation of hydrolyzed gurru containing 4.5% corn syrup by *L. plantarum*.

organoleptic spoilage did not occur. The commercial process involved the use of entrails at room temperature for proteolysis over a number of days following acidification with phosphoric acid to a pH of 3.5 and the addition of 0.2% propionic acid. The lack of pasteurization apparently resulted in the selective development and eventual dominance of a contaminating yeast resistant to the level of propionic acid used. We were able to readily isolate this organism which we designated isolate PR-1 and found it to be rather unique. Small well defined colonies 3 to 5 mm in diameter were produced on Orange Serum Agar plates (Fig. 7) whereas on Mycological agar and Tryptic Soy Agar (containing 0.5% dextrose) giant colonies developed which completely filled the plate after 24 to 48 hr (Fig. 8). Static growth of isolate PR-1 in media containing glucose results in extensive surface film formation which characteristically rises about 1 cm above the culture broth along the walls of the vessel. The organism is primarily oxidative but does produce detectable amounts of ethanol. Its morphological characteristics are quite unique in that it produces cells with bipolar budding with multiple polar buds, particularly on acetate agar (Fig. 9). Ascospores are not formed nor are pseudomycelium observed. The

yeast like organisms were assessed for their relative resistance to various concentrations of propionic acid. Representative isolates and data are given in Table 3. Isolate PR-1 was clearly the most resistant (Table 3). It is of interest that *S. cerevisiae*

Table 3. Static growth of yeasts in filter clarified cod hydrolysate containing 4.0% glucose and varying concentrations of propionic acid at pH 4.0.^{a,b}

Culture	PERCENT PROPIONIC ACID					
	0.0%	0.1%	0.2%	0.3%	0.4%	0.5%
PR-1	8.6	9.1	2.7	0.50	0.38	0.00
<i>S. cerevisiae</i>	8.0	6.6	4.1	0.31	0.00	0.00
<i>D. hansenii</i>	7.5	0.86	0.08	0.00	0.00	0.00
<i>C. lipolytica</i>	5.3	0.79	0.06	0.00	0.00	0.00
<i>Z. priorianus</i>	3.3	2.2	1.0	0.00	0.00	0.00
<i>E. fibuliger</i>	1.3	0.22	0.00	0.00	0.00	0.00
<i>H. saturnis</i>	0.44	0.00	0.00	0.00	0.00	0.00

^aIncubation was at 32°C for 72 hr.

^bNumerical values are absorbance readings at 600 nm.

exhibited the next highest level of tolerance to propionic acid (Table 3). When 50 ml volumes of filter clarified and sterilized hydrolysate in tubes (with no glucose added) containing increasing concentrations of propionic acid from 0 to 0.5% at a pH of 4.0 were inoculated with isolate PR-1 and incubated statically, a concentration of 0.5% propionic acid was required to inhibit the organism (Fig. 10). When this study was repeated with the addition of 4.0% glucose to tubes of hydrolysate, growth was more rapid and 0.5% propionic acid was again required to inhibit growth (Fig. 11). When isolate PR-1 was incubated with rotary agitation (200 rpm) in 200 ml baffled flasks containing 100 ml of filter clarified and sterilized hydrolysate to which 4.0% glucose was added at a pH of 4.0, both 0.1% sodium benzoate and 0.1% potassium sorbate were notably inhibitory (Fig. 12). With static incubation of isolate

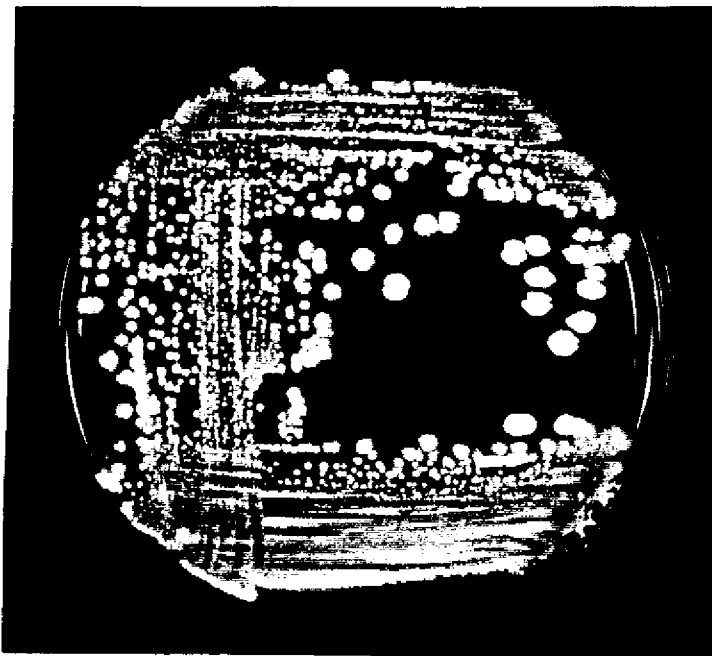


Fig. 7. Colonies of yeast isolate PR-1 on Orange Serum Agar. Incubation was for 48 hr at 32°C.



Fig. 8. Giant colony of isolate PR-1 on Tryptic Soy Agar containing 0.5% glucose. Center of plate (1 mm) was inoculated. Incubation was for 48 hr at 32°C.

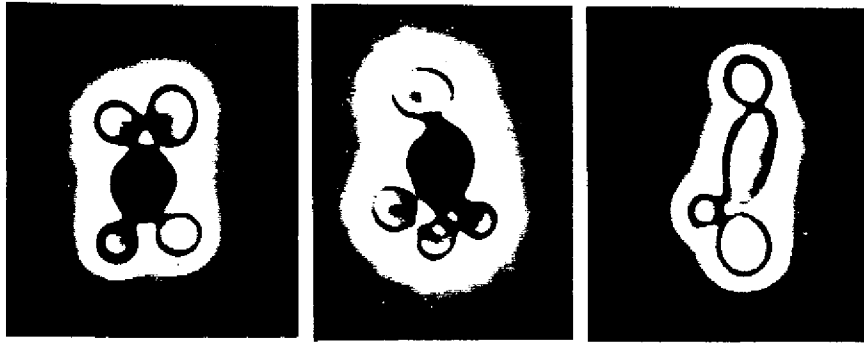


Fig. 9. Dark-phase photomicrographs of isolate PR-1 grown on acetate agar. x 1,400.

organism grows from 15°C to 45°C which is an unusually wide temperature range for a yeast. Among 36 carbon sources tested using the auxanographic plate technique only glucose, fructose, mannose, succinic acid, and glycerol were utilized. Neither disaccharides nor pentose sugars were used, and the organism is neither lipolytic nor proteolytic. Although nitrate is not utilized as a sole nitrogen source the organism is able to readily utilize urea. The characteristics of the organism are given in Table 2. A number of yeasts and

Table 2. Characteristics of yeast isolate PR-1

Colony morphology:	Small colonies on orange serum agar. Giant colonies on Mycological Agar and Tryptic Soy Agar.
Film formation at surface of static growth media:	Positive
Metabolism:	Primarily oxidative with weak fermentation.
Morphology:	Bipolar budding with multiple polar buds. No pseudomycelium.
Temperature range for growth:	15°C - 45°C.
Ascospore formation:	Negative.
Carbon sources utilized of 36 tested:	Glucose, fructose, mannose, succinic acid, & glycerol. Disaccharides and pentoses not utilized.
Nitrate utilized as a sole N source:	Negative
Urea utilized as a sole N source:	positive.
Lipase:	Negative
Protease:	Negative

PR-1, in filter sterilized hydrolysate (with no glucose) at pH 4.0, 0.1% K sorbate was notably inhibitory while 0.1% Na benzoate was significantly less inhibitory (Fig. 13).

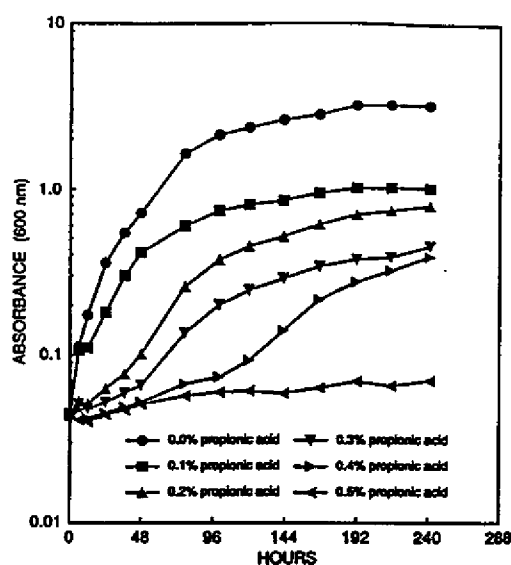


Fig. 10. Growth of isolate PR-1 in filter clarified and sterilized cod gurry hydrolysate containing increasing levels of propionic acid. Hydrolysate (pH 4.0, 50 ml) contained no added glucose, and was incubated statically in tubes.

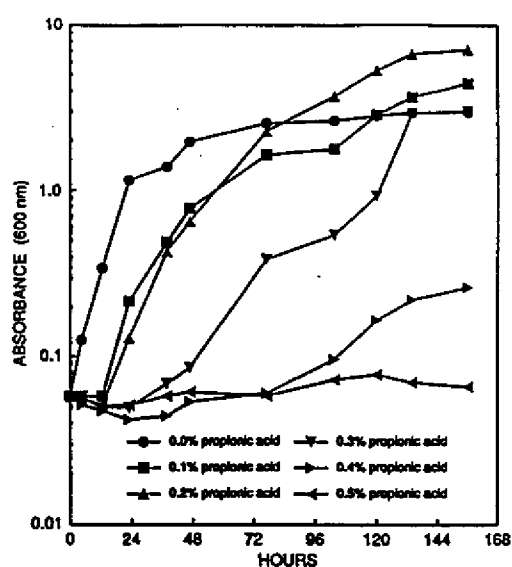


Fig. 11. Growth of isolate PR-1 in filter clarified & sterilized hydrolysate containing increasing levels of propionic acid. Hydrolysate (pH 4.0, 50 ml) contained 4.0% glucose, and was incubated statically in tubes.

When *L. plantarum* was inoculated into tubes of steam sterilized whole hydrolysate containing 4.0% glucose neither 0.1% Na benzoate nor 0.1% K sorbate exerted an inhibitory effect on the resulting rate of pH decline (Fig. 14). When the same study was performed with *P. acidipropionici* ATCC 4875, 0.1% K sorbate had no effect, whereas 0.1% Na benzoate significantly increased the rate of pH decline so as to yield a pH of 4.5 in 48 hr compared to 77 hr for the control (Fig. 15).

CONCLUSIONS

The propionic acid fermentation by itself is too slow to prevent significant numbers of contaminants from developing when non sterile hydrolysates are inoculated with propionic acid bacteria. The lactic acid fermentation is rapid and can result in a pH of 4.5

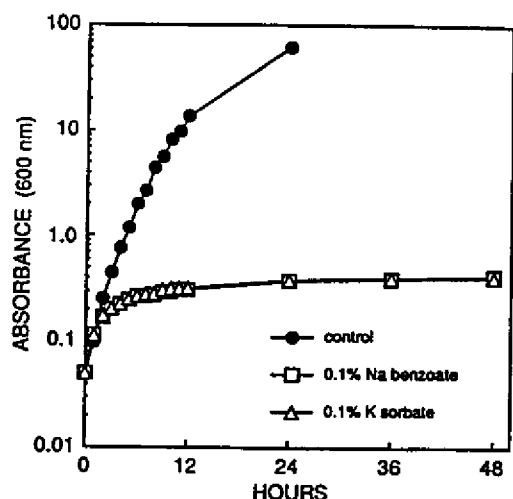


Fig. 12. Growth of isolate PR-1 in filter clarified & sterilized cod gurry hydrolysate containing 0.1% Na benzoate or K sorbate. Hydrolysate (100 ml), pH 4.0) contained 4.0% glucose & was in 250 ml baffled flasks which were incubated with rotary agitation (200 rpm).

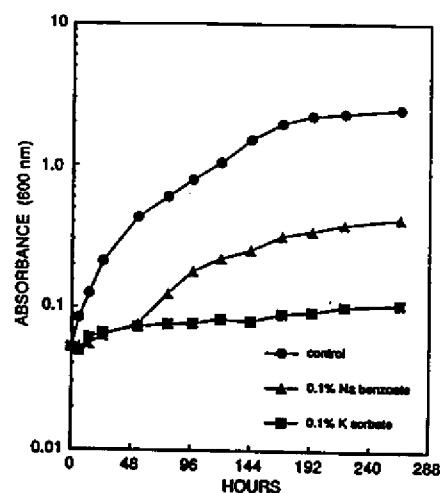


Fig. 13. Growth of isolate PR-1 in filter clarified & sterilized cod gurry hydrolysate containing 0.1% Na benzoate or 0.1% K sorbate. Hydrolysate (50 ml in tubes, pH 4.0) contained no glucose. Incubation was static.

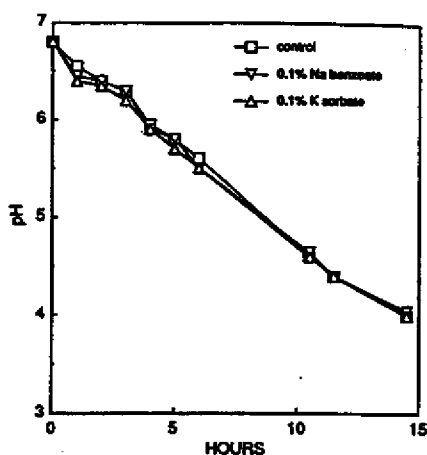


Fig. 14. Rates of pH reduction by *L. plantarum* in hydrolyzed cod gurry containing 4.0% glucose & 0.1% Na benzoate or K sorbate. Hydrolysate (50 ml in tubes) was steam sterilized. Inc. was static.

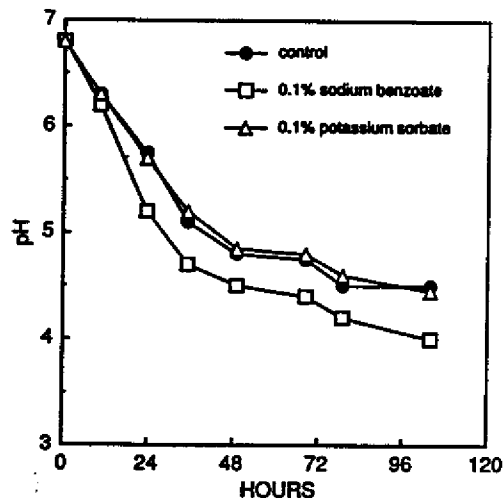


Fig. 15. Rates of pH reduction by *P. acidipropionici* in hydrolyzed cod gurry containing 4.0% glucose & 0.1% Na benzoate or K sorbate. Hydrolysate (50 ml in tubes) was steam sterilized. Inc. was static.

being reached in 7 hr. If both a lactic acid bacterium such as *L. plantarum* and a propionic acid bacterium such as *P. acidipropionici* are used as inoculum plus 3.0% to 4.0% glucose along with 0.1% K sorbate, a rapid reduction of pH will occur with preservation resulting from the presence of K sorbate (at reduced pH). As lactic acid is formed, it will be slowly converted to propionic acid. At the end of the fermentation, about 1.0% propionic acid will result so as to achieve an extremely high level of preservation in combination with the 0.1% K sorbate.

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**CAPILLARY ZONE ELECTROPHORESIS OF FRESHWATER WHITEFISH
(*COREGONUS CLUPEAFORMIS*) SARCOPLASMIC PROTEINS DURING
MODIFIED ATMOSPHERE PACKAGING**

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Demand for seafood (24) has increased primarily because of today's consumer interest in freshness, convenience, taste, nutritional value, and potential health benefits related to the presence of ω -3 fatty acids. The present trend in exports of either fresh or saltwater fish is toward increased high quality, chilled product. Current industrial competitiveness can be improved with the application of advanced technology (6) to help the congruent development of both fresh and saltwater fishery resources. Of these advanced technologies, modified atmosphere packaging (MAP) (7) and shelf-life extension have been ranked as top Canadian priorities; of the Western Canadian freshwater fish species, industry has identified whitefish (*Coregonus clupeaformis*) due to its abundance, quality, and sales value. It is widely accepted that if the high quality of this labile product could be extended, then both whitefish delivery to local markets, as well as exports to the United States, Europe and Asia could be better developed. Conversely, a longer shelf-life might permit fish products to be sea or road freighted to closer markets thus saving the high cost of air freight. These considerations have led to research on extension of the shelf-life of packaged fresh fish using MAP (1, 4, 8, 23, 25, 27) or controlled atmosphere packaging technology (CAP-TECH) (3, 22), the latter having been originally developed for packaging of red meats (10, 11). However, minimal work has been done with these packaging technologies to assess improvement in the shelf-life of freshwater fish species (2), particularly those such as whitefish with a moderate-high fat content. To date, researchers (1, 4, 8, 25) have reported that MAP of fish results in a significant increase in shelf-life but its maximum storage life may not have been attained because of insufficient CO₂. Under optimal conditions for red meat (10), a recent study (22) using CAP-TECH for medium microbiological quality snapper (*Chrysophrys auratus*) fillets gave a storage life of ca. four weeks at -1.0°C. Studies (10) of CAP of red meat at -1.5°C±0.5°C gave a storage life of >24 weeks due to the superior microbiological quality of the red meat. Moreover, other investigations (9, 20, 26) on the efficacy of MAP of fish, under variable packaging and storage conditions, have focused on spoilage microflora; however, important nonbacterial changes which influence resultant fish quality also occur during extended storage (23). Some studies (8, 25) on sensory properties of MAP of stored fish have been flawed by

inappropriate sensory evaluation techniques (13, 29); hence data on color, odor, flavor and texture may not give a true indication of this most important quality index. There is a paucity of published reports on the composite sensory, physical, biochemical and microstructural attributes of stored, fresh fish processed via MAP or CAP-TECH systems. Indeed, a systematic investigation of this type seems warranted to identify which is the preferable system for commercial application.

Conventional electrophoresis and high performance liquid chromatography (HPLC) methods based on sarcoplasmic protein (SAR) profiles of raw and/ or cooked fish are limited in their effectiveness for routine quality control and storage change tests. However, capillary electrophoresis (CE) (17) is now becoming an important technique for fast and efficient separation, characterization, and quantitation of proteins. It offers a gentle, non-denaturing environment, making it a technique that is well suited to explore subtle differences in protein structures in their native state (12, 14). Recent work by Chen *et al.* (5) established potential capillary zone electrophoresis (CZE) conditions for analysis of model proteins, serum, and complex milk proteins. LeBlanc *et al.* (16) optimized a CZE method to analyze SAR for fish species identification and to detect changes in product during frozen storage. The purpose of the present work, a component of a major project that encompasses sensory, physical, biochemical, microbiological, and microstructural assessments, was to apply the CZE method (16) to monitor changes in freshwater whitefish SAR during Aligal or CO₂ modified atmosphere packaging storage at -2°C for 21 days.

MATERIALS AND METHODS

Experimental design

For the study, whole whitefish (*Coregonus clupeaformis*) was obtained through Billingsgate Fish Co. Limited, Head Office, Calgary, AB. Same day, landed, gutted, bled, iced whitefish from Lake Wabamun were butterfly-filleted, packed appropriately to maintain premium grade quality and transported from the Billingsgate plant in Edmonton to the Alberta Agriculture Food Processing Development Centre, Leduc, AB. A Multivac packaging machine (Model M855E PC, Knud Simonsen Industries Limited, Rexdale, ON) with Winpak high barrier top and bottom films (ESXE 1250 S - composition 0.48 mil PVDC coated polyester/2 mil EVA copolymer; APETXE-37550 S - 15.0 mil polyester/PVDC/ 2.0 mil polyethylene, Winpak Ltd., Winnipeg, MN) was used at the latter location for packaging the fillets. The respective forming and seal temperatures were 120°C and 130°C. The gas flush time after evacuation was 7 s with i) Aligal certified mixture (10.1% O₂, 40.5% Ar, 49.4% CO₂) or ii) CO₂ (Canadian Liquid Air, Edmonton, AB) from individual cylinders. The top and bottom films had the following gas transmission rates: O₂, 7.2 cm³/m²/24 h, dry, 23°C and water vapor, 6.0 g/m²/24 h, 90% RH, 37.8°C; O₂, 5.5 cm³/m²/24 h, dry, 23°C and water vapor, 2.9 g/m²/24 h, 90% RH, 37.8°C. A Mocon Oxytran (Model DL 200) was used to measure the O₂ transmission rate on empty but intact formed packages and gave average readings of 0.232 cc/pkg/day under atmospheric conditions (20% O₂), ambient temperature (22.5°C), and 20% RH.

Prior to packaging, all fillets were prescreened using a portable pH meter with a surface electrode and weighed on a Mettler balance to ensure an initial pH range of 6.8-7.0 and weights between 450-500 g. A total of 384 kg butterflyed, skin-on whitefish fillets were randomly allocated into 2 groups of 192 kg - Group 1 for MAP with the Aligal gas mixture with argon (21) and Group 2 for CO₂ modified CAP-TECH (10, 11) processing. Whitefish fillets designated to each treatment Group were

randomly assigned to each of four different, evenly spaced storage intervals (0, 7, 14, 21 days) with 48 kg of whitefish fillets assigned to each of six replications per gas mixture X storage interval combination. Fillets (ca. 500 g) were packed using the appropriate gas (Algal or CO₂) treatment with an inflation volume of 1.5 L of gas mixture/0.5 kg (11) of whitefish fillets to give a residual O₂ concentration of <0.5%. All samples for each of the six replications were stored in a Bally walk-in cooler (Model 4884-11) at -2°C for the specified storage interval. Temperatures were monitored in the cooler with two Delphi model 861 temperature loggers. Head space analysis was carried out on each sampling day on four packages of each gas treatment/replication using a Hewlett Packard gas chromatograph (Model 5890A) equipped with a 6' Porapak Q column 80/100 mesh (CO₂ air separation) and a 10' molecular sieve 13X column 45/60 mesh (O₂ N₂ separation). Oven temperature was 40°C, helium carrier gas flow rate was 24 cc/min and the detector was thermal conductivity. The area under the peaks was measured using a Hewlett Packard (Model 3390A) integrator calibrated with a Scotty I analyzed gas mix #237 (7.0032% CO, 14.9992% CO₂, 4.5039% methane, 7.000 O₂ ± 2%).

Upon completion of these analyses at each specified storage interval (Days 0, 7, 14, 21), the packages from the various replications were transported in coolers to the Department of Food Science and Nutrition, University of Alberta. Packages from each replication were randomly assigned to the various sensory, physical, biochemical, microbiological, and microstructural analyses that were to be done within the overall study of which this component was part of the protein assessment by CE.

pH of whitefish muscle

pH was determined on duplicate samples of fish muscle from each of the six replications of both gas treatments at each storage interval according to the procedure of Woyewoda *et al.* (28). Representative samples were cut into 2 cm³ cubes from each side of the fillet and 60 g of muscle was homogenized for 1 min in a Cuisinart (Model RC1). The walls of the food processor were scraped down after 30 s and the sample was placed into a 25 mL glass beaker. A Fisher Accumet pH meter (Model 815MP) with a combination electrode standardized at pH 6.0 and 7.0 was inserted into the center of the sample and the pH value obtained.

Preparation of SAR for CE

Duplicate samples of fish fillets from each of the six replications of both gas treatments at each storage interval were cut into 1 cm³ pieces, mixed, and 50 g samples were homogenized in 50 mL cold double-distilled, deionized water using a Virtis S23 homogenizer. The samples were blended at high speed for four 15-sec intervals with a 30-sec cooling interval between each 15-sec run. The homogenization container was continually cooled in an ice bath. The homogenates were kept cool on ice and weighed immediately into pre-chilled centrifuge tubes. The latter were ultracentrifuged at 45,000 X g for 30 min in a Beckman Model L-2 preparative ultracentrifuge with an SW 27 rotor at -5°C. The supernatant was used immediately for protein profile separation by CE.

CZE of SAR

Phosphate buffer of pH 7.4 was prepared by mixing appropriate amounts of 0.5M mono-, di-, and/or trisodium phosphate. All buffers were made with double-distilled, deionized water and Milli-Q treated prior to filtering through a 0.22 µm disposable filter before use. Protein standards were

obtained from Sigma Biochemicals as lyophilized powders. Protein standards were dissolved in sample diluent buffer containing 75 mM sodium chloride, 20 mM potassium phosphate, 0.01% sodium azide, pH 7.0 (PBS). Each protein concentration was between 0.3-1.0 mg/mL. All fish SAR samples were diluted 1:10 in PBS. CZE was performed with a Beckman P/ACE System 2100 controlled with a DELL 316 SX expanded computer and post-run data analysis was carried out with Beckman System Gold Version 7.1 software (Beckman Instruments, Fullerton, CA). A standard untreated, fused silica capillary of 27 cm length (20 cm to detector window) X 20 μ m i.d. (Beckman part no. 338475) was used with on-column UV absorbance detection with the P/ACE system set at 200 nm with a 50 X 200 μ m aperture in the P/ACE cartridge (Beckman part no. 338462). The temperature of the capillary during electrophoresis was programmed and maintained at 20°C. The inlet was held at 10 kV and the samples were introduced by pressure injection for 30 s. Between runs, the capillary was sequentially washed with two column volumes of 1.0 N sodium hydroxide and water (0.3 min high pressure rinsing each), followed by reconditioning with 5-10 column volumes of run buffer (1.5-2.0 min high pressure rinsing).

Protein content of SAR extracts

Protein concentration of the extracted SAR was determined by the Lowry *et al.* (18) method with BSA as a standard.

RESULTS AND DISCUSSION

pH of whitefish muscle

Figure 1 shows that the pH of the fillets ranged from 6.40 ± 0.13 for the CO₂ treatment (Day 7) to 6.66 ± 0.17 for the Aligal (Day 7). The Aligal packaged fillets exhibited a significantly ($p \leq 0.05$) higher mean pH during storage than that found for the CO₂ packaged fillets. This difference may reflect the lower per cent of CO₂ in the Aligal gas mixture (49.5%). In addition, the CO₂ treatment had a significantly ($p \leq 0.05$) lower pH on Day 21 compared to that found on Day 7. These values demonstrate the solubility of CO₂ and its concentration in this packaging treatment. Other researchers found similar pH ranges during storage for snapper (22), salmon (1), and swordfish steaks (15, 19) packaged in modified atmospheres that contained gases with 100%, 90% 70% or 40% CO₂. The extent to which pH decreases is proportional to the concentration of CO₂ in the package, the buffering capacity of the muscle, and the composition of the microbial flora (19). An increase in pH indicates bacterial growth which results from microbial deamination and the formation of basic amine compounds such as ammonia and total volatile base nitrogen.

CZE of SAR

Electropherograms of SAR extracts of replicate whitefish fillets from Day 0 are presented in Figure 2. The profiles of four representative extracts show the reproducibility of the optimized CZE method (16) in that all replicates give similar electropherograms and the ratios among the peaks are comparable. The relative standard deviation (R.S.D.) of the migration time for each of the samples analyzed is <1%. Washing of the capillary between runs enhanced the reproducibility in conjunction with the other CZE operating variables used. It is noteworthy that the SAR profiles were obtained using an injection volume of ca. 2.5 nL and a maximum absorbance of 0.02 units at 200 nm. Peak elution occurred in ca. 5 minutes within a run time of <10 minutes. The electropherograms (Fig. 2)

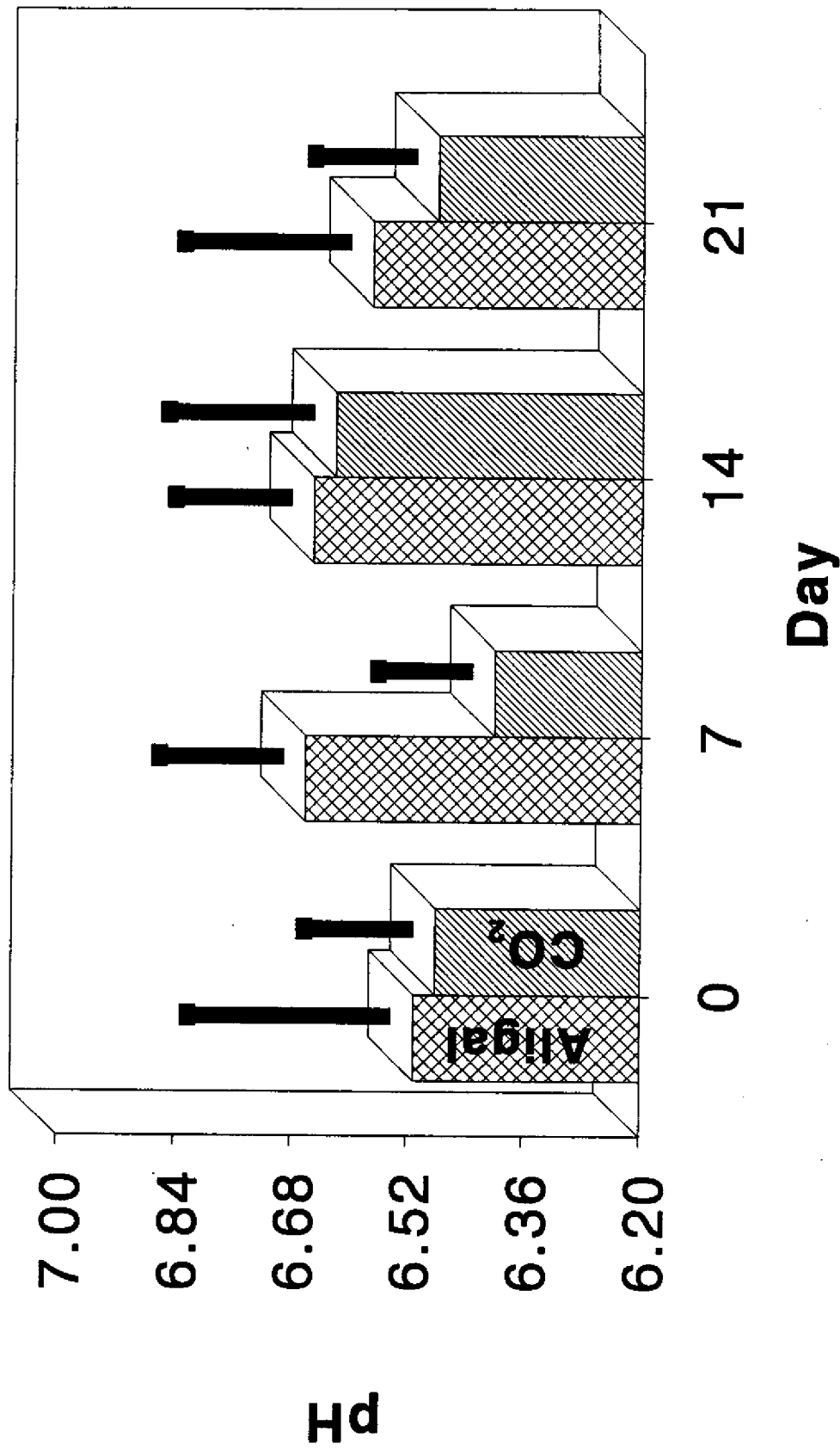


Figure 1. Changes in pH of Algal or CO₂ modified atmosphere packaged whitefish fillets during storage at -2°C for 21 days.

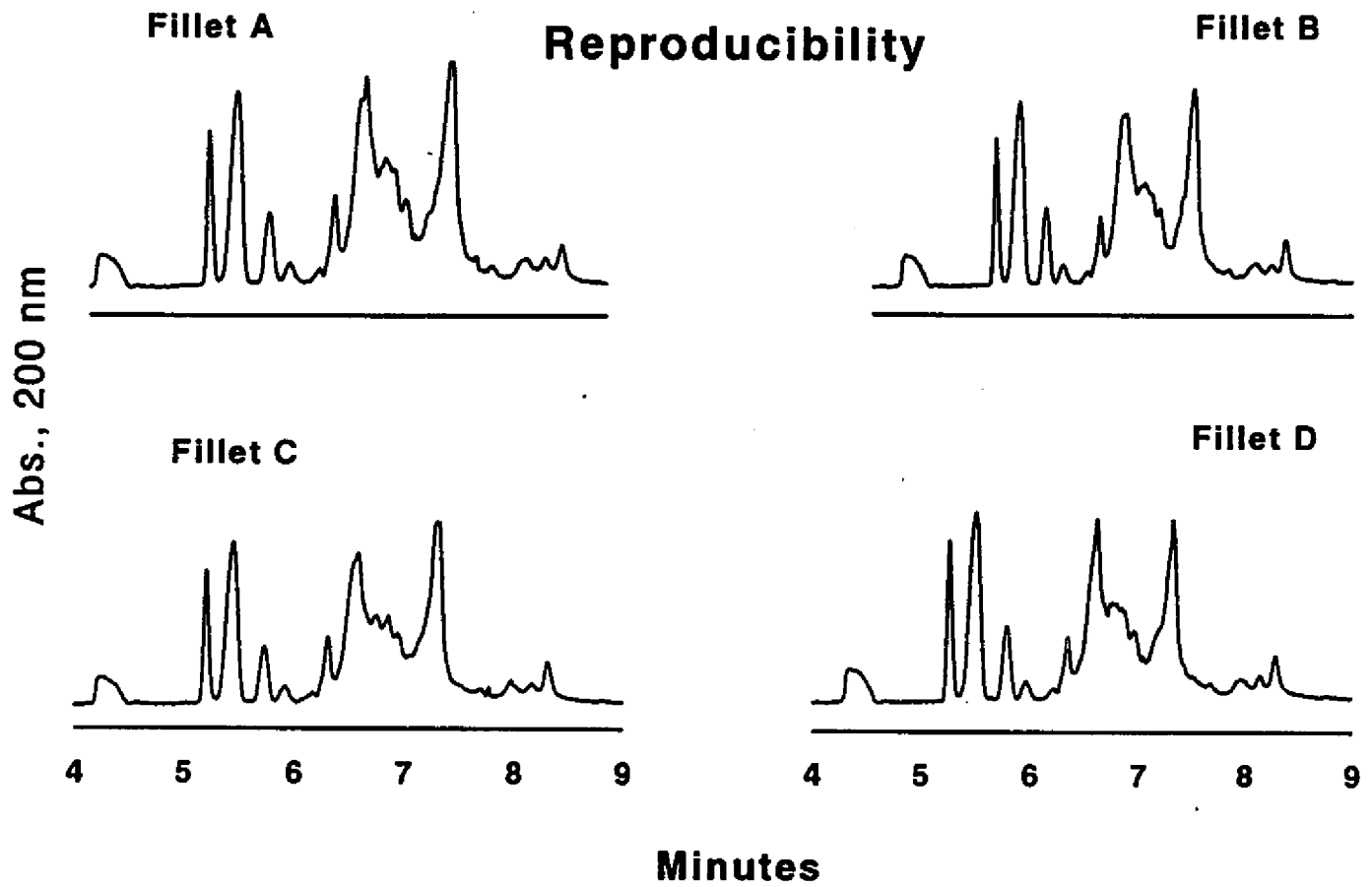


Figure 2. Electropherogram profile reproducibility of four replicate whitefish fillet sarcoplasmic protein extracts at Day 0.

contain ≥ 15 well resolved peaks under these CZE conditions. As well, the use of a high NaCl concentration in the phosphate buffer did not result in either a noisy electropherogram or poor UV detection.

Figure 3 shows the SAR profiles of Algal or CO₂ packaged whitefish fillets during modified atmosphere storage at -2°C for 21 days. Whitefish fillets were of comparable initial quality as shown by the SAR profiles on Day 0. The Algal packaged whitefish fillets show a decrease in the SAR between 6 and 8 min. migration time for Days 7, 14, and 21. Conversely, the CO₂ packaged whitefish fillets exhibit an increase in the SAR between 4.8 and 5.6 min. migration time for Days 14 and 21. The electropherograms also show three peaks having similar ratios at ca. 4.8 min. migration time; a fourth peak becomes apparent at 6 min. migration time in the electropherograms for Days 14 and 21. The major peak at 7.2 min. migration time is absent only in the Day 21 Algal electropherogram. It is of interest to note that the SAR profiles do not change substantially until Day 14 for Algal and Day 21 for CO₂; however, there is a notable decrease by Day 21 in the number of SAR peaks with either gas treatment. It seems likely that any of the degradative changes apparent are associated with bacterial activity and hydrolysis by the naturally occurring proteolytic enzymes of the muscle. These are retarded more effectively by the CO₂ packaging treatment because of its greater effect on pH. The electropherograms (Fig. 3) support the fact that fewer changes occurred in the SAR profiles of the CO₂ packaged fillets during storage thereby resulting in a higher quality product at Day 21.

Protein content of SAR extracts

Figure 4 shows that there was no significant ($p \leq 0.05$) difference in protein content of the SAR extracts from the Algal or CO₂ packaged whitefish fillets during storage at -2°C for 21 days. Therefore, no apparent relationship can be made with the changes observed in the SAR electrophoregrams (Fig. 3).

CONCLUSION

The CZE method optimized for SAR profile analysis for species identification and determination of changes in fish products during frozen storage by LeBlanc *et al.* (16) was effectively used to monitor changes in fresh Algal or CO₂ modified atmosphere packaged whitefish fillets during storage at -2°C for 21 days. The present results show that CZE gives very reproducible data and that changes in SAR were monitored quickly by this technique using nL of SAR extract and μ L of buffer. Differences in the SAR profiles of either the Algal or CO₂ packaged whitefish fillets during storage were detectable by CZE even though there was no significant ($p \leq 0.05$) difference in the protein content of the extracts determined by the Lowry (18) analysis. Fewer changes occurred in the SAR profiles of the CO₂ modified atmosphere packaged whitefish fillets during storage and these findings are supported by the decrease in pH found in the muscle for this treatment. CO₂ packaging appears to result in better quality product as shown by the CZE data. This advantageous CZE method may potentially be used to monitor SAR profiles for quality assessment of fresh fish and the application of this approach is presently under investigation.

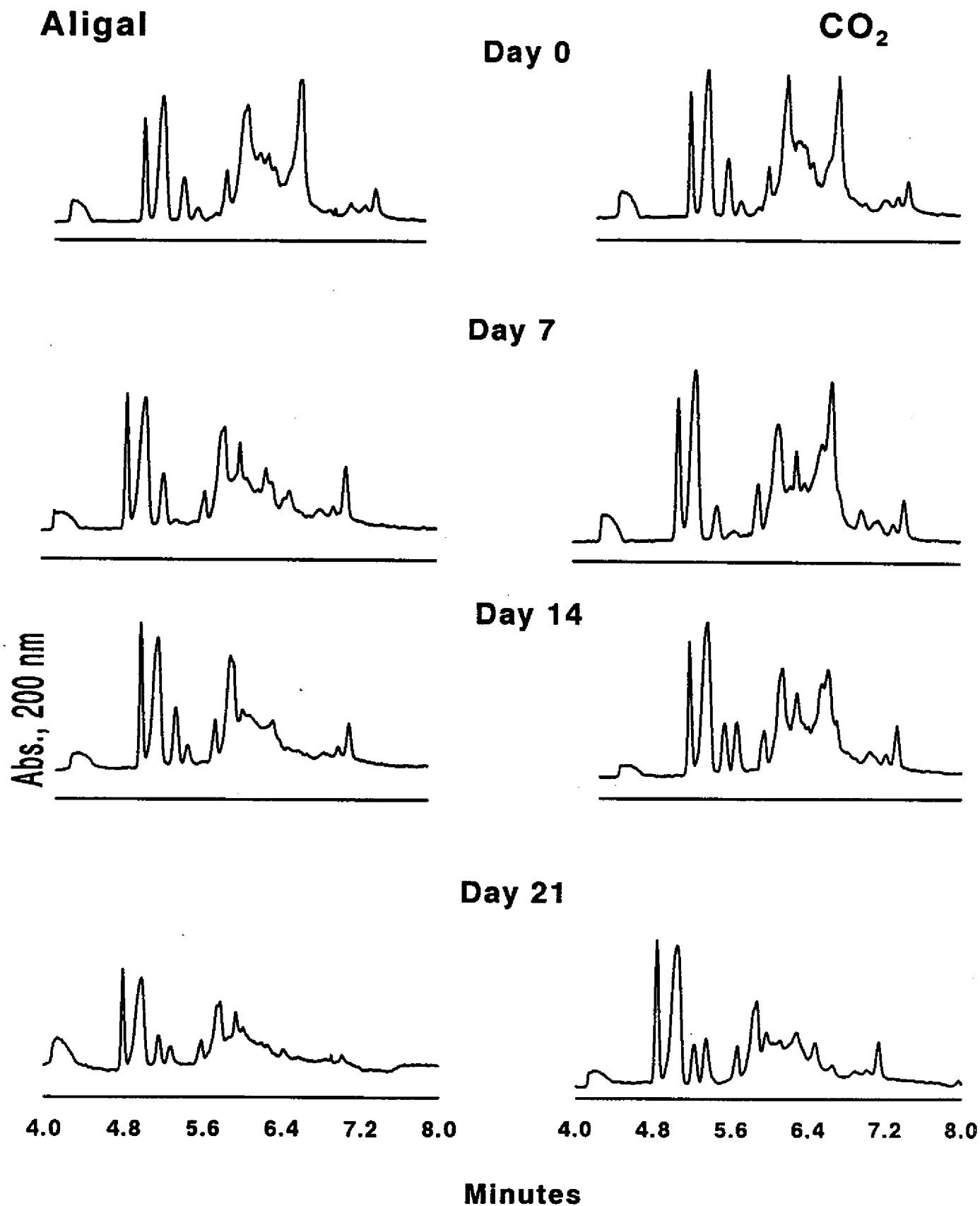


Figure 3. Electropherograms of sarcoplasmic protein profiles of Algal or CO₂ modified atmosphere packaged whitefish fillets during storage at -2°C for 21 days.

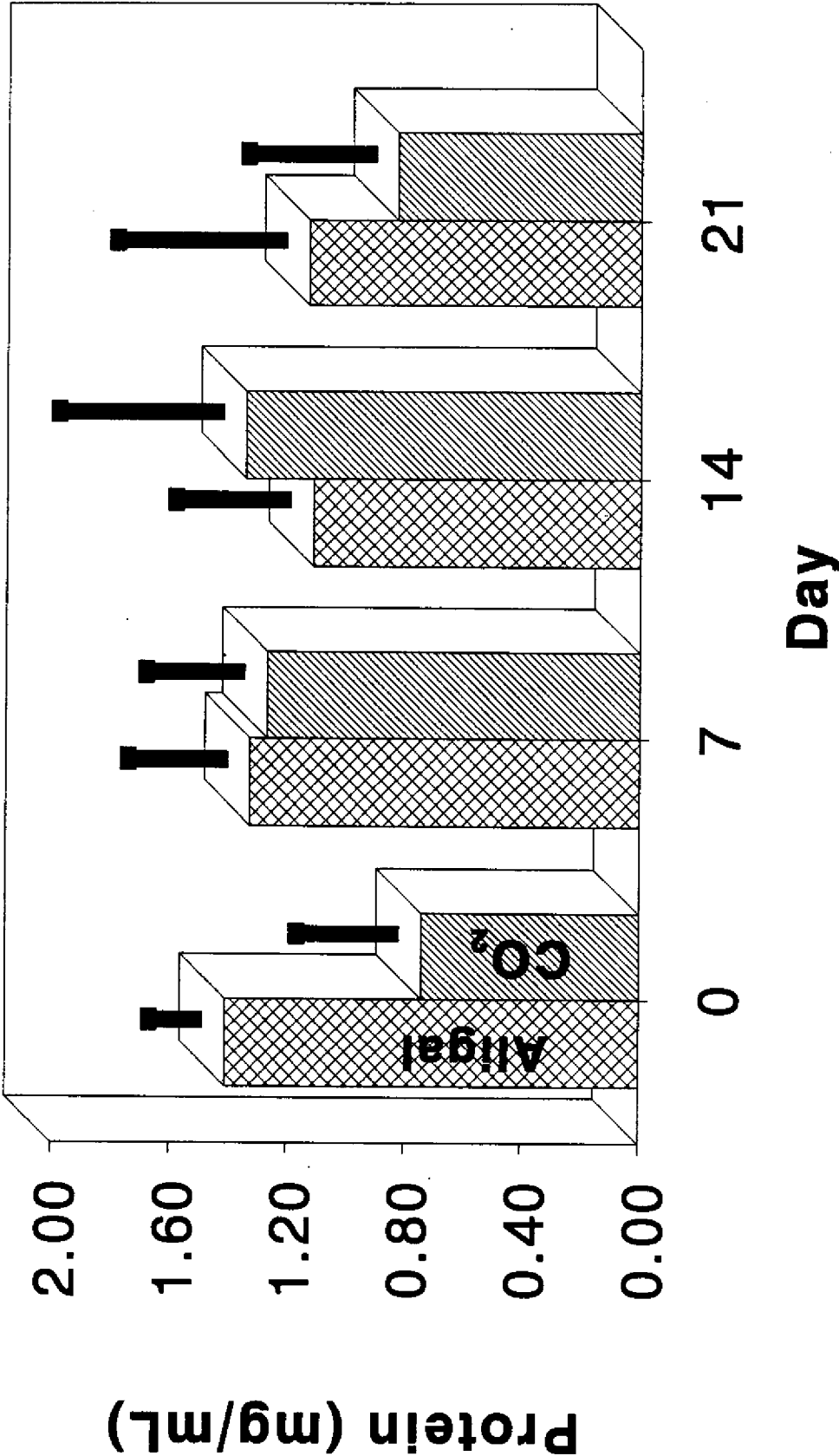


Figure 4. Protein content of sarcoplasmic protein extracts from Algal or CO₂ modified atmosphere packaged whitefish fillets during storage at -2°C for 21 days.

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PROTEIN HYDROLYZATES FROM AQUATIC SPECIES

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Enzymatic hydrolysis is carried out for improving nutritional and/or functional properties of protein ingredients as well as to retard quality deterioration and off-flavour development. These products may be prepared from a variety of protein sources originating from plant, animal and fungi. Underutilized aquatic species as well as processing discards of muscle foods may also serve as raw materials for production of hydrolyzates (Shahidi, 1993).

Protein hydrolyzates may be used in a variety of food formulations intended for hypoallergenic and immune-compromised individuals since peptides are involved in the initiation of the immune response and in the process by which the body is able to differentiate self from non-self (Mills *et al.*, 1992). Their use in infant foods and for patients with digestive tract complications is also commonplace. In fact short peptides are among the most potent pharmacologically active agents known. Furthermore, protein hydrolyzates may be used as growth enhancing components for juvenile fish in aquaculture operations.

Amongst the aquatic species common to Newfoundland waters are capelin (*Mallotus villosus*) and harp seal (*Phoca groenlandica*). Capelin is a small silvery fish related to smelt which is found abundantly in the offshore and inshore beaches of Newfoundland and Labrador in months of June and July. Spawning stocks consist of 3 to 4 years old capelin. Female roe capelin is an important product in the Japanese market, referred to as "shishamo". However, male and spent capelin are generally dumped or are reduced to silage and meal. Small amounts of capelin is also used for production of corned products, fish sauce and pet food. Harp seal carcasses are also generally dumped or reduced to silage, although research from our laboratories has demonstrated that seal meat is superior to other types of meat with respect to its protein content, amino acid profile, omega-3 fatty acid constituents as well as mineral and vitamin contents.

As part of an on-going research program in our laboratories for preparation of value-added products from processing discards and underutilized aquatic species (Shahidi *et al.*, 1990, 1991a; Shahidi and Synowiecki, 1991), we have investigated production of protein hydrolyzates from capelin and seal meat. Some characteristics of the

resultant protein hydrolyzates referred to as Biocapelin and Biosea-L have been studied and their potential applications in a variety of food formulations is being evaluated.

PRODUCTION OF HYDROLYZATES

Production of protein hydrolyzates involves comminution of raw material followed by their dissolution or suspension in water. Enzyme is added to the slurry and the reaction proceeds for 2 hours to 1 week, depending on the activity of the enzymes employed as well as other factors. After separation of solids, the aqueous layer is clarified and dehydrated (Figure 1). The process may include sterilization at different stages, if necessary.

Table 1 summarizes typical processing conditions for preparation of hydrolyzates from processing discards and underutilized species using selected enzymes. Typical yield of protein and proximate composition of Biocapelin and Biosea-L are given in Table 2. Although many factors affect the yield of hydrolysis, the type of enzyme employed has a marked effect on this and also on the characteristics of the final product.

A close scrutiny of the results presented in Table 2 indicates that hydrolyzates generally have a much lower lipid content than their original protein source, on a dry weight basis. As hydrolysis proceeds, elaborate membrane system of the muscle cells tend to round up and form insoluble vesicles, thus allowing the removal of membrane structural lipids. Therefore, based on highly unsaturated nature of lipid fatty acids from marine species, the resultant products are considerably more stable towards oxidative deterioration.

SOME CHARACTERISTICS OF HYDROLYZATES

The amino acid composition of Biocapelin produced by Alcalase-assisted hydrolysis was determined and compared with that of the original capelin. Although sensitive amino acids such as methionine and tryptophan were affected, in general the nutritional value of proteins in the hydrolyzate were reasonably well preserved. This is well demonstrated by using an amino acid scoring procedure to calculate protein efficiency ratio (PER) values of the resultant products (Lee *et al.*, 1978). Thus, the PER values of the hydrolyzates were 2.61-3.11 as compared with 2.86-3.25 for the starting material. A similar observation was made when the results for mechanically separated seal meat and Biosea-L were compared. Furthermore, all protein preparations so obtained had an ivory-white colour with Hunter L values ranging from 74.2 to 84.6.

The hydrolyzates so prepared had excellent fat absorption, water solubility and moisture absorption and whippability characteristics (Shahidi *et al.*, 1991b). In addition, when Biocapelin was added to meat model systems, a large reduction in the amount of drip water was noticed. Furthermore, Biocapelin had excellent ability in preventing cooking loss and development of

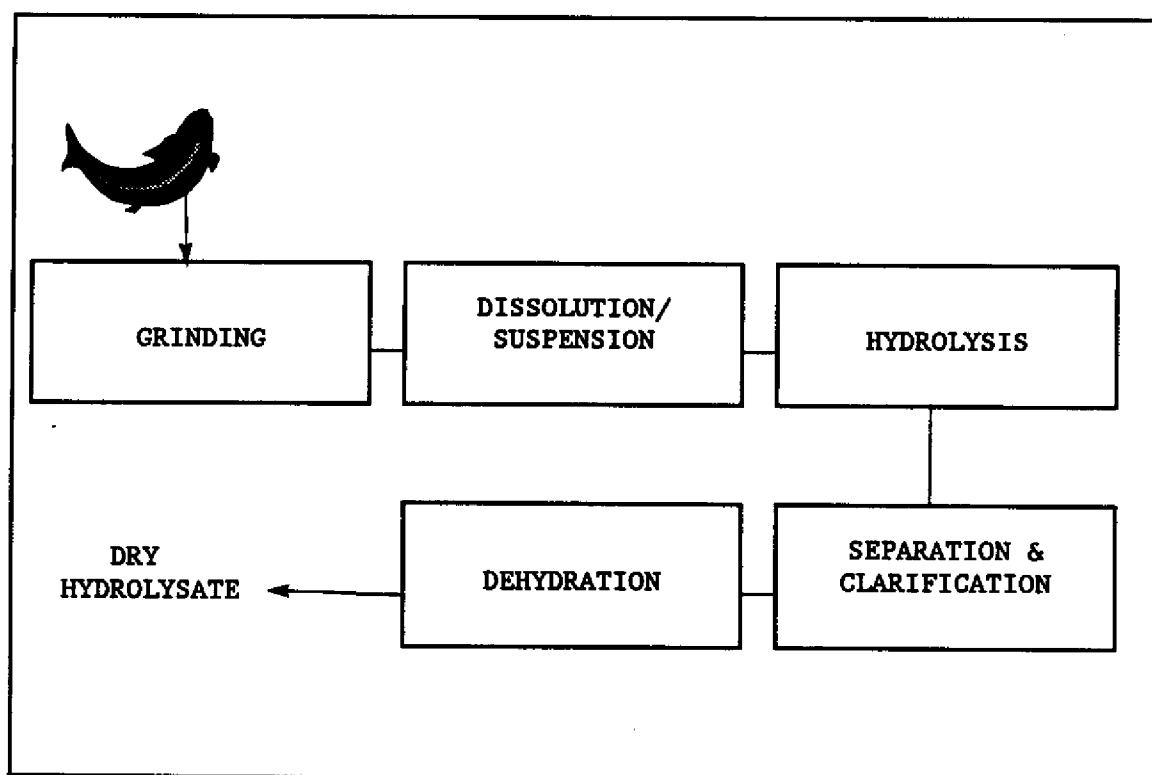


Figure 1. Scheme for preparation of protein hydrolysate from aquatic species.

Table 1. Processing conditions for preparation of protein hydrolyzates from aquatic species.

Processing Conditions	Enzyme			
	Alcalase	Neutrase	Papain	Endogenous
Temperature, °C	40 - 65	40 - 65	40 - 65	25
Reaction pH	8.0 - 9.0	6.5 - 7.5	5.6 - 6.6	3.0
Inactivation pH	4.0	3.0	3.0	As is
[E]/[S] ^a	30 - 100	30 - 100	1 - 4	—

^aAnson units/100g protein for Alcalase and Neutrase and as % of raw material for papain.

Table 2. Typical protein recovery and composition of hydrolyzates from capelin (Biocapelin) and harp seal (Biosea-L).

Raw Material/Product	Protein Recovery, %	Composition, %			
		Protein	Lipid	Moisture	Ash
Capelin	—	13.9±0.2	3.6±0.3	78.1±0.1	2.4±0.0
Biocapelin	51.6-70.6	65.9-73.3	0.2-0.4	5.3-6.3	14.9-22.6
Seal	—	23.2±0.5	3.7±0.2	70.8±0.1	2.0±0.2
Biosea-L	58.0-72.4	64.1-84.9	0.2-2.0	6.1-6.2	5.3-15.6

Table 3. Selected amino acids and calculated PER values of capelin and Biocapelin.

Amino Acid/PER value	Capelin	Biocapelin
Arginine	6.0 ± 0.8	5.7 ± 0.0
Histidine	2.4 ± 0.0	2.1 ± 0.0
Isoleucine	4.7 ± 0.8	4.3 ± 0.0
Leucine	8.2 ± 0.1	7.6 ± 0.0
Lysine	8.5 ± 0.1	8.5 ± 0.1
Methionine	3.1 ± 0.0	2.1 ± 0.0
Phenylalanine	3.8 ± 0.0	3.2 ± 0.0
Proline	3.7 ± 0.2	3.7 ± 0.0
Threonine	4.8 ± 0.1	4.6 ± 0.0
Tryptophan	1.1 ± 0.0	0.4 ± 0.0
Tyrosine	3.3 ± 0.0	2.5 ± 0.1
Valine	5.7 ± 0.1	5.8 ± 0.0
PER Value	2.86 - 3.25	2.61 - 3.11

Table 4. Effect of Biocapelin on cook yield enhancement and inhibition of oxidation over a 5-day refrigerated storage period (%).

Biocapelin	Cook Yield Enhancement	Inhibition of Oxidation
0.5	0.5	17.7
1.0	1.2	21.8
2.0	3.1	44.4
3.6	4.0	60.4

Table 5. Inhibitory effect (% IE) of Biocapelin and Biosea-L against bleaching of β -carotene in model systems.*

Hydrolyzate Concentration, %	Incubation Period, min		
	30	60	90
BIOCAPELIN			
0.02	11.5	8.5	5.0
0.04	16.7	12.2	8.0
0.10	33.3	19.5	13.0
0.20	38.5	24.4	16.0
BIOSEA-L			
0.02	9.0	3.7	2.0
0.04	12.8	7.3	3.7
0.10	2.6	-2.4	-4.0
0.20	-2.6	-7.3	-7.0

*Calculated as $100 (B-A)/(C-A)$ where A = absorption of the control sample, B = absorption of the sample containing hydrolyzate, and C = absorption of the control sample prior to incubation.

rancidity in meat model systems as summarized in Table 4. However, a study of the inhibitory effects of Biocapelin and Biosea-L against bleaching of β -carotene in a β -carotene/linoleate (Miller, 1971) system demonstrated that while the former always exhibited an inhibitory effect, the latter had pro-oxidant activity when used at higher concentrations (Table 5). Chromatographic separation of the hydrolyzates indicated that while their antioxidant activity was due to ninhydrin-positive components, prooxidant components were ninhydrin-negative. Further studies towards structural identification of the biologically active components of the hydrolyzates are in progress.

ACKNOWLEDGEMENTS

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AN ECONOMIC ANALYSIS OF THE
SOUTHEAST U.S. SEAFOOD PROCESSING INDUSTRY, 1973-90¹

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A variety of species are harvested in the Southeast United States, i.e., the coastal states of North Carolina through Texas. These regional catches, together with domestic and foreign imports, provide the raw material product needed to support a processing sector valued at \$1.4 billion (includes only the production of edible products) in 1990. This equates to about 20% of the \$7.0 billion national industry (edible.).

Despite the size of the Southeast processing industry and its importance to many of the local communities, economic information concerning its structure, conduct, and performance is not readily available. The purpose of this paper is to provide a brief economic analysis of the Southeast edible seafood processing sector. The analysis covers the 1973-90 period and is based on information collected and maintained by the National Marine Fisheries Service, Fisheries Statistics Division. The information presented is derived from a final report by Keithly et al. (1), and the reader is referred to that report for a more detailed analysis which examines processing at a much more disaggregated and area specific level.

In order to achieve the stated objective, the paper proceeds as follows. First, some general industry characteristics are presented followed by a presentation of issues related to the structure, conduct, and performance within the industry. Then, historical Southeast edible seafood landings are briefly reviewed. The paper concludes with an examination of processing activities in relation to landings.

RESULTS

GENERAL INDUSTRY CHARACTERISTICS

As indicated in Table 1, the reported number of Southeast edible seafood processors clearly increased when evaluated in three-year intervals. In 1973-75, for example, about 500 firms were engaged in seafood processing on an annual basis and this number increased to 623 during 1988-90. These 623 firms, while representing a 25% increase above the number reported during the initial three-year period, represented a decrease of more than twenty from 1982-84, when a maximum 646 firms were reported. As indicated, the 1979-81 period was one of particularly pronounced growth in firm numbers.

Production of processed edible seafood, expressed on a product weight basis, averaged 400 million pounds annually in 1988-90 compared to 273 million pounds in 1973-75, an annualized growth rate of about three percent. Overall, production increased during each three-year interval of analysis except for the most recent when production fell slightly, i.e., less than two percent. Observed changes in aggregate seafood processing activities, by and large, represented changes in shrimp processing activities. As will be shown shortly, this reflects the dominance of this single species in the aggregate.

For purposes of analysis, the product weight poundage was also converted to an equivalent edible-meat-weight basis and live-weight basis and the conversions are presented in Table 1. As indicated, the estimated edible-meat-weight poundage consistently averaged from about 75%-85% of the product weight poundage, the difference reflecting shell and additional ingredients that are included in the calculation of product weight. The estimated live-weight poundage, on the other hand, traditionally exceeded the product weight poundage by 80%-90%.

The value of Southeast U.S. seafood processing activities increased by a factor of two during the period of analysis, when examined in three-year intervals, from \$459 million annually in 1973-75 to \$1.37 billion annually in 1988-90 (Table 1). Most of this increase, however, was inflationary based. After removing the effects of inflation, the value of Southeast seafood processing activities has remained relatively stable since 1976-78 with the exception of about a 10% decline during the most recent three-year period. An increasing production in conjunction with a stable deflated value indicates a declining deflated processed price. This decline is clearly identified in Table 1. The average annual deflated price in 1988-90, \$3.63 per product-weight pound (expressed in 1990 dollars), was less than 80% of the 1973-75 average price of \$4.50 per pound and less than 70% of the peak price of \$5.62 per pound which occurred during the 1976-78 period.

Table 1. Selected Statistics on the Production of Processed Edible Seafood in the Southeastern U.S., 1973-90 (three-year averages).

TIME PERIOD	NO. OF FIRMS	POUNDS PROCESSED			PROCESSED VALUE			PROCESSED PRICE	
		Product weight	Edible-meat Weight ^a	Live Weight	Current	Deflated ^b	Current	Current	Deflated
	#	-----	----- Mills. -----	-----	----- \$ Mill. -----	-----	----- \$/lb. -----		
1973-75 avg.	503	273.1	221.7	499.7	458.6	1,229.8	1.68	4.50	
1976-78 avg.	519	309.4	246.0	546.6	757.3	1,619.7	2.45	5.23	
1979-81 avg.	609	316.6	246.8	567.7	995.7	1,597.1	3.14	5.04	
1982-84 avg.	646	376.2	299.1	679.8	1,273.1	1,663.2	3.38	4.42	
1985-87 avg.	612	404.6	313.0	761.6	1,377.7	1,632.3	3.40	4.03	
1988-90 avg.	623	398.3	304.9	748.2	1,373.2	1,444.2	3.45	3.63	

^a See Keithly et al. (1) for a list of conversion factors used in deriving edible-meat weight poundage and live weight poundage.

^b The 1990 Consumer Price Index, i.e., 1990=100, was used to deflate values and prices.

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

The composition of aggregate Southeast processing activities, expressed as a percent of total value by key species (or groups), is presented in Table 2. As indicated, shellfish consistently represented in excess of 90% of aggregate Southeast processing activities (edible) when measured by value and, in earlier years, exceeded 95%. Shrimp represented, by far, the largest component of aggregate activities, from about 70% to 80%. Much of the remaining shellfish processing activities were attributable to blue crabs and oysters. The contribution to the total by finfish species, while relatively small, increased from 4.2% during 1973-75 to 7.8% during 1988-90, or by 85%.

Table 2. Contribution to the Total Value of Southeast U.S. Seafood Processing Activities, Expressed on a Percentage Basis, 1973-90.

TIME PERIOD	SHRIMP	BLUE CRAB	OYSTER	OTHER SHELLFISH	TOTAL SHELLFISH	TOTAL FINFISH
-----% of Total-----						
1973-75	76.5	8.6	5.4	5.3	95.8	4.2
1976-78	80.7	6.4	5.1	4.2	96.4	3.6
1979-81	77.6	6.1	4.0	7.1	94.8	5.2
1982-84	74.1	6.8	4.9	7.8	93.6	6.4
1985-87	73.9	7.9	4.7	5.6	92.1	7.8
1988-90	73.7	7.9	3.6	7.0	92.2	7.8

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

ECONOMIC STRUCTURE, CONDUCT, AND PERFORMANCE

Several aspects of the economic structure, conduct, and performance of the Southeast processing industry are presented below. They include (i) productivity within the Southeast processing industry and changes therein, (ii) the size distribution of firms within the industry and changes therein, and (iii) industry concentration and changes therein.

Productivity of the Southeast Processing Industry

Productivity within the Southeast seafood processing industry was evaluated using two methods. The first was the estimation of production per firm. The second was the estimation of production per worker.

Production per Firm. Production per firm among Southeastern seafood processors is given in Table 3 for the 1973-90 period. Production per firm over the 18- year period of analysis averaged 590.4 thousand pounds, when evaluated on a product weight basis. Production averaging 639.7 thousand pounds annually during 1987-90 exceeded the comparable statistic for the 1973-75 period by almost 100 thousand pounds (18%) but was below peak production of 661.2 thousand pounds per firm (1985-87 avg.) by about three percent.

Table 3. Per Firm Production of Edible Processed Seafood in the Southeastern United States, 1973-90 (three-year averages).

TIME PERIOD	POUNDS/FIRM ^a	VALUE PER FIRM	
		Current	Deflated ^b
	--- 1,000s ---	----- \$1,000s -----	
1975-75 avg.	543.0	911.8	2,445.0
1976-78 avg.	596.2	1,459.2	3,120.8
1979-81 avg.	519.9	1,634.9	2,622.4
1982-84 avg.	582.7	1,971.8	2,575.9
1985-87 avg.	661.2	2,251.2	2,667.1
1988-90 avg.	639.7	2,205.3	2,319.3

^a Given on a product weight basis.

^b The deflated values are expressed in 1990 dollars and are based on the Consumer Price Index.

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

Evaluated on a deflated value basis (1990 base year), production per firm averaged \$2.63 million during the 18-year period of analysis. Production peaked during 1976-78 at \$3.12 million which exceeded the low production of \$2.32 million by about 35%. The low production occurred during the most recent three-year period and, in general, the deflated value of production per firm has been declining since 1976-78.

In general, there was an increase in production per firm since the mid 1980s, when evaluated on a poundage basis, compared to earlier years. Because of the decline in the deflated per pound price of the processed product, however, a concurrent increase in the deflated value of processing activities per firm was not identified.

Production per Worker. As indicated by the information contained in Table 4 the average Southeast seafood processing firm employed 28 workers on an annual basis³ during 1973-75. Each of these workers produced an average of 19.7 thousand pounds of seafood

(product weight) valued at \$88.6 thousand when expressed in 1990 dollars.

Table 4. Estimated Productivity per Worker in the Southeastern United States Edible Processed Seafood Industry, 1973-90 (three-year averages).

TIME PERIOD	AVERAGE/ EMPLOYEE	POUNDS/ WORKER ^a	VALUE PER WORKER	
			Current	Deflated ^b
		- 1,000s -	-----	\$1,000s -----
1975-75 avg.	28	19.7	33.1	88.6
1976-78 avg.	27	22.0	54.1	115.7
1979-81 avg.	23	23.1	72.8	116.7
1982-84 avg.	24	24.0	81.2	106.0
1985-87 avg.	27	24.9	84.9	100.5
1988-90 avg.	25	25.7	88.7	93.3

^a Given on a product weight basis.

^b The deflated values are expressed in 1990 dollars and are based on the 1990 Consumer Price Index.

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

As suggested by the information contained in Table 4, production per worker, expressed on a product weight basis, increased throughout the 18-year period of analysis when evaluated in three-year intervals. Overall, production of 25.7 thousand pounds per worker during 1988-90 exceeded 1973-75 average annual production per worker by slightly more than 30%.

When examined on a deflated value basis, production per worker grew from \$88.6 thousand annually during 1973-75 to \$116.7 thousand during 1979-81. This growth reflects increased pounds processed per worker and an increasing deflated price of the processed product. Since the 1979-81 period, however, production per worker, expressed in terms of the deflated value of processed product, has fallen sharply. Overall, the estimated per worker production of \$93.3 thousand annually in 1988-90 was only about 80% of that estimated during 1979-81.

To evaluate production per worker in the Southeast seafood processing industry in greater detail, Southeast seafood processing establishments were separated into four mutually exclusive size categories: (i) those with annual deflated processed sales of <\$250 thousand, (ii) those with annual deflated processed sales from \$250 thousand to \$1.0 million, (iii) those with annual deflated processed sales from \$1.0 million to \$10.0 million, and (iv) those with annual processed sales of \$10.0

million or more. Selected information pertaining to new worker productivity among firms in each of these four groupings is presented in Table 5.

As indicated, employment per firm with annual deflated sales less than \$250 thousand generally averaged from about six to eight workers annually. The number increased in relation to firm size with the largest firms, i.e., those with annual processed sales in excess of \$10 million, generally reporting in excess of 150 workers annually.

An examination of the information contained in Table 5 indicates that production per worker, expressed in terms of either pounds or value, increased rapidly with firm size. For example, production per worker in 1988-90 averaged 4.8 thousand pounds among the smallest firms, 7.4 thousand pounds among firms with annual sales from \$250 thousand to \$1.0 million, 22.5 thousand pounds among firms with annual sales from \$1.0 million to \$10.0 million, and 43.7 thousand pounds among firms with annual sales 10.0 million or more.

Four hypotheses can be offered to explain the differences in productivity per worker in relation to firm size. First, workers in the smaller firms may function in more than one role. For example, they may carry administrative duties in addition to time spent actually involved in processing. Second, many of the larger companies tend to be shrimp oriented which tend to be more mechanized (see Keithly et al., 1). Third, larger firms are likely to have a steady supply of raw product and, hence, workers tend to be more full-time oriented and may in some instances work more overtime hours. Finally, there may be some economies to scale in relation to firm size.

Overall, there was a clear increase in production per worker (measured in pounds) during the 1973-90 period among the two larger classification of firms. Among firms in the \$1.0 to \$10.0 million processed sales range, production per worker increased one-third, from 16.6 thousand pounds annually during 1973-75 to 22.5 thousand pounds during 1988-90. Among firms with annual sales greater or equal to \$10.0 million, production per worker rose 40%, from 32.2 thousand pounds to 43.7 thousand pounds. Because of a decline in the per pound processed price, however, the deflated value of processed sales per employee has fallen sharply since the 1979-81 period.

Size Distribution

To examine the size distribution of seafood processing firms in the Southeast, and changes therein through time, firm's were grouped into four categories based upon deflated value of processed sales: (i) firms with annual sales <\$250 thousand, (ii) firms with annual sales from \$250 thousand to \$1.0 million,

Table 5. Estimated Productivity per Worker in the Southeast U.S. Processed Seafood Industry (Edible), by Size of Firm, 1973-90 (three-year averages).

TIME PERIOD	WORKERS/FIRM	POUNDS/WORKER (1,000s)*	OUTPUT VALUE PER WORKER	
			Current (\$1,000s)	Deflated (\$1,000s)
----- < \$250,000 -----				
1973-75 avg.	6	3.7	5.4	14.4
1976-78 avg.	7	3.7	6.9	14.7
1979-81 avg.	6	4.1	10.3	16.5
1982-84 avg.	7	3.3	9.6	12.5
1985-87 avg.	7	3.9	10.9	13.0
1988-90 avg.	6	4.5	13.4	14.0
----- \$250,000 - \$1.0 Million -----				
1973-75 avg.	17	8.1	12.2	32.1
1976-78 avg.	17	6.5	13.9	29.8
1979-81 avg.	16	7.9	20.5	32.7
1982-84 avg.	18	7.9	22.8	29.8
1985-87 avg.	20	7.4	23.8	28.1
1988-90 avg.	18	7.4	28.9	30.5
----- \$1.0 Million to \$10.0 Million -----				
1973-75 avg.	42	16.6	28.9	76.7
1976-78 avg.	37	16.7	39.6	85.0
1979-81 avg.	33	20.6	60.2	95.7
1982-84 avg.	36	21.6	69.0	90.2
1985-87 avg.	36	21.5	75.1	88.9
1988-90 avg.	36	22.5	80.0	84.4
----- ≥ \$10.0 Million -----				
1973-75 avg.	186	31.2	52.5	141.4
1976-78 avg.	141	37.9	95.6	204.2
1979-81 avg.	155	38.5	127.6	205.4
1982-84 avg.	161	42.1	148.6	194.1
1985-87 avg.	163	44.4	151.0	179.0
1988-90 avg.	164	43.7	147.5	154.9

* expressed on a product weight basis

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

(iii) firms with annual deflated sales of \$1.0 million to \$10.0 million, and (iv) firms with annual deflated processed sales of \$10.0 million or more.

As indicated from the information contained in Table 6, little change occurred in the size distribution of the Southeast seafood processing sector during 1973-90 when evaluated on the basis of the deflated value of processed sales. From about 40% to 46% of the establishments consistently exhibited sales of less than \$250 thousand when examined in three-year intervals. Another 27% to 32% exhibited sales in the \$250 thousand to \$1.0 million range. From 20% to 23% of the firms consistently reported sales in the \$1.0 to \$10.0 million range. The remaining 5% to 8% of the establishments reported sales of \$10.0 million or more.

Table 6. Size Distribution Among Firms in the Southeastern U.S. Seafood Processing Industry, 1973-90 (three-year averages).

TIME PERIOD	DEFLATED VALUE OF PROCESSED SALES			
	< \$250,000	\$250,000-\$1.0 Million	\$1.0 Million-\$10.0 Million	≥ \$10.0 Million
	----- % -----			
1973-75 avg.	43	28	23	6
1976-78 avg.	39	32	21	8
1979-81 avg.	44	30	20	6
1982-84 avg.	46	27	21	5
1985-87 avg.	43	28	23	6
1988-90 avg.	46	28	21	6

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

It is noteworthy that size distribution of firms within the industry remained relatively constant during 1973-90, despite a sharp increase in the number of firms. This suggests that raw product may not have been a limiting factor in industry growth during 1973-90. If it were, one would expect to find that a larger percentage of firms would fall into the smaller size classes through time as the total number of firms expanded.

Industry Concentration

Concentration among firms in the Southeast seafood processing industry during 1973-90 and changes therein can be evaluated using several alternative methods. One method commonly

employed in applied research is the market share approach in which the market shares accumulated by the largest x firms are analyzed ($x \in N$; N is the total number of firms comprising the industry). Starting with the firm with the largest market share, measured in value of processed seafood sales, and adding the shares of the next largest firms in succession, produces an estimate of accumulated market shares in the Southeast seafood processing industry. These shares, evaluated in terms of the largest five, ten, and twenty establishments for the 1973-90 period are given in Table 7.

Table 7. Market Shares of Largest Seafood Processing Firms in the Southeastern U.S., Ranked by Value of Sales, 1973-90 (three-year averages).

TIME PERIOD	LARGEST FIVE FIRMS	LARGEST TEN FIRMS	LARGEST TWENTY FIRMS
----- % of Industry Value -----			
1973-75 avg.	24	37	53
1976-78 avg.	23	38	55
1979-81 avg.	24	38	56
1982-84 avg.	24	37	53
1985-87 avg.	22	36	52
1988-90 avg.	22	35	50

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

As indicated, the largest five Southeast seafood processing establishments consistently represented from 22% to 24% of total Southeast activities when measured by value, while the largest ten firms represented from 35% to 38% of the total. The largest 20 firms consistently accounted for more than one-half of the total value of seafood processing activities in the Southeast.

In general, the information contained in Table 7 suggests that industry concentration on the southeast seafood processing industry was relatively stable during 1973-90 despite about a 25% increase in firm numbers.⁴ This finding is intuitively appealing when consideration is given to the fact that the industry size distributions was also relatively constant during 1973-90 (see Table 6).

SOUTHEAST LANDINGS

Southeast landings of fish and shellfish, excluding menhaden, are presented on an annual basis for 1973-90 in Table 8. As indicated, pounds landed increased throughout the 18-year period of analysis when examined in three-year intervals, with the exception of a slight decline (3%) during the most recent three-year period. Overall, average annual production of 673.2 million pounds in 1988-90 exceeded average annual production of 631.8

million pounds in 1979-81 by about 7% and exceeded 1973-75 average annual production of 524.0 million pounds by 28%.

Table 8. Southeast Seafood Landings (less menhaden), 1973-90 (three-year averages).

TIME PERIOD	POUNDS ^a	VALUE	
		Current	Deflated ^b
	--- 1,000s ---	----- \$1,000s -----	
1973-75 avg.	524,028	286,444	699,885
1976-78 avg.	585,583	439,706	942,354
1979-81 avg.	631,787	607,856	975,923
1982-84 avg.	634,284	705,111	925,364
1985-87 avg.	690,540	848,825	968,959
1988-90 avg.	673,161	812,653	875,153

^a Expressed on a live-weight basis

^b The deflated values are expressed in 1990 dollars and are based on the 1990 Consumer Price Index.

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

The current value of Southeast fish and shellfish landings (excluding menhaden) increased from \$286.4 million annually in 1973-75 to \$812.7 million annually in 1988-90 (Table 8). After adjusting for inflation, however, the value of Southeast landings has remained relatively stable in the \$925 million to \$975 million range since 1976-78 with the exception of a decline in 1988-90 to \$875 million. This decline reflects both a decline in harvest and a decline in deflated price.

PROCESSING ACTIVITIES IN RELATION TO LANDINGS

Southeast edible seafood processing activities, expressed on a live-weight basis, were presented in Table 1 while Southeast edible seafood landings, also expressed on a live-weight basis, were given in Table 8. A comparison of the relevant information in the two tables indicates that processed quantity averaged from 90% to 95% of Southeast landings (less menhaden) through 1979-81. Since 1982-84, however, processed quantity has exceeded landings and the margin has been increasing. While difficult to document with the limited available data, the information suggests that most reported domestic landings in the earlier time periods went into processing activities. With increased imports throughout the decade of the 1980's, particularly shrimp, processing activities advanced in relation to landings, i.e., a portion of the increased imports were used in Southeast processing activities. This increased usage in shrimp processing activities was documented by Roberts et al (2) and the reader is referred to Keithly et al. (1) for additional insight as to the relationship between processing activities and landings of individual species processed in the Southeastern United States.

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ENDNOTES

1. The authors acknowledge support from the National Marine
Fisheries Service, Saltonstall-Kennedy Project # NA90AA-H-
SK053.
2. Farm-raised catfish was excluded from the analysis.
3. Administrative employees are not included in this estimate.
Rather, the estimated number includes only those workers who
come in contact with the product during processing
operations, i.e., the production workers. The number of
workers used in calculations reflects the yearly average
which tends to be below the maximum number of workers
employed by firms during peak production months.
4. More advanced techniques to measure concentration were also
presented by Keithly et al. (1993). In general, they all
agreed with the findings associated with the market share
approach.

SOME QUALITY CHARACTERISTICS OF FARMED COD (*GADUS MORHUA*)

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Atlantic cod (*Gadus morhua*) is an important source of income for the fishermen and processors in Maritimes and Newfoundland. Depletion of cod stocks, particularly in the Grand Banks, has prompted the Federal Government to introduce a two-year moratorium in certain areas. Sea Forest Plantation has initiated a cod-farming operation in several locations in Newfoundland. Undersized cod from inshore trap fishery during June-August are transported to holding pens by boat. They are then fed intensively on live or frozen capelin or on pelleted feed formulations.

The intensively fed cod generally grow at a much faster rate than wild cod. The high growth rates obtainable in holding pens are due to higher temperature in the shallow waters and the feeding regimes. Although cod may be relatively easily bred and grown to a marketable size in approximately two years, the high cost of raising fish up to approximately 500 g size makes the operation uneconomical at current prices.

Harvesting of farmed cod generally begins in December and may continue until January-February. They may be sold as prime fish fillets in the U.S. and Canadian markets or could be frozen in special custom-designed packages. The present paper reports on changes in the chemical composition and characteristics of pen-held cod over a 20-week period during intensive feeding.

MATERIALS AND METHODS

All fish used in this study were obtained from the Experimental Offshore Cod Farm of the Sea Forest Plantation, Bay Bulls, NF. They were held in pens located near the shore where the depth of water was approximately 30-50 m. They were fed on formulated diets with a typical ingredient composition given in Table 1. For sampling, fish were caught using a "dip net", stunned by a blow to their head and bled by severing gills. They were then eviscerated. The length and weight of fish as well as the weight of the liver was recorded. However, for growth measurement, tagged fish were caught in a similar manner and weight in a dip net and their length was quickly measured using a measuring tape.

Proximate composition of fish fillet and liver was determined using the AOAC (1984) protocols, except for total lipids which were extracted into a chloroform/methanol/water mixture according to the procedure outlined by Bligh and Dyer (1959). Moisture was determined by oven drying at 102°C until a constant weight was reached. The crude protein content was determined and recorded as N x 6.25. The ash content of samples was determined after charring of samples over a Bunsen burner followed by ashing in a muffle furnace at 550°C (AOAC, 1984).

The hepatosomatic index of fish, defined as percentage weight ratio of liver to whole fish after slaughtering and eviscerating, was recorded. The content of glycogen in samples was determined according to the method described by Carroll *et al.* (1956). A 5 g sample tissue was placed in a vial containing 10 mL of 30% (w/v) KOH for determinations, as described by Ang and Haard (1985).

The total lipids extracted according to the method of Bligh and Dyer (1959) were dried directly. For fatty acid analysis, the lipid extracts were subjected to transmethylation in acidified methanol (Hitchcock and Hammond, 1978). Methyl heptadecanoate was used as an internal standard. The methyl esters were separated on a 30 m x 0.25 mm capillary SP2337 column (Supelco Inc., Bellefonte, PA) using a Perkin Elmer 8500 gas chromatograph. Oven temperature was 180°C and the injection port and flame ionization detector oven temperatures were 230°C. Quantification was accomplished with the data handling system and the control unit of the instrument (Shahidi and Synowiecki, 1991).

RESULTS AND DISCUSSION

Table 1 summarizes the typical ingredient composition of formulated pellets used for feeding pen-held cod to satiation. A number of fish in each pen (1 to 6) were tagged. The pen growth data for length and weight of 6 to 23 fish were recorded in different time intervals over a 20-week period. Results summarized in Table 2 indicate that percent monthly growth of fish as reflected in their length and weight was 3.8 - 4.9 and 18.1 - 29.8%, respectively. Based on this data, a 3.5 to 5.5 months period is required for doubling up the weight of pen-held cod.

Proximate composition of fillet and liver tissues of pen-held cod are given in Table 3. The content of protein in cod muscles increased from 16.90 to 18.30% while in the liver a decrease from 7.50 to 3.05% was noted. The content of lipid in the muscles increased from 0.74 to 0.94% and corresponding values for liver were 58.90 and 75.92%, respectively. The moisture content in the muscles showed little change, from 81.90 to 80.70%, however, for liver tissues a decrease from 32.60 to 18.60% was evident. Meanwhile, the weight of liver of pen-held cod increased from 45.8 to 211.0 g and hepatosomatic index increased from 4.41 to 12.70% (Table 4). The increase in the content of liver glycogen over the same period was insignificant.

Tables 5 and 6 summarize the Omega-3 fatty acid composition in the muscle and liver tissue of wild and pen-held cod. There was a modest increase in the content of omega-3 PUFA in the muscle tissues, from 52.13 to 55.82%. The corresponding change in the omega-3 PUFA in the liver tissues of cod was from 17.94 to 26.12%.

Table 1. Typical Feed Formulations for Pen-Held Atlantic Cod (*Gadus morhua*).

Component	%
Capelin / Cod Offal / Silage / Meal	70 - 80
Wheat Middling	5 - 15
Fish Oil / Vegetable Oil	5 - 12
Whey Powder / Starch	2
Flavour Components	0 - 2
Minerals	0.2
Vitamins	0.2

Table 2. Comparison of mean growth in different pens over a 20-week period of atlantic cod (*Gadus morhua*).

Pen No.	No. of Tagged Fish	Monthly Increase (%)	
		Length	Weight
1	14	3.8 ± 1.4	20.8 ± 11.2
2	6	3.8 ± 1.4	20.5 ± 6.0
3	23	4.2 ± 1.6	19.8 ± 11.7
4	12	4.5 ± 1.3	22.6 ± 8.2
5	15	4.3 ± 2.1	18.1 ± 11.0
6	13	4.9 ± 1.7	29.8 ± 9.9

Table 3. Proximate composition (%) of muscles and livers of wild and pen-held cod (*Gadus morhua*).^a

Fish	Moisture	Protein, N x 6.25	Lipid	Ash
<i>FISH FILLETS</i>				
Wild	81.90 ± 0.53	16.90 ± 0.65	0.74 ± 0.05	1.17±0.06
Pen-Held	80.70 ± 0.84	18.30 ± 0.82	0.94 ± 0.07	1.12±0.04
<i>FISH LIVER</i>				
Wild	32.60 ± 7.48	7.50 ± 2.02	58.90 ± 0.77	0.29±0.02
Pen-Held	18.60 ± 2.24	3.05 ± 0.57	75.92 ± 2.03	0.31±0.02

^aResults are mean values of triplicate determinations for a dozen fish upon harvest or fed for 18-20 weeks in pen on formulated diets.

Table 4. Changes in liver weight, hepatosomatic index and glycogen content of pen-held Atlantic cod (*Gadus morhua*).^a

Feeding Period	Weight of Liver, g	Hepatosomatic Index, %	Glycogen, mg%
0	45.8 ± 17.9	4.41 ± 2.52	1.79 ± 0.10
2 - 3	64.1 ± 37.0	4.23 ± 1.70	1.62 ± 0.41
13 - 14	194.0 ± 98.9	12.00 ± 3.19	1.87 ± 0.22
16 - 18	211.0 ± 85.7	12.70 ± 1.89	2.04 ± 0.41

^aMean values of triplicate determination from mix fish liver ± standard deviation.

Table 5. Omega-3 fatty acid composition of fillet lipids of pen-held and wild Atlantic cod (*Gadus morhua*).

Selected Fatty Acids	Wild	Pen-Held (16-18 wk)
18:3	0.19	0.66
18:4	0.40	1.37
20:5	18.02	17.50
22:5	1.79	1.79
22:6	31.73	34.50
Saturates	17.63	17.05
Monounsaturates	17.78	17.67
Polyunsaturates	56.07	59.47
Omega-3 Polyunsaturates	52.10	55.82

Table 6. Omega-3 fatty acid composition of liver lipids of pen-held and wild Atlantic cod (*Gadus morhua*).

Selected Fatty Acid	Wild	Pen-Held (16-18 wk)
18:3	tr	1.48
18:4	1.10	3.24
20:5	7.60	8.30
22:5	1.37	1.16
22:6	7.97	11.94
Saturates	22.17	21.39
Monounsaturates	53.96	45.09
Polyunsaturates	19.03	28.21
Omega-3 Polyunsaturates	18.04	26.12

CONCLUSIONS

Based on the results presented in this work, it is apparent that cod farming is a viable alternative to production of cod. The composition of fish and fatty acid composition of muscle and liver tissues may be modified by using appropriate feed formulations. Further work on post-mortem physiological changes in muscle tissues and quality of fillet has been carried out. Results will be comminuted elsewhere.

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MANAGEMENT OF BLUE CRAB PROCESSING WASTES

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As environmental legislation becomes more stringent, the blue crab (*Callinectes sapidus*) industry is being challenged to develop technically and economically feasible methods to manage their liquid and solid wastes. The blue crab industry is one of the largest seafood processing industries in Virginia. More than 50 blue crab processing firms are presently certified by the Division of Shellfish Sanitation, Virginia Department of Health (Croonenberghs, 1991).

The industry is economically vital to many communities of the Chesapeake Bay and its tributaries. In 1989, there was a total landing of 206.7 million pounds of blue crab in the United States valued at 81 million dollars. The Chesapeake region produced 89 million pounds of this total (U.S. Department of Commerce, 1990).

In the 1970's, the United States Environmental Protection Agency (EPA) developed documents which defined effluent limits for segments of the seafood processing industry. The regulations were then adopted by the states. Compliance with these limits was obtained, in most cases, with minor plant modifications that did not require large capital investments and operating costs. During the last decade, citizen and environmental groups have encouraged more stringent legislation in an effort to achieve a cleaner environment. Individual states have established waste disposal standards that exceed current U.S. EPA requirements.

The restoration of water quality in the Chesapeake Bay has become a goal of states which border the Bay and its tributaries. In Virginia, the Department of Environmental Quality (DEQ) has initiated a vigorous program to remove pollutants from the Bay. As a result, new standards for the disposal of liquid and solid wastes have been established. The new standards, many of which substantially exceed previous standards, have resulted in: the loss of public landfills; highly regulated waste transportation regulations; the inability of municipal systems to accept industrial wastes; waste disposal surcharges; and perhaps most importantly, new liquid effluent standards for discharge into receiving waters. Many blue crab processors have reported serious waste disposal problems as they are unable to consistently comply with their current allowable pollutant discharge limits.

Waste management is difficult for blue crab processing firms for several reasons. In general, crab firms are less capitalized and do not have the economics of scale when compared to some other seafood industries. Treatment systems with the ability to produce the desired effluent quality may not be economically feasible. Chemical, biological and physical treatment may be required to reduce the strength of processing plant effluents (Geiger *et al.*, 1985; Wheaton *et al.*, 1984). These treatment processes will be expensive and require adequate planning to avoid unfavorable economic impact.

Secondly, the location of processing plants also creates problems. Most blue crab processing facilities are located in rural areas on bodies of water. These facilities usually do not have access to municipal waste treatment plants. Land application and lagoon treatment are often not viable treatment options due to the high water table and wetland limitations. Some larger crab plants are located in cities with access to municipal treatment systems. However, these plants are often assessed substantial surcharges unless the concentration of their waste is reduced. There is also a possibility that municipal treatment systems may be forced to reject crab wastes due to the rapid population and industrial growth that is pushing municipal waste treatment systems to maximum operating capacity. Furthermore, plants located in metropolitan areas often lack the space needed for treatment systems. The plants are usually tightly bordered by water, parking lots and/or neighboring industries.

Finally, in Virginia, as the DEQ is in a period of transition in implementing new standards, the wastewaters from different crab processing firms are often monitored for different effluent constituents. This discrepancy can even appear in two adjacent crab plants. The inconsistency of the regulations makes it difficult for the industry as a group to address its waste disposal alternatives.

The blue crab processing industry faces serious solid and liquid waste disposal problems. Approximately 14% of the live crab results in food for human consumption with the remainder as by-products or waste (EPA, 1974). Consequently, processing firms will need to develop in-plant programs that will include: water conservation and recovery processes, improved by-product recovery systems and the development of industrial products from wastes (including foods, feeds, or biologics). Solutions to the waste management problems are essential for the future of many blue crab processing facilities. Some of the solutions will require the application of innovative technology, while others will result in substantially increased production costs. The achievement of such a goal presents substantial economic and technical obstacles and may be one of the greatest challenges in the future.

The objectives of this project were to evaluate the use of an anaerobic, biological process and air stripping as means of treating crab processing waters. In a previous paper, the authors presented information about the characterization of crab processing waters and the use of various other treatment methods (Harrison *et al.*, 1992).

MATERIALS AND METHODS

Anaerobic Treatment Experiment

The reactor vessel was made from a 4 liter (L) high density polyethylene (HDPE) bottle. The reactor top was plugged with a size 13 rubber stopper, through which two 0.5 inch (in.) glass tubes were fitted. This allowed feeding, capture of biogas, and sampling of effluent, while maintaining an anaerobic environment in the reactor. The effluent/feeding tube was connected to a 1 foot (ft.) length of 0.5 in. Tygon® tubing which was clamped off at all times except during feeding and sampling. The biogas was passed through Tygon® tubing to the collection system. This consisted of a calibrated 4 L HDPE bottle which was filled with water and inverted in a water bath. The biogas tubing was passed into this bottle, and its volume was measured via water displacement. Figure 1 shows a schematic of the reactor setup.

Retort wastewater was collected in a 5 gallon (gal.) HDPE bucket held under the outlet of a retort. The wastewaters from the Harris Claw reel wash, brine bath, and conveyor wash operations were collected in similar manners. Once obtained, the feeds were stored at 4°C in HDPE containers until used. Total chemical oxygen demand (COD) of the feed was monitored during storage to check for degradation.

Four reactors were operated over the course of the study. Reactors A and B treated waters derived from cooking crabs (retort water) retort water and were maintained at food to microorganism (F/M) ratios, stated in units of lb COD/lb MLVSS/day, of 0.35 and 0.25, respectively. Reactor A was operated at an F/M ratio of 0.40 from day 1 through day 40, but the ratio was decreased to 0.35 after reduced treatment was observed. Reactors C and D, meanwhile, were fed a mixed wastewater, the composition of which was based on daily production flows determined for the processing facility. These reactors were maintained at F/M ratios of 0.10 and 0.07, respectively. The wastewaters from the Harris Claw reel wash, brine bath, and conveyor wash operations were combined with the retort process water in a 2.0:4.9:8.6:3.4 ratio to make this mixed wastewater. Reactor A had been operated at F/M ratios of 0.15 and 0.25 over a total of 55 days prior to the beginning of this study. Similarly, reactor C was operated at an F/M ratio of 0.05 for a period of 55 days prior to the beginning of this study. Data on reactor performance during this earlier period is presented in the interim report by Harrison *et al.* (1992). For these reactors, day 1 is the first day on which the F/M ratio was increased to the levels tested in this study. In the case of reactors B and D, day 1 is the day on which the reactors were started.

In an attempt to achieve a start-up mixed liquor volatile suspended solids (MLVSS) level of between 3,000 and 4,000 mg/l, each reactor was seeded with 0.4 L of anaerobically stabilized municipal sludge. This was obtained from the Peppers Ferry Sewage Authority treatment plant. This was added to 1.6 L of warm tap water, which had been purged with pure nitrogen in order to remove any dissolved oxygen, for a total reactor volume of 2.0 L. Calcium carbonate, in the amount of 4.0 grams, was added to each reactor to guard against upset during start-up.

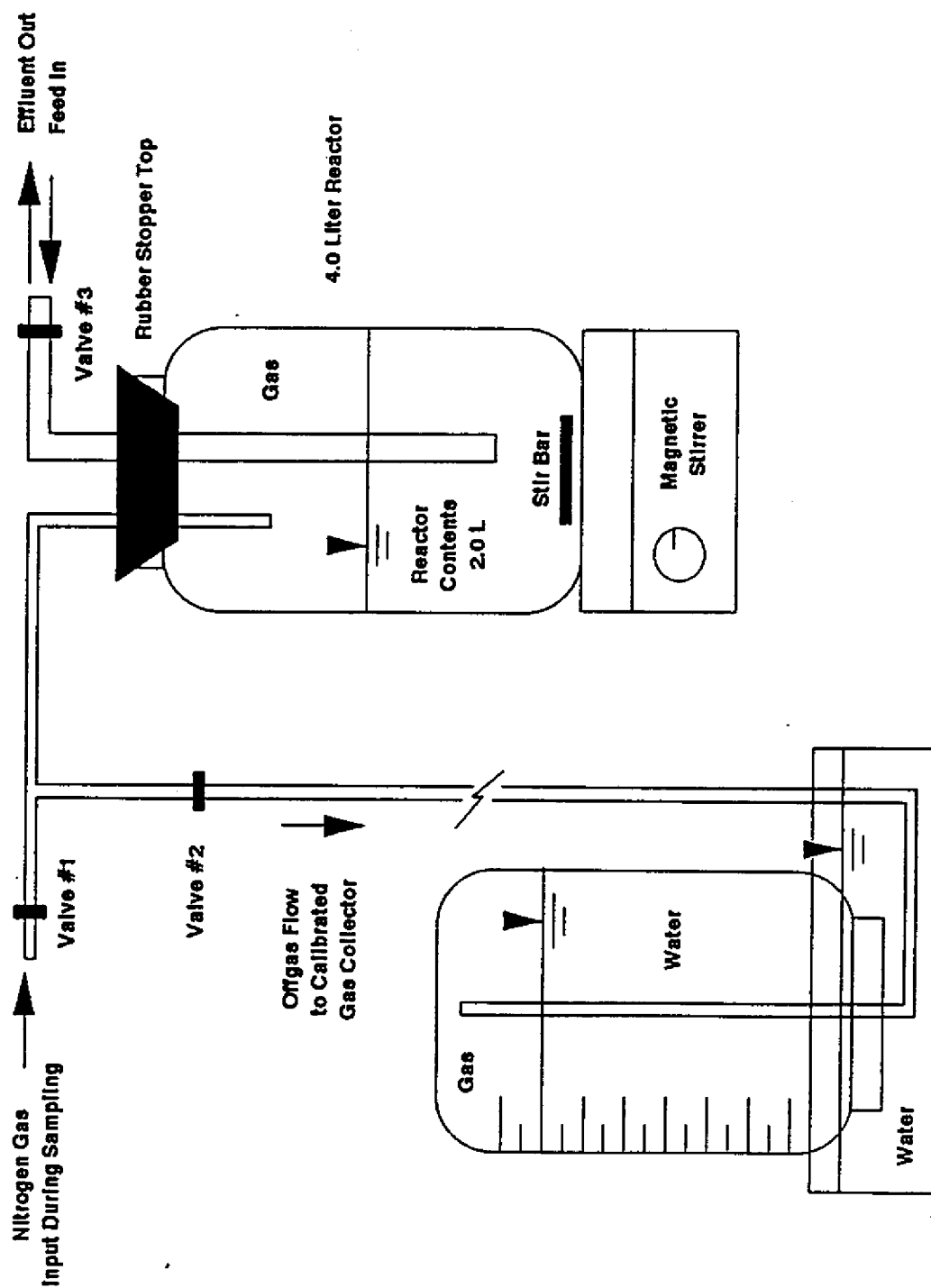


Figure 1. Experimental arrangement used in the anaerobic reactor studies.

In order to maintain a relatively constant temperature, the reactors were kept in an incubating cabinet at approximately 35°C. This temperature was chosen in order to maintain the reactors in the mesophilic range recommended for anaerobic digestion (McCarty, 1964). The reactors were maintained in a continuously-mixed state through the use of magnetic stirrers. These stirrers were turned off only for a period of 45 minutes to an hour prior to the daily wasting and feeding of the reactors to minimize the loss of MLVSS. This settling period was increased to 1 hour for reactors C and D after the MLVSS concentrations in each began to decrease.

Due to changes in the MLVSS concentrations of the reactors and in the strength of the wastewater used as feed, the hydraulic retention times (HRT) of the reactors varied considerably over the course of their operation. Average HRTs for reactors A, B, C, and D were found to be 12.2, 18.7, 36.3, and 32.4 days, respectively.

Reactor performance was tracked by monitoring temperature, biogas production volume, pH, alkalinity, and MLVSS. In addition, concentration levels of the following factors were determined for both the feeds and the reactor effluents at intervals necessary to define reactor health and treatment effectiveness: total and soluble biochemical oxygen demand (BOD₅), total and soluble COD, total and volatile suspended solids (TSS and VSS, respectively), Total Kjeldahl and Ammonia Nitrogen (TKN-N and NH₃-N, respectively), Total Phosphorus (TP), and the cations, calcium, magnesium, potassium, and sodium.

With the following exceptions and clarifications, all of the aforementioned tests were performed in accordance with Standard Methods for the Examination of Water and Wastewater (1992):

- Alkalinity titrations utilized 0.02 N H₂SO₄ solution as a titrant.
- TKN-N determinations were performed using Semi-Macro Kjeldahl method with 0.02 N H₂SO₄ titration.
- NH₃-N tests utilized distillation into boric acid indicating solution followed by titration with 0.02 N H₂SO₄.
- COD determinations were performed utilizing dichromate reflux digestion at 150°C for two hours followed by ferroin addition and titration with 0.05 N ferrous ammonium sulfate.
- Initial and final dissolved oxygen (DO) levels for BOD₅ tests were determined using a YSI DO meter.
- Samples for soluble COD and soluble BOD₅ were filtered using 1.05 micron glass microfiber filters.
- TP was tested by the ascorbic acid method with persulfate digestion.
- pH was determined with a glass electrode probe.

- All cation concentrations were determined through ion chromatography using a Dionex 2010i chromatograph at a flow rate of 1.0 ml/min with a CS-12 column with a self regenerating suppressor utilizing 20 mM methane sulfonic acid as a carrier liquid.

Air Stripping Experiment

Figure 2 shows a schematic of the air stripping tower design.

The tower was constructed from three sections of 0.625 in. thick PVC pipe with an inside diameter of 18 in. PVC piping was chosen for its heat and chemical resistance properties. The base of the tower was constructed from a 2.5 ft. section of the pipe which was secured to a 20 in. square Plexiglas base with a thickness of 0.625 in. The joint was sealed with a silicone-based caulk to prevent leakage. A shelf of 1 in. chicken wire was inserted into a 6.5 ft. pipe section and was held in place with a PVC support ring. By using this type of packing support, adequate strength was supplied, while back pressure was kept to a minimum. The 6.5 ft. section was then inserted into the flanged top of the first section and filled to a 6 ft. depth with 2.3 in. LANPAC® packing material. A 1 ft. section of the pipe was then attached to the top of the tower in order to prevent splashing when the blowers were in operation.

The wastewater was pumped from a HDPE holding basin by a centrifugal pump resistant to temperatures up to 93°C and pH levels up to 11. In order to prevent damage from higher pH levels, the pump was flushed with tap water after use. This pump was powered by a 0.04 hp motor. A combination of high temperature 0.75 in. diameter PVC piping and Tygon® tubing was used to transport the water from the basin to the high temperature PVC distribution system at the top of the 6.5 ft. section.

The tower was of a countercurrent design with the air supplied by two two-speed blowers which were both operated at their high speed setting and generated a combined air flow-rate of approximately 500 cfm. These were attached to the base of the tower via 4.25 in. by 8.5 in. galvanized steel ducts inserted into ports in the side of the bottom section. The treated wastewater left the tower via two 0.75 in. ports located in the side of the base section.

The wastewater used in the testing of the tower was collected in a 5 gal. HDPE bucket held under the retort process waste outlet. Although every attempt was made to recover the entire retort waste stream, a small percentage was lost. It is believed, however, that a representative sampling of the stream was recovered.

This water was then transferred to the holding basin where pH adjustments necessary for the different tests were done using 50% NaOH w/w (weight ratio). The pH of the waste was checked using a Fisher Model 160, portable pH meter with a glass electrode probe. After the pH of the waste in the basin was adjusted, the basin was covered in order to minimize ammonia loss before treatment.

After the waste was brought to the appropriate pH level, ranging from 10 to 12.2 depending on the trial, the pump was primed and the tower was allowed to operate until a consistent effluent flow-rate was observed (approx. 10 minutes).

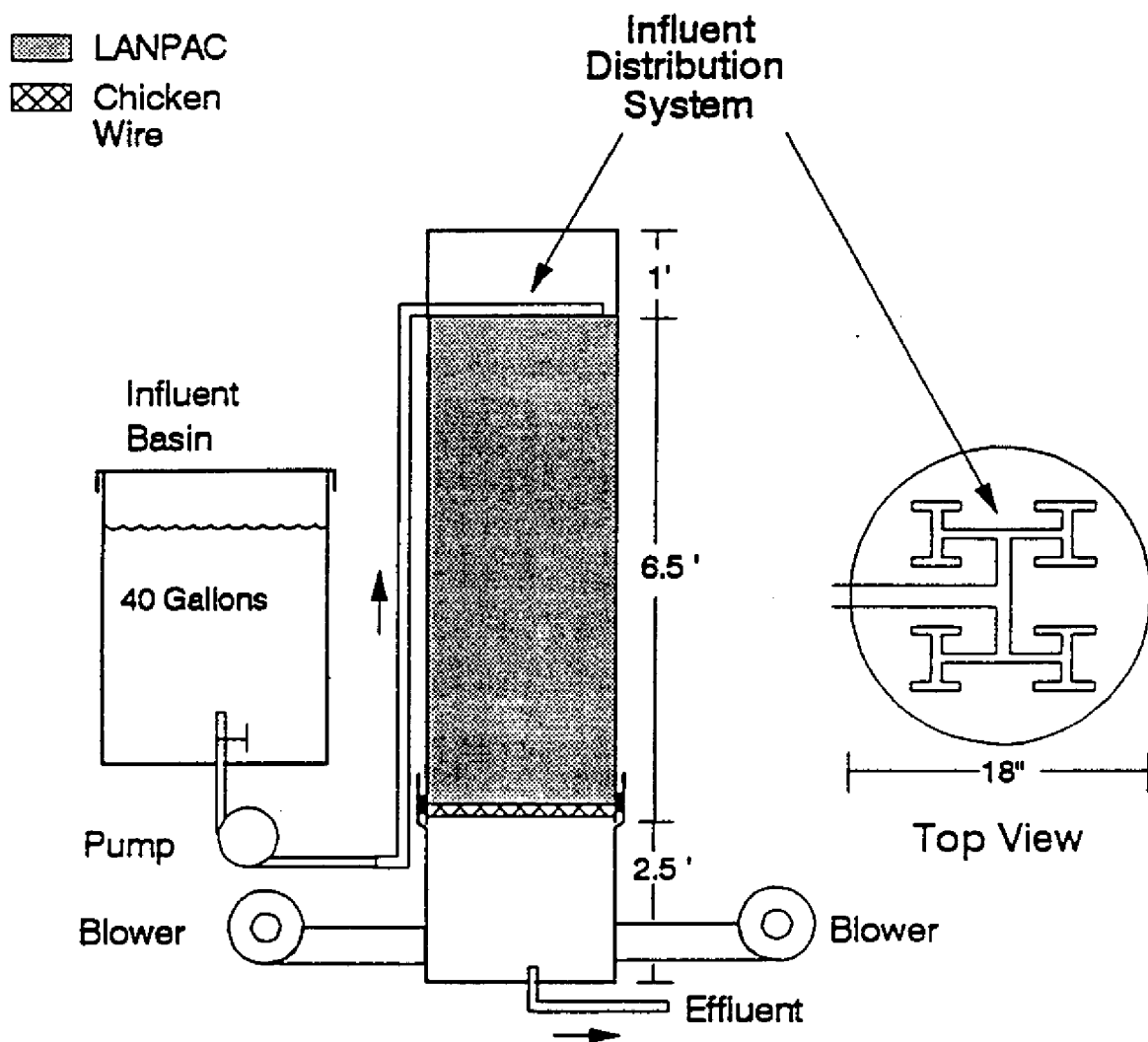


Figure 2. Air stripping tower schematic.

Once a constant flow rate was observed, grab samples of both influent and effluent were taken at 5 minute intervals. Influent and effluent temperature readings were also taken at these intervals in order to determine any cooling benefits of the air stripping process. In order to track the tower performance over the course of each trial, individual effluent samples were taken within 30 seconds of the corresponding influent sample. In addition, samples of the retort effluent were taken prior to pH adjustment to ensure that the tower influent ammonia levels were consistent with the retort effluent levels. All samples were taken and stored in 500 mL HDPE bottles and were acidified to pH < 2 through the addition of 15% sulfuric fuming acid. The samples were transported to Virginia Tech Environmental Engineering Laboratory where ammonia levels were determined according to the Standard Methods (1992) procedure stated in the previous section.

The efficacy of the treatment scheme was determined through the comparison of the tower effluent and influent (retort wastewater after pH adjustment). To ensure that the main portion of the ammonia removal occurred as a result of the air stripping process and not the pH adjustment itself, occasional samples were also taken of the retort process waste prior to the pH adjustment and compared to the tower effluent.

In order to examine the potential benefits of operating two towers in series, two trials were conducted. In the first, conducted at pH 12.2, the tower effluent from one test was collected, combined in a 3:1 ratio with a portion of that test's influent, and used as the influent for the tower. This combination was attempted in order to test the potential benefits of increasing the temperature of the effluent before recycling it through the tower. In the second, performed at pH 11.0, the effluent was collected and run back through the tower without any mixing. Samples from these tests were taken, preserved, and analyzed, as described above.

The trials were geared toward determining the effects that changes in three variables would have on the tower performance. These factors were influent temperature, pH, and the air-to-water ratio at which the tower was operated. This last parameter was adjusted by varying the input liquid flow rate. The values of the operating parameters for each test are summarized in Table 1. The trials are presented in the order in which they were performed.

In all tests the air flow rate was held steady at approximately 495 - 500 CFM. This flow-rate was calculated by multiplying the average air speed through the tower, measured across the tower diameter, by the area of the tower opening. The average air speed was measured with a TSI Model 1650, air velocity meter.

Table 1. Influent Variables for Air Stripping Tests

TRIAL	pH	Liquid Flow Rate GPM	Air-To-Water Ratio ft ³ /gal	Influent Temperature °C
1	11.0	2.2	225	56
2	11.0	1.2	412	58
3	10.0	1.2	412	59
4	11.1	1.2	412	65
5	12.2	0.6	825	58
6	12.2	0.6	825	20
7	12.2	0.6	825	50
8	11.0	0.6	825	58
9	11.0	0.6	825	20
10	10.0	0.6	825	58

RESULTS AND DISCUSSION

Anaerobic Treatment Experiment

The first two of the four reactors (A and B) were fed retort process wastewater and were operated for 216 and 94 days, respectively. Reactor A was fed at a 0.40 F/M ratio from day 1 through day 40, but after decreased treatment was observed, the reactor's F/M ratio was reduced to 0.35. Reactor B operated at a 0.25 F/M ratio over the course of its operation. Both reactors were able to achieve substantial reductions in the level of organics in the treated waste, but each eventually exhibited signs of failure and was shut down.

From day 48 through day 161, reactor A achieved COD removal rates ranging between 85% to 91% with an average of 87%. This removal translates to an effluent with a soluble COD largely in the range of 1550 to 2520 mg/L with an average of 2103 mg/L. Similarly, the reactor exhibited BOD₅ removal resulting in soluble effluent concentrations ranging between 1365 and 1497 mg/L over this time with an average removal of 88%, translating to an average effluent soluble BOD₅ of 1405 mg/L. Data on Effluent COD and BOD₅ concentrations are presented in Figure 3. Soluble material accounted for an average of 87% of the COD and 95% of the BOD₅ observed in reactor A effluent. These levels imply that the effluent solids contained a higher portion of inorganic material than would be suggested by the ratio found in solids from the secondary effluent of activated sludge systems (0.65 mg BOD₅/mg TSS). During this period of operation, the reactor produced biogas at an average rate of 1430 mL/day which is somewhat higher than the 1061 mL/day level that would correspond

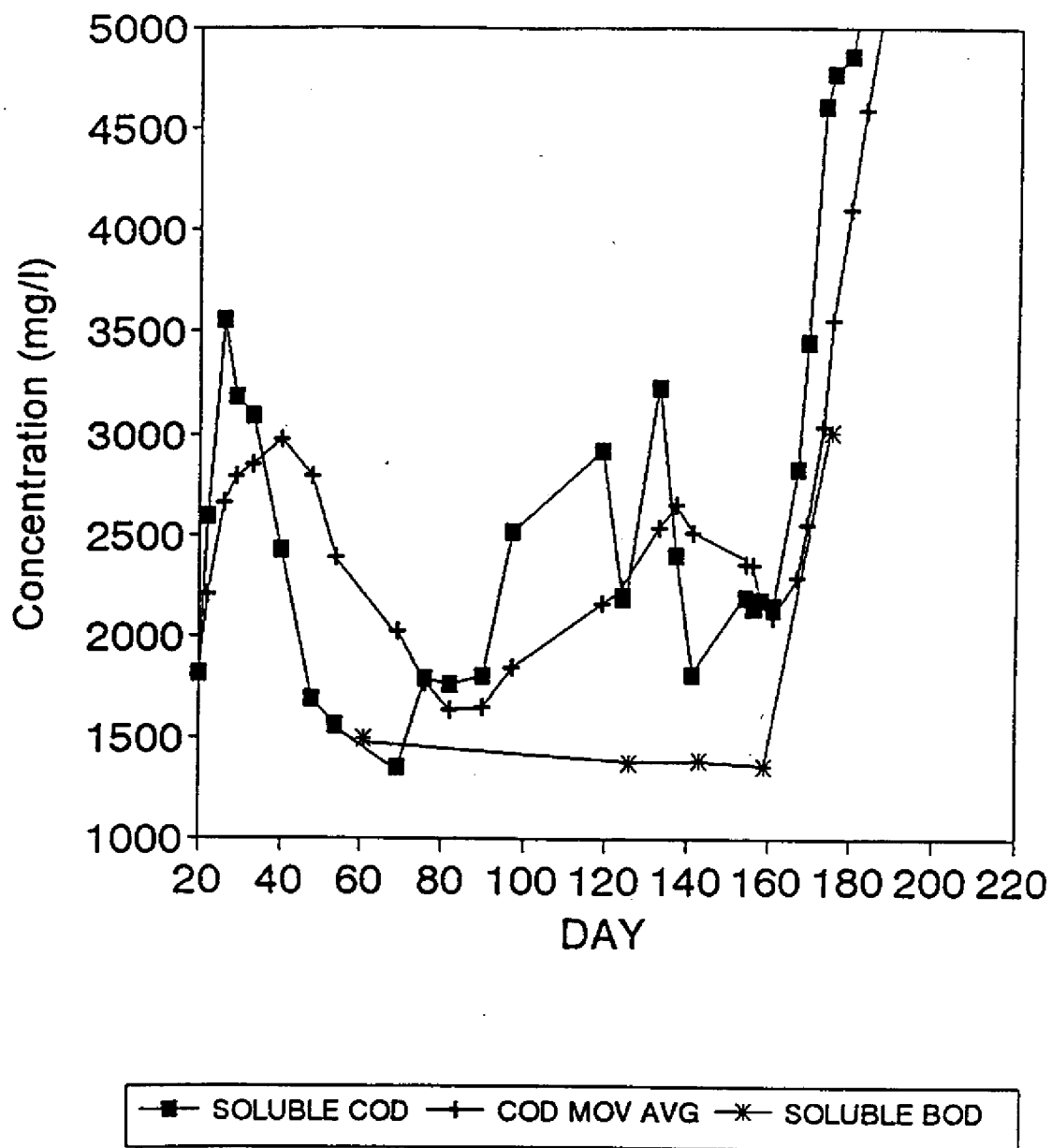


Figure 3. Effluent BOD₅ and COD levels for Reactor A.

to the aforementioned removal rates ($17.049 \text{ g/L COD} * 0.180 \text{ L of feed/day} * 88\% \text{ removal} * 0.393 \text{ L of gas produced/g of COD digested}$). Over this same period, effluent total suspended solids (TSS) varied between 400 and 520 mg/l with an average of 473 mg/L while MLVSS ranged from 3600 to 4800 mg/L with an average of 4042 mg/L. Solids retention time (SRT) varied between 96 and 248 days over the life of the reactor with an average value of 154.6 days. Hydraulic retention time (HRT) was maintained at an average of 12.2 days over the life of the reactor. Beginning on day 167, COD and BOD₅ levels steadily increased while biogas production decreased until the reactor was shut down on day 216. By this time the effluent COD had reached a level of over 9300 mg/L.

Reactor B similarly obtained substantial reduction in COD and BOD₅ levels from day 26 through day 67 of operation. Over this period of time, the reactor achieved COD removal rates of between 89% and 93% with an average of 91% and BOD₅ removal at rates that remained between 93% and 94%. These removal rates translate to effluent COD levels ranging from 1120 to 1875 mg/L with an average of 1461 mg/L and BOD₅ concentrations that varied from 558 to 603 mg/L with an average of 578 mg/L. Soluble material in reactor B effluent accounted for an average of 87% of all observed COD and BOD₅. As with those found for reactor A, these levels imply that the effluent solids contained a higher portion of inorganic material than the 0.65 mg BOD₅/mg TSS suggested by the ratio found in solids from the secondary effluent of activated sludge systems.

Over the course of the previously mentioned time, effluent TSS levels were maintained in the range of 240 to 500 mg/L with an average of 408 mg/L, and the reactor's MLVSS concentration increased from just under 3000 mg/L on day 33 to over 3500 mg/L by day 64. SRT ranged between 215 and 448 days over the course of reactor operation and had an average value of 263.4 days. HRT was maintained at an average of 18.7 days over the life of the reactor. Shortly after day 67 this reactor began to exhibit a decreased effectiveness in the removal of organics. This decrease is characterized by the steady increase in both COD and BOD₅ levels shown in Figure 4. Together with the rise in COD and BOD₅ came a decrease in the reactor's biogas production. This deterioration continued until the reactor was shut down on day 94 of operation.

In an attempt to determine a cause for the failure of these reactors, various operating parameters were studied. At the time of failure, both alkalinity and MLVSS in reactor A were found to be at levels (6560 mg/L and 4740 mg/L, respectively) which were consistent with those found in earlier tests conducted when the reactor had been achieving the previously mentioned removal rates. Similarly, neither alkalinity nor MLVSS levels (5258 mg/L and 3340 mg/L respectively) in reactor B were found to be abnormally low at the time of the reactors failure. Tests for total effluent phosphorus of both reactors showed concentrations (50 mg/L in A and 31 mg/L in B) which indicate levels sufficient to meet the nutrient needs of each reactor.

Ammonia toxicity has also been cited as a possible cause for disruption in anaerobic digestion processes and was therefore investigated. Ammonia nitrogen concentrations in reactor A at the time of failure remained in the 1680 to 1780 mg/L range through day 177. While ammonia levels between 1500 and 3000 mg/L have been shown to be inhibitory at pH levels above 7.4 to 7.6 (McCarty, 1964), the fact that the reactor was operating at a pH of 7.50 and that no decrease in pH, usually found during reactor failure (McCarty, 1964), was observed would tend to suggest that ammonia toxicity was not the primary cause of the failure. While reactor B operated in a higher pH range of

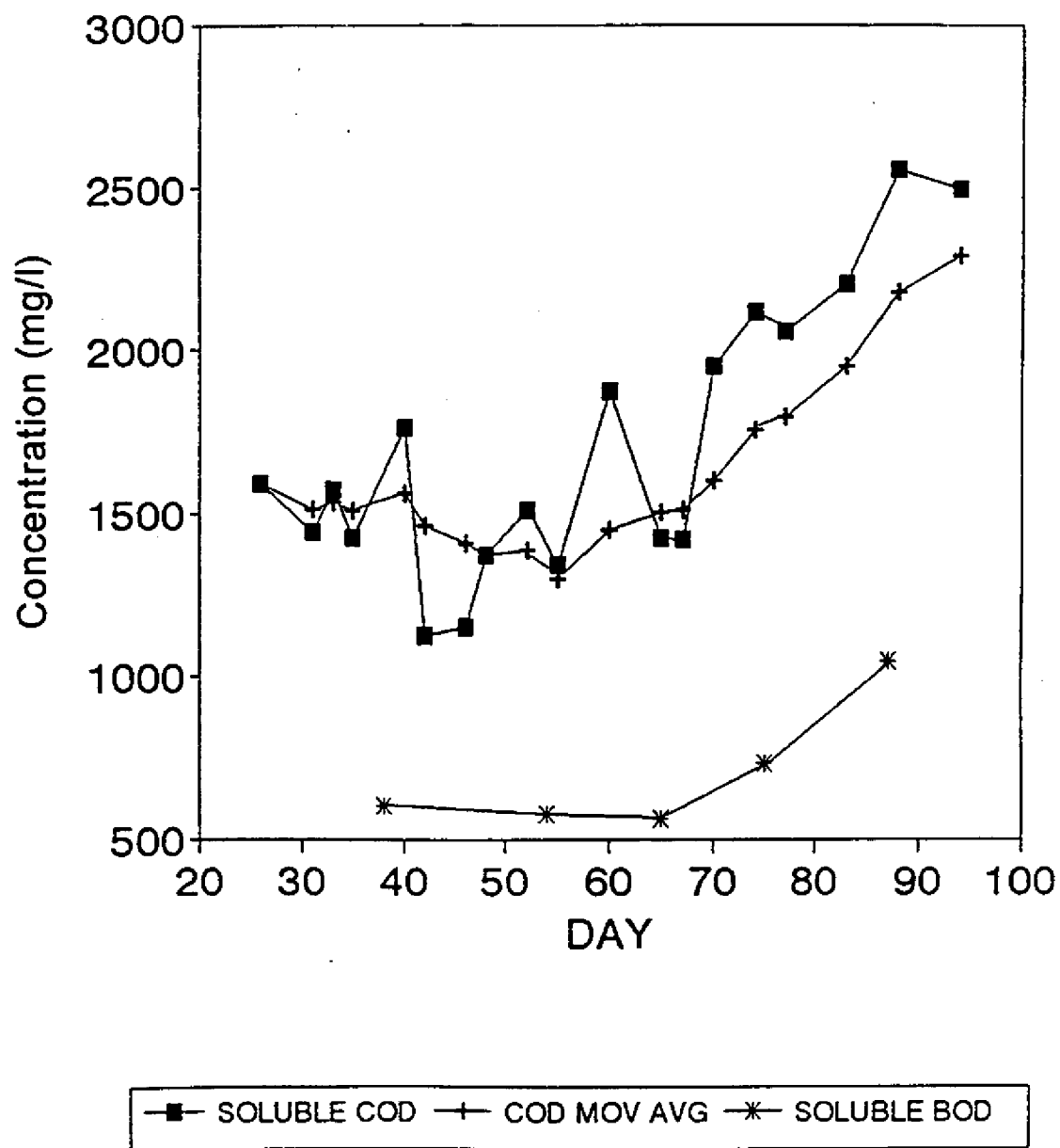


Figure 4. Effluent BOD₅ and COD levels for Reactor B.

7.62 to 7.75 during failure, the ammonia concentration never reached the inhibitory 1500 mg/L level during operation.

Finally, cation toxicity was explored as a possible cause for the failure of the reactors. At the time of failure, sodium, potassium, magnesium, and calcium levels within reactor A had reached 5080 mg/L, 526 mg/L, 224 mg/L, and 162 mg/L, respectively. Tests on effluent taken from reactor B during failure also revealed these four cations to be present in concentrations of 4175 mg/L, 495 mg/L, 210 mg/L, and 177 mg/L, respectively. In each case, sodium is present in levels which have been found to be moderately inhibitory (McCarty, 1964). Inhibition observed in these reactors may have been mitigated through the presence of cations (potassium, calcium, and magnesium) which are often seen as antagonists in situations of sodium toxicity (Kugelman and Chin, 1971). Even with the antagonistic effects of these additional cations, high sodium levels together with the high ammonia concentrations present are believed to be the cause for the unstable manner in which these reactors operated and the eventual cause of their failure.

The last two reactors (C and D) were operated at F/M ratios of 0.10 and 0.07, respectively. These two reactors were both fed a wastewater mix representative of the processing facility's overall wastewater output and were operated for periods of 287 and 94 days, respectively. As with the retort fed reactors described previously, these reactors showed signs of failure, and were subsequently shut down, after having earlier exhibited reductions in COD and BOD₅ levels of the waste.

For the period of time from day 22 through day 247, effluent soluble COD levels in reactor C remained between 1250 and 3310 mg/L with an average effluent COD of 2290 mg/L. These levels correspond to removal rates ranging from 55% to 83% with an average of 69%. This reactor also exhibited BOD₅ removal rates, over the same period, varying between 72% and 84% with an average of 79%. These rates translate to effluent soluble BOD₅ concentrations between 893 and 1596 mg/L with an average of 1180 mg/L. Also over this period of time it was observed that MLVSS of the reactor declined from 3240 mg/L on day 28 of operation to 1340 mg/L by day 235. Effluent TSS levels for this reactor ranged from 700 to 2180 mg/L over this same time with an average of 1577 mg/L. Solids retention time varied between 41 and 183 days over the life of the reactor with an average value of 98.6 days. HRT was maintained at an average of 36.3 days over the life of the reactor. The period from day 248 through day 276 was marked by an increase in average COD concentration to 2730 mg/L, and was followed by substantial increases in both soluble COD and BOD₅ levels. As a result, the reactor was shut down on day 287 after effluent COD had reached 4100 mg/L, and biogas production had fallen to less than 100 ml/day. Soluble material accounted for an average of 85% of all COD and 94% of all BOD₅ observed in reactor C effluent. As with those found for the retort water reactors, these levels imply that the effluent solids contained a higher portion of inorganic material than that suggested by the ratio found in solids from the secondary effluent of activated sludge systems (0.65 mg BOD₅/mg TSS).

Reactor D operated with effluent soluble COD levels between 985 and 1445 mg/L from day 26 through day 46 with an average COD of 1281 mg/L. This translates to COD removal rates ranging from 79% to 86% with an average of 84%. Over this period, the biogas production from the reactor proceeded at the rate of 195 ml/day, but this rate decreased during failure to a level of less than 100 mL/day. During the observed reactor failure, effluent COD levels steadily increased after day 48 to

over 4200 mg/L by shut down on day 94. Soluble BOD₅ levels remained between 833 and 891 mg/L from day 26 through day 65 with an average of 870 mg/L for a BOD₅ removal rate of 83%. After day 65, BOD₅ concentrations also began a rise, which continued until reactor shut down. Soluble material accounted for an average of 84% of all COD and 99% of all BOD₅ observed in reactor D effluent over the course of reactor operation. As with the preceding reactors, these levels imply that the effluent solids contained a higher portion of inorganic material than what could be expected given the ratio found in solids from the secondary effluent of activated sludge systems (0.65 mg BOD₅/mg TSS). Effluent TSS increased over the course of the reactor operation from 240 mg/L on day 26 to 2320 mg/L on day 83, and while MLVSS remained between 2780 and 2960 mg/L from day 26 to day 46, their levels fell to 2460 mg/L by day 85. After an initial reading of 686 days, solids retention time for the reactor decreased rapidly until it stabilized in the 102 - 112 day range. The average value of the SRT over the life of the reactor was 138.6 days. HRT was maintained at an average of 32.4 days over the life of the reactor.

As with the retort-fed reactors, an attempt was made to discover the cause of failure in these two reactors. Alkalinity and pH were not seen to be problems for either reactor, because alkalinity was 3828 mg/L in a pH range of 7.50 to 7.65 for reactor C, and 3000 mg/L in a pH range of 7.45 to 7.55 for reactor D, consistent with those values observed during the aforementioned period of organics removal. Loss of MLVSS was, as mentioned earlier, a problem for both mixed wastewater reactors; however, since failure of each occurred during periods of stability in this parameter and not during periods of decline, this loss is not seen as a primary cause of reactor failure in either reactor C or D. Effluent phosphorus concentrations found during operation and failure in each reactor (70 to 120 mg/L for reactor C and 55 to 120 mg/L for reactor D) indicate that it was present in sufficient quantities as not to be a limiting factor for either reactor.

Ammonia levels in the reactors, ranging from 748 to 949 mg/L in reactor C and from 592 to 787 mg/L in reactor D, were not seen to be problems for either reactor as they never reached the previously mentioned inhibitory range of 1500 to 3000 mg/L.

Cation toxicity, and specifically sodium toxicity, is seen as the primary cause of failure in both reactors. As sodium has been found to be strongly inhibitory at a concentration of 8000 mg/L (Kugelman and McCarty, 1965; McCarty and McKinney, 1961), the fact that the sodium level in each reactor far exceeded this amount clearly suggests sodium toxicity. The question of how these reactors performed as long as they did, albeit at a somewhat lower than hoped-for removal efficiency, given this sodium toxicity, can be answered via the phenomena of cation toxicity antagonism. Potassium concentrations found in both reactor C and D were consistent with those needed to act as an antagonist for sodium toxicity (Kugelman and Chin, 1971). Additionally, the presence of significant concentrations of calcium and magnesium, which are often found to be stimulatory in the presence of another antagonist (McCarty, 1964), suggests that a multiple antagonistic effect (Kugelman and Chin, 1971; Kugelman and McCarty, 1965) may have been responsible for the extended performance exhibited while in a state of sodium toxicity.

Cation toxicity problems in any treatment system for either of these wastewaters must be addressed in order for such a system to operate successfully. Such provisions may include an

elutriation step where the reactor sludge is periodically "washed" with fresh water which has been purged of dissolved oxygen. After a settling period, this water would then be flushed out of the system in the same manner in which reactor effluent is removed, thereby removing excess sodium. The reactor would then be refilled with fresh, purged water and treatment could continue. Alternatively, a periodic dumping and restarting of the reactor with new biomass could be employed when toxicity effects are observed; however, this method may lead to excessive start-up times.

Based on the results of this experiment, it is suggested that processing facilities which fall under the "existing" classification, which does not have BOD₅ discharge limits, do not employ an anaerobic system for the treatment of their wastewaters unless they are prevented, in some other manner, from discharging directly to the environment. In this case anaerobic treatment could be used as a pretreatment for disposal to the sewers. This recommendation is due to the high effluent ammonia concentrations (592 - 1780 mg/L) resulting from the anaerobic deamination of proteins. In light of the projected ammonia discharge limits, these levels would make direct discharge of wastewaters very difficult even if the waters were subsequently treated for ammonia removal, and would eliminate the possibility of direct discharge without such ammonia removal treatment.

In the case of facilities which fall under the "new" facilities classification, and are subsequently subject to BOD₅ discharge limits, the data from these experiments suggest that anaerobic treatment would be an effective tool for the treatment of wastewaters prior to discharge to a sewer system or an aerobic, biological treatment step. It is further suggested that, if only retort process waters are to be anaerobically treated, this system should be operated at an F/M ratio of 0.35 or less (with improved removal rates to be balanced against larger required reactor volumes at lower F/M ratios). It is also recommended that the anaerobic system include a settling chamber to increase the solids retention time of the reactor, and thereby improve the reactor's performance. A reactor of the type stated could be expected to provide approximately 88% removal of soluble BOD₅, and result in an effluent soluble BOD₅ concentration of approximately 1400 mg/L.

If it is decided that the total plant wastewater stream, such as the mixed wastewater studied here, is to be anaerobically treated, the above system should then be operated at an F/M ratio between 0.07 and 0.10. In this treatment scheme, soluble BOD₅ removal rates of between 79% and 83% can be expected, resulting in effluent concentrations between 870 and 1180 mg/L.

As stated previously, any anaerobic treatment system used to treat these processing waters must be monitored for cation and ammonia toxicity problems. Additionally, provisions, such as the elutriation step mentioned previously, should be made to counteract any such toxicity problems that arise.

Air Stripping Experiment

Although the retort processing and sample collection methods remained the same throughout the testing period, a great variability was found in the ammonia levels of the retort effluent; i.e., the tower influent. With the exception of one test, which had an ammonia level of about 50 mg/L, the range of tower influent ammonia concentrations was approximately 80 to 160 mg/L. These levels are consistent with those found in previous research involving this waste (Harrison *et al.*, 1992). As a

result of this variability, percent reduction in the ammonia levels was the main criteria used in the comparison of tower performance at the various operating parameter combinations.

In the testing of the ammonia concentrations of the retort process effluent, it was found that there was no significant difference in the levels found in the pH adjusted tower influent as compared to those found in the unadjusted retort process effluent. For this reason, it is believed that the ammonia removal achieved in these tests can be attributed to the air stripping process alone.

Figure 5 shows the impact of varying the influent liquid flow rate, and thereby the air to water ratio, for the tower on the treatment efficiency of the air stripping process. The graph shows that at pH 11, decreasing the liquid flow-rate from 2.2 GPM to 1.2 GPM led to an approximate doubling in removal efficiency, but a further decrease in flow-rate to 0.6 GPM did not provide any increase in ammonia removal. In tests performed at a pH of 10, however, a 7% increase in treatment was observed when the flowrate was decreased from 1.2 GPM to 0.6 GPM. All tests were performed at average influent temperatures of approximately 56°C to 58°C. As a result of these findings, it is suggested that while the lower flow-rate of 0.6 GPM may not improve treatment efficiency at higher pH levels (11 and above), the higher air-to-water ratio may offset, to some extent, the deleterious effects of the lower pH levels (10 and below). This may allow a choice of treatment schemes which would permit chemical costs to be balanced against the problems associated with lower processing rates.

Figure 6 displays the effect of temperature variation on ammonia removal effectiveness at pH 11.0 and pH 12.2. From this data it can be seen that an increase in influent temperature can be expected to bring about some increase in treatment efficiency. This would suggest that any system of this type be operated in such a manner that the retort effluent be processed through the tower with the minimum possible cooling time allowed. One cautionary point that must be stated is the fact that the LANPAC® packing material used in the study is not recommended for use at liquid temperatures above 71°C (S. Joyce, LANTEC Products, personal communication, 1993).

Figure 7 compares the treatment achieved by the tower as the pH of the influent stream was varied. It shows a small improvement (from 61% to 63%) in removal efficiency as the influent pH was raised from 10 to 11, while temperature and air-to-water ratio were held constant. A further test conducted at pH 12.2 and the same temperature and air-to-water ratio showed an increase to a 71% removal efficiency.

Finally, tests performed to establish the added treatment achieved by recycling the tower effluent back through the system showed 32% treatment for the tower effluent recycled without mixing and 51% removal of the ammonia in the mix of tower and retort effluent. These figures translate to effluent ammonia concentrations of 23.8 mg/L and 30.4 mg/L, respectively. These levels of treatment were achieved even though the treated liquid entering the tower had an average temperature of 20°C.

An additional benefit of the air stripping process was found in the cooling effect that it imparts upon the wastewater. In the tests conducted, while the influent average temperatures ranged from 50°C to 65°C, the tower effluent was cooled to essentially the ambient air temperature (13°C - 20°C). The majority of this reduction is attributed to the cooling effects of the tower because influent temperature changed very little (0°C-4°C) over the course of each test.

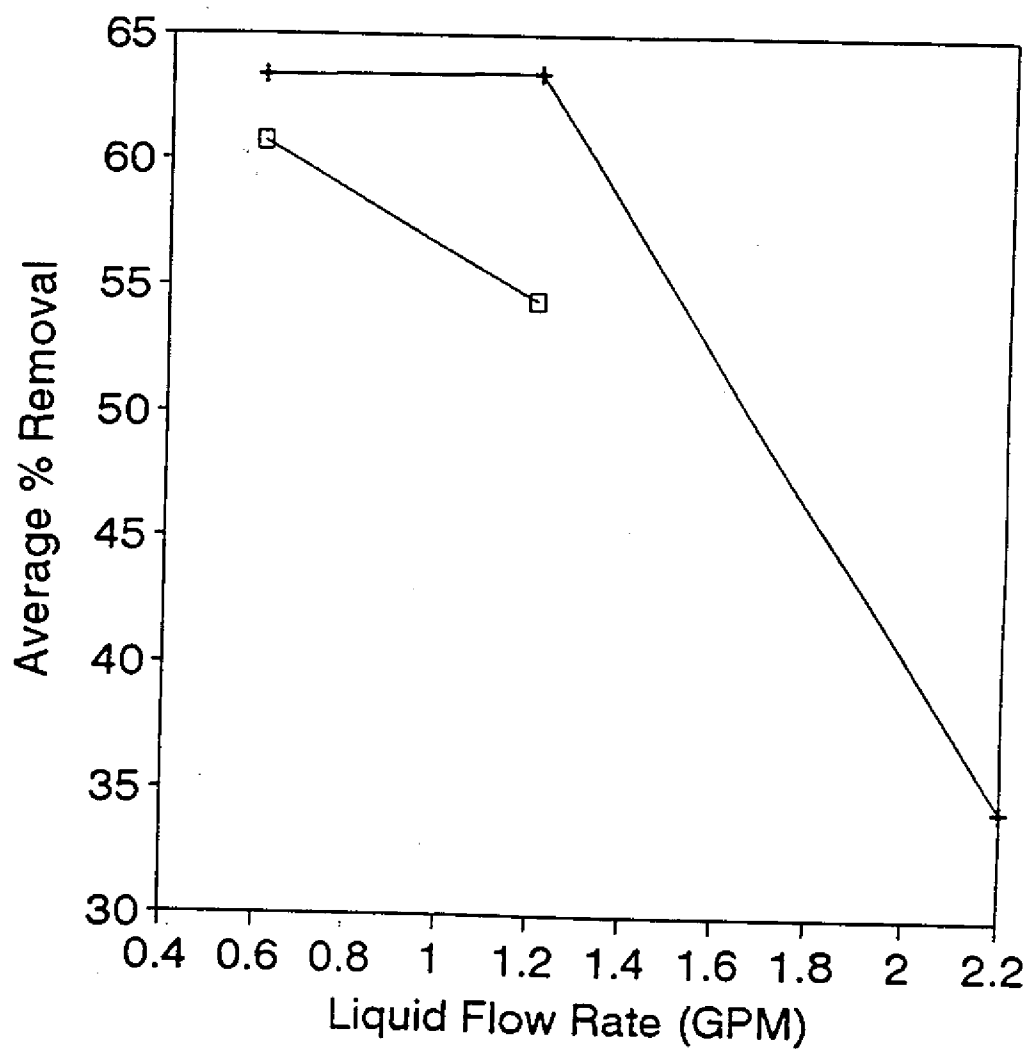


Figure 5. Air stripping ammonia removal rates as a function of liquid flow-rate; + = pH 11.0, □ = pH 10.0.

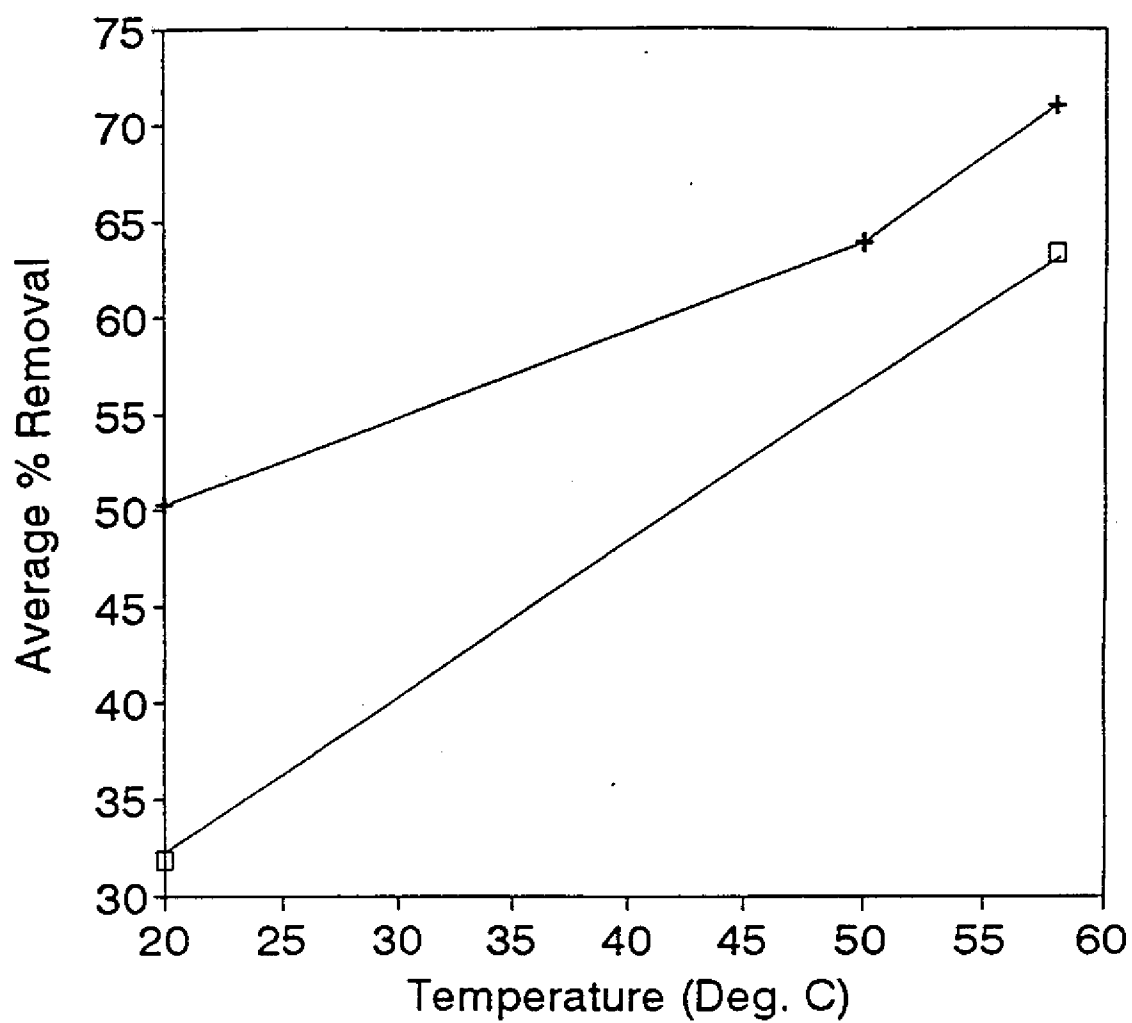


Figure 6. Air stripping ammonia removal rates as a function of influent temperature;
+ = pH 12.2, □ = pH 11.0.

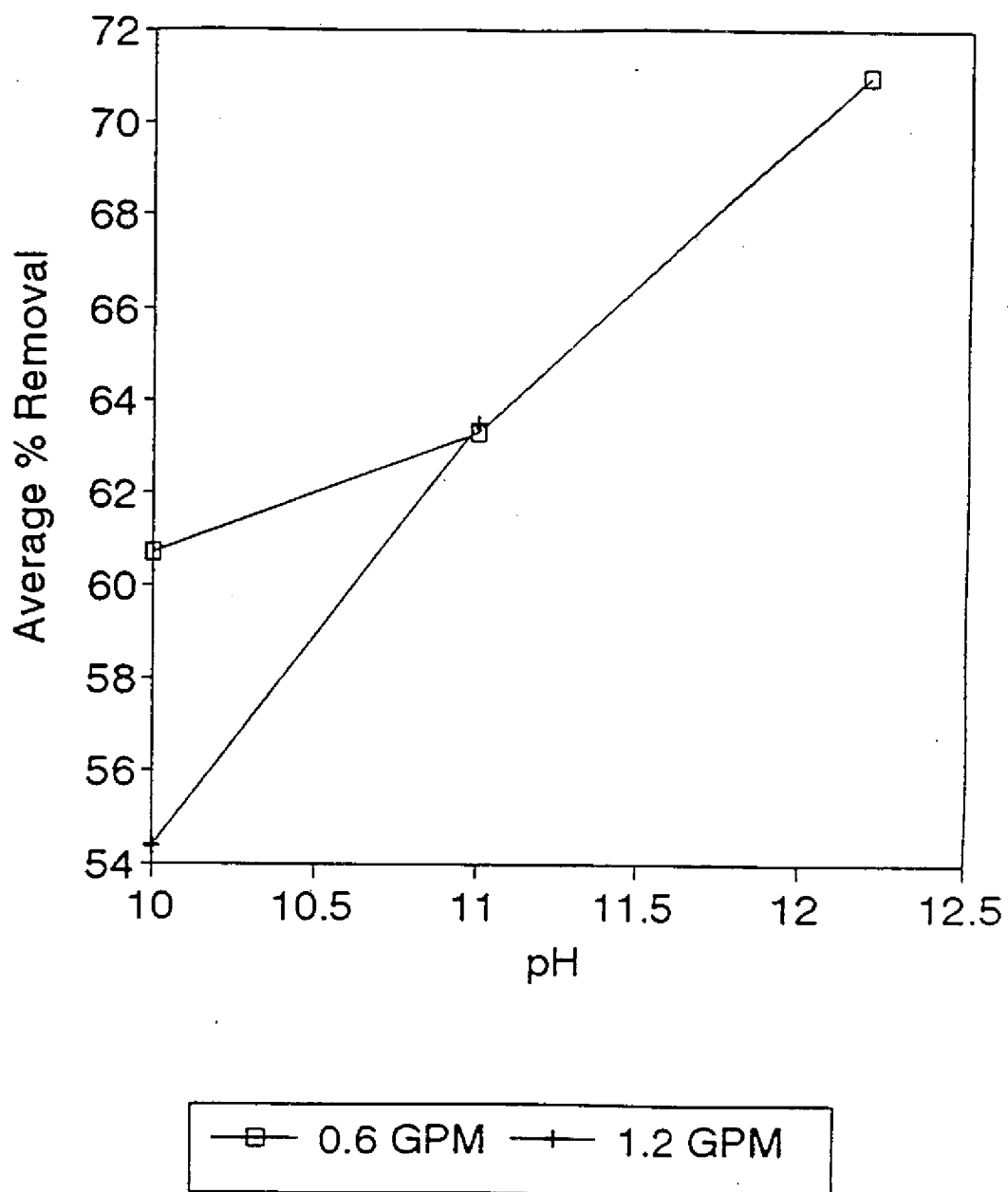


Figure 7. Air stripping ammonia removal rates as a function of influent pH level.

With the exception of the trial performed at an air-to-water ratio of 225 ft³/gal, the air stripping tower produced an effluent with ammonia concentrations between 19.5 mg/L and 58.3 mg/L.

While these tests showed significant reductions in wastewater ammonia levels as a result of the air stripping process, the reductions were not as great as those stated in literature sources (Kuhn, 1956; Slechta and Culp, 1967; Culp and Culp, 1971). While this reduced efficiency could be attributed to the higher ammonia concentrations dealt with in this study, it is more likely due to a shortcoming in one or more aspects of the tower design employed here.

Specifically, it is believed that a liquid distribution system that is capable of introducing the wastewater into the tower as fine droplets could improve system performance. Spraying the waste over the top of the tower would increase the surface area of water that would come in contact with the air at a point before the benefits of the high waste temperature have been lost.

Additionally, while the blower system utilized in these tests was able to maintain a sufficiently high average volumetric air flow, it was observed that the air flow rate varied greatly across the diameter of the tower. This may have caused a certain portion of the waste to channel through "dead zones" where a lower gas to liquid loading ratio may have resulted in less than optimal treatment. It is believed that the use of an in-line blower system, in which a fan, located at the top of the tower, would draw air up from the bottom of the tower, would remedy this problem and lead to higher removal rates.

Another possible design modification which could be employed in order to improve removal efficiencies is an increase in the effective tower packing depth. This could be accomplished through the use of either one tower of a greater packing depth or two towers operated in series.

A concern which must be addressed before the implementation of any ammonia stripping process is the potential for air pollution problems. The odor threshold for ammonia is approximately 35 mg/m³ (Culp, 1978). Care should be taken to ensure that levels present in the stripper discharge do not exceed this concentration. At an air-to-water ratio of 500 ft³/gal (3.7 m³/L), up to 129 mg/L can be stripped without exceeding this threshold. At the 825 ft³/gal (5.9 m³/L) ratio tested here, it would be possible to achieve removals of up to 207 mg/L of ammonia before the odor threshold became a problem. Removals of concentrations greater than these would most likely require the capture and treatment of the tower off-gas. Odor was not a problem in any of the trials performed here.

Based on the data obtained in these experiments, it is suggested that an air stripping could be used to reduce retort effluent ammonia concentrations to a level where direct discharge would be possible. This tower would need to be operated at an air-to-water ratio greater than 400 ft³/gal in order to ensure sufficient ammonia removal, while minimizing the potential for air pollution problems. In the event that direct discharge is not possible, the tower could still be used as a pretreatment to reduce sewer charges.

CONCLUSIONS

The anaerobic reactors used in the treatment of crab retort process waters have shown significant levels of organics removal, as shown by high removal rates observed in reactors A and B. Similarly, the performance of reactors C and D suggest that anaerobic digestion may present an alternative for preliminary treatment of the total waste stream from a mechanized plant. Any use of this technology in the treatment of either wastewater must, however, include provisions to diminish the effect of the sodium toxicity found in this study. Additional thought must also be given to the implications of the high concentration of ammonia found in the effluent from each reactor. Finally, while the retort water reactors produced an effluent with TSS levels in the 200 to 650 mg/L range, which may be acceptable, the reactors which were fed the mixed wastewater produced effluent TSS levels ranging on average from 1391 to 1587 mg/L. These levels could be reduced through the extension of the settling period beyond the one hour allowed in these experiments. It should also be noted that these solids contributed a relatively small (1% to 15%) portion of the total BOD₅ and COD found in the reactor effluent.

The air stripping experiments showed that substantial reductions in the ammonia concentrations found in the retort process wastewater are possible through the use of this technology, and that the high temperature of the retort process wastewater aids in the accomplishment of this reduction. Consequently, it is suggested that the wastewater be treated as quickly as possible after the retort process in order to minimize temperature loss. Additionally, while the tests performed showed higher removal rates were obtained as the pH of the wastewater was increased, this benefit must be weighed against the costs of chemicals needed to achieve this increase together with those needed to return the pH of the treated waste to a level that may be discharged directly to the environment. While design modifications are needed for the development of a full-scale air stripper, it is believed that the system considered in this work demonstrated that air stripping of ammonia is a possible treatment alternative for the crab industry.

ACKNOWLEDGEMENT

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PREVENTIVE MAINTENANCE AND PARTS INVENTORY CONTROL PROGRAMS

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As the title states, this paper deals with what a preventive maintenance and parts inventory control program is and what qualities a good program contains. Therefore I start with a few definitions. Looking up and combining the definitions of preventive maintenance and parts inventory control in the New College Edition of the American Heritage Dictionary, I found that **preventive maintenance** is to thwart or ward off illness by keeping a system in proper condition. For my purposes, this means using a computer to facilitate checking a machine to keep it working within set conditions so it will not fail. **Parts inventory control** means to use a computer to regulate the periodic survey of all goods and materials used by or for the above mentioned machines in stock. It's important to remember that parts inventory control refers to the inventory of parts for your machines not the inventory you produce.

Preventive maintenance programs are usually combined with parts inventory control programs since it is useful to keep an inventory of the parts needed for maintenance then update the inventory. Typically, a preventive maintenance & parts inventory control program covers: **Work Orders, Parts Inventory, Equipment, Purchasing and Personnel.**

The work order area or preventive maintenance area as it is sometimes called includes a work order form that you fill out using the keyboard. Just as with a normal preventive maintenance plan, the work order includes the item needing work, what needs to be done, how long it should take to do and how long it actually takes, equipment needed and who's available to work on the order. The computer's advantage is to allow you to look up information about a work order in the program's database. For example, you could select an appropriate part from a list of parts in a machine. The computer would then fill in the appropriate area on the form for you.

The work order area should also contain a section on periodic or regularly performed maintenance. This area allows you to record a normal maintenance schedule. The computer then automatically prints work orders for you when periodic maintenance is due on a machine. Some programs allow hook-up of sensors for detecting when a machine is working improperly then print work orders based on that information. All programs should allow printout of emergency work orders.

```

Editing Work Order  [Alt-F1]-help, [F3]-vendor labor, [F4]-employee labor,
                    [F10]-menu, [F2]-done.

+Work Orders-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
|No #: 801226 Equipment #: 20-BOIL-76 Status: A Down Time:|
|Task: REPLACE IGNITER RODS IN KOMEX BOILER 3|
|Work Order Type: CORRECTIVE Complete Date: Complete Time:|
|Employee #: Last name First name Date Reg Ot Ot|
|hrs hrs factor|
|-----|
|Item # Description Qty Date Qty|
|10-0630-15 460 VOLT FIREKIX IGNITER RODS 2 Reg Used Used|
|-----|
|Comments|
|How let's go to Page 2 and enter an estimate|
|of how long the job might take.|
|-----|
|Vendor ---Performed by---Date---Reg Ot Ot---Rate---|
|hrs hrs factor|
|-----|
+ *Press [F1]-lookup table, [CtrlPgDn]-next record, [CtrlPgUp]-prev record +

```

Figure 1: Work Order Screen from MP2 by Datastream Systems demo

Figure 1 above shows a work order placed for heating rods in a boiler. The screen after this shows approximate times for repairing the boiler. After entering a time estimate, the work order is printed and given to maintenance personnel. After completing the repair job, the maintenance personnel give the work order sheet or a copy back to their manager with remarks and completion time/date. The manager enters the new information into the PM system. Later, the manager could access this information for more accurate estimates or to check on the amount of overtime (OT) used.

The parts inventory section keeps track of the amount in stock of a particular part such as a ball bearing, how much the part costs, the storage location of the part, the part number, possible suppliers of parts and work orders currently "out" using that part. When you close a work order which used a part, the program reduces the stock of the part. Similarly, if you withdrew a part from inventory during maintenance but returned it when you closed the work order, the inventory section temporarily reduces the number of available parts then returns the inventory to its original level when the part is returned. Of course, the stock increases automatically when you purchase new parts.

```

Issue/Return Stock [Alt-F1]-help, [F3]-return stock, [F4]-issue stock, [F2]-done.
+--Inventory-----+
| Item #: 28-0900-02      Type: SAFETY Stock item [y/n]: y
| Description: SAFETY FULL-FACE SHIELDS W/ HEADBAND
| Order point: 6      Default order qty: 10      Unit of measure: EACH
| Single source [y/n]: n      Account code: SAFETY      Manufacturer #: ANCHOR
| Current Stock-----+
| Location      Qty on hand      Unit cost      Extended cost
| ROW # BIN 1      5      13.52      67.60
| ROW # BIN 4      2      13.00      26.00
| Suppliers---(---primary vendor)-----Lead-----Contract-Contract-----
| Vendor # Vendor's item #      Unit cost Time Contract # Page # Item #
| ANCHOR  SPST-43
| Here's the record you're looking for. If
| it hadn't been the right one, you would
| continue to press [Alt]-2 until the correct
| record was displayed. Now, press [F4] and
| we'll issue the face shield.
|-----Press [F4]-----
+-- Press [F1] for lookup table -----+

```

Figure 2: Parts Inventory Screen from MP2 by Datastream Systems demo

Figure 2 shows the location of safety masks for use in the repair job in Figure 1. You can see by the re-order quantity that it is almost time to order more. When the total quantity reaches six, the program automatically orders more from the vendor list (which is partially obscured by the demo instructions).

Equipment is similar to parts inventory in that it too keeps track of parts, but instead of checking a part's stock it tracks a specific part's date of purchase, company of purchase, the cost of a part, and the status of the equipment. Some PM & PIC computer programs also include a section for the part's warrantee, suitable alternate replacement parts, and generation of reports (including graphs) based on price, company or repair history showing the frequency of repairs.

Figure 3 (next page) shows an Equipment screen where the user searches for all equipment with the word "cartoner" in the description. This avoids looking for a specific part in a list of many but rather lists only those associated with a cartoner. If he had known the exact part number he could have entered that number and found the exact part he needed.

The purchasing section processes orders for new parts. Most programs alert you if your stock is low on a part, some even automatically generate a purchase request if you wish.

Figure 4 (next page) shows a typical Purchase Order screen with the parts to be ordered, their equipment number, location and comments. Other available information is easily accessed by using the cursor keys or a mouse to activate the scroll bars on the right and bottom of the parts list. When highlighted, each item displays all available information in the area below the parts list.

Equipment

Form Table Field Next! Previous! Database Options Screens Run Help

Equipment #: **MAXIMO**

Belongs to:

Eq Class: Failure Code: Location: Drawings:

Calendar: Meter Reading: Date: Meter Avg:

Serial #: Asset #: Cost Center:

Manufacturer:

Vendor:

Part #:

Costs

Total:

YTD:

Budgeted:

Uncharged:

Total Uncharged:

Equipment Status

Up?: ☐

Date:

Total Downtime:

Modified

Date: By:

Warranty

Date Installed: Expiration Date:

Purchase Price: Replacement Cost:



QUERY  Form is in QUERY mode.
Record cannot be inserted. 

Figure 3: Equipment Screen from a MAXIMO demo by Project Software Development Inc.

Purchase Order Line Items

Form Table Field Next! Previous! Database Options Screens Run Help

PO #: 12333 **CARTONER BEARING ORDER**

Parts: Total Cost: 131.60

?	Part #	Description	Location	Model #	Remark
>	217215-A	BLOCK BEARING PRIMARY		TIMKEN	SPECIAL CARTONER

Part #: 217215-A **BLOCK BEARING, 2" ID TAPPED ROLLER, TIMKEN**

Location: PRIMARY Cost Center: 7000-99 Entered By: MAXIMO Date: 09/28/90

Remarks: SPECIAL CARTONER BEARING, QUOTED DELIVERY 10/10/90

Quantity: 2 Model #: TIMKEN

Order Unit: PAIR Catalog #: 76865

Conversion: 2.00 Back Order:

Unit Cost: 65.80 Taxed?: N

Line Cost: 131.60

Received

Quantity: 0

Rejected: 0

Unit Cost: 0.00

Actual Cost: 0.00


MODIFY 

Figure 4: Purchasing Screen from a MAXIMO demo by Project Software Development Inc.

The **personnel** section covers information on maintenance workers usually found in a payroll system. This section includes names, address, pay rate, annual and sick leave total and used. Additionally, a PM & PIC system includes information on their areas of expertise and how much time they have scheduled or available for working on preventive maintenance. This allows prevents overworking someone or choosing the wrong person for a job. Usually, the program accesses this information automatically then requests you to choose a worker and change their schedule.

In addition to the those five areas, many programs also include simple graphics capabilities, job plans, networking capability, bar code capability and data conversion. Two examples of graphics are the ability to draw graphs from a history of part purchases or schematics of machines. Job plans show how to run maintenance on a machine and what you'll need to do the job. A networked version of a program is one that allows multiple people to use the program at the same time but prevents people from giving conflicting orders to the program. For example, no two people could withdraw the same part from inventory, so when one person is requesting a part on the computer another would not have access to it. Bar code reading and printing allows quick access to parts, work orders or any area of the program by using a light pen. This helps to quickly enter most or all of the important information on a part or job without having to type it into the computer. Data conversion facilities allow the user to access information from a variety of commercially available databases.

Areas of Concern with Computerized Maintenance & Parts Inventory Control Programs

All of these capabilities won't help you in the least if you don't find a program that fits your needs. There are several qualities you should look for to find a good program. They are: Cost, Ease of Installation, Ease of Use, Ability to produce output, Accounting Needs & Program Support. Even though the list isn't in any special order, the most important feature of any PM & PIC computer program is Cost.

Cost: Costs range from \$145 for General Energies Technologies' Predictive Maintenance Control System to \$75,000 for EDS/Scicon's Teroman. Because of the wide range in costs, it is crucial to know how much money you can spend on a system before you go shopping. However, the price tag does not always reflect the true cost of a system. There are a few hidden costs that you must consider before purchasing a program. If your company is just starting to use computers, check the computer requirements of the PM package you're considering. You may have to buy more memory for your computer, a larger hard disk and possibly you'll need a different (that is faster or more recent) computer. Make sure the programs you look at run on the operating system you use. Many systems run only on DOS or only under Windows. Both of those operating environments run on IBM compatibles. A few PM programs expect a UNIX environment. Most of these will not run on IBM compatibles. Of course, any programs written for Macintosh

(Apple) computers will not run on IBM's. The manufacturer of these programs most likely has an IBM version.

If you run a moderate to large sized company you may want the program to operate on a network. The main advantage of running a networked PM program is many people from different locations in the plant can access the program at the same time. If this is an advantage you need, you may need to pay for installing wires for a computer network. You'll also need a separate computer to act as a file server. This is where the PM program will reside. Some companies charge extra for a network capable PM program or may charge for the number of computers you hooked up to the network. The number of computers connected in a network is the number of nodes on the network. Don't forget to include a way to back up all that new information you'll be entering into the PM program - tape backups are a necessity!

Ease of Installation: This is actually a hidden cost as how long it takes to install and enter information into the PM program directly effects your office's productivity. The actual installation time for the PM program shouldn't take more than a week including working any bugs out of the new installation and basic training. If a program takes longer to install than this, look for a different program.

Installation of a networked version of a PM program usually monopolizes the file server. Other computers on the network may be unusable during the installation depending on the network or PM programs implementation. Entering information into the PM system will take longer than this. You'll lose the services of a secretary or two for a week, a month, or if you're a big company or have much information to enter, half a year or more! That's because they're entering all the item numbers, warrantee information, costs, all the information the preventive maintenance program needs. This virtually monopolizes the computer being used. It's a good idea to hire a temp at this time. Possibly, the software company you bought the PM program from will have someone to handle this. Alternately, if you don't mind taking a long time to get all your information onto the system, enter your data for a few hours each day. This frees you to use the computers normally during the rest of the time but, as I said, will take longer to get all the information into the system.

Ease of Use or User Friendliness: Once your system is up, it will take you a while to get use to running it properly; to enter work orders quickly, to find a substitute part on the computer, to get printouts when and how you want them. The more "user friendly" a program is, that is the more intuitive the program is to use, the faster you'll learn to use it well. Before you buy a PM program, test it out. Have the people you expect to use it play with a demonstration version. I feel it's a good idea to hold a meeting and discuss their thoughts on the program. If the program is difficult for them to use, if they can't get the demo to run through some simple commands after a few hours of playing around, it will take a long time for them to run the program efficiently. If this is the case I'd seriously think of buying your PM program from a different company.

If the future users of the PM program are at all familiar with Windows, I'd use a PM program made for Windows simply because the user interface will be familiar to them easy to understand. With Dos programs, every new program had a different look to it, there was no standard way of doing things. Since there is a standard for the "user interface" in Windows, you know the basics of any Windows program. This makes learning to use a new program much easier. Several companies make versions of their programs for Windows, Project Software & Development Inc. for example. An equally good alternative to this is to have a PM program custom designed for your company because there you can work with the designers to produce an interface you like. There are companies that do this, C.K. Systems being one.

Occasionally, you'll need help using the PM program. When you do, you'll want good documentation. All companies should provide you with documentation as part of the cost. Documentation should include the installation procedure, basic instructions on how to use the program, descriptions on how to use each area in detail, a tutorial, an index with many entries and extensive cross listing, a glossary of terms and phrases, some dos and don'ts and how to get ahold of company for support. Instructions in the documentation should be concise with any explanatory notes easily differentiated from the instruction. This makes it easy to perform the task without having to search through several paragraphs to find the instructions. Each area should have usable examples to illustrate procedures listed in the text. The examples should be practice; you should be able to do the example yourself. The tutorial does not have to be a complete course on the software package but should at least run through all basic procedures you're likely to perform. Many companies provide an on-line tutorial: a computer program that runs through exercises prompting you to make the appropriate entries and telling you when you make a mistake. I prefer on-line tutorials since they actually demonstrate proper usage of the program avoiding any ambiguities from confusing printed documentation. As a whole, the documentation should be logically organized. That is the glossary should either be all the way in the back or the front, installation instructions should be in the front of the book after the table of contents and/or glossary, the areas of the program you are most likely to use should follow and be in the order of use - that is, an example should start in the first section and build upon itself in each successive section. For example, you wouldn't remove a new type of pressure gauge from inventory when you haven't ordered it yet.

Lastly, many programs now have extensive on-line help. Some are even context sensitive. If the PM programs run via Windows, the help is most likely context sensitive. This means that when you press the help key, the first thing that appears on the screen is related to where you are in the program. For example, if I was entering a work order and pressed the help key, I'd get a screen listing the procedures for processing work orders. If this is not what I wanted help on, I should be able to get help on another subject by running through a computerized index. On-line help is very useful. The time spent on help drops when you don't have to grab a book but can access the same information through a few

keystrokes or mouse clicks. The help system should be quick to respond. Response time will depend on your computer's processor speed but if you meet the program's suggested requirements you shouldn't wait more than 5 seconds.

Output: A good PM & PIC program supports a wide variety of printers. A good networked PM program has no problems printing to two different types of printers from two separate locations. All good PM & PIC programs print work orders, purchase requests and periodic reports of inventory. In addition, most programs allow you to print any screen in any area you have access to within the program. Any output from the program should be very close to its final form requiring only a signature or the entry of information not already in the computer. Though effected by the type of computer, printer and network your system is on, 10 seconds should be the longest you have to wait for one page of text. Graphics, such as a parts diagram, take longer to print, ranging from 30 seconds to 2 minutes depending on the complexity of the drawing. Many PM programs now include provisions to allow bar code printing. Bar codes typically print on label size stickers and take few seconds to print. If the program supports bar code printing, it should support bar code reading also. This is usually accomplished with a light pen attached to either a portable hand held unit or a stationary computer.

Accounting Support: This is the area I'd rate as the second most important in a PM & PIC program. A good PM programs automatically updates account balances everytime you purchase a part. It also warns you of negative balances before they occur. A good PM program tracks other maintenance and labor costs allowing you to print this information in periodic reports (periodic meaning weekly, monthly, quarterly, etc). The program should contain some facility for projecting future costs and help you locate areas where improvements may be made whether that's in changing maintenance schedules or switching to another vendor for a part. This is the meat and potatoes of a PM & PIC program. Without good accounting support, the program is worthless. After all, the idea behind a PM & PIC program is to save you money. If you have to keep track of all the costs associated with parts inventory control or preventive maintenance by hand instead of using a computer, most of the time you save is lost. Therefore, you should look for a program with as many automatic accounting capabilities as possible and as many facilities to display cost and labor information as you can get.

Program Support: All good PM & PIC programs will have phone support available. Some companies provide toll free 800 numbers for such calls, some provide 24 hour service, some both. All should provide an address for user support. If they can't fix your problem over the phone, they should send a person out to your company to help. Support and service should be available from 90 days to 1 year, on site, **for free**. Any less than this and you're getting a raw deal! Some software companies sell use a third party to provide support. This usually works out fine but there are occasions when they will not be able to answer your questions. When this occurs, they should provide you with a telephone number and address of someone in the software company who can help.

The company you buy your PM program should notify you when new versions of their program come out. They should discount the price of these new versions. Your warantee on the program should be at least one year if not longer due to the time it takes to install and enter in all the data.

When you buy a PM & PIC program, the company you bought the program from or an authorized third party should provide an instructional class. These usually cost around \$500 if they are provided by a third party but could be free if offered by the software company. These programs may seem expensive but are worth the price in a shorter learning period for the eventual users. Sometimes the classes are offered on-site or off with the on-site costing a more expensive set fee as opposed to a cost per individual for off-site classes. With enough people, the on-site classes are actually less expensive and save you travel time too.

There are so many Preventive Maintenance and Parts Inventory Control systems available, with a little effort, you should be able to find one that suits your needs. About the only thing a good PM & PIC program will not do for you is enter basic information into your system. With the price of CD ROM drives dropping, lists of companies stocks may take care of equipment entering for you!

Included is Chilton's Industrial Maintenance & Plant Operation's Buyer's Guide to Maintenance Management Software from December 1992.

December 1992
For more information from the developers listed, circle the appropriate number on the IMPO Reader Service Card.

Company & program	Program features:																Operates on:					Suggested price for basic package	MSP No.	
	Equipment usage by type	Engineering data & operating specs	Failure & repair histories	On-line series of standards & procedures	Bill of materials parts lists for equipment	Employee skills and training data	Time keeping & labor distribution	On-line work requests and approvals	Work order scheduling worksheet	Work order planning with parts & labor	Work order status tracking	Work order priority backlog planning	Inventories tracking	Catalog of stock & non-stock parts	Automatic purchase reqs for stock	Purchase order tracking	IBM PC & compatibles	Other PC's	Minis	Micros	Mainframes			Program can be expanded by user
HSB Reliability Technologies, Lakeland, FL 33803 Program: Micro Maint [®]	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	\$9,950	646
Inova Systems, Minnetonka, MN 55305 Program: Opti [™]	*											*			*							*	\$10,000	647
Integrated Software Design, Inc., Mansfield, MA 02048 Program: On-Tag [™]												*			*		*	*	*	*	*		\$195	648
IRD Mechanalysis, Inc., Columbus, OH 43229 Program: PM Power	*	*	*																		*			649
JB Systems, Inc., Woodland Hills, CA 91367 Program: Manager	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$3,000	650
J&H Software, Inc., Toledo, OH 43608 Program: Maintenance Manager/Equipment	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$1,950	661
Jesall Inc., Erika, NC 28728 Program: Preventive Maintenance System	*									*	*	*	*	*	*	*	*	*	*	*	*		\$795	652
Life Cycle Engineering Inc., Charleston, SC 29407 Program: Orion	*	*	*	*	*									*									\$4,995	653
M ² Limited, GaitHERsburg, MD 20879 Program: Main Plan E/O	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$3,995	654
Management Planning Systems, Eugene, OR 97401 Program: Argus [™]	*	*	*	*	*									*	*	*	*	*	*	*	*		\$9,500	655
MicroWest Spaceseaver Software Systems, Inc., San Diego, CA Program: Advanced Maintenance Management System	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$5,995	656
Minneapolis Software, Inc., Roseville, MN 55113 Program: PM Manager	*	*	*	*	*															*		\$3,485	657	
OmelComp, Inc., State College, PA 16801 Program: Service Call Maintenance Management	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$2,990	658
OSHA-Soft, Inc., Amherst, NH 03031 Program: Fasttag															*								\$550	659
Pearl Computer Systems, Moorestown, NJ 08057 Program: uMAINT/Pro	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$5,495	660
Penguin Computer Consultants, San Jose, CA 95180 Program: Maintenance & Inspection System	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$1,450	661
Peregrine Systems, Inc., Pittsburgh, PA 15227 Program: OOPS! Once Of Prevention System	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$2,995	662
Performance Associates, Inc., Danville, CA 94526 Program: Performance Manager	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*			663
Phoenix Data Systems, Inc., Southfield, MI 48034 Program: AMS	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$3,500	664
PMS Systems Corp., Santa Monica, CA 90405 Program: SmartMMS	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*			665
Precision Maintenance System, Inc., East Hartford, CT 06027 Program: CMMS	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$8,300	666
Prism Computer Corp., Costa Mesa, CA 92626 Program: Farnis [™]	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$24,000	667
Process Analyzer Resources, Inc., New Florence, PA 15944 Program: Maintenance Management Software Series	*																			*		\$2,450	668	
Project Services International, Pittsburgh, PA 15205 Program: Totally Integrated Maintenance Management	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$1,750	669
Project Software & Development Inc., Cambridge, MA 02136 Program: Maximo Series 3	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$12,600	670
Prototype, Inc., Greenbrae, CA 94904 Program: EMS/PC, EMS/DX	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$8,000	671
QBIC III Systems, Inc., Columbia, MD 20146 Program: QBIC III Facility Management System	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$6,985	672
Qgest Software Systems, Murray, UT 84157 Program: Qgest Maintenance Management	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$595	673
Rainbow Enterprises, Redmond, WA 98052 Program: Maintenance Management	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$499	674
Albert Raymond & Associates, Inc., Hinsdale, IL 60521 Program: Remstar 2000	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$7,500	675
Remstar, Inc., Miami, FL 33178 Program: Maintenance Management System	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$5,000	676
Revere, Inc., Birmingham, AL 35243 Program: Revere Software	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$50,000	677
RMS Systems, Philadelphia, PA 19113 Program: Tristar Maintenance Management System	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$10,000	678
Rubicon Technology, Inc., Mountain View, CA 94043 Program: Support Manager	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$4,950	679
Spectec General, Inc., Newport Beach, CA 92663 Program: AMOS-D	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$14,100	680
Team Tech Systems, Inc., Red Bank, NJ 07701 Program: TEAM Maintenance Management System	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$1,495	681
Thomas Marketing Information Center, New York, NY 10001 Program: CMMS Directory & Comparison Guide																								682
Timesaver Systems, Portland, OR 97208 Program: Computerized Maintenance Manager, Phase III	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$995	683
Titan Software Corp., Morrisville, PA 15148 Program: TMS Total Maintenance System	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$9,500	684
TMA Systems, Inc., Tulsa, OK 74136 Program: The Maintenance Authority	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$11,995	686
Trow Consulting Engineers, Brampton, Ontario Program: Focus 2000 Maintenance Info. Mgmt. System	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$8,000	685
UE&C Catalytic, Philadelphia, PA 19101 Program: TRAC Vector	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$50,000	687
Walsh Automation Inc., Montreal, Quebec Program: Mainman	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$5,500	688
Western Software Solutions, Portland, OR 97223 Program: "X-Site"																							\$15,000	689
Wintercress Development, Renton, WA 98055 Program: Maintenance Director 1.5	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		\$7,000	690

MAINTENANCE MANAGEMENT SOFTWARE

December 1992

*This Buyer's Guide to Software is based upon survey responses from developers.
For more information from those listed, circle the appropriate number on the IMPO Reader Service Card.*

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OXIDATION AND LIPOLYSIS OF LIPIDS IN CHANNEL CATFISH DURING FROZEN STORAGE

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With sales of frozen channel catfish making up about 57% of total sales domestically, it is essential to ensure that quality of the frozen product be maintained throughout storage. Untreated channel catfish, however, have a limited shelf-life (3-6 months) due to oxidation of lipids and development of rancid off-flavors. Consequently, to combat this problem commercially, the catfish processing industry routinely applies an ascorbic acid-based antioxidant to their product.

Efforts to further promote stability in catfish muscle tissue have centered on defining the contributions of individual lipid classes to oxidation. While Silva (10) and Freeman et al. (6) have submitted that the non-polar lipids were the major site of oxidation in catfish, the study by Erickson (4) indicated that non-polar lipids served as the primary oxidative substrate in early phases of storage only. Acceleration in the rate of oxidation, on the other hand, was associated with oxidation of phospholipids. To explore the modifying influence of lipid hydrolysis and ascorbic acid treatment on contribution of lipid classes to oxidation, analyses for tocopherol, ascorbic acid, headspace volatiles and lipid class fatty acid composition were conducted in this study on frozen catfish fillets.

MATERIALS AND METHODS

Sample Preparation

Forty channel catfish, obtained from a local fish farm, were divided into two groups, and filleted. One group designated as 'treated' was vacuum tumbled in the presence of ascorbic acid (50 mg/150 ml deionized water) while the other group designated as 'control' was left untreated. Fillets were packaged and sealed in polyethylene bags (Product No. Curlon 550; Cryovac Co., Duncan, SC) under atmospheric conditions before subjecting to varying periods (0, 1.5, 3, 4.5 and 6 months) of frozen storage (-6°C). A second batch of fish, obtained one week later, were handled similarly for the second replication of the study.

Tocopherol Analyses

Muscle tissue (1.0 g) was saponified and extracted for tocopherols according to the procedure of Erickson (3). Quantification of alpha- and gamma-tocopherol was achieved with reverse-phase high performance liquid chromatography (HPLC) and fluorescence detection (2). Gamma-tocopherol was converted into alpha-tocopherol equivalents using a conversion factor of 0.31 (1).

Ascorbic Acid Analyses

Extraction of ascorbic acid from muscle tissue and subsequent ion-pairing HPLC was performed as described by Thed and Erickson (12).

Headspace Analyses

Minced tissue (1.5 g) was heated at 95°C for 15 min in a vial sealed with a teflon-lined rubber septum. Gas chromatography of headspace (1.5 ml), using conditions described by Young and Hovis (13), were used to quantify propanal and hexanal volatiles.

Lipid Characterization

Chloroform:methanol (2:1, v/v) extraction of muscle tissue was used for isolation of a lipid extract (3). Phospholipid, triacylglycerol and free fatty acid (FFA) fractions were obtained following thin layer chromatography of the lipid extract in 80:20:1 hexane:ethyl ether:acetic acid. The isolated fractions were esterified with H₂SO₄ in methanol and fatty acid methyl esters were separated by gas chromatography (3).

Moisture Analyses

Microwave drying was used to measure moisture content in samples. Oxidative measurements were then expressed on a dry weight basis to compensate for introduction of water into treated samples.

RESULTS AND DISCUSSION

No significant differences between treatments or between storage times were found in triacylglycerol levels of channel catfish (135.2 ± 19.6 mg/g dry wt) due to the large variability in triacylglycerol levels which occurred between fish. This measurement was therefore unable to detect lipolysis of triacylglycerols.

Lipolysis of lipid, however, was supported by the increase in free fatty acids during frozen storage (Fig. 1). Initially accounting for 0.5-0.6% of total lipids, FFA's increased throughout storage such that by the end of storage, 3 to 5 fold higher levels were found. The high temperature used for frozen storage (-6°C) would account for the extensive hydrolysis observed in these samples, higher temperatures having been associated with increased lipolysis rates (8). In contrast to the fairly steady increase in FFAs during storage, over 50% of phospholipid fatty acid losses were observed during the first 1.5 months. Decreased extractability of phospholipids and/or phospholipase activity could account for these losses. Net losses of phospholipid fatty acids, however, could not account for the major portion of FFA generated, except at 1.5 months. These results would therefore provide indirect support for the lipolysis of triacylglycerols in catfish muscle.

Lipid oxidation, as measured by the formation of hexanal and propanal, exhibited only slight increases during the first 3 months of storage for both control and treated samples (Fig. 3). In later stages of storage, greater increases

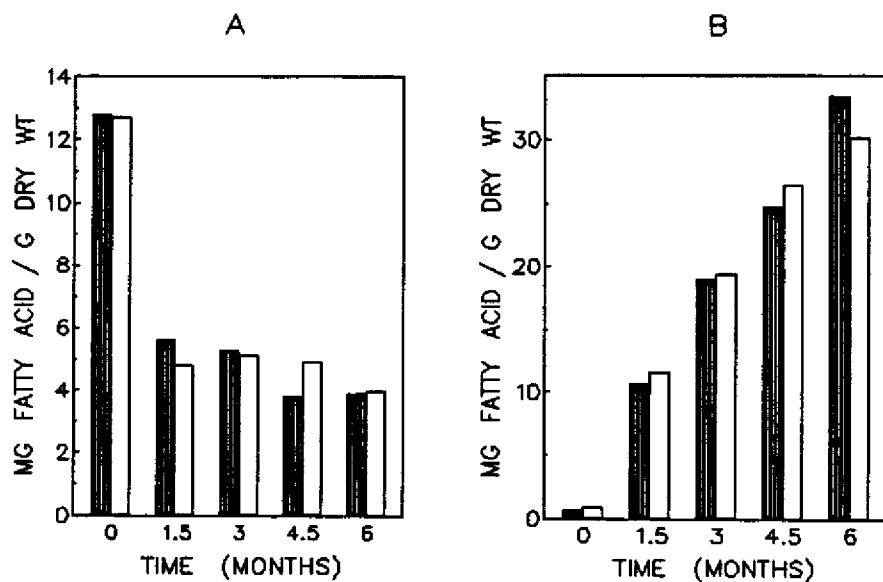


Fig. 1. Fatty acid content in phospholipid (A) and FFA (B) fractions of channel catfish muscle tissue following frozen storage. Striped bar = control; Empty bar = treated.

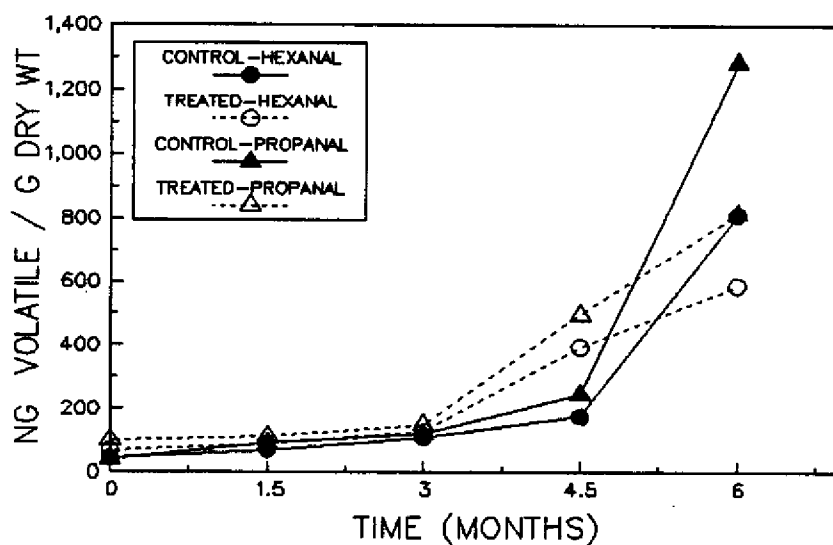


Fig. 2. Hexanal and propanal content of channel catfish muscle tissue following frozen storage.

in propanal than hexanal were noted, which is consistent with the lower apparent activation energies associated with propanal formation from w3 PUFA than with hexanal formation from w6 PUFA (5). Differences in the volatile profile of control and treated samples were also evident after 3 months. While the control sample remained stable for up to 4.5 months, thereafter the oxidative rate was much greater than the treated sample which began accelerated formation of volatiles after 3 months of storage. Thus, the prooxidant character of ascorbic acid in effect altered the mechanism by which lipid oxidation was occurring.

Examination of the tocopherol and ascorbic acid levels during storage supported the idea that oxidative mechanisms differed between samples. In the control sample, tocopherol losses were observed up to 4.5 months, whereas tocopherol losses in treated samples did not occur until after 3 months of storage (Fig. 3). A reverse trend occurred for ascorbic acid, with losses observed in treated samples occurring over the first 4.5 months and losses in control samples not occurring until after 3 months of storage (Fig. 4). A possible explanation for these results would be the regeneration of tocopherol by ascorbic acid in treated samples. Levels of ascorbic acid in control samples, however, appeared to have been insufficient to regenerate tocopherol, in which case, membrane fatty acids would be expected to degrade soon after tocopherol reached a critical level (7, 9, 11).

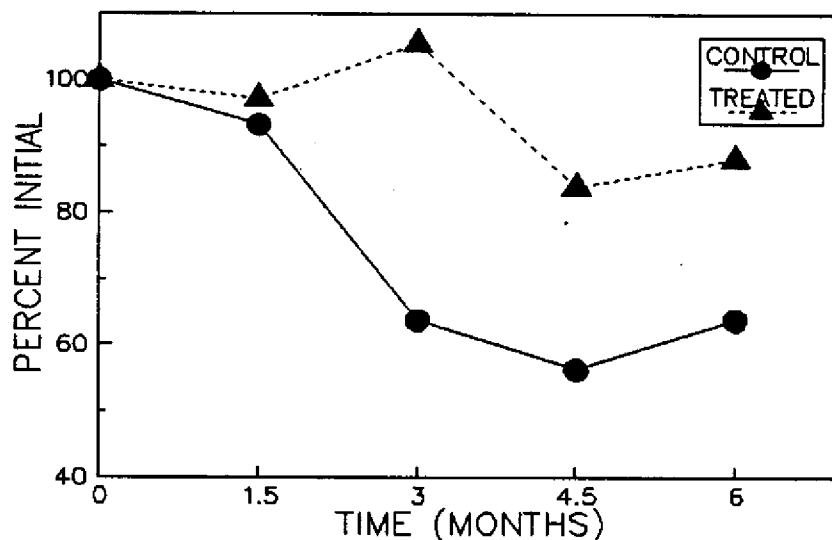


Fig. 3. Tocopherol content of frozen channel catfish in relation to initial (0-month) values.

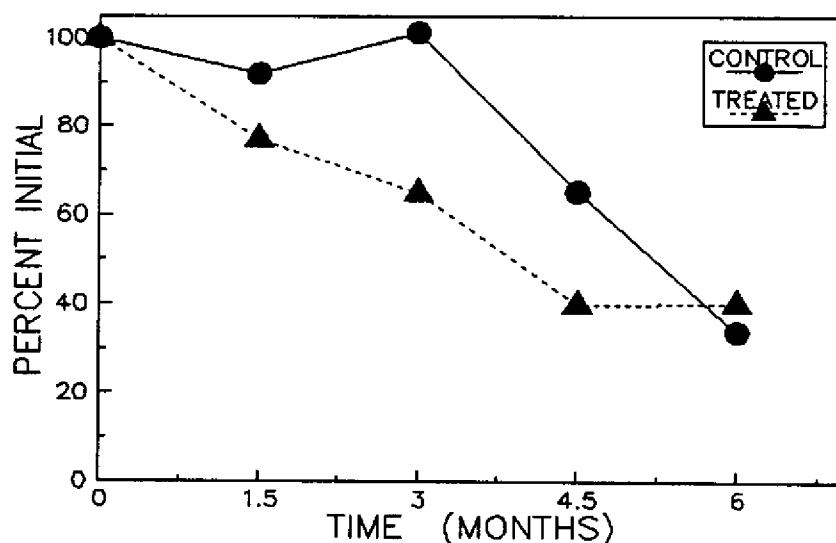


Fig. 4. Ascorbic acid content of frozen channel catfish in relation to initial (0-month) values.

In line with total fatty acid changes, changes in phospholipid and FFA palmitic acid (16:0) were similar for control and treated samples (Fig. 5). In contrast, lower concentrations of arachidonic acid (20:4 w6) in both FFA and phospholipid fractions were seen for control samples compared to treated samples at 4.5 months (Fig. 6). Similarly, a lower concentration of docosahexaenoic acid (22:6 w3) was seen in the phospholipid fraction of control samples than treated samples at 4.5 months (Fig. 7). Such results contrast to the lower hexanal and propanal concentrations observed in control samples than treated samples (Fig. 2) implying that the triacylglycerol fraction was the principal substrate of oxidation in ascorbic acid treated samples. The reduced levels of 20:4 w6 and 22:6 w3 in the control FFA and phospholipid fractions, on the other hand, may account for the acceleration in generation of oxidative products at 6 months.

CONCLUSION

By monitoring the lipid and antioxidant levels in frozen channel catfish, the role of tocopherol in protecting membrane phospholipids and free fatty acids from oxidation was supported. Ascorbic acid treated samples protected membrane fatty acids through regeneration of tocopherol. Simultaneously, ascorbic acid acted as a prooxidant toward triacylglycerols. Consequently, the significance of treating channel catfish with ascorbic acid was to shift the primary oxidative substrate from phospholipids to triacylglycerols.

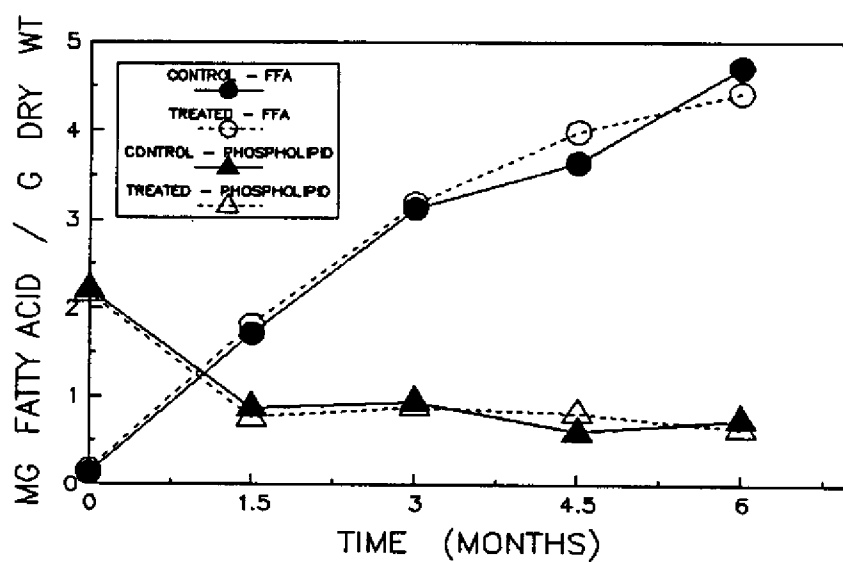


Fig. 5. Palmitic acid content of lipid fractions in frozen channel catfish.

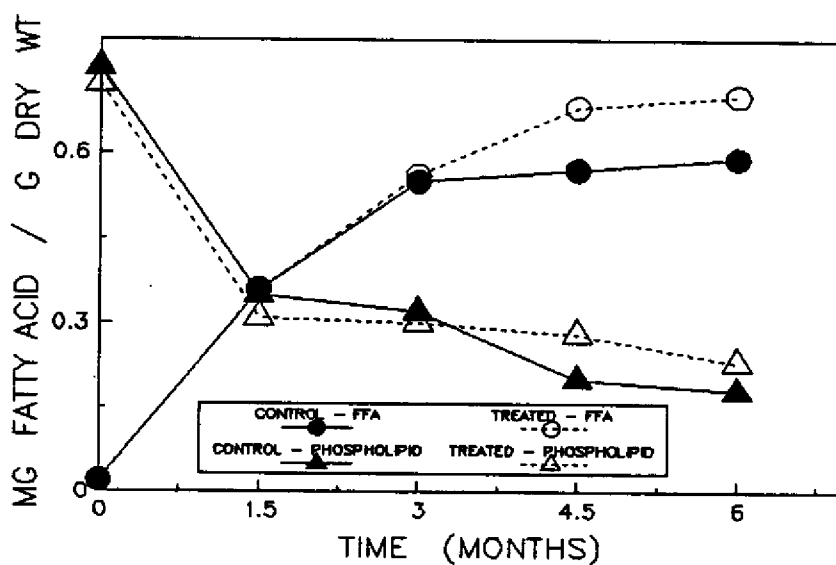


Fig. 6. Arachidonic acid content of lipid fractions in frozen channel catfish.

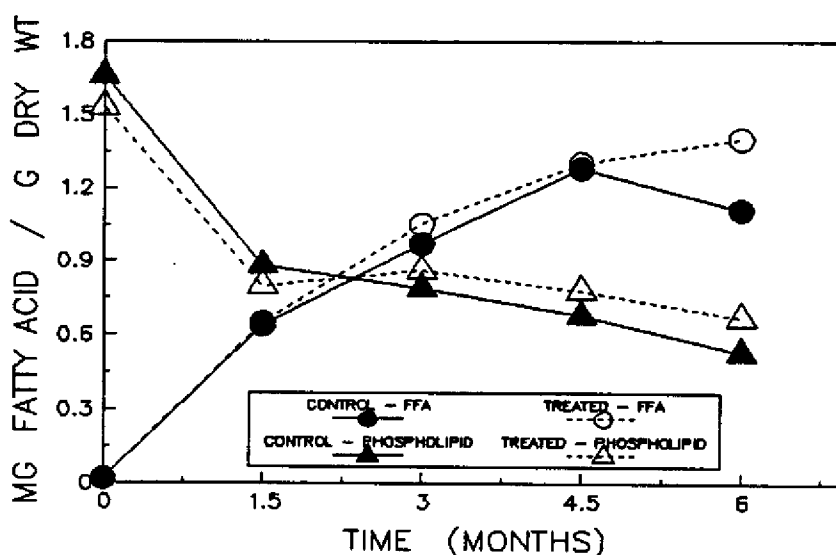


Fig. 7. Docosahexaenoic acid content of lipid fractions in frozen channel catfish.

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A SIMPLE, RAPID SOLVENT EXTRACTION METHOD FOR DETERMINATION OF TOTAL LIPIDS IN FISH TISSUE

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Several solvent extraction methods have been available for fish lipid extraction. Some of them are as follows. (i) Chloroform-methanol (CHCl₃-MeOH) system : Folch et al. (4) used 2 CHCl₃-1 MeOH system where 40g tissue was blended with 760ml and 400ml solvent in two steps ; Later, Bligh and Dyer (2) used 1 CHCl₃-1 MeOH in a two-step extraction where 100g tissue was blended with 200ml MeOH and 100ml CHCl₃, followed by 100ml CHCl₃, and the residue blended with 100ml CHCl₃ and rinsed with 50ml CHCl₃ ; Hubbard et al. (6) used 2 CHCl₃-1 MeOH at a 20 solvent - 1 tissue ratio by weight as a streamlined version for simplicity and rapidity ; (ii) n-Hexane-isopropanol system : Hara and Radin (5) used 3 hexane-2 isopropanol where 1g tissue was blended 18ml solvent and nonlipid fraction was removed with 12ml 6.25% sodium sulfate. However, incomplete extraction of proteolipid protein and gangliosides was noted ; and (iii) Methylene chloride-methanol system was tried by Swaczyna and Montag (7) where methylene chloride replaced chloroform to reduce toxicity. Nevertheless, methylene chloride poses a problem because of its low boiling point (39.7C) compared to chloroform (61C). Such a low boiling point may result in solvent losses due to rapid evaporation during blending and filtering leading to somewhat erroneously increased yield.

Recently, Erickson (3) compared 9 different solvent systems on catfish tissue lipid extraction using a screw-cap test tube (16 x 125mm) and a vortex with about 20 volume of solvent. The size of the test tube used limits the sample size to 1g based on a solvent to tissue ratio of 20. She concluded that CHCl₃-MeOH was preferred to other solvent systems (hexane : isopropanol and CHCl₃ : isopropanol) which required longer evaporation time. She also noted that exposure of the tissue to methanol prior to chloroform was necessary for the methanol-chloroform solvent extraction system. Although this method offers low volume solvent usage, simplicity and rapidity, it has some drawbacks, namely, limited sample size (not more than 1g) will require extremely uniform sample to minimize variability, and pressure build-up inside the tube and two-minute holding during vortexing could accompany safety hazards. Also the vortexing may well result in inadequate disintegration of tissue compared to mixing in the blade-equipped blender. Such an inadequate breakup of sample tissue may prevent complete extraction of lipid from the tissue.

Since one way or another all previous methods appear to have some drawbacks, either lack of reproducibility and precision or laborious and time and solvent-consuming nature, we decided to develop a simple, rapid extraction

method with a high reproducibility and precision based on the CHCl_3 -MeOH solvent system through a thorough examination of various procedural variables.

MATERIAL AND METHODS

Sample Preparation

Cod (*Gadus morhua*) and mackerel (*Scomber scombrus*) were chosen for lean and fatty species. Fish was filleted, cut into small pieces and homogenized in a kitchen Waring blender. The resulting paste free of bones, skins and scales was used as sample for solvent extraction.

Procedural variables

A single extraction with the CHCl_3 -MeOH mixture was employed for lipid extraction throughout the study. The following procedural variables were examined to determine the optimum conditions for maximum allowable extraction of lipids. The solvent volume to sample weight ratio was varied from 2 to 14. The CHCl_3 to MeOH ratio was varied from 1 to 16 plus 100% CHCl_3 . Phase separation time was varied from 30 min to 6 hrs.

Lipid extraction procedure

The paste weighing approximately 5 g was placed into the 250 ml capacity Eberbach homogenizer which has a narrow stem and a wide upper body and opening. Such geometry allows efficient blending with a small amount of solvent and large headspace which prevents solvent spilling. The varying amounts of solvent were added to vary the solvent to sample ratios at the varying ratios of CHCl_3 to MeOH. The mixture was blended for 1.5 min at a moderate speed using a rheostat to maintain constant speed. The high speed resulted in solvent vaporization and temperature rise. The homogenate was filtered through a coarse, fast speed filter paper (12.5 mm diameter) and funneled into 100 ml glass stoppered graduated cylinder. The wet cake was pressed with a round tip of spatula to squeeze out the remaining solvent. The final volume of filtrate varied with the batch of sample and species. The losses of solvent in the form of residue left in the homogenizer after blending-pouring out and soaked into the filter paper after filtering were taken into account in yield determination.

To separate the filtrate into two phases, namely, methanol and chloroform, 20 ml 0.5% NaCl solution was added and gently shaken by tilting the graduate cylinder four times, and the mixture was let stand for 30 min or until a clear separation is visible. The NaCl solution was added to prevent the formation of a stable emulsion. At this point the volume of the chloroform layer should be equal to the theoretical value minus the losses that occurred during blending and filtering. The theoretical volume of chloroform for 2 CHCl_3 :1MeOH is $50 \times \frac{2}{3} = 33$ ml.

For the determination of the yield of lipid extracted, 5 ml of chloroform layer was removed with a 10 ml pipet and transferred into a preweighed (to one mg) 10 ml beaker, and evaporated off on a Corning hot plate (PC-35) at the setting between low - 2, avoiding excessive heating and drying.

Calculation of yield determination

Lipid content (%)

$$= \frac{\text{lipid extracted (g)}}{\text{sample weight (g)}} \times \frac{(\text{chloroform layer} + \text{amounts lost})(\text{ml})}{5 \text{ ml}} \times 100$$

* The combined volume should be close to the theoretical calculated value.

Performance analysis

The analysis of variance was employed to determine the significance of variations due to treatments, namely, solvent ratio and sample size. The performance of the proposed method was evaluated for its reproducibility, precision and rapidity. Standard deviation and % variability from the mean were used to determine the degrees of reproducibility and precision, respectively.

RESULTS AND DISCUSSION

Table 1 shows how phase separation time after addition of 0.5% NaCl solution affected the amount of fat measured.

Table 1. Effect of phase separation time on the amount of lipid measured

TIME (hour)	CLARITY	LIPID measured (%)
1/2	opaque, but visible separation	16.50
1	slightly clear	16.84
3	clear	16.28
6	very clear	16.34

The amount of lipid measured remained constant regardless of the time of sampling as long as a visible separation had occurred. This indicates that there is no added advantage of lengthy waiting for clear separation. Although the opacity was a result of emulsion which contained some level of protenaceous substances, based on the above result no significant impact of such protenaceous matter was noted on the amount of lipid measured. Therefore, for time saving it is advisable to take sample out as soon as a visible separation occurs.

Table 2 and 3 present the differences in lipid extraction behavior between lean (cod) and fatty (mackerel) in relation to solvent ratio and sample size. For lean fish, the more polar solvent extraction system (2 MeOH : 1 CHCl₃) was more effective than less polar solvent extraction system (1 MeOH : 2 CHCl₃) especially as the sample size got larger. Compared to lean fish, fatty fish reacted sharply to the solvent ratio and sample size. As the polarity of the solvent increased with increased sample size, the efficiency of lipid extraction dropped drastically. For instance, at 1 MeOH : 2 CHCl₃ the lipid extracted from mackerel ranged from 11.1% to 9.96% while at 2 MeOH : 1 CHCl₃ it dropped from 10.6% to 1.90%.

This was clearly supported by much greater F values for effects of solvent ratio and sample size as well as their interaction for fatty fish than lean fish.

Table 2. Variations in lipid extraction for cod(lean fish) as affected by solvent ratio and sample size

SOLVENT RATIO (MeOH:CHCl ₃)	SAMPLE SIZE (gm/50 ml solvent)					F _{4,15}
	1	3	5	7	9	
2 : 1	1.20 a	1.12 ab	1.05 b	1.04 b	1.03 b	4.23
1 : 1	1.12 a	0.94 b	0.93 b	0.93 b	0.90 b	32.79
1 : 2	1.34 a	1.04 b	0.86 c	0.74 d	0.72 d	36.43

Effect of solvent ratio (F_{2,15}) : 30.12

Effect of sample size (F_{4,15}) : 51.28

interaction (F_{8,15}) : 10.94

* Different letters in the same row are significantly different (P < 0.05)

Table 3. Variations in lipid extraction for mackerel (fatty fish) as affected by solvent ratio and sample size

SOLVENT RATIO (MeOH:CHCl ₃)	SAMPLE SIZE (gm/50 ml solvent)					F _{4,15}
	1	3	5	7	9	
2 : 1	10.6 a	10.1 a	4.42 b	2.53 c	1.90 d	901.4
1 : 1	10.8 a	10.2 b	10.1 b	6.59 c	5.65 d	432.1
1 : 2	11.1 a	10.3 b	10.1 b	10.0 b	9.96 b	12.8

Effect of solvent ratio (F_{2,15}) : 1021

Effect of sample size (F_{4,15}) : 827

interaction (F_{8,15}) : 284

* Different letters in the same row are significantly different (P < 0.05)

The observation of lean fish favoring a polar solvent (2 MeOH:1 CHCl₃) is explained by the following lipid class analysis where lean fish lipid is mostly composed of membrane-bound phospholipids whereas the fatty fish is composed of triacylglycerols.

LIPID CLASS	COD (1) MACKEREL*	
	(% DISTRIBUTION)	
POLAR(phospholipids)	88.1	13.2
NON-POLAR(triacylglycerol)	11.8	86.8

* B. Trevino, 1991, Univ. of Rhode Island, unpublished data

Our earlier study (8) showed that maximum lipid extraction was achieved in the solvent ratio range from 2 CHCl₃:1 MeOH to 5 CHCl₃:1 MeOH and the solvent to sample ratio of equal to or above 10. No significant increase in lipid extraction was noted beyond 10 up to 17, but at the ratio of 50 (50 ml solvent to 1 g sample), significant increases in lipid extraction (Tables 2 and 3) was observed at most solvent ratios. However, the use of such a small sample will run into an unacceptable level of variability unless the homogeneity of the sample is assured.

Based on the above results, it was decided to use 2 CHCl₃:1 MeOH as an optimum solvent ratio for fatty fish such as mackerel whose lipid content is higher than 6% and composed of largely triacylglycerols, and 1 CHCl₃:2 MeOH for lean fish such as cod whose lipid content runs less than 2% and composed of mostly phospholipids; and to use 10 (50 ml solvent to 5 g sample) as an optimum solvent to sample ratio for both lean and fatty fish. It is possible to see incomplete extraction at this ratio when the lipid content is very high (> 20%), the portion not included in the measurement is likely insignificant.

The level of reproducibility and precision of the proposed method when mackerel was tested is shown in Table 4.

Table 4. The degree of reproducibility and precision of the proposed solvent extraction method.

SAMPLE WT(g)	CHCl ₃ LAYER(ml) + CHCl ₃ lost blending and filtering*	LIPID extracted(mg)	LIPID content (%)
5.01	29 + 4	141.68	18.66
5.01	29 + 4	141.32	18.67
5.02	29 + 4	142.56	18.74
5.00	28.8 + 4	141.57	18.57
MEAN ± S.D.			18.66 ± 0.069
VARIABILITY RANGE			± 0.37%

Mackerel was tested using 2 CHCl₃:1 MeOH at 50 ml solvent to 5 g tissue.

* 1 ml left in the homogenizer and 3 ml soaked into the filter paper during filtering

The average value of actual volume of CHCl₃ came out to be quite close to the theoretical volume, 33 ml, which is obtained from calculation. This suggests that if all procedural steps are taken carefully under the precise control, one can use the theoretical volume on a routine basis without getting the precise volume of CHCl₃ by hard squeezing every drop out of the wet homogenate. However, a close follow-up of the procedure is advised for the good monitoring purpose.

**A SINGLE SOLVENT EXTRACTION METHOD
FOR TOTAL LIPID DETERMINATION IN FISH TISSUE**

1. CUT THE SAMPLE INTO SMALL PIECES or GRIND IF LARGE.
2. HOMOGENIZE(or CHOP) IN A WARING BLENDER.
3. WEIGH OUT THE PASTE (5.0 - 5.1g; RECORD THE EXACT AMOUNT) and PLACE IT INTO AN EBERBACH HOMOGENIZER(8580)(250 ml capacity; FISHER 14-509-25).
4. ADD 50 ml SOLVENT : **1 MeOH - 2 CHCl₃** (for HIGH FAT FISH > 6%;
LIPID COMPOSED OF MOSTLY TRIACYLGLYCEROLS)
2 MeOH - 1 CHCl₃ (for LOW FAT FISH < 2%;
LIPID COMPOSED OF MOSTLY PHOSPHOLIPIDS)
5. BLEND FOR 1.5 MIN AT A MODERATE SPEED (USE A RHEOSTAT TO CONTROL THE SPEED)
6. FILTER THE HOMOGENATE THROUGH A COARSE FILTER PAPER (FAST SPEED), AND FUNNEL INTO AN 100 ML GLASS STOPPERED GRADUATED CYLINDER (FISHER 08-5650) (TOWARD THE END OF DRAINING, PRESS THE CAKE WITH THE ROUND TIP OF SPATULA TO SQUEEZE OUT THE REMAINING SOLVENT; BE SURE TO INCLUDE THE SOLVENT LOSSES DURING BLENDING (1 ml) AND FILTERING (3 ml) IN THE FINAL AMOUNT OF FILTRATE FOR CALCULATION)
7. ADD 20 ml 0.5% NaCl SOLUTION AND GENTLY SHAKE BY TILTING 4 TIMES.
8. STAND UNTIL A VISIBLE SEPARATION OCCURS (TAKES 30 MIN OR MORE)
9. USING A 10 ml PIPET, REMOVE AN ALIQUOT OF MORE THAN 5 ml OF CHLOROFORM LAYER AND TRANSFER AN EXACT 5 ml INTO A PREWEIGHED (TO ONE mg) 10 ml BEAKER.
10. EVAPORATE OFF THE SOLVENT ON A CORNING HOT PLATE AT THE SETTING BETWEEN LOW - 2, AVOIDING EXCESSIVE HEATING AND DRYING.
11. WEIGH THE BEAKER (TO ONE mg) AND OBTAIN THE WEIGHT GAIN AS A WEIGHT OF LIPID EXTRACTED.

Lipid content (%)

$$= \frac{\text{lipid extracted(g)}}{\text{sample weight (g)}} \times \frac{(\text{chloroform layer} + \text{amounts lost}) \text{ (ml)}}{5 \text{ ml}} \times 100$$

* should be close to the theoretical calculated volume.

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MOISTURE CONTENT OF NORTH CAROLINA BAY, CALICO AND SEA SCALLOP MEATS AT HARVEST, PROCESSING AND RETAIL

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During the fall of 1992, the U.S. Food and Drug Administration (FDA) issued an interim labeling policy for scallops treated with sodium tripolyphosphate (STP) and water. The policy required that scallops found to have a moisture content exceeding 80 percent but less than 84 percent water be labeled as an "X Percent Water Added Scallop Product" in addition to ingredient declarations (3). The new policy was designed to provide consumers with a better indication about the amount of water in the scallop products they purchased. Shortly after release of the new labeling policy, the U.S. Dept. of Commerce (USDC) Voluntary Seafood Inspection Program issued guidelines to its field inspectors for compliance with the FDA policy (2). Most major U.S. sea scallop processors were made aware of this policy and took the appropriate steps to monitor and label their products accordingly. A few smaller processors, namely southeastern U.S. bay and calico scallop processors, were less aware of the FDA interim labeling policy.

In March of 1993, a North Carolina scallop processor contacted the N.C. State University Seafood Laboratory for assistance in moisture determination of calico and bay scallop meats. The processor had requested USDC lot inspection but was not equipped to perform required moisture analyses for inspection services. Fortunately for the processor, samples sent to the NCSU Seafood Laboratory in March were found to contain less than 80 percent water. The product was USDC lot inspected, stamped and shipped to the buyer. Concern was expressed by other NC scallop processors for the 80 percent moisture labeling policy, especially for bay scallops whose water content was known to fluctuate with season and environmental conditions.

As a result of these concerns, NCSU Seafood Laboratory personnel, supported with a rapid response grant from the UNC Sea Grant College Program, began a study to assess moisture levels in North Carolina bay, calico and sea scallop meats. The study consisted of two parts: a random survey of scallop meat moisture at harvest, after processing and in retail establishments; and a controlled study using live bay scallops to determine the influence of salinity at harvest on natural meat moisture levels.

MATERIALS AND METHODS

Sample selection--Bay (*Argopecten irradians*), calico (*Argopecten gibbus*) and sea (*Placopecten magellanicus*) scallops were sampled during the months of April, May and June in 1993. Both shellstock and commercially shucked meats were obtained when available from North Carolina dealers and processors. Sea scallop meats were sampled from retail establishments only. Live bay scallops were purchased from a commercial mariculture facility on Harkers Island, North Carolina in order to study influence of salinity fluctuations (e.g., rainfall) on meat moisture at harvest. All shellstock was transported live above ice in coolers to the NCSU Seafood Laboratory, Morehead City. Scallops were hand-shucked, rinsed lightly with water to remove grit and particulate matter and patted dry with a paper towel. A minimum of 25 scallops was shucked for sample preparation. Commercially shucked scallops purchased at wholesale and retail stores were composites obtained from one gallon containers of scallops. All scallops sampled (shellstock and shucked meats) during the random survey and the controlled salinity studies were obtained in duplicate unless otherwise noted.

Sample preparation--A preliminary study of sample preparation was performed on scallop adductor muscles. Methods using a Waring commercial blender (No. 7011), Waring commercial food processor (No. FPC10) and Hobart commercial mixer (No. D-300-T) equipped with a grinder attachment and 3 mm perforated plate were compared. Fresh sea scallops were purchased from a local market for use in the preliminary study. A composite was made by mixing 3-one gallon containers of scallops. Scallops were subdivided into four equal portions. Three portions of the scallops were frozen (-23°C) for later use. Each method of preparation was performed in triplicate for each of the four replicates. Approximately one hundred grams were placed into a blender jar (Eberbach Corp., 500 cc capacity) and chopped, using the Waring blender, speed set on low, for 4-five second intervals. Approximately three hundred grams were placed into the Waring food processor and chopped for 20 seconds. Approximately three hundred grams were ground 3 consecutive times through the 3mm perforated plate attached to the Hobart mixer operating on a speed setting of two. All subsequent samples analyzed during the random survey of North Carolina scallops and the controlled salinity studies were prepared using the Waring blender method.

Sample analyses--Immediately after comminution, three 1-2 gram subsamples of scallop meat were placed in pre-dried, aluminum pans and weighed on a Mettler (No. H51AR) analytical balance. Total moisture was determined using the AOAC procedure with a preheated forced air convection oven (Blue M, Series JT-5747) set at 125°C for 4 hours (1). Dried samples were placed in desiccators to cool, then reweighed and the percent moisture calculated. The blender preparation method, analytical balance and forced air convection oven were used throughout the remainder of this study.

Random Survey of North Carolina Scallops

Harvest--Bay scallops (shellstock) were obtained from North Carolina dealers and processors during April and May. Additional bay scallops were purchased live from Carolina Cultured Shellfish, Harkers Island in May and June for use in controlled salinity experiments. Salinities occurring at harvest were recorded when available using an American Optical T/C Refractometer (Buffalo, NY). Calico scallops (shellstock) were obtained during off-loading of tractor trailer shipments from the

west coast of Florida during April and May.

All shellstock was hand-shucked in the laboratory, rinsed with water and patted dry with paper towels. All meats obtained were counted and weighed to determine the relative size of scallops (i.e., counts per pound) used for moisture determinations.

Processing-- Commercially processed bay (hand shucking) and calico (atmospheric steam shucking) scallops were obtained at the point of packing in one gallon containers. All samples were kept on ice in whirltop bags for transport. Commercially shucked scallops were treated in the same manner (rinsed with water and blotted dry) as shellstock in the laboratory. The number of adductor muscles per sample weight was noted for each sample analyzed.

Retail-- Random samples of bay, calico and sea scallops were purchased at various retail markets across North Carolina. Information concerning the source of scallops (i.e., wholesale distributors and processors) was obtained when possible. Samples were placed in whirltop bags on ice for transport and moisture analyses were performed within 36 hours of sample collection.

Controlled Study of Salinity Effects on Scallop Moisture

Bay scallops, harvested from natural surrounding areas, were placed in concrete raceways supplied with continuous running seawater at Carolina Cultured Shellfish. Natural salinity of the seawater during the study period was 30 ppt. Salinity conditions were modified by mixing seawater with continuous running well water. Live scallops were subjected to reduced salinities for 24 hours prior to sampling. Modified salinities conditions were 13, 15, 18 and 20 ppt. Scallops exposed for 24 hours to reduced salinities of 13 ppt (all) and 15 ppt (most) had perished. Only live scallops from modified salinities of 15, 18, 20 ppt and controls (30 ppt) were used in the study. All scallops were transported above ice in coolers and shucked immediately upon returning to the laboratory. Scallops were rinsed with water and patted dry with paper towels. For controlled salinity experiments, enough scallops were harvested to prepare samples in triplicates. During all other sample collections, enough scallops were harvested to prepare duplicate samples.

RESULTS AND DISCUSSION

A summary of results obtained during the preliminary study of sample preparation is given in Table 1. No significant effect between methods of preparation and moisture content determined for scallop meat was found. As a result of these findings, the blender method was selected for use in this study because smaller sample size and less time were needed in sample preparation.

Data obtained during the 1993 random survey of moisture in North Carolina scallops are summarized in Table 2. The study confirmed previously reported research that showed moisture content in bay scallops routinely exceed the 80 percent moisture level set under the FDA interim labeling policy (4). Mean moisture values for bay and calico scallops were within previously reported ranges for North Carolina harvested bay (74.15 - 83.66 percent) and calico (76.12 - 81.86 percent) scallops. Mean

values for sea scallops (86.311 percent) collected at retail under the present study were found to exceed the 84 percent maximum moisture content allowable under the interim labeling policy. These data support FDA's concern for excessive moisture content of sea scallops entering interstate commerce. The range for moisture in sea scallops previously reported was 74.63 - 80.97 percent (4).

Table 1. Comparison of preparation methods used for moisture analyses.

Method	Sample	¹ Mean \pm S.E.	² Mean \pm S.E.
Waring blender	1	87.537 \pm 0.026	87.495 \pm 0.222
	2	87.282 \pm 0.172	
	3	87.720 \pm 0.201	
	4	87.440 \pm 0.160	
Waring food processor	1	87.705 \pm 0.027	87.774 \pm 0.177
	2	87.835 \pm 0.105	
	3	87.922 \pm 0.238	
	4	87.630 \pm 0.073	
Hobart grinder	1	87.783 \pm 0.022	87.801 \pm 0.220
	2	87.813 \pm 0.209	
	3	88.070 \pm 0.076	
	4	87.536 \pm 0.040	

¹Values are mean \pm standard error of means for 3 replicates.

²Values are mean \pm standard error of means for 12 replicates.

During the Webb *et al.* (1969) study at N.C. State University, bay scallops were sampled at monthly intervals from known locations in Bogue and Core sounds, North Carolina. Calico scallops were sampled from the Atlantic Ocean, 10-20 miles southeast of Beaufort Inlet. All samples were analyzed for total moisture, protein, crude lipid, ash and glycogen. Total moisture was determined in a vacuum oven at 65 °C for 16 hours.

The study concluded that proximate composition varied widely among locations and months of harvesting for the species studied. Coefficients of variances were low for moisture and protein but high for lipid, glycogen and ash. Ranges for components were similar for each species, but the variation for any selected component (e.g., moisture) was large. As a result, variations in proximate composition were large enough to prohibit the establishment of standards for the U.S. scallop industry. The results supported the hypothesis by the authors that environmental factors contribute to differences in proximate composition.

Table 2. Summary of random survey of North Carolina scallop moisture.

Type	Value	Form		
		Shellstock	Processed (Dry)	Retail
Bay	N	24	9	3
	$\bar{X} \pm \text{S.E.}$	80.324 ± 1.274	83.043 ± 0.825	82.573 ± 1.358
	Range	77.471 - 82.547	81.815 - 84.294	80.791 - 84.085
Calico	N	10	11	1
	$\bar{X} \pm \text{S.E.}$	77.736 ± 1.051	79.279 ± 1.365	78.620 ± 0.142
	Range	76.134 - 79.179	77.700 - 81.181	-
Sea	N	0	0	13
	$\bar{X} \pm \text{S.E.}$	-	-	86.311 ± 1.630
	Range	-	-	82.883 - 88.133

To test the hypothesis, a series of controlled salinity experiments was conducted to study the influence of salinity on scallop meat moisture at harvest. Bay scallops were used for study because of proximity to the natural resource and documented meat moisture exceeding the minimum labeling requirement. Results from this study are summarized in Table 3.

Scallop meat moistures were found to be inversely related to salinity at time of harvest. A correlation coefficient of -0.951 was calculated from a linear plot of water salinities versus meat moisture (Figure 1). These data support the hypothesis that environmental conditions at harvest significantly contribute to differences in proximate composition of scallops. Data also support industry observations that bay scallop moisture can vary widely at harvest as a result of severe weather conditions such as heavy rainfall.

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Figure 1. Correlation of Meat Moisture with Water Salinity

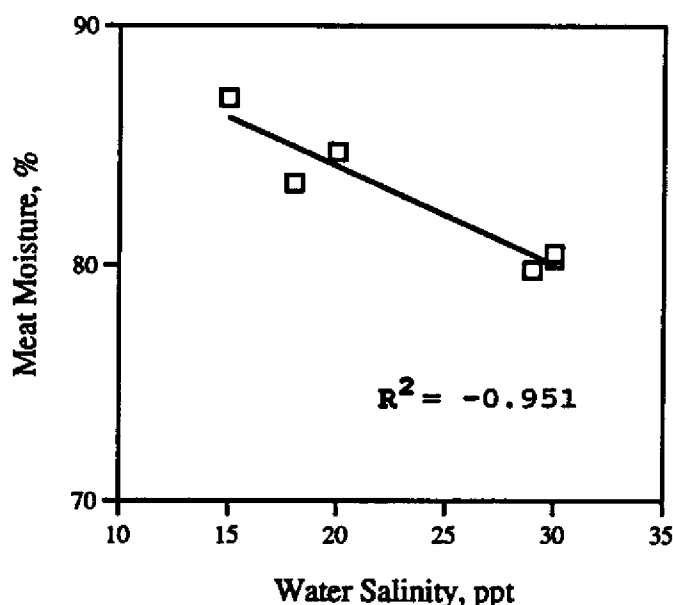


Table 3. Summary of controlled studies on the effects of salinity on bay scallop moistures.

Date	Temp (°F)	Salinity (ppt)	Add.s (No.)	Weight (g)	Counts (lb.)	% Moisture X ± S.D	% Moisture X ± S.E.
5-21	69	15	25	92.3	123	87.119 ± 0.023	87.010 ± 0.089
	69	15	30	105.9	129	87.010 ± 0.074	
	69	15	24	95.4	114	86.902 ± 0.001	
	70	29	25	60.7	187	79.932 ± 0.047	79.807 ± 0.089
	70	29	26	66.1	179	79.754 ± 0.093	
	70	29	23	61.9	169	79.734 ± 0.056	
5-25	76	20	25	93.0	122	84.568 ± 0.113	84.747 ± 0.195
	76	20	27	104.3	118	84.656 ± 0.061	
	76	20	24	86.0	127	85.019 ± 0.039	
	77	30	25	66.6	170	80.652 ± 0.118	80.260 ± 0.286
	77	30	25	74.0	153	80.187 ± 0.019	
	77	30	29	82.6	159	79.967 ± 0.059	
5-27	72	18	26	96.0	123	83.545 ± 0.038	83.438 ± 0.177
	72	18	25	83.7	136	83.581 ± 0.046	
	72	18	14	42.8	149	83.188 ± 0.223	
	72	30	25	72.4	157	80.744 ± 0.038	80.507 ± 0.237
	72	30	26	80.8	146	80.184 ± 0.044	
	72	30	31	78.1	180	80.594 ± 0.148	

Implications from this study are: meat moisture at harvest for North Carolina bay scallops contradicts current interim labeling requirements enforced by the FDA and USDC inspection programs; handling practices in commercial processing of scallops can significantly increase amount of moisture content without direct soaking of scallops in phosphate solutions; and regulatory policies based on moisture content must take into account both natural fluctuations in moisture and incidental water uptake in processing on a species by species basis.

CONCLUSIONS

This study verified previously reported research on natural fluctuations in moisture content associated with North Carolina bay scallops. It correlated the fluctuations in meat moisture to salinity conditions at harvest. The study provides evidence both in favor of and against the current interim labeling policy issued by the U.S. FDA. Further study of natural and incidental pickup of meat moisture appears warranted, based on the current regulatory policy and species to species variation demonstrated in this study.

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EXOPEPTIDASES AS BY-PRODUCTS FROM SHELLFISH VISCERA

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The hepatopancreas tissue from crustacean and molluscan processing is a rich source of exopeptidase enzymes. However, the digestive organ also contains endopeptidases and a relatively low ratio of exo- to endo-peptidase activity with azocasein substrate. Most of the endopeptidase activity of extracts was inhibited by serine proteinase inhibitors, especially soy bean trypsin inhibitor. Leucine amino peptidase, carboxypeptidase A and carboxypeptidase B were not retained by a SBTI-agarose gel at pH 7.5. This fraction had a relatively high $DH\%/A_{440\text{ nm}}$ and yielded high molecular weight product with azocasein substrate. These data show that the ratio of exo- to endo-peptidase activities in simple hepatopancreas extracts was enriched by Agarose-SBTI affinity chromatography.

INTRODUCTION

More than half of all industrial enzymes used today are proteolytic. (Godfrey and Reichelt 1983) Recently, digestive proteases from fish processing wastes have

been used as industrial process aids. (Liu and Pigott 1981; Haard, Feltham et al. 1982; Haard, Shamsuzzaman et al. 1983; Simpson and Haard 1984; Gildberg and Almas 1986; Simpson and Haard 1987; Reece 1988; Almas 1990; Olson, Johansen et al. 1990; Raa 1990; Simpson, Smith et al. 1991; Borresen 1992; Haard 1992) Commercially available proteolytic enzymes typically have a high ratio of endo-/exo-peptidase activity. A limitation to using proteolytic enzymes as food and other industrial process aids in some applications is that endopeptidases may cause defects like bitter off-flavors and excessive deterioration of the physical characteristics of the product. (Chobert et al., 1992) For example, acceleration of Cheddar cheese ripening normally causes quality defects in the product. (Law and Wigmore, 1983; Fox, 1982). Exopeptidases are also important agents to accelerate fermentation processes like fish sauce. (Vo, Kusakabe et al. 1983; Raksakulthai, Lee et al. 1986; Raksakulthai and Haard 1992; Raksakulthai and Haard 1992)

The digestive system of marine invertebrates appears to have a relatively high content of exopeptidases. For example, protein digestion with extracts from krill hepatopancreas results in release of 15% of the amino acids in casein as free residues (Osnes 1979), and the hepatopancreas of Atlantic short finned squid is a rich source of dipeptidylaminopeptidase I. (Hameed and Haard, 1985) Carboxy- and amino-peptidases isolated from the hepatopancreas of various crustacean species have recently been reviewed. (Haard 1993)

In this study we report the characterization of the exopeptidase activity of hepatopancreas extracts from two decapod species, crayfish and langostilla crab and a method to enrich exo-peptidase activity.

MATERIALS AND METHODS

Raw material

Crayfish (*Pacifistacus astacus*; CF), harvested in the wild near Sacramento, were obtained live from the California Crayfish Marketing Association, Sacramento, CA. Hepatopancreas's were removed from decapitated animals, frozen and stored at -70°C prior to enzyme

extraction. Langostilla crab (*Pleuroncodes planipes*; LC) were obtained during an experimental catch by "B/O El puma" vessel in Vizcaino Bay, Baja California Sur, Mexico; 28°40'61'' N and 114°35'54'' in September, 1991, at 125 m depth. The crabs were processed by pressing to obtain a proteolytic enzyme extract. (Garcia-Carreño 1992) The extract was frozen at -10°C until arrival at La Paz Harbor (BSC, Mexico).

Preparation of crude enzyme extracts

The preparation of crude proteolytic enzyme extracts was done as described elsewhere. (Garcia-Carreño 1992; Garcia-Carreño and Haard 1993) Briefly, the langostilla crab extract (LC) was obtained by centrifuging the press liquid, obtained on board the collection vessel, at 2500 x g for 15 min. to obtain a lipid free supernatant that was stored at -70°C. Crayfish hepatopancreas tissue was homogenized three times at 10 sec intervals in a Waring blender and centrifuged at 2500 x g for 15 min. to obtain a lipid free supernatant (CF) that was stored at -70°C.

Partial purification of exopeptidases

The LC and CF extracts were fractionated by affinity chromatography to obtain exopeptidase enriched preparations (LC-exo; CF-exo) and endopeptidase enriched fractions (LC-endo; CF-endo). To a chromatography column containing 5 ml Agarose 4%- SBTI (T-0637; Sigma Chemical Co., St. Louis, MO) in 50 mM Tris-HCl, pH 7.5, was added 1.5 ml of either LC or CF extract. After 10 min. at 25°C, the column was washed with 20 ml of 50 mM Tris-HCl and the A_{280nm} of 1.0 ml effluent fractions was recorded. The unbound fractions obtained from LC and CF affinity chromatography with A_{280nm} were pooled and designated LC-exo or CF-exo. The bound protein was then eluted from the column by washing with 20 ml of 0.10 N HCl. The fractions with A_{280nm} and pH of 1.5 or higher were collected, pooled, adjusted to pH 7.5 with solid Trisma base and designated LC-endo and CF-endo, respectively. The column was regenerated by washing with 50 mM Tris-HCl, pH 7.5 until the pH was 7.5. Fractions from repeated affinity chromatography runs were pooled and freeze dried prior to assay.

Protease assays

The appropriate conditions of time and enzyme concentration were selected to give a constant rate of reaction over the course of the assays.

The increase in absorbance of trichloroacetic acid (TCA)-soluble material after hydrolysis of azocasein at pH 7.5 (Tris-HCl, 50 mM; 25°C) was measured as described elsewhere. (Garcia-Carreno and Haard 1993) Normally the reaction was stopped 10 min after the addition of enzyme. One unit of activity was defined as the amount which increased the absorbance at 440 nm of TCA soluble material by 1.0 in 1 min.

Hydrolysis of azocasein was also monitored by pH-stat. The degree of protein hydrolysis (DH%) is the percentage of peptide bonds hydrolyzed during the course of the reaction and was calculated from the amount of standard alkali (0.02 N NaOH) required to maintain the pH of the reaction at 8.0. (Dong, Hardy et al. 1993) The reaction mixture consisted of 62.5 mg azocasein and enzyme in a 13 ml volume adjusted to pH 8.0 with NaOH at 12°C.

The increase in absorbance of trichloroacetic acid (TCA)-soluble material after hydrolysis of 1.5% casein at pH 7.5 (Tris-HCl, 50 mM; 25°C) was measured at 25°C. The reaction (0.5 ml volume) was normally stopped 10 min. after adding the enzyme by addition of 0.5 ml 20% TCA. The mixture was centrifuged for 5 min. at 6500 x g. One unit of activity was defined as the amount which increased the absorbance at 280 nm of TCA soluble material by 1.0 in 1 min.

The increase in absorbance at 410 nm of a solution of 1 mM benzoyl-arginine-p-nitroanilide (BAPNA) in Tris-HCl (50 mM, pH 7.5, 20 mM CaCl₂; 37°C) was measured by the method of Erlanger et al. (Erlanger, Kokowski et al. 1961). Bovine trypsin (1 mg/ml) from Sigma Chemical Co. (St. Louis, MO; type IX) was used as a control. One unit of amidase activity was defined as the amount of enzyme which would hydrolyze 1 μ M of BAPNA in one min (E₄₁₀ nm of p-nitroaniline = 8.8 cm²mmole⁻¹). Leucine aminopeptidase (LAP) activity was evaluated using freshly prepared 1.6 mM L-Leu-p-nitroanilide (LeuNA) in 60 mM sodium phosphate buffer, pH 7.0. The increase in absorbance at 405 nm was recorded at 25°C. LAP (1 mg/ml) from Sigma Chemical Co. (St. Louis, MO; L-5006) was used as internal control. One unit of LAP was the amount of enzyme which would release 1 μ M p-nitroaniline in one min.

Carboxypeptidase A (CPA) was evaluated using 1 mM hippuryl-L-Phenylalanine in 50 mM Tris-HCl, pH 7.5 containing 0.50 M NaCl. The increase in absorbance at

254 nm was recorded at 25°C. CPA (1 mg/ml) from Sigma Chemical Co. (St. Louis, MO; C-9762) was used as internal control. One unit of CPA was the amount of enzyme which would release 1 μ mole of hippuric acid in one min.

Carboxypeptidase B (CPB) was evaluated using 1 mM hippuryl-L-Arginine in 50 mM Tris-HCl, pH 7.5 containing 0.10 M NaCl. The increase in absorbance at 254 nm was recorded at 25°C. CPB (1 mg/ml) from Sigma Chemical Co. (St. Louis, MO; C-7261) was used as internal control. One unit of CPB was the amount of enzyme which would release 1 μ mole of hippuric acid in one min.

Chymotrypsin was assayed using 0.02 mM Succinyl-Ala-Ala-Pro-Phe-p-nitroanilide (SAPNA) in Tris-HCl, pH 7.8 containing 10 mM CaCl_2 . Bovine chymotrypsin from Sigma Chemical Co. (St. Louis, MO; C-4129) was used as a control. One unit of chymotrypsin was the amount of enzyme which caused an increase in $A_{410\text{nm}}$ in one min.

Inhibition Assays

SBTI, N-tosyl-L-Phe chloromethyl ketone (TLCK), N-p-tosyl-L-Lys chloromethyl ketone (TPCK), ethylenediaminetetraacetate disodium salt (EDTA) and 1,10 phenanthroline (PT) (Sigma Chemical Co., St. Louis, MO) were used according to Garcia-Carreno. (Garcia-Carreno 1992) The inhibitor and enzyme preparation were preincubated at 25°C for 60 min. prior to measurement of activity using one of the above enzyme assay methods.

Substrate- SDS PAGE

Electrophoresis and gel activity zymograms for casein hydrolysis by CF, CF-endo and CF-exo was done according to a previously described method. (Garcia-Carreno, Dimes et al. 1993)

Molecular size of azocasein hydrolysis products

Azocasein was assayed by method 2 and at one h intervals, a 20 μ L sample was removed from the reaction mixture and chromatogrammed on an Ultrasperogel SEC 2000 column (7.5 x 300 mm; Beckman, San Ramon, CA). The eluant was 100 mM KH_2PO_4 , 100 mM Na_2SO_4 and 0.05% NaN_3 at a flow rate of 1 ml/min and approximately 1000 PSI using a BioRad 1330 HPLC system (Richmond, CA).

RESULTS AND DISCUSSION

Inhibition of hepatopancreas endopeptidase activity.

Previously we reported that most of the digestive, protease activity in extracts of langostilla and crayfish was arrested by serine- and metallo- but not by aspartyl- and cysteine-proteinase inhibitors. (Garcia-Carreno and Haard 1993) Similar results were obtained in this study with LC and CF extracts using assay method 3 for casein hydrolysis (Table 1).

Table 1: The effect of serine and metallo-proteinase inhibitors on casein hydrolysis by crude enzyme extracts (LC and CF)

Inhibitor	Activity (%Inhibition)				
	LS	CF	Trypsin ^a	Chymotrypsin ^a	Thermolysin ^a
Control	0	0	0	0	0
SBTI, 10 μ M	75	69	93	25	ND
TLCK, 100 μ M	37	32	90	0	ND
TPCK, 100 μ M	1	0	0	75	ND
PT, 2 mM	21	14	ND	ND	94
EDTA, 1 mM	10	32	ND	ND	93

^a Standard enzymes were purchased from Sigma Chemical Co., St. Louis, MO. All assays were conducted using assay method 3. Data are average of 3 determinations.

Casein hydrolysis in both extracts was most sensitive to trypsin inhibitor indicative of serine proteases, particularly trypsin and/or trypsin-like enzymes. The activities of both extracts was inhibited somewhat less by TLCK, a more specific trypsin inhibitor, and was not significantly affected by TPCK, an inhibitor specific for the serine protease chymotrypsin. Part of the casein hydrolytic activity of these extracts was also inhibited by the metallo-proteinase inhibitors EDTA and PT.

Inhibition of hepatopancreas exopeptidase activity.

Assay of LC and CF extracts for specific exopeptidases (LAP, CPA, CPB) revealed the presence of relatively large amounts of activity. The specific exopeptidases were not inhibited by SBTI (Table 2).

Method to measure exo- to endo-peptidase activity ratio with azocasein substrate

The degree of hydrolysis (DH%) of azocasein by CF extract was similar to that observed with LAP standard (>12% after 4 h reaction) and considerable higher than that of trypsin standard (7%) (Fig. 1). Using the same reaction conditions, the increase in A440nm of these enzymes is shown in Fig. 2. Since the dye bound to azocasein is more or less randomly distributed on the peptide chain, hydrolysis of a given amount of peptide bonds by an endopeptidase like trypsin would be expected to yield much more dye in the TCA soluble fraction than would an exopeptidase like LAP. The ratio of DH% (method 2) to the activity using A440nm of the TCA soluble material (method 1) was much higher for the exopeptidase standard LAP (>80) than was that for the endopeptidase standard (≈ 34) (Fig. 3). The DH%/A440nm was taken as an indice of the ratio of exopeptidase/endopeptidase activities. The CF extract exhibited a relatively low ratio of exopeptidase/endopeptidase based on this criterion (Fig. 3). This is not surprising in view of the abundance of endopeptidases in hepatopancreas tissue of decapods. (Osnes 1979; Galgani and Nagayama 1988; Sakharov and Litvin 1990; Garcia-Carreño 1992; Garcia-Carreño and Haard 1993) The endopeptidases of hepatopancreas are mainly serine proteases, including trypsin, trypsin-like and digestive collagenases and elastases.

Table 2. The effect of SBTI on the exopeptidase activities of crude enzyme extracts (LC and CF).

Activity ^a	Inhibitor	LS	CF	LAP	CPA	CPB
(% Control)						
LAP	-	100	100	100	-	-
	SBTI, 10 μ M	100	103	100	-	-
CPA	-	100	100	-	100	-
	SBTI, 10 μ M	101	93	-	105	-
CPB	-	100	100	-	-	100
	SBTI, 10 μ M	108	100	-	-	100

^a The specific activities (Units/mg protein) of control enzymes were: LAP (LAP standard-0.500; LC- 0.004; CF-0.071); CPA (CPA standard- 0.210; LC- 0.086; CF-0.107); CPB (CPB standard-0.36; LC-0.12; CF-0.15). Data are average of 3 determinations.

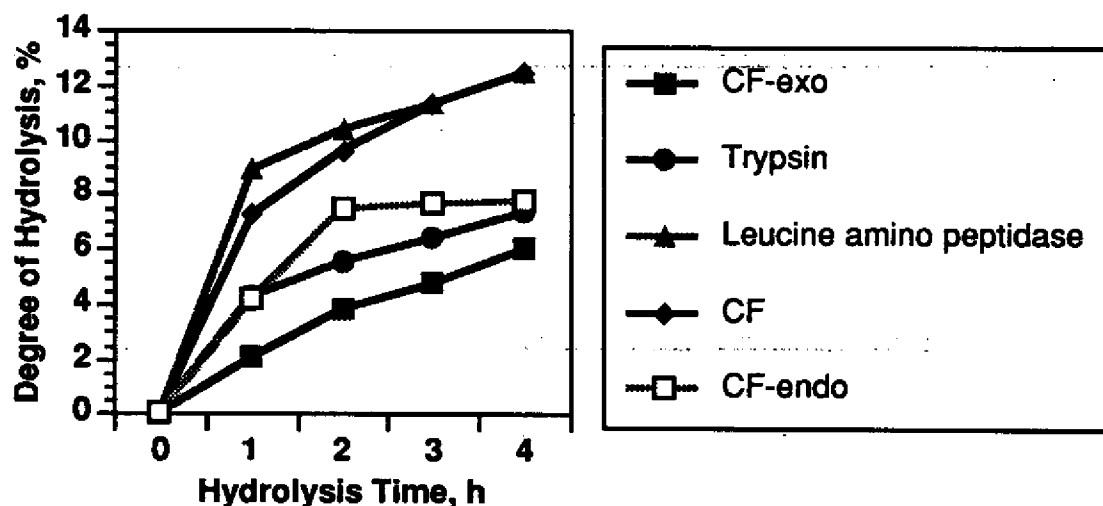


Figure 1. Degree of protein hydrolysis (DH%) of azocasein digested with leucine amino peptidase, crayfish extract, CF-endo fraction, trypsin, and CF-exo fraction.

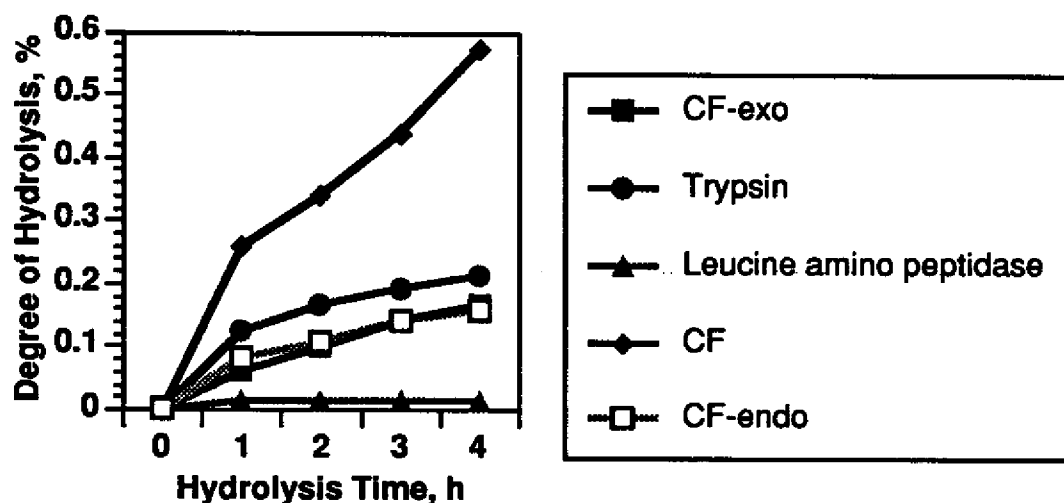


Figure 2. A440 nm of trichloroacetic acid soluble fraction of azocasein after digestion with leucine amino peptidase, crayfish extract, CF-endo fraction, trypsin, and CF-exo fraction.

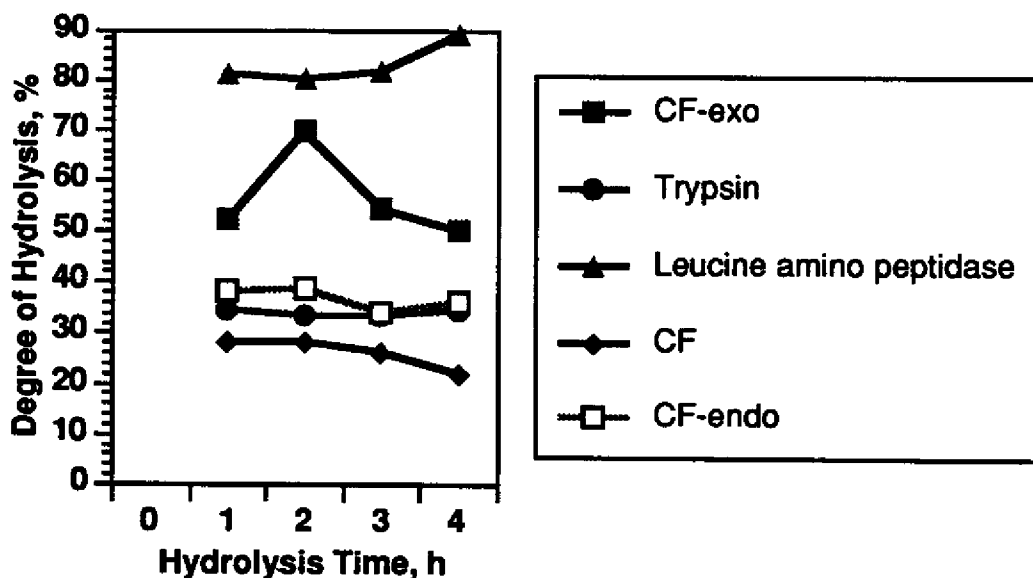


Figure 3. Ratio of DH% to A₄₄₀ nm during course of hydrolysis of azocasein by leucine amino peptidase, crayfish extract, CF-endo fraction, trypsin, and CF-exo fraction.

Method to increase ratio of endo- exo-peptidase activity of hepatopancreas extracts

On the basis of these results, we employed SBTI-affinity chromatography with the aim of reducing the endopeptidase activity of LC and CF extracts. The material which did not bind to the affinity ligand was enriched 13.7 and 4.8 fold in LAP specific activity and 342 and 2.7 fold in CPA specific activity for LC-exo and CF-exo, respectively. On the other hand, the enzymes which were bound to SBTI-Agarose were enriched 3.1 and 865 fold for chymotrypsin specific activity and 16.5 and 41.7 fold for trypsin amidase activity for LC-exo and CF-exo, respectively (Table 3).

To assess the ratio of exopeptidase/endopeptidase we measured azocasein hydrolysis using the pH stat (method 2) and A₄₄₀nm (method 1) for the affinity fractions from CF extract (Fig. 3). The ratio was increased from 21-28 for the CF extract to 50-69 for the CF-exo fraction. The CF-endo fraction also had a somewhat higher ratio than the starting material. The poor recovery of endoproteases after affinity chromatography was probably caused by the poor stability of trypsin and trypsin-like enzymes at acidic pH

.(Simpson and Haard 1984; Simpson and Haard 1985; Simpson and Haard 1987; Garcia-Carreno 1992)

Table 3: Specific activities of exopeptidase and exopeptidase fractions from LC and CF extracts after SBTI-affinity chromatography.

Fraction ^a	Activity(U/mg)				
	LAP U/mg	CPA U/mg	SAPNA U/mg	BAPNA U/mg	Assay 1 U/mg
LC	0.54	0.03	15.9	6.60	0.069
LCexo	8.00	8.88	0.00	0.00	0.100
LCendo	0.00	0.00	50.0	0.40	0.100
CF	0.26	0.07	0.37	0.36	0.005
CFexo	1.25	0.19	0.08	0.04	0.009
CFendo	0.00	0.00	320	15.0	0.144

^a Results are average of 3 determination. Recovery is the % of total units in the material applied to the column that was recovered in the material not bound to the column (LC-exo; CF-exo) and the bound material eluted with 0.1 N HCl (LC-endo; CF-endo).

Qualitative analysis of endopeptidases in hepatopancreas extracts.

SDS-PAGE analysis of endoproteinase activity of the CF extract revealed several active zones ranging in molecular weight from 12 to 72 KDa (Fig. 4) as reported by us previously. (Garcia-Carreno and Haard 1993) Both CF-exo and CF-endo fractions exhibited bands which are presumably indicative of endoproteinases. (Garcia-Carreno, Dimes et al. 1993) The CF-exo fraction retained active bands in the low molecular weight range, i.e., 40, 37, 30, 27 and 12 KDa, while the CF-endo fraction contained active bands of 57, 54, 40, 37, 33, and 30 KDa. We were not able to detect exopeptidases as active bands on these zymographs.

Molecular size of azocasein hydrolysis products.

HPLC analysis of the reaction products carried out by method 2 revealed that the CF extract and the CF-endo fraction, but not the CF-exo fraction significantly reduced the molecular weight of the azocasein substrate. Digestion of azocasein with the CF-exo fraction resulted in product with a relatively short retention time (7.2

min.), indicative of high molecular weight, compared to the untreated extract (CF). Digestion with the CF-endo fraction yielded product with the substrate peak mostly depleted and containing products with a relatively long retention time (17.5 min.). These data are consistent with the conclusion that exo- and endo-peptidases have been partitioned in these respective fractions.

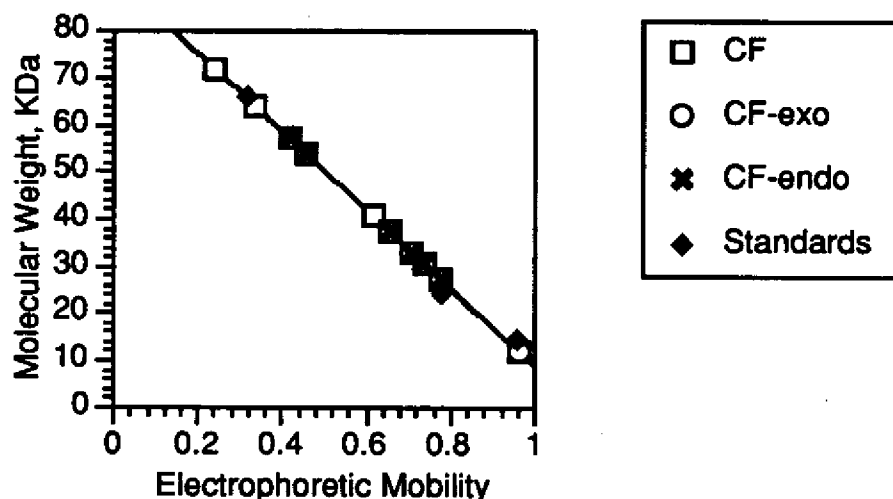


Figure 4. Polyacrylamide gel zymograms for casein hydrolysis. Molecular weights of active endo-proteinases: crayfish extract, CF-exo fraction, CF-endo fraction, and molecular weight standards.

CONCLUSIONS

Crude extracts from langostilla crab and crayfish hepatopancreas contained significant amounts of the specific exopeptidases assayed, LAP, CPA and CPB. However, the ratio of exopeptidase/ endopeptidase, evaluated by comparing the relative number of peptide bonds hydrolyzed (DH%) in azocasein with the A_{440nm} of TCA soluble product revealed a high percentage of endopeptidase activity during the course of the reaction. Most of the endopeptidase activity in these extracts are serine proteinase (Garcia-Carreno and Haard 1993), and this observation was confirmed in the present study. We observed that the specific exopeptidases examined were not sensitive to the serine proteinase inhibitor SBTI. Fractionation of extracts by Agarose-SBTI affinity chromatography resulted in a fraction

enriched several fold in specific exopeptidase activities and having a relatively high ratio of peptide bond hydrolysis to formation of TCA soluble peptides. The latter measure was shown to be an indication of high exopeptidase activity and low endopeptidase activity and should be of use in further work characterizing the exopeptidase/endo-peptidase ratio of complex mixtures of proteolytic enzymes. The reason for the poor recovery of endopeptidase activity from the bound fraction of the affinity column is not known at this time. It is possible that some enzymes were not released from the column and/or were inactivated by the low pH used in the second column eluant. Further research is needed in order to clarify this point. However, the demonstration that an enriched exopeptidase fraction can be simply recovered from shellfish digestive tissue has promise for production of exopeptidases for accelerating cheese ripening and other industrial applications.

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OXIDATIVE STABILITY OF VARIOUS OILS AS DETERMINED BY RANCIMAT METHOD

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Fats and oils are essential ingredients in the formulation and processing of foods in the food industry. The oxidative stability of fats and oils plays an important role in determining the quality of lipid-containing products (3). The stability of the omega-3 fatty acids and other essential fatty acids contained in oils is crucial to obtaining their beneficial health effects.

Oxidative stability is an important parameter for the quality assessment of fats and oils (5). Presently, the conditions and keeping qualities of an oil or fat, as well as the effectiveness of antioxidants may be assessed in terms of induction time (3). Autoxidation of fats or oils is a complex process initiated by free radical reactions involving unsaturated fatty acids. Hence, fats and oils containing unsaturated fatty acids are highly susceptible to oxidation. The oxidation process is affected by atmospheric oxygen, heat, heavy metals, exposure to light, and porphyrins. These factors generate free radicals which lead to formation of peroxide radicals and subsequent chain reactions leading to the formation of secondary products (5).

Natural and synthetic antioxidants can be added to oils or fats to inhibit autoxidation. The effectiveness of the antioxidants vary according to type and stability of an antioxidant as well as the microenvironment into which it is placed. Improved antioxidant activity can be obtained by addition of synergistic agents, such as citric acid and ascorbic acid, as well as reduction of factors favoring the formation of free radicals.

Therefore, it is the objective of this study to assess the oxidative stability of various oils and the effectiveness of selected natural and synthetic antioxidants to inhibit oxidation as measured by the Rancimat method and changes in fatty acids profile.

MATERIALS AND METHODS

The following oils were investigated at 100° C using a Rancimat 617 (METROHM, Herisau, Switzerland): peanut oil, rice bran oil, corn oil, encapsulated salmon oil, Menhaden oil (both industrial and dietary grades). The fish oils were donated by Zapata Haynie Corporation, Reedsville, Virginia.

Quadruplicate determinations of each sample were made by weighing 2.5 g into thoroughly cleaned reaction vessels, adding 50 ml of distilled water to the reaction vessels, and adjusting the air flow to 18 liters per hour. The conductivity of the breakdown products was measured on a stripe chart recorder of the instrument.

FATTY ACID ANALYSIS

The fatty acid composition of the oils was determined before and after oxidation on the rancimat to determine change in the fatty acids profile of the oils. The oils (25mg) were transesterified at 50° C overnight using 5% methanolic hydrochloric acid. Fatty acid methyl esters (FAME) were purified by eluting through pasteur pipets packed with Florisil (2 cm) with 95:5 Hexane: diethyl ether solution (2). Purified FAME were analyzed on an HP 5890 Hewlett Packard (Avondale, PA) gas liquid chromatograph containing a DB 225 capillary column (J & W Scientific Co., Folsom, CA) as described by Boyd et al. (1). Fatty acids were determined by relative retention time using 23:0 as an internal standard in both the samples and references standards (NuChek Prep, Elysian, MN).

ANTIOXIDANT ACTIVITY

The antioxidant activity of butylated hydroxytoluene (BHT), phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), and sphingomyelin (SPH) was determined by their addition to salmon oil and menhaden oil.

The BHT was added at 0.02% of 2.5g samples whereas phospholipids were added at 0.5% of 2.5g quantities of salmon and menhaden oils. Antioxidant activity of the oils was measured as the activity index (AI), and was defined as the ratio of the time required to oxidize a given sample heated at 100° C with and without the presence of an antioxidant. Therefore, quadruplicate analyses of the antioxidant property of all samples were determined by measuring AI and the change in fatty acid profile following heating.

RESULTS AND DISCUSSION

The induction time of the various oils with and without antioxidants is shown in Table 1. Corn oil and salmon oil showed the longest induction time whereas the menhaden oils showed the shortest induction time. The induction time appeared to be a function of the PUFA content and the degree of unsaturation of the oils as both menhaden oils had relatively short induction values when compared to the corn oil and the salmon oil. The PUFA content of the peanut and rice bran oils were similar (31.5 and 38.7%) compared to a much higher amount (60.5%) found in the corn oil.

Table 1: Induction Time of Various Vegetable Oils using the Rancimat*

<u>Oil</u>	<u>Induction Time (hrs)</u>
Salmon	8.10
Menhaden (dietary)	0.22
Menhaden (Industrial)	0.35
Corn	9.32
Peanut	6.75
Rice Bran	7.93

*Induction time- Time required for fatty acids to break down upon heating at 100° C

Table 2 shows the induction time and the AI of the fish oils. Comparisons of the induction times (Table 1) of the fish oils prior to the addition of the antioxidants and following addition (Table 2) shows that the effectiveness of the antioxidant varied with the type of oil. The BHT was more effective in the dietary menhaden oil showing only modest increases in stability when added to the salmon oil. The PL differed considerably in their ability to stabilize the oils showing differences between individual PL as well as differences between oils. Sphingomyelin appeared to be most effective antioxidant, followed by PC and PE, respectively. The SPH exhibited more antioxidant property in the salmon oil model system than in the menhaden oil systems. Comparisons of AI values and induction times of both SPH and PC to those of BHT in the salmon oil model system showed that both PL were more effective antioxidants under these test conditions. The SPH appeared to be a highly effective natural antioxidant having an induction time of more than 100 hr and an AI value approximately 10 times that of BHT.

The fatty acid profiles of the fish oils before and after oxidation, as well as with and without added antioxidants are shown in tables 3-5. The PUFA contents decreased with increasing oxidation. Salmon oil model systems containing BHT and SPH retained the greatest amount of PUFA following oxidation (Table 3). Both the PL and the BHT appeared to perform differently in the two grades of menhaden oils. In the dietary grade of menhaden oil (Table 4), the BHT was most effective, followed by PC and SPH with PE being least effective. Samples containing PC and PE were equally ineffective in preventing the extensive loss of PUFA. Only the BHT was used in the industrial grade of menhaden oil (Table 5). The BHT performed at an antioxidant level comparable to that of PE in the dietary grade of menhaden oil.

Table 2: Induction Time and Antioxidant Activity Index of Various Oils Following the Addition of Natural and Commercial Antioxidants(a)

<u>Oil</u>	<u>Induction time (Hrs)</u>	<u>Antioxidant Activity Index(b)</u>
Salmon+ BHT	10.93	1.3
Menhaden + BHT (Dietary)	1.53	7.2
Salmon + PC	60.00	7.4
Salmon +PE	21.50	2.7
Salmon + SPH	100.00	12.3
Menhaden + PC	0.20	1.0
Menhaden + PE	0.10	0.5
Menhaden + SPH	0.40	2.0

a- Induction is time required for fatty acids to breakdown upon heating at 100° C; b- Antioxidant Activity Index= Ratio of time required to oxidize sample with and without antioxidant present. BHT= butylated hydroxytoluene; PC=phosphatidylcholine; SPH= sphingomyelin PE=phosphatidylethanolamine

Table 3: Changes in fatty acid composition of salmon oil with and without

FATTY ACID	SALMON OIL	OXIDIZED SALMON OIL	SALMON OIL + BHT	SALMON OIL + PC	SALMON OIL + PE	SALMON OIL + SPH
Saturates	35.2	48.5	41.9	48.4	50.5	36.4
Monoenes	39.1	46.9	44.0	46.5	45.9	37.3
Dienes	2.2	0.1	1.5	2.4	1.6	2.0
n-3 PUFA	17.8	2.0	4.9	1.1	0.0	21.2
Total PUFA	23.1	4.6	14.1	2.5	3.1	26.2

Each row value is the mean of 4 determinations. BHT= butylated hydroxy-toluene; PC= phosphatidylcholine; SPH= sphingomyelin; PUFA = polyunsaturated fatty acid.

Table 4: CHANGES IN FATTY ACID GROUPS OF MENHADEN OIL (DIET.GRD.) WITH AND WITHOUT ADDED BHT AND PHOSPHOLIPIDS

FATTY ACID	M.O. DIETARY GRADE	OXIDIZED M.O. DIETARY GRADE	M.O. DIETARY GRADE + BHT	M.O. DIETARY GRADE + PC	M.O. DIETARY GRADE + PE	M.O. DIETARY GRADE + SPH
Saturates	33.7	56.0	39.6	45.1	46.9	44.0
Monoenes	25.9	37.7	31.3	32.8	33.7	32.2
Dienes	1.9	2.9	4.6	2.0	1.9	1.6
n-3 PUFA	27.8	3.4	20.6	15.6	13.7	13.1
Total PUFA	40.8	6.3	29.1	22.1	19.8	23.8

Each row value is mean of 4 determinations. BHT = butylated hydroxytoluene; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PUFA = polyunsaturated fatty acid

Table 5: Changes in Fatty Acid Groups of Menhaden Oil (Industrial Grade) With and Without BHT

FATTY ACID	M.O. IND. GRADE	OXIDIZED M.O. IND. GRADE	M.O. IND. GRADE+ BHT
Saturates	45.0	70.4	50.7
Monoenes	21.6	29.0	25.3
Dienes	1.9	0.5	4.8
n-3 PUFA	23.7	0.0	14.0
Total PUFA	33.4	0.5	24.0

Each row value is mean of 4 determinations. BHT= butylated hydroxytoluene; Ind= industrial grade; PUFA= polyunsaturated fatty acid; MO= menhaden oil

In general, the BHT and SPH were most effective in protecting the unsaturated fatty acids from oxidation. However, the effectiveness of the antioxidants appeared to be dependent on the fatty acid composition of the oils as well as the micro-environment of the oil. For example, the corn oil contained a very high content of 18:2 but had a relatively high AI value comparable to the more saturated fish oils. However, the fish oils contained more omega-3 PUFA with a greater degree of unsaturation per molecule. The presence of natural antioxidants, such as tocopherols, could explain the unusual stability observed in corn oil. The natural tocopherol levels as well as the mineral content of the oils were not determined and is still under study.

CONCLUSION

The Rancimat method appears to be suitable for the determining of the oxidative stability of oils as well as for measuring and comparing the relative effectiveness of different antioxidants. The effectiveness of antioxidants, however, appears to be dependent on a number of factors including the structure of the antioxidant as well as the micro-environment of the model system or food system. Changes in fatty acids profile of an oil also appears to be an effective tool that can be used to monitor the oxidation and deterioration of oils.

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CORRELATION OF SENSORY DECISIONS WITH CHEMICAL INDICATORS OF SEAFOOD ACCEPTABILITY

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The term "decomposition" when applied to seafood, refers to the breaking down of normal tissue in the fish into new products either by the action of bacteria, endogenous enzymes or the direct action of environmental abuse. The change from an acceptable state to a decomposed state may manifest itself by abnormal odors, taste, texture, color, physical breakdown of tissue, or other change in appearance. Seafood requires careful handling after removal from their normal aquatic environment if decomposition, or spoilage, is to be avoided. Despite decades of research, training and discussions with producers of fishery products, the commercial harvesting of fish is still frequently undertaken without sufficient cooling capacity on fishing vessels and spoilage begins before the fish reach port. Much of the spoilage found in commercial products is caused by the growth of bacteria resulting in numerous changes which are affected by the temperature at which decomposition occurs. Even in the presence of ice, bacteriological growth can advance if good sanitation practices are not observed.

A traditional approach for detecting the presence of decomposition in seafood has been the sensory analysis for odors. A determination of decomposition by organoleptic analysis is defined as that point when the first definite odors of decomposition are detected. Raw/ frozen fishery products are often classified as Class 1 (acceptable), Class 2 (decomposed), or Class 3 (advanced decomposed) while a Pass/Fail approach is used for processed products such as cooked shrimp or canned tuna.

The training of organoleptic analysts is a specialized area and is long and difficult. Moreover, analysts trained by different organizations can develop significant variations in the reject threshold point which can lead to disagreements over product acceptability. One consequence has been an intensified effort to develop mechanisms to support organoleptic

decisions by alternative measurements such as chemical tests. In addition, such tests should also be able to detect decomposition in adulterated fishery products where odors have been removed or masked by processing. Many tests have been explored as potential tests of decomposition but few have worked effectively at the rejection point as determined by sensory analysts. Some tests have been based on the volatile acids and amines while others have been directed at nonvolatile compounds such as hypoxanthine and long chain amines.

In this report, we will summarize some of our findings regarding the relationships between sensory evidence of decomposition and the presence of elevated amounts of cadaverine and putrescine for frozen products such as mahimahi and for canned tuna.

MATERIAL AND METHODS

Frozen Fish

Samples of mahimahi were prepared under contract with the Department of Food Science, University of Hawaii. Freshly caught fish were permitted to spoil in sea water at controlled temperatures ranging from ice water to 90 F. Fillets were removed, frozen and shipped to organoleptic experts for sensory analyses followed by chemical analyses for a variety of chemical indicators of decomposition. Samples of cod, scallops and plaice were prepared under contract for the Canadian Department of Fisheries and Oceans. The products were allowed to decompose only in ice. The frozen samples were later examined by organoleptic experts for their acceptability.

Canned Tuna

Samples designated as "authentic" were prepared by canning fish which were obtained from known sources after examination by experts. The canned fish were prepared in commercial plants under supervision. Samples designated as "commercial" were collected at the retail level or at import. The collected cans were analyzed by organoleptic analysts for odors of decomposition and then submitted to chemical analyses.

Methods

Samples were analyzed for cadaverine and putrescine by the gas chromatographic method published in 1981 (1) and updated in a Laboratory Information Bulletin (2) in 1993. All results are expressed as ug/g (ppm).

RESULTS AND DISCUSSION

Fresh\Frozen Seafood

The fillets from the mahimahi pack were randomly numbered and sent to three expert sensory analysts who assigned either class 1 (acceptable) or class 2 or 3 (decomposed) designations to the samples. The fillets were submitted to chemical analyses for a series of compounds including histamine, putrescine and cadaverine. The organoleptic results on the frozen fillets were then compared to the known spoilage treatment conditions, the sensory category of the fish in the round prior to freezing, filleting and storage, and to the chemical results. The fillets taken from fish spoiled at low temperatures were correctly identified as acceptable or decomposed in accordance with the decisions made on the whole fish prior to filleting. However, for fillets taken from fish decomposed above 70 F, a significant number of decomposed units were labeled as acceptable by the sensory analysts. Sixty-five percent of the fillets which contained scombrotoxic levels of histamine (>50 mg histamine/100g) were accepted by the analysts based solely on odors in the cleaned fillets.

Figures 1 and 2 show the rates of formation for histamine and cadaverine at 3 spoilage temperatures. Figures 3 and 4 show the variability in amine content between individual fish decomposed at the same temperature, 90 F. Similiar differences in organoleptic characteristics were also observed between individual fish. Histamine was frequently found in the samples spoiled at temperatures above 70 F while increases in cadaverine were observed in samples spoiled at all temperatures.

This data demonstrates that sensory testing faces limitations when applied to those samples most likely to cause toxic reactions in consumers. Intensified training on samples decomposed at high temperatures reduced the differences found in these experiments. Chemical testing, however, provides an improved level of safety in contrast to odor evaluation alone.

The potential extension of chemical testing to other seafood products was explored in preliminary experiments on samples of cod, scallops, and plaice decomposed on ice. Portions of a product, such as cod, were collected from ice storage at various times up to 18 days and frozen. After thawing, the fillets were examined by organoleptic analysts who passed or failed each unit. The samples were then analyzed for cadaverine and putrescine and the results compared to the sensory decisions. Figure 5 shows cadaverine levels in cod with increasing degree of spoilage. Both % rejection and cadaverine

Figure 1.

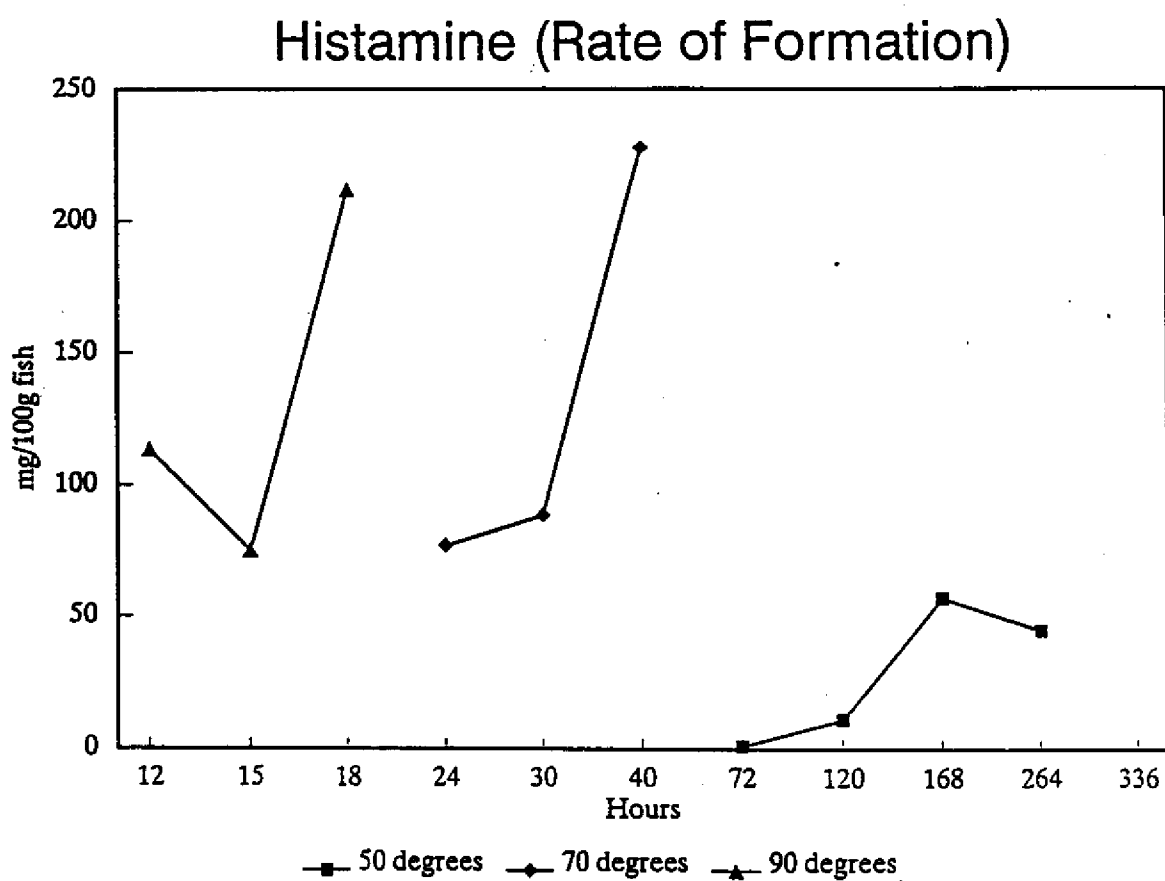


Figure 2.

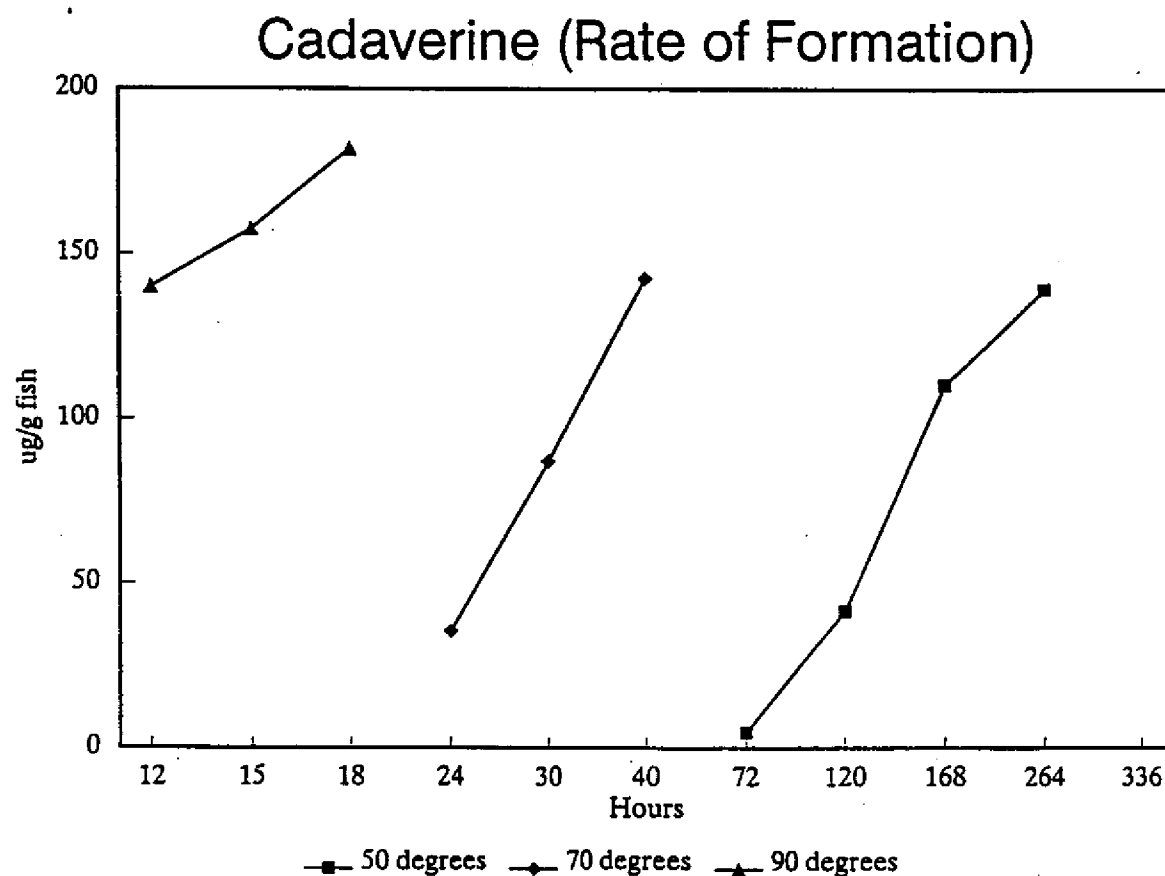


Figure 3.

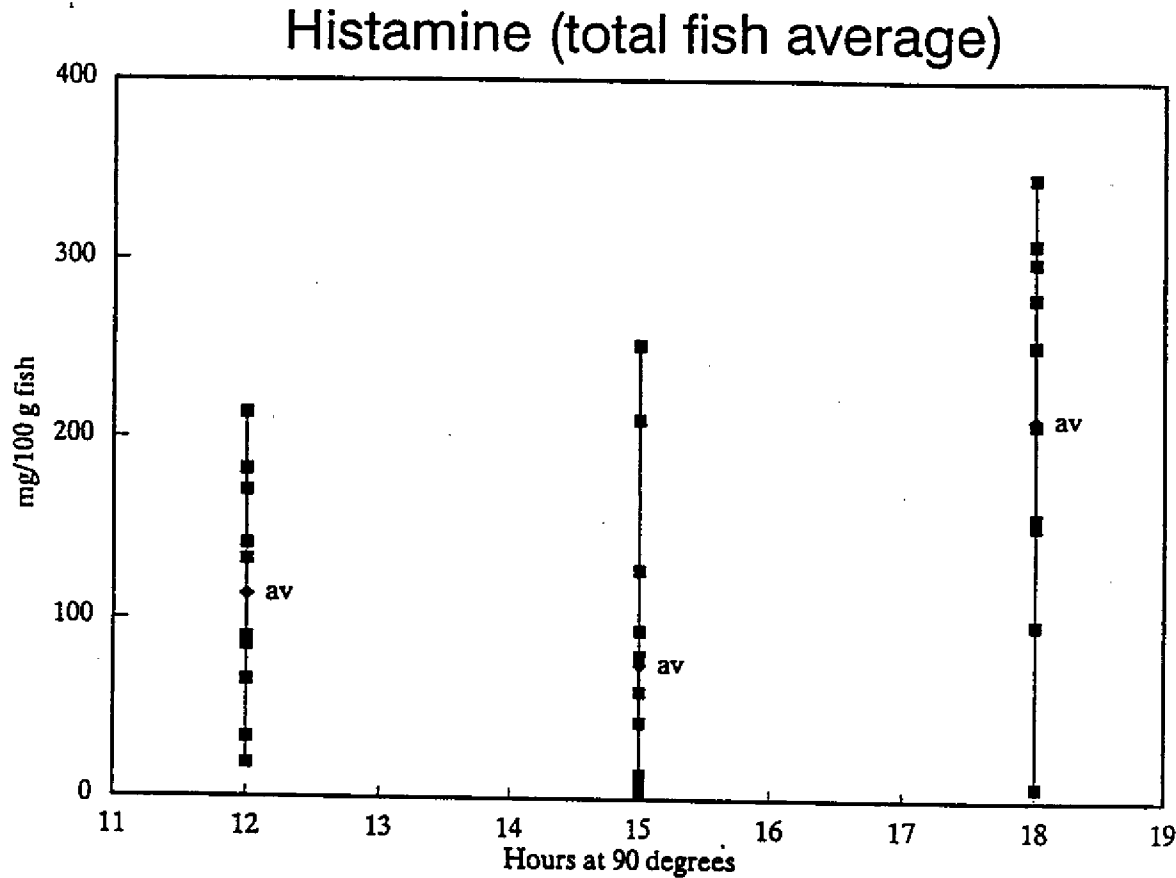
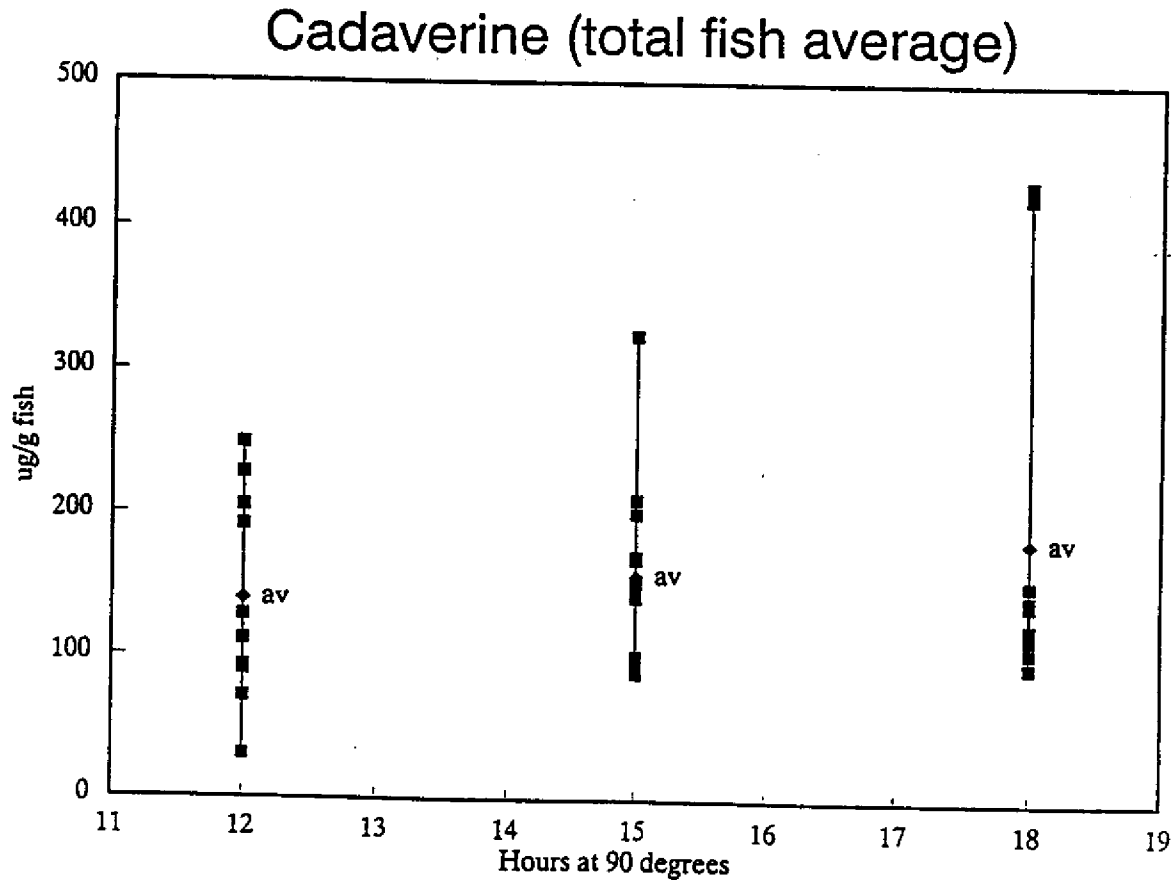


Figure 4.



increased as spoilage proceeded. Table 1 summarizes data from a second pack of cod. There was good correlation between increases in putrescine and sensory rejection of fillets during the first stage of deterioration. The greatest change in sensory rejection occurred when increases in both cadaverine and putrescine were observed.

Table 1. COD					
Sensory				ppm	
Spoilage Increment	#Subs	# Failed	% Failed	Put	Cad
1	4	0	0	0.4	0
2	16	2	13%	1.1	0
3	21	6	29%	1.1	0.2
4	23	21	91%	2.1	5.1

For fillets of plaice decomposed on ice, rejection by sensory analysts began when both cadaverine and putrescine were above 1 ppm (Table 2).

Table 2. PLAICE					
Sensory				ppm	
Spoilage Increment	# Subs	# Failed	% Failed	Put	Cad
1	8	0	0	0.6	0
2	22	9	41%	1.3	2.6
3	20	17	85%	1.6	4.1
4	20	18	90%	2.1	3.1
5	14	14	100%	5.9	13.6

For scallops, Table 3 shows that cadaverine was the first compound to increase when odors of decomposition were initially detected. A large change in rejection was observed when putrescine also was formed. The odor characteristics changed dramatically at this point and suggested that a second mode of decomposition had become predominant.

elevated levels of histamine or other spoilage metabolites. Table 4 summarizes the levels of these two compounds as determined in cans of tuna prepared from good quality fish which were examined prior to processing into government packs for workshops and the levels found in samples of commercial canned tuna which have not been found to contain levels of decomposition or other defects sufficient to warrant

TABLE 4					
LEVELS OF DIAMINES IN PASSABLE CANNED TUNA					
		PUTRESCINE		CADAVERINE	
	n	<u>AV</u>	<u>SD</u>	<u>AV</u>	<u>SD</u>
Workshop Samples ^a	41	0.3	0.2	0.2	0.2
Commercial Samples ^b	504	0.6	0.3	0.3	0.5
a. samples of passable examined prior to canning b. samples not containing sufficient evidence of decomposition to cause rejection					

regulatory action. For government samples where the initial condition of the fish was known, putrescine averaged 0.3 ppm and cadaverine averaged 0.2 ppm. Commercial products contained an average of 0.6 ppm putrescine and 0.3 ppm cadaverine. A frequency distribution of these data is given in Figure 6.

Cans of tuna prepared from samples containing decomposed tuna usually contained increased amounts of the diamines. Exceptions can originate from cans originating from acceptable parts of commingled fish and those circumstances arising from decomposition mechanisms which do not produce amines. Table 5 summarizes data from packs of decomposed tuna prepared by the industry, and exhibit changes representative of high temperature decomposition, and a sample pack prepared by FDA at temperatures below 70 F. A frequency distribution

Figure 5.

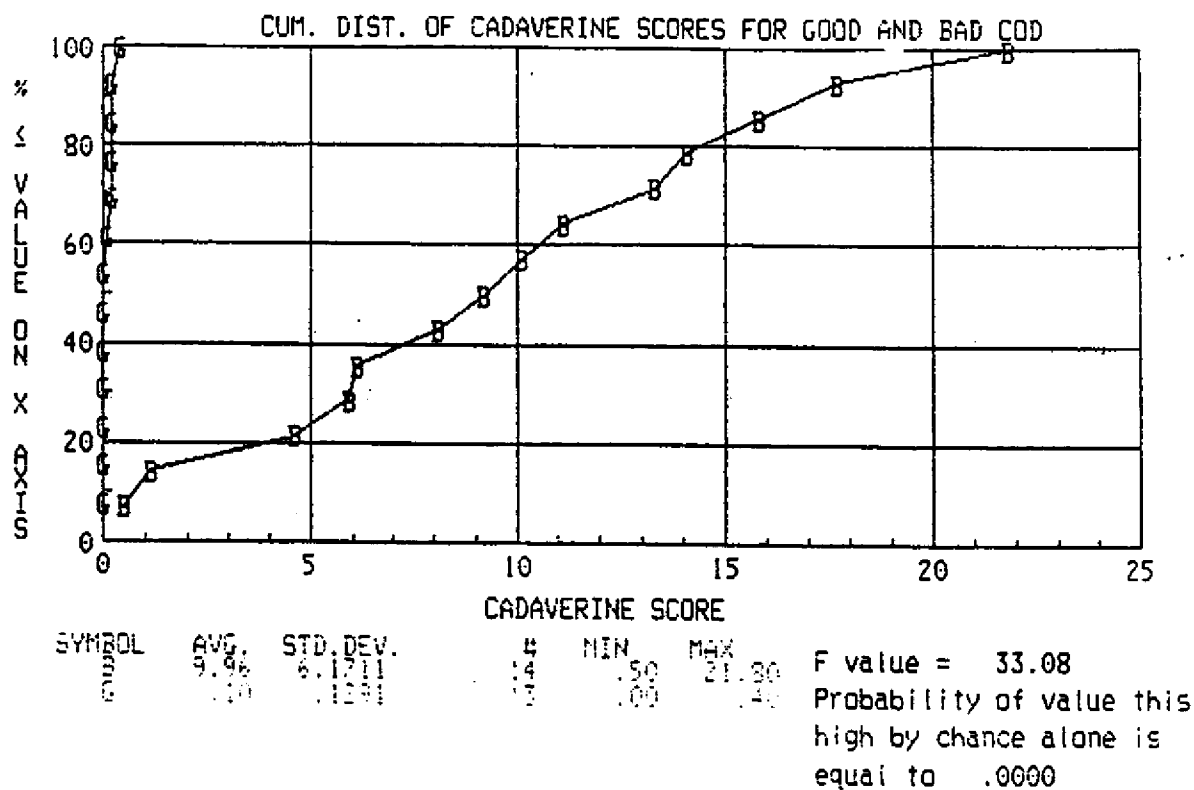
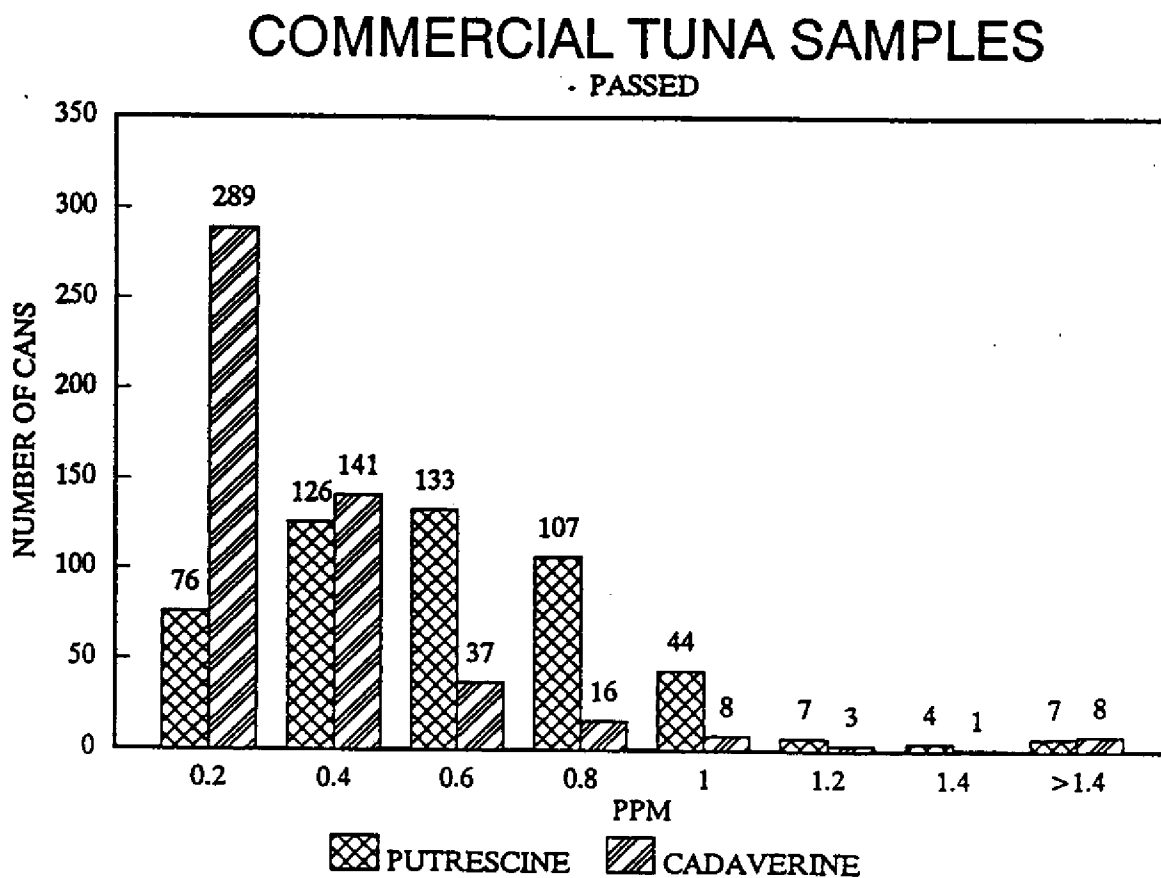


Figure 6.



Much more work is needed but the initial results are encouraging.

Table 3. SCALLOPS					
Sensory				ppm	
Spoilage Increment	# Subs	# Failed	% Failed	Put	Cad
1	6	0	0	0	0
2	14	3	21%	0	3.3
3	17	2	12%	0	1.5
4	17	14	82%	12.4	4.2

Canned Tuna

The use of chemical testing for the support of sensory decisions on canned tuna acceptability has had a long history. Groups of tests have been evaluated for correlations with sensory scores with mixed results. Only histamine has been routinely applied because of its relationship to scombrototoxic poisoning. Other compounds which have undergone evaluation have included formic and acetic acids, volatile acid number, total volatile bases, hypoxanthine, volatile reducing substances, and ethanol. It was found that the most effective approach was to apply 2 or 3 tests in order to cover several potential spoilage routes. A particular difficulty revolved around the heterogenous distribution of some spoilage metabolites within decomposed fillets which exemplified themselves as wide variations between cans of product which contain only a small section of a fillet. An ideal chemical indicator of decomposition would need to form over a large area of a decomposing fillet so that increases could be detected in many of the cans prepared from such fish. As we have described in talks before this association in the 1991 meeting, the use of cadaverine and putrescine most closely fills these needs.

A summary of available data shows that the baseline levels of the two amines in samples of good quality acceptable canned tuna are low. Such samples do not exhibit odors of decomposition, honeycomb,

TABLE 5						
DIAMINE LEVELS IN SAMPLE PACKS OF CANNED DECOMPOSED TUNA						
			PUTRESCINE (ppm)		CADAVERINE (ppm)	
TYPE	QUALITY	# DETNS	<u>AV</u>	<u>RANGE</u>	<u>AV</u>	<u>RANGE</u>
I	DECOMPOSED	42	2.0	0.6 - 7.2	20.4	1.7 - 145
	ADVANCED DECOMPOSED	94	3.6	0.4 - 35	55.5	1.9 - 280
II	DECOMPOSED	24	0.9	0.3 - 1.5	4.4	0.8 - 8.0
	ADVANCED DECOMPOSED	10	1.3	1.1 - 1.6	9.2	4.3 - 16.9
I Industry Prepared Pack II FDA 1992 Sample Pack						

of the data from the industry packs is given in Figure 7 and from the FDA pack in Figure 8. In the industry packs, approximately 86% of the values determined for cans prepared from decomposed tuna and 94% of the values determined for cans prepared from advanced decomposed fish exceeded 5 ppm cadaverine. The analyses of cans prepared from the FDA pack exhibited cadaverine values generally in the range 1 to 3 ppm. The importance of the lower range of cadaverine values is underscored by data collected from regulatory samples in which organoleptic analysts reported odors of decomposition, and in which diamine levels in the 1 to 2 ppm range are frequently found. On occasion, some of the individual can values will remain low because of anatomical differences in metabolite levels, differences in rates of spoilage between individual fish, and alternative routes of decomposition in tuna.

The GLC method has been applied to more than 200 cans of tuna collected from samples failed by FDA analysts due to the presence of decomposition odors. In such samples, the spoilage odors and the maximum diamine levels are sometimes found in different cans within a sample for reasons previously discussed. Can to can correlations are not practical for any group of indicators of decomposition on a routine basis. The objective is to identify CID's that are present in a minimum

Figure 7.

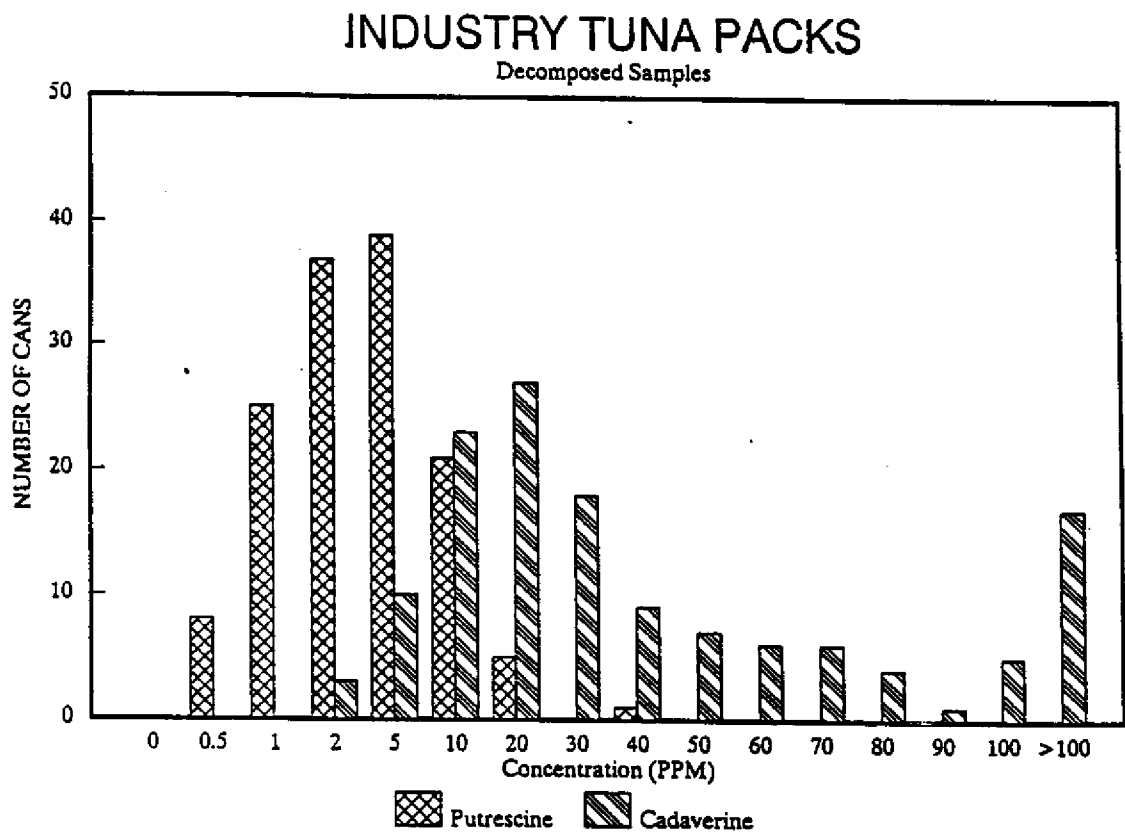
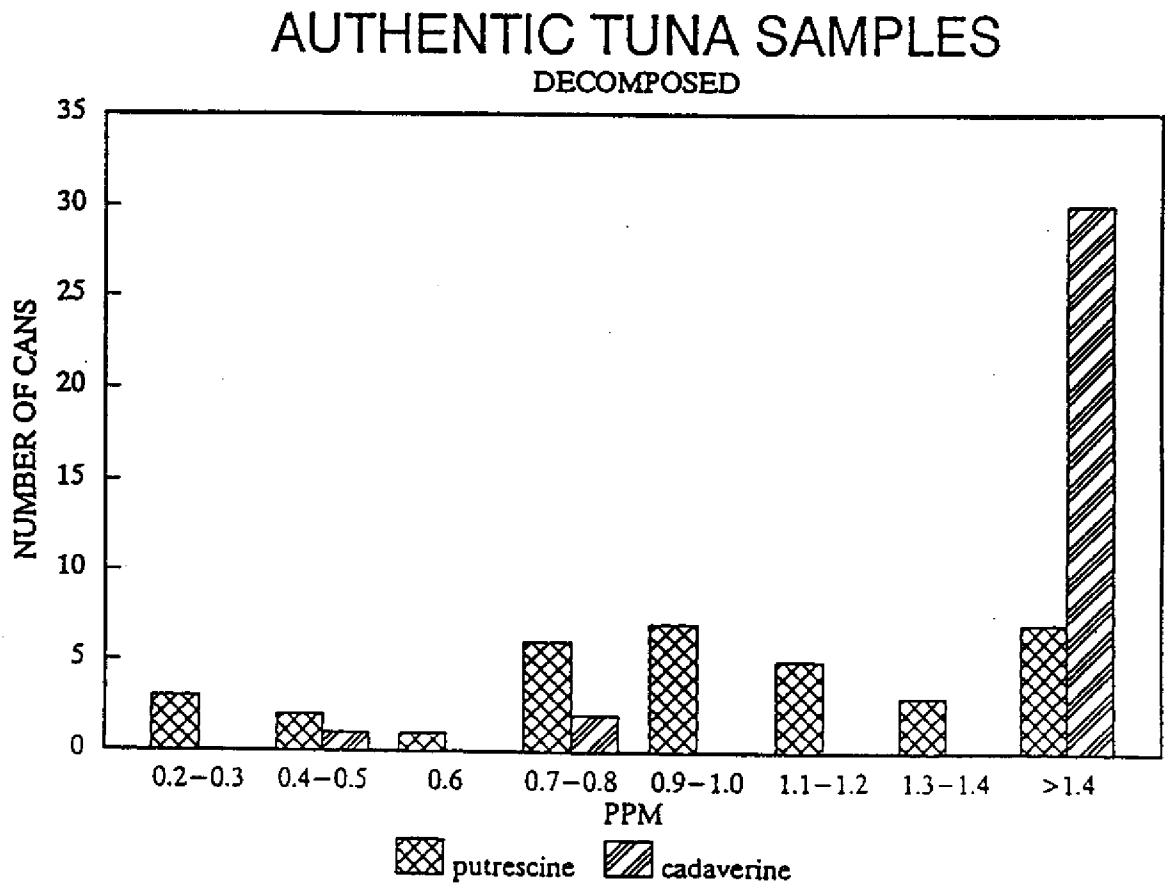


Figure 8



of 2 cans when odors of decomposition are found in 2 or more cans within a sample of 24 cans. However, as a special exercise a can to can comparison was conducted on a set of 128 cans collected from commercial samples rejected for the presence of decomposition odors. One hundred seven units were failed by organoleptic experts and increased levels of the diamines (> 0.6 ppm) were found in 98 of the same cans. An additional 128 analyses were conducted on other samples and for the combined 256 data points, over 90% of the cans contained > 0.6 ppm of at least 1 diamine. For regulatory purposes, 2 of 24 cans (8.3%) in a sample must contain evidence of decomposition to support a recommendation or to confirm action. A graphical representation of the data is given in Figure 9. Cadaverine is the more predominant compound present at increased levels in these samples.

Approximately 68% of the cadaverine values in cans from failed samples exceeded 1.4 ppm and 23% were in the range 0.6 to 1.4 ppm. The relationship between cadaverine and putrescine levels is shown in Figure 10. Generally, cadaverine is more frequently found at increased levels than is putrescine. Several values from cans collected from passed samples exceeded 0.6 ppm. These represent samples that did not meet the criteria for rejection but may have contained some amount of substandard material. Similarly, some cans from failed samples contained less than 0.6 ppm. They may represent commingled lots of fish as well as the anatomical variations expected in large fish. Regardless of the efficiency of any chemical test, only a battery of chemical tests will cover all spoilage possibilities. The sample size selected and degree of homogeneity of a commercial lot will remain limiting factors.

CONCLUSIONS

The use of cadaverine and putrescine levels to confirm sensory findings of decomposition appear very promising. The addition of these 2 compounds to the applications in which histamine is currently used should help resolve questions where different sensory decisions are at issue, assist in detecting decomposition when adulteration or processing has obscured odors, and improve the selection and evaluation of training samples for organoleptic analysts. These compounds form under many decomposition temperatures and should provide improved detection of spoilage in frozen products such as mahimahi. Interlaboratory testing of the chemical methodology is underway.

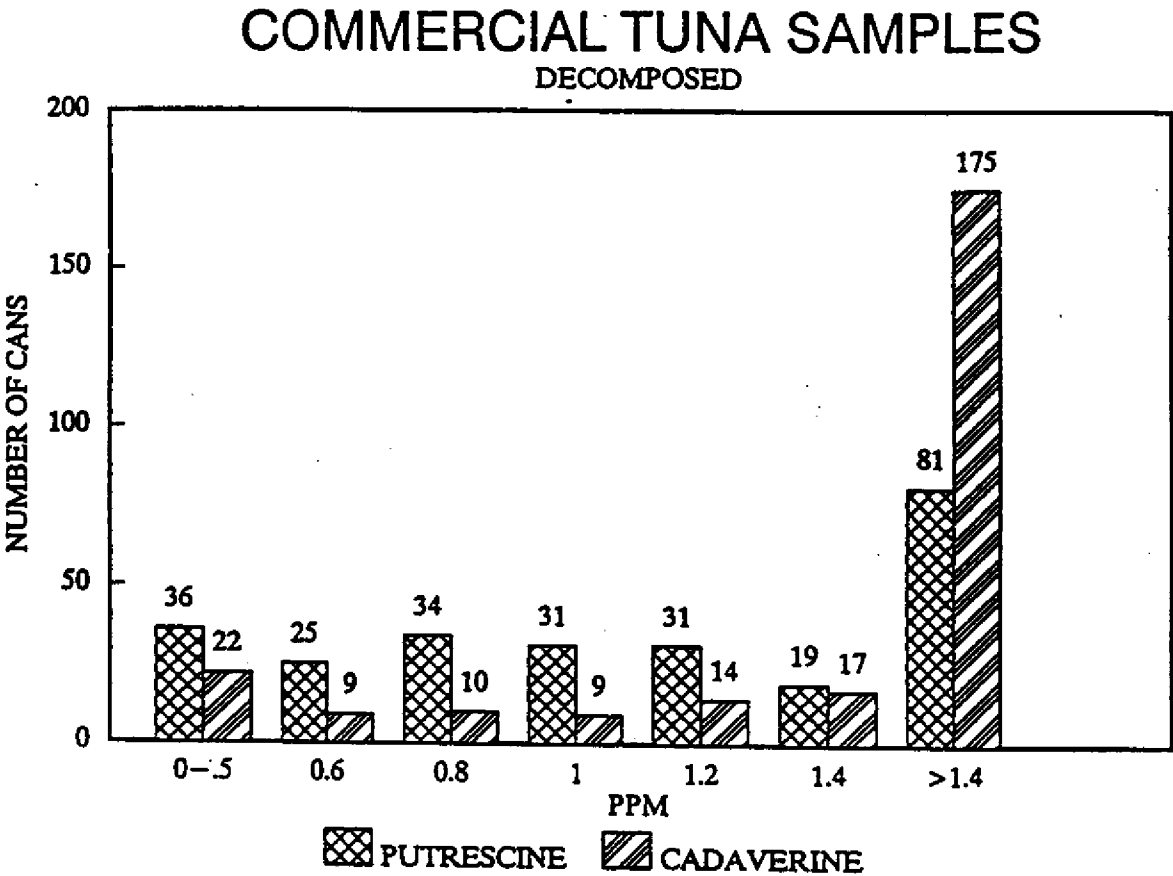
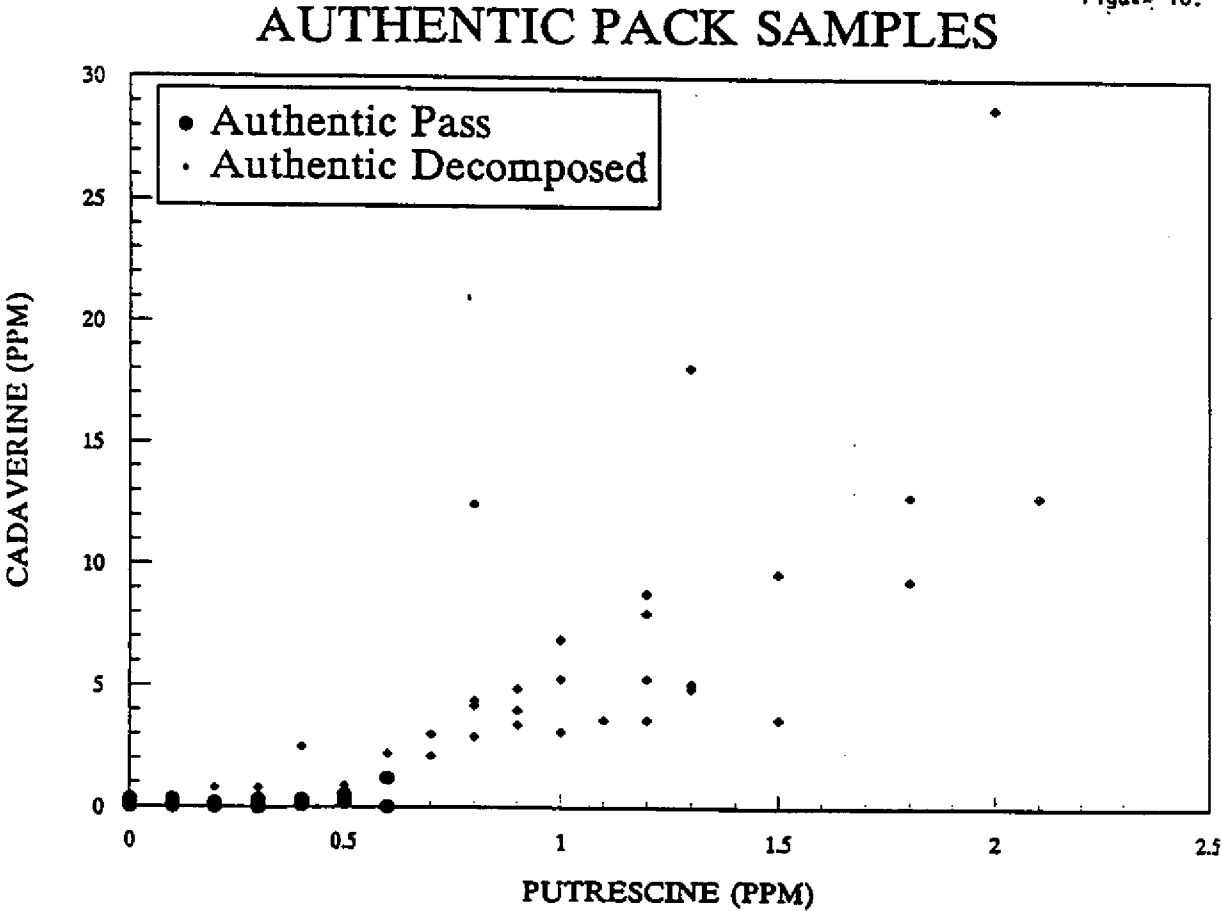


Figure 10.



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EFFECT OF DIFFERENT LIPID LEVELS ON THE UPTAKE AND
DEPURATION OF PETROLEUM HYDROCARBONS BY ATLANTIC SALMON*

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Salmon in their natural environment are known to avoid parts of the water column contaminated with the water-soluble fraction (WSF) of crude petroleum (18), but farmed, pen-reared salmon cannot avoid this type of oil pollutant. Abnormal situations that could be encountered by this species are high doses of WSF for short periods of time or low doses for prolonged periods. The first possibility was in fact the case in the Shetland Islands in January of 1993 where salmon stocks were exposed for several days to the oil spill of the Braer tanker, and could certainly occur in Canadian salmon farms located on shores where heavy tanker traffic increases the chances of oil spills. Low-level exposure can also be found along shorelines where normal vessel activity can contribute to low-level pollution (20) or where hydrocarbons are spilled into natural drainage systems or leached from beaches, marshes or other contaminated sites.

Previous research in our laboratory (9,10) of exposing cod and scallops to the WSF showed that lean (< 1% fat) fish or shellfish muscle could be tainted through picking up aromatic hydrocarbons ranging from benzene to approximately the methyl naphthalenes. Such edible muscles were rapidly freed of the resulting objectionable hydrocarbon flavors by holding the animals for a few days in clean seawater. It is generally accepted that the more fat in a tissue, the greater the potential for uptake and storage of lipophilic chemicals (5,8,12,27,28). To test this hypothesis, we performed a series of short-term exposures to different concentrations of the WSF to establish the threshold levels that could lead to tainting in Atlantic salmon (*Salmo salar*). With this background, we then studied the uptake and depuration rates of hydrocarbons in edible muscle, and determined the relationship with the lipid content of whole muscle and different muscle types.

MATERIALS AND METHODS

Materials and Exposures

Flotta North Sea crude oil (Esso Petroleum Canada) was stirred with cold seawater in a ratio 1:99 v/v for 24 h followed by 48 h settlement to obtain a WSF of about 10 to 13 ppm hydrocarbon. This was diluted with fresh seawater to obtain different exposure levels ranging from 0.1 to 3 ppm WSF. Salmon were starved for 24 h before the exposures. Typically, during the

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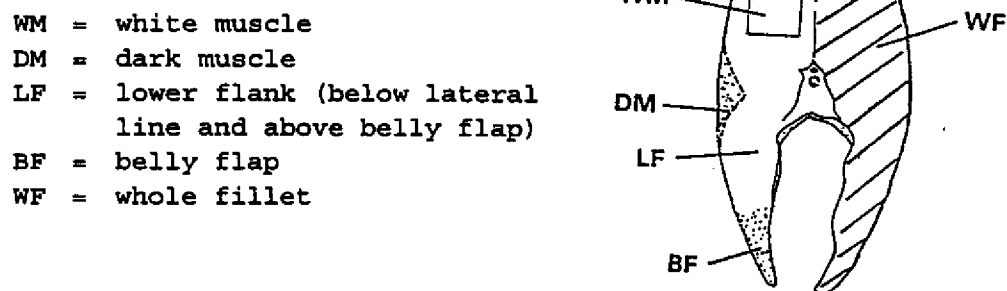


Figure 1. Cross section of salmon muscle showing the different parts sampled.

experiments to find the threshold level of tainting, 3 salmon were placed in flow-through seawater of an appropriate concentration, and a control group was held in clean seawater. Exposure time was fixed at 8 h. During the depuration experiments, fish of two different lipid contents (average weights 650 g and 1,470 g, respectively) that had been exposed to 1.5 ppm WSF for 8 h, were depurated for 4 weeks in clean seawater. Fish were sacrificed for analysis at various times during the elimination period. All exposure experiments were done at the Aquatron Laboratory, Dalhousie University with a seawater temperature of 5-9 °C and dissolved oxygen levels of 80-110 % saturation.

Fish analyses

Fish were killed by a blow on the head, washed and transported on ice to the Canadian Institute of Fisheries Technology at the Technical University of Nova Scotia. Skin-off muscle fillets from half of each fish were pooled and frozen for sensory evaluation. The other pooled halves was used for hydrocarbon analysis by gas chromatography (GC), taking samples of whole fillet muscle, belly flap, the flank portion below the lateral line and above the belly flap (henceforward called lower flank), dark muscle and white muscle (Figure 1). All samples were frozen at -35 °C until analysis. In the case of the low-lipid fish, hydrocarbon content was only determined on whole muscle. The hydrocarbons were recovered from muscle tissues by a modification of the steam distillation technique of Ackman and Noble (3).

Lipid content was determined gravimetrically by CHCl_3 -MeOH extraction of each sample following the method of Bligh and Dyer (6). The lipid classes were examined by chromatography on silica gel (Chromarods-SIII) followed by quantification on an Iatroscan TH-10. About 20 µg of the extracted lipids, dissolved in chloroform, was spotted on each Chromarod. The Chromarods were conditioned in a constant humidity chamber for 5 min before putting into developing tanks. The development was carried out for 50 min in hexane/chloroform/isopropanol/formic acid (80:14:1:0.2 v/v). The quantification of lipid classes was based on the calibration of Chromarods using authentic standards (4). Sensory tests were performed on samples thawed at room temperature, minced in a food processor, and cooked in a microwave oven before presentation to the sensory panellists. Preferences were ranked among the samples by the triangle test (15).

RESULTS AND DISCUSSION

Threshold level

The hydrocarbons of the WSF were dominated by monoaromatic compounds, and exhibited a pattern similar to those previously reported (13). There were no mortalities or signs of fish distress over the 8 h of exposure in any of the experiments, and fish rapidly took up the hydrocarbons from the seawater. However, during the depuration stages fish only ate occasionally, possibly being disturbed by the added effect of handling, and also by the bioaccumulated hydrocarbons (22). Figure 2 shows the relationship between the sensory panel results and hydrocarbon concentration in the exposure water. The threshold level of WSF that produced tainting was around 0.4 ppm. In agreement with this result, Brandal et al. (7) found that salmon exposed to 0.04-0.05 ppm WSF were tainted only after 4 days of continuous exposure. Experiments currently being carried out in our laboratory have shown that after 96 h exposure to 0.15 ppm, the fish were significantly tainted, but that fish exposed to 0.1 ppm for the same time, were not. This suggests that the threshold level of WSF that can taint salmon depends, among other reasons discussed below, on the exposure time as well as the concentration of WSF. The amount of hydrocarbon in the whole muscle determined by GC was correlated with the sensory panel results and could be ranked in accord with sea-water concentrations (Figure 2). Fish that were found by the sensory panellists not to be tainted still had a hydrocarbon content between 0.9 and 4 ppm, and tainted fish contained approximately 5 to 31 ppm hydrocarbon in muscle. As reported earlier (13), monoaromatics, naphthalene and methyl naphthalenes were the major components accumulated (results not shown).

Uptake and depuration

A) Low vs high fat fish

When fish of ~3 % and ~10 % muscle lipid content were exposed, the uptake was correlated with lipid content (Figure 3). In the lower-fat fish, the total uptake was 30 ± 1 ppm while the fattier fish accumulated 53 ± 7 ppm. Clearly, whole muscle bioconcentrated the hydrocarbons by several orders of magnitude with respect to the surrounding WSF. This relationship between muscle lipid content and amount concentrated was supported by the results found in other fish such as cod by Ernst et al. (9). In those studies, it was observed that cod exposed to 1.38 ppm WSF did not have detectable levels of hydrocarbons and with concentrations as high as 3 ppm WSF cod accumulated only 0.7 ppm hydrocarbons. Cod is a lean fish with 1 % or less muscle lipid.

The major hydrocarbons assimilated were in all circumstances the low molecular weight aromatics with one or two rings. Aromatics with higher molecular weights than dimethyl naphthalenes were virtually absent. Alkane concentrations were not very much affected by the exposure, essentially because alkanes also were not present in the WSF due to their low solubility in seawater. Alkanes are relatively odourless and almost tasteless, and are not involved in the tainting of muscle. High molecular weight hydrocarbons were found to be more slowly depurated than the more volatile benzene to xylene range. The same trend of different rates of depuration for low vs high molecular weight aromatics was also found by Rice et al. (24) in coho salmon fry.

Fish took a minimum of 11 days to depurate, a result similar to that of McKeown (18) with juvenile coho salmon. The depuration rate was slower in the fatty fish. In both experiments, panellists were unable to detect the taint after 11 days, and as

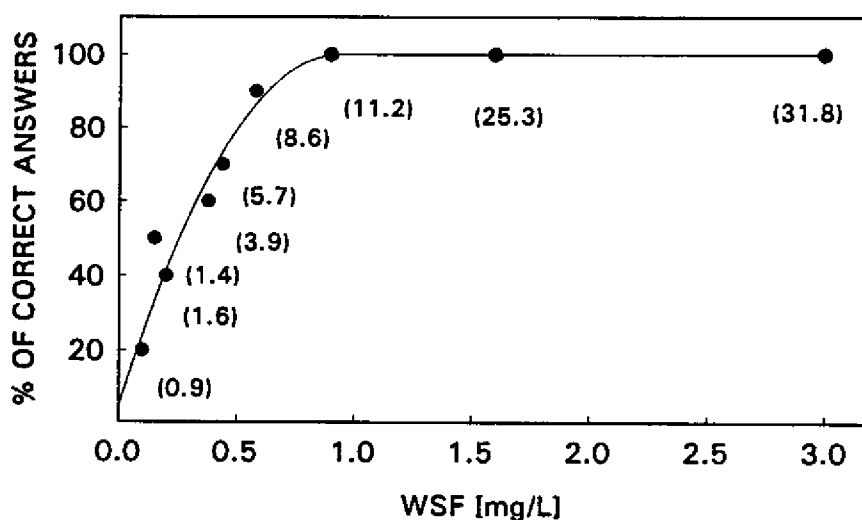


Figure 2. Relationship between sensory panel results and hydrocarbon concentrations of the exposure water and salmon muscle (figures in parenthesis). 70% correct answers are needed for 5% significance.

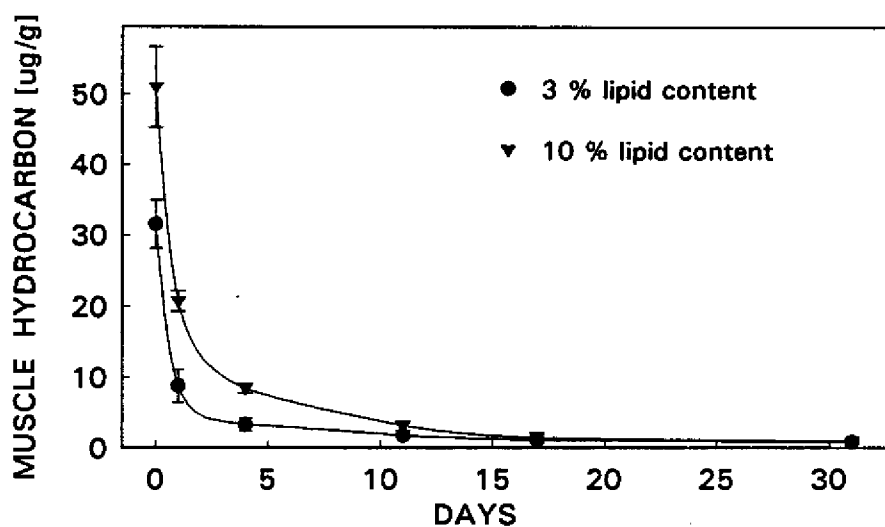


Figure 3. Depuration of Atlantic salmon whole muscle for 31 days following 8 h exposure to 1.5 ppm WSF (average of duplicate analyses). After day 10 no off flavor samples could be identified by sensory evaluation.

found in the threshold experiments, muscle was not freed of hydrocarbons even after 31 days of depuration. After almost exactly the same time frame sensory panellists could still detect low levels of hydrocarbons in petroleum-tainted arctic charr *Salvelinus alpinus* (16)

B) Different muscle types

When we compared the uptake and the depuration rates of different sections of the muscle tissue (Figure 4), the hypothesis was again corroborated. Salmon used in these experiments contained 3-10 % muscle fat, which is within the normal range of salmon (2,21). However, the fat distribution is quite dependent on the part of the muscle analyzed. Figure 5 shows the total lipid content of the different parts of muscle. Belly flap contains the highest lipid content (32.3%), while white muscle has only 3.5% fat. Dark muscle and the lower flank muscle have an intermediate lipid content of 15.7% and 12.7%, respectively. The relationships among fish lipid classes (Table 1) in different tissues have been discussed elsewhere (1). Although dark muscle had more lipid than white muscle (Figure 5), it is reputed to be metabolically more active. This and the fact that there was a high proportion of triacylglyceride in all tissues (Table 1) means that depuration of stored hydrocarbons will not always be related directly to total lipid.

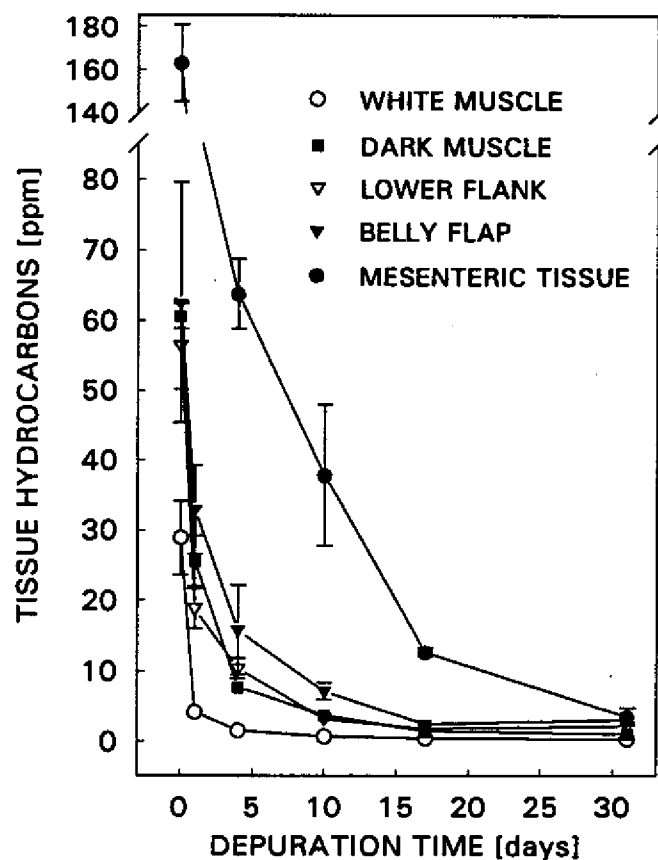


Figure 4. Depuration of different muscle types of Atlantic salmon for 31 days following 8 h exposure to 1.5 ppm WSF (average of triplicate analyses).

Table 1. Lipid Classes of Salmon Muscles and Mesenteric Tissue (as percentages of total lipid).

Tissues	TG	FFA	CHO	DG	PL
White muscle	81.39	0.62	1.14	0.61	16.25
Dark muscle	90.34	0.60	0.73	0.56	7.76
Lower flank	94.69	0.26	0.54	0.36	4.15
Belly flap	98.13	TR ^b	0.26	ND ^c	1.61
Mesenteric tissue	99.30	TR	0.21	ND	0.47

a TG-triglyceride, FFA-free fatty acid, CHO-cholesterol, DG-diglyceride, PL-polar lipids.

b TR-trace (<0.1%)

c ND-not detected

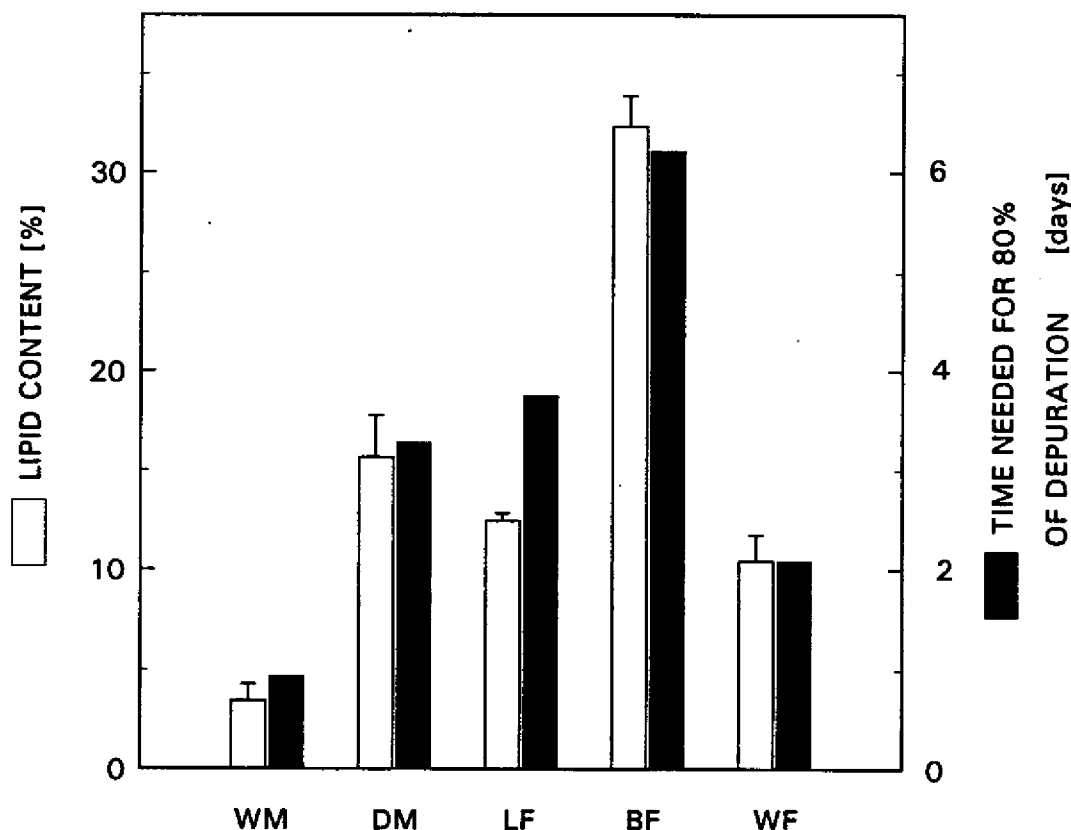


Figure 5. Relationship between lipid content of whole fillet and different muscle types and the time needed to depurate 80% of the hydrocarbons (average of triplicate analyses. WM = white muscle, DM = dark muscle, LF = lower flank, BF = belly flap, WF = whole fillet).

The results of the uptake and depuration of tainting hydrocarbons by these muscle types are shown in Figure 4. At the end of the 8 h exposure, it was found that the higher the lipid content of the tissue, the more the hydrocarbons were accumulated. This has also been observed by Stegeman (25) when studying the tainting of oysters by petroleum hydrocarbons. This was not only observed in muscle but also in other tissues of the fish, hence the mesentery is included in Figure 4. The bioconcentration clearly paralleled the fat content in the order belly flap > dark muscle > lower flank muscle > white muscle. The white muscle which contains only one fourth the lipid content of dark muscle or lower flank muscle, shows one half the level of hydrocarbons (28 ppm compared with 60 ppm). On the other hand, the difference in the uptake of hydrocarbons between belly flap and dark muscle is quite small (63.4 ppm compared with 60.6 ppm of dark muscle), although the lipid content of belly flap is two times higher than that of dark and lower flank muscle. This may suggest that the accumulation of hydrocarbons into the salmon tissues on this short term exposure had not reached a saturation plateau, and there is also the possibility that the blood circulation of hydrocarbons to the belly flap may be slower than to other muscle tissues. This last hypothesis is further sustained by looking at the hydrocarbons depurated after one day; more than half of the tissue hydrocarbons were released from the different parts of the muscle, except for the belly flap which needed 1.9 days. However, differences in the lipid class composition may also be involved.

After 17 days of depuration, most of the hydrocarbons in the muscle tissues had been depurated. The monoaromatics depurated more quickly than the higher molecular weight di- and polyaromatic hydrocarbons (PAH). The higher solubility in body fluids of monoaromatics and also their faster enzymatic metabolism (14,26) may be responsible for their relatively swift depuration compared to the higher molecular weight hydrocarbons that could be sequestered in lipid tissues.

The longer-retained hydrocarbons were even better correlated with the lipid contents of the tissues. Thus, at day 17 there were only 0.38 ppm hydrocarbons present in the white muscle, while in dark muscle and belly flap the levels were 1.35 ppm and 2.36 ppm respectively. The depuration of the remaining polyaromatic hydrocarbons may well take months.

Figure 5 also illustrates the time needed to depurate 80% of the hydrocarbons in different muscles types. It is clear that the tissues with a higher lipid content should display a lower depuration rate than tissues with a lower lipid content. The belly flap needs 6.2 days, while white muscle needs only 0.94 days to reach the same extent of hydrocarbon removal. Muscle with intermediate lipid values (i.e. dark muscle and lower flank) with similar lipid levels (~14%) accumulate similar amounts of hydrocarbons (60.6 ppm for dark muscle and 56.4% for lower flank). They also depurated to 80 % at a similar rate (3.3 days for dark muscle and 3.8 days for lower flank muscle).

Our findings are different from those of Miller et al. (19) who compared the PCB content of relatively lean (5.5-17.1% lipid) lake trout (*Salvelinus namaycush namaycush*) with that of the much fatter (21-27% lipid) siscowet (*S. namaycush siscowet*). In their opinion there were similar rates of PCB bioaccumulation on a basis of mg/kg of PCBs in skin-on edible portion fillets. At this time we simply draw attention to our results for depuration of different tissues. Although PCBs are commonly held to be readily stored in marine animal body depot fats, this view is not always

based on sound data, methods, samples, and understanding of fats (5). Intestinal absorption of organochlorines has also been split into two possible routes, lipid co-transport and chemical diffusion (11). Long-term, low-level exposure to PCBs may not be the same as our "pulse" exposure to WSF hydrocarbons. Similarly exposure of cod *Gadus morhua* to petroleum oils in production areas on a presumed long-term basis showed little evidence of tainting by sensory evaluation but uptake and retention of naphthalene and similar high molecular weight molecules could be detected (23). Since we have actually recovered WSF hydrocarbons from isolated salmon muscle adipocytes (Zhou et al., unpublished results) we are confident that these cells and their stored hydrocarbons are indeed the source of tainting in the longer term.

CONCLUSIONS

Regardless of the tissue analyzed and the lipid content of the fish, the rates of depuration were compatible with two pools of tainting hydrocarbons. One, fairly rapidly depleted, could represent the hydrocarbons in body fluids. The second pool, more long-lasting, could be that held in fat storage cells (adipocytes).

Although the hydrocarbons accumulated correlated with the tissue total lipid content, some variation not explained by this variable factor was also observed (i.e. belly flap vs dark muscle). It is felt that different lipid class compositions and/or the different circulation in the tissues may also be involved. These possibilities are now being studied.

A major question confronting the salmon aquaculture and processing industries is the influence that petroleum may have on the edible quality of exposed fish (17). The belly flap seems to be the main storage site of hydrocarbons in the muscle and was the major contributor to the retention of hydrocarbons observed in the whole muscle, so the frequent consumer practice of excluding the belly flap when consuming salmon steaks would eliminate the major part of any lipophilic pollutant present in the salmon fillet. Commercial size salmon can have more muscle fat (up to 12-14 % lipid) than the fish so far analysed in this experiment, and short and long term exposure of these fish will be the subject of our ongoing research.

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QUANTITATIVE DESCRIPTIVE ANALYSIS OF AMPHIPOD BROTHS.

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Amphipods were harvested during the fifties and the sixties as a rich source of proteins and pigments for the provincial government fish breeding operations. Commercial dry diets stopped the amphipod business because they were easier to use and stock. We are searching for ways to give potential commercial outlet to this underexploited marine crustacea, mainly for human consumption. A second goal is to help the diversification of catches from the maritime region of Québec. One of the main objectives of this work is to gather more information about the amphipod biomass, since there is very little in the literature concerning compositional or nutritional aspects.

People are suspicious of the amphipod external appearance, thinking they are insects because of their small size, their cuticle or dark color in some cases. This attitude changes when they are informed this animal is a crustacean, as indicated in table 1. Amphipods contain a multitude of soluble components, mainly proteins and lipids giving characteristic flavor similar to those of higher crustaceans when submitted to thermal treatments. This product could be made directly at the seafood factory and sold as a flavor concentrate. The main interest of this transformation is the constitution of an important source of natural flavors.

Table 1. Classification of the amphipods studied and main flavor characteristics.

		<u>TWO MARINE SPECIES</u>	<u>TWO FLAVORS</u>
Phylum	arthropod		
Class	crustacea	Family: Gammaroid	
Sub-class	malacostraca	<i>Gammarus oceanicus</i>	crustacea
Superorder	peracarida	Family: Lysianassid	
Order	amphipoda	<i>Anonyx sarci</i>	mollusc

The first objective concerning flavors was to develop a precise descriptive vocabulary to characterize amphipod broths. Another objective was to differentiate

between the broths from the two amphipod species and to try to define the similarities with higher crustaceans or molluscs during tasting sessions.

MATERIALS AND METHODS

1. Sensory methods

a. Choice and principle of test.

A quantitative descriptive analysis (QDA) was used, including two parts: a brainstorming session to identify sensory properties of the products and tasting sessions to evaluate each of the stated attributes. An unstructured line scale was chosen to get repeated measures for statistical analysis (6), thus ensuring objective conclusions to the test.

Well identified broth samples and a series of references were presented to the panel, one for each characteristic, the location of which was readily indicated on the scale (7). Panelists were asked to taste the first reference before the broths, to verify its situation on the scale and then to proceed with each broth, tasting, rinsing, positioning them both on the same scale. Results were measured as distances in cm from the "none" side to the mark left by the judges (1, 4).

b. Panel members.

Essentially, judges must know the product, be available, have good memory and verbal capacities (10). The test group included people of both sexes, aged between 25 to 40 years. They were workers at the provincial Ministry of Food, Fisheries and Agriculture (MAPA-Québec) in Gaspé city and were excused from their regular duties for a 30 min period each day to take part in the sensory evaluation program. They had little training in descriptive tests, but were chosen from a previous triangle test and showed good skills. The triangle test allowed a first contact of members with amphipod samples and the selection of twelve members for the QDA.

c. Test room.

The test room was equipped with regular, well ventilated, individual booths and a stainless steel sink. The trays were passed through a vertical sliding door, which separates the preparation area from the test room. The test area was always odor-free and cleaned before each working session. A white lighting was used for the test.

2. Raw material.

a. Amphipods.

The species *Anonyx sarci* was harvested on July 1st, 1992 in the St-Lawrence river beside Grandes-Bergeronnes in the province of Québec. The animals were then cleaned, immediately frozen at -30 °C and kept at this temperature until cooking. *Gammarus oceanicus* were sampled from Rimouski on July 20th, 1992 and similarly treated.

b. References for QDA.

To guide the panelist, all attributes were accompanied by a reference sample, except for three words: marshy, flavor intensity and flavor persistence. The varied references were made from food grade compounds or fresh food (see Table 2).

c. Preparation of broths.

Dilution of the broths was tried, but only a strong algal note was then detected. To achieve the best description during tasting sessions, dilution was limited to one part biomass with two parts water. This material was brought to a boil and cooked for 10 min. The mixture was grinded for 1 min in a Waring blender and finally, the cuticles were strained out. Broths were always freshly made for each block of criteria.

d. Preparation of sample trays.

The samples and all references required were served in small plastic glasses containing about 50 g of broth; judges were served as needed. All liquids including rinsing water, were kept at room temperature (23°C). The references were not served at random, but in order of increasing strength. A ruler, a pencil, a napkin, a stick or spoon to mix the settling solids were also supplied. Rusks (Grissols) were added to avoid fatigue caused by persistent flavors and the large number of attributes.

Table 2. Concentration of reference substances.

ATTRIBUTES*	REFERENCES	CONCENTRATION (g/l)
Acid	Citric acid, cristals	0.4
Algal	Dried algae, Japanese	20.0
Astringent	Strong tea	24.0
Bitter	Quinine sulfate	0.1
Boiled corn	Without the kernels	cob cuts
Hepatopancreas	Commercial paste (incl. liver)	10.0 g
Iodide/iodine	Lugol	1.0
Milk	Lukewarm	30.0 ml
Mushrooms	Fresh	1.0
Pepper	Boiled and filtered	0.8
Potatoes	Raw, peeled	pieces
Salty	NaCl	1.4
Snow peas	Cooked 5 min	few pods
Sunflower seeds	Shelled	6-10 seeds
Sweet	Glucose	18.0
Umami	Monosodium glutamate	2.0
Urchin gonads	Fresh, grinded	86.5

* Terms translated from French.

3. Experimental design and statistical analysis.

The selected design and relevant statistical analysis should ensure the validity and accuracy of results and conclusion (5). The main objective was to develop a precise descriptive vocabulary to characterize the broths. Also, by using the same scale for both broths, comparison of data by ANOVA analysis was possible.

At the round table session, panelists narrowed down the list of attributes to twenty, which were divided in three blocks, each making up a different test form: 1st= 7, 2nd= 7 and 3rd= 6 words. Six tasting sessions took place between august 4 and 11 1992, panelists being convened between 2 and 3 PM each day. Sessions

lasted around 30 min. Table 3 shows how the experimental design was organized to perform the ANOVA analysis. Attributes were named criteria, repetitions were called experiments (Exp), while the treatments (Trt) were the broths. Blocks #1 and #3 were evaluated twice by 12 panelists, but for block #2, the first session was done with 11 people and the repetition was done with only 8. To ease analysis of these results (block #2), we only kept the evaluations from the eight judges that came to both repetitions. The distance on the scale between the zero side to the mark left by the panelist was measured and a group mean was computed. Through the SAS system, a program giving an evaluation of normality, data homogeneity and the analysis of variance was used.

We proceeded criterion by criterion for the ANOVA, as for repeated measures. Differences between the two broths can be drawn with a high d.f. of 23 at the error. It also gives differences between repetitions (Exp), which must be null, since broths and all tasting sessions were made according to specific standards. Another part of ANOVA shows the variability between the 12 judges and Judges*Exp shows variability of the individual judge from one repetition to the other. A value of $\alpha \leq 0.05$ was considered an acceptable level of significant difference. Type I errors, i. e., rejecting H_0 when in reality there is no difference between treatments, are unlikely to happen at this or lower levels.

Table 3. Experimental design for the SAS program.

Treatments (Trt)	broths	2 = A, G
Experiments (Exp)	repetitions	2
Judges	tasters	12, except block #2 = 8
Criteria	attributes	20

General Linear Models Procedure: PROC GLM
 Example of Criteria = Acid "AC"
 Dependent variable: Evaluation
 Number of observations per group = 48

Source	d.freedom	Mean square	F	Pr > F
Model	24	8.94979167	1.93	0.0594
Exp	1	0.15187500	0.03	0.8579
Judges	11	13.62929924	2.94	0.0140
Judges*Exp	11	5.52823864	1.19	0.3440
Trt	1	3.91020833	0.84	0.3676
Error	23	4.62977355		
Total	47			

- H_0 : null hypothesis, there is no significant difference between the two studied broths for the same criteria or qualifier.
 H_1 : alternative hypothesis, is when H_0 is rejected meaning a difference exists between treatments.

RESULTS AND DISCUSSION

Dr R. York of Manitoba Fisheries and Oceans Dept. helped over the choice of test and confirmed that better results would be obtained with regular consumers of marine products, even if the training to QDA was short. Attributes called to mind during the brainstorming depends on personal experience of involved members. Gaspé, a fishing region in the province of Québec, was chosen for the sensory evaluation of crustacean broths. The panel members all work at the MAPAQ and the tasting sessions are seen as a part of their job. This insures a responsible attitude towards the tests, which was maintained to the end of the study.

The first results are from the round table list of words; if these were improper, there would be no point in quantifying them. At the round table, up to sixteen persons were invited to decide on the terminology to be used. No interference was made during this session and all remarks and attributes were written down, reducing the number of words with panel agreement only. There was no time for exhaustive training and the round table period was used for that purpose, but this was insufficient (see below).

This study was done in French and the different attributes had to be translated for the present publication. Many of the twenty attributes are found in the literature (2). No term received a mean lower than 3.3, so we may say no attribute was eliminated by the quantitative test. There were never differences between repetitions and seldom did significant variations in judgments happen between the repetitions for the same criteria and by the same judge (Judges*Exp). The values from the entire group of judges show great disparities: the worst example is the boiled corn with a minimum of 2.1 and a maximum of 14.1 on the 15-cm scale. Either some panelists had problems detecting or identifying the flavor, else it could be they had different opinions on the definition of this term, even if agreed upon previously. This emphasizes the need for more training.

Out of the twenty criteria or studied attributes, only seven showed significant differences ($\alpha < 0.05$) between the broths: astringency, hepatopancreas, flavor intensity, flavor persistence, milk, pepper and salty taste. The other thirteen criteria, though not differentiating broths from one another, are nonetheless important in distinguishing amphipod broths from other marine products. To ease discussion and allow analysis of prominent features of broths, results were arbitrarily classified according to types of flavors.

The next five tables show results concerning attributes: the **bold letters** indicate the significant differences between broths and the corresponding α values. The qualitative evaluation was given *a posteriori* to ease discussion of results: 0 is null, > 0 to 3 is very weak, > 3 to 6 is weak, > 6 to 9 is average, > 9 to 12 is strong and then up to 15 would be extreme. Some attributes can be found in more than one category.

The first category comprises all basic tastes (Table 4), including umami because it is also produced by non volatiles. Only one of the basic tastes gave a significant difference between the amphipod broths: it was the salty criteria. Using the standard procedure with a Chloride Analyser 926 from Corning, the salt concentration of the broths was found to be 0,30 % for *Anonyx* and 0,49 % for *Gammarus*. The standard deviations are low, but the judges did not quite agree on the absolute value on the scale.

Table 4. Category: basic tastes.

ATTRIBUTES	Pr α (*)	MEAN, STD DEVIATION AND QUALITATIVE EVALUATION OF BROTHS			
		<i>ANONYX</i>		<i>GAMMARUS</i>	
Acid	0.3676	4,3 \pm 0,3	weak	4,8 \pm 0,2	weak
Bitter	0.9727	5,1 \pm 0,4	weak	5,1 \pm 0,2	weak
Salty	0.0007	7,1\pm0,1	average	8,3\pm0,4	average
Sweet	0.1289	5,0 \pm 1,3	weak	5,6 \pm 0,9	weak
Umami	0.5238	7,7 \pm 0,5	average	8,0 \pm 1,1	average

* Significant differences in bold letters. N = 48.

The sweet criteria did not show an acceptable difference ($\alpha > 0.05$), but there was a tendency for the members to find one broth sweeter. All means were higher for *Gammarus* except for bitter, which shows equal values for both broths. This could be due to the strong flavor intensity of this species (Table 5).

Table 5. Category: mouthfeel.

ATTRIBUTES	Pr α (*)	MEAN, STD DEVIATION AND QUALITATIVE EVALUATION OF BROTHS			
		<i>ANONYX</i>		<i>GAMMARUS</i>	
Astringency¹	0.0097	4,7\pm0,4	weak	6,9\pm0,0	average
Intensity	0.0001	8,3\pm0,2	average	10,9\pm0,2	strong
Pepper¹	0.0673	3,8\pm0,2	weak	3,3\pm0,5	weak
Persistence	0.0623	10,0\pm0,3	strong	11,2\pm0,2	strong
Raw potato ¹	0.3562	8,2 \pm 1,2	average	7,8 \pm 1,1	average

* Significant differences in bold letters. ¹N = 32; otherwise N = 48.

Table 5 presents the means for the criteria intensity of flavor and the unquestionable significant difference between the broths. The mouthfeel category is statistically the most informative: four of the five words gave significant differences between broths with varying values of α . Pepper and persistence are accepted as

different, even if values slightly exceed the 0.05 level; care must be taken however when conclusions are drawn. *Gammarus* was always rated higher, except for raw potato and pepper flavors. Judges experienced difficulties with pepper, since we find disagreement for the same members between repetitions. This definition should be better specified.

The various categories were introduced after treatment of data, only from looking at the nature of the words, not from the ANOVA results. Still, it may not be quite the way the members saw it, particularly for raw potato: on tasting *Anonyx a posteriori*, we felt a similar texture to that of starch granules, but this was not defined at the round table. Instead, panelists specifically chose that term for the raw potato flavor.

In the sea category (Table 6), there is no difference between broths, except for salty. No words like fishy, cheesy or ammonia came out in this category. Instead words like urchin, hepatopancreas and umami (Table 8) are found, which support the resemblance with cooking liquids from molluscs or crustaceans. Some panel members felt the broths were too concentrated, but dilution made it impossible to distinguish any other notes besides a predominant algal flavor.

Table 6. Category: sea.

ATTRIBUTES	Pr α (*)	MEAN, STD DEVIATION AND QUALITATIVE EVALUATION OF BROTHS			
		<i>ANONYX</i>		<i>GAMMARUS</i>	
Algal ¹	0.5454	8,6±0,6	average	8,7±0,5	average
Iodide/iodine ¹	0.3312	4,4±0,2	weak	5,0±0,6	weak
Marshy ¹	0.9175	5,8±1,0	weak	5,9±0,3	weak
Salty	0.0007	7,1±0,1	average	8,3±0,4	average

* Significant differences in bold letters. ¹N = 32; otherwise N = 48.

There was a controversy at the round table about iodide/iodine. Some members said it felt similar to an algal flavor for them, others arguing they were totally different. After discussion and reference tasting, the members agreed iodide/iodine represented the closest reference to sea breeze, sea shore smell. It received weak rates, much lower than algal; these words are very different. According to Whitfield *et al.* (9), the 2,6-dibromophenol would provide the salty-marine or iodine-like impression from fish and seafood. This chemical, specific to marine animals, allows to differentiate them from freshwater ones (3).

From table 7, the evaluations seem to be higher four times out of six for the *Anonyx* species, but no significant difference was found in the vegetable category. Concerning *Anonyx*, the reason for all the vegetable words could be explained by

their high lipid content (15.8 % of dry wt, unpublished data). We compared our results with reports and articles and found many vegetable-based descriptors. Many carbonyls and alcohols are yielded by specific lipoxigenases, responsible for the existence of similar compounds in plants (3).

Table 7. Category: vegetables.

ATTRIBUTES	Pr α	MEAN, STD DEVIATION AND QUALITATIVE EVALUATION OF BROTHS			
		ANONYX		GAMMARUS	
Algal ¹	0.5454	8,6 \pm 0,6	average	8,7 \pm 0,5	average
Boiled corn	0.7432	7,8 \pm 0,3	average	8,4 \pm 0,8	average
Mushroom	0.6267	5,6 \pm 0,3	weak	5,3 \pm 0,4	weak
Raw potato ¹	0.3562	8,2 \pm 1,2	average	7,8 \pm 1,1	average
Snow pea	0.4080	3,9 \pm 0,1	weak	3,6 \pm 0,4	weak
Sunflower seed	0.3872	5,7 \pm 0,3	weak	5,0 \pm 0,4	weak

¹N = 32; otherwise N = 48.

Algal notes are the strongest in this category also, with means around 8.7. *Gammarus* species directly feed on algae and there could be some in their stomach that would impart the algal note. Concerning the boiled corn, the panel talked about the cob without the grains, involving a sulfur compound that resulted in average marks for this flavor. The fresh mushroom aroma would essentially come from 1-octen-3-ol, an eight-carbon component (8). Preliminary GC-MS unpublished results show the presence of a whole family of enol substances, on which no sniffing was done. More work is planned to correlate the volatile compounds identified by GC-MS with terms used by the panel.

For the meat flavor category (Table 8), *Gammarus* was given all the high marks with a good significant difference for hepatopancreas. The justification for working with a panel of regular seafood eaters is very clear here; city dwellers avoid this part of the lobster. We are presently looking at soluble non-protein nitrogen, like nucleotides and quaternary ammoniums. A high amount of these compounds could explain the stronger values for hepatopancreas notes and umami taste of *Gammarus* broth.

The qualifier milk did not fit in the previous categories, but it could be related to the nutty/buttery flavor, as identified by Edmunds and Lillard's panel (2). According to these authors, it characterizes crustacea as well as molluscs. However, broths were parted by this attribute with a significant level of 0.0333. Milk notes were detected as significantly stronger for the *Anonyx* broths; it concerns milk at room temperature, as specified by panel members.

These are preliminary results, giving a basis for future work. The choice of the experimental place was excellent and all panel members were very interested, professional, motivated and patient. Still more work is needed, plus another round table session to discuss more appropriate terms if adequate differences between the products are to be determined. In fact, repeated measures would lower variation coefficients, the standard deviations and provide greater confidence in the conclusions. Absolute values for each studied attribute could probably be obtained.

Table 8. Category: meat flavor.

ATTRIBUTES	Pr α (*)	MEAN, STD DEVIATION AND QUALITATIVE EVALUATION OF BROTHS			
		ANONYX		GAMMARUS	
Hepatopancréas	0.0203	4,4±0,1	weak	5,6±0,3	weak
Umami	0.5238	7,7±0,5	average	8,0±1,1	average
Urchin gonads ¹	0.7756	5,7±0,6	weak	5,9±0,1	weak
MISCELLANEOUS					
Milk	0.0333	8,5±0,3	average	6,7±0,1	average

* Significant differences in **bold** letters. ¹N = 32; otherwise N = 48.

CONCLUSION

The project main objective was attained, since both broths were specifically defined. *Anonyx* broth was qualified a milky juice with nutty, pepper, green, raw potato and mushroom notes. It leaves a long lasting mouthfeel, probably caused by the high lipid content. *Gammarus* broth also possessed a strong persistent flavor; it is more astringent, saltier and characterized by a hepatopancreas note. More testing would be necessary to validate a correct terminology and establish the minimum words required to characterize our products.

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**U.S. DEPARTMENT OF AGRICULTURE PROGRAMS SUPPORTING
AQUACULTURAL PRODUCTS SAFETY AND QUALITY**

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The U. S. Department of Agriculture (USDA) has traditionally supported and participated in programs and projects that help assure that U. S. agricultural commodities and crops provide consumers with an affordable, wholesome food supply. Since 1990, USDA's involvement with food safety and quality issues related to aquacultural products has increased. Numerous USDA programs now recognize that aquatic foods, which include finfish and shellfish, are regarded as agricultural derived foods. USDA's Agricultural Marketing Service, Cooperative State Research Service and Extension Service, in addition to the USDA Regional Aquaculture Center program, have supported research and extension education projects related to food safety and quality.

With an increased awareness of and expanding involvement in aquaculture issues, USDA can be expected to continue supporting food safety and quality research and educational projects that have regional and national impact and relevance to enhancing our Nation's commercial aquatic food sector.

This paper provides specific information on USDA funded and supported initiatives and opportunities for extending participation to those persons and institutions involved with aquatic foods safety and quality programs.

Agricultural Marketing Service

This USDA program funded a project through a cooperative agreement with a university that evaluated physical distribution procedures to maintain product quality of hybrid striped bass from pick-up at the point of production, delivery to the processing plant, and final delivery to the retail store. Funding of cooperative agreements are based on projects of mutual interest and need for facilitation of critical linkages with private sector businesses. Only research projects are funded. The output from this project was a publication (1) and an educational video titled, "Sunshine Bass from Tank to Market". Note that sunshine bass is the same as hybrid striped bass.

Cooperative State Research Service

The National Competitive Research Initiative Grants Program was initiated in fiscal year 1991. Since that time one funded program area has been "Food Safety". For fiscal year 1994 the program area name has been changed to "Ensuring Food Safety". Annual funding for Food Safety Program area projects has been about \$2 million. For fiscal year 1991, a grant was awarded to fund a project titled, "Effects of Refrigeration on Virulence and Viability of Vibrio vulnificus". For fiscal year 1992, a grant was awarded to fund a project titled, "Effects of Storage and Depuration Temperature on Pathogenic Vibrios in Shellfish". Future annual appropriations are expected as food safety remains a high priority issue within USDA.

Extension Service

The Extension Service began a national initiative, "Food Safety and Quality" in 1991. This is a competitive grants program that provides funding to state Cooperative Extension Services that are involved with the development and delivery of educational programs related to this topic. Funded projects are anticipated to have regional or national impacts.

In fiscal year 1991 the following projects were funded: development of an easy to use hands-on manual for implementing HACCP in deli operations (2); development of a video educational program on the HACCP system applied to the catfish industry (3); and development of an aquaculture database as a component of USDA's Food Animal Residue Avoidance Databank (FARAD) located at the University of California, University of Illinois and University of Florida.

The aquaculture database project had continued funding in fiscal year 1992. This project will provide information on the regulatory status of EPA registered pesticides for aquatic site use and FDA approved new animal drugs for aquaculture use. Bibliographic support is also available to individuals or organizations to fulfill FDA informational requirements associated with Investigational New Animal Drug applications (INADs). Data and information are also available for incorporation into producer developed quality assurance programs.

The Catfish Farmers of America has developed a quality assurance program (4) and the U. S. Trout Farmers Association is in the process of developing a similar quality assurance program for trout producers that is expected to be completed at the beginning of 1994. The American Tilapia Association is also developing plans for an industry quality assurance program that meets the needs of U. S. tilapia growers. The Washington Fish Growers Association was one of the first finfish organizations in the U. S. to develop a program to promote high facility operating standards (5). These voluntary industry initiatives follow earlier efforts directed at developing Hazard Analysis-Critical Control Points (HACCP) guidelines and model programs for U. S. aquaculture producers (6,7).

For fiscal year 1993, two projects related to seafood/aquaculture safety and quality have been funded by the Extension Service. The first involves a multi-institutional team approach to develop a comprehensive educational program that will enable aquaculture firms (catfish and oyster processors) to utilize TQM and HACCP concepts for the processing of safe and wholesome products. Another aspect of this project is to develop a suggested strategy for national implementation.

The second project also employs a multi-institutional team approach to develop and distribute educational products that will support producer-based quality assurance programs in the U. S.. Materials to be developed include an educational fact sheet, video and slide set. Another component is the broadcast of a national satellite videoconference that will educate a broad aquaculture constituency about the importance and guidance for participating in producer oriented quality assurance programs. The last objective in this project is to evaluate the access and services of the aquaculture database associated with the FARAD program. The results of this evaluation will be shared with the database managers to improve services as needed. Both Extension Service projects use an industry advisory committee to assist with project planning and implementation.

Regional Aquaculture Center Program

The five USDA funded Regional Aquaculture Centers were established in 1987 and 1988. Project priorities are identified by an Industry

Advisory Council with private-sector members and a Technical Committee composed of research and extension education scientists. Project funding recommendations are made by a Board of Directors and final approval is given by USDA. There have been numerous projects and publications funded by this program that relate to food safety and quality of aquatic food products.

The Southern Regional Aquaculture Center (SRAC) has developed several educational fact sheets (8,,9,10,11,12,13) that address various safety and quality related topics. Two multi-year projects have also been funded. One addresses aquaculture food safety residues and the other focuses on aquaculture food safety microbials. The residues project addresses the following objectives; i) develop a database for chemical contamination information in farm-raised catfish, crawfish and rainbow trout, ii) develop guidelines and protocols for a residue monitoring program at processing facilities, iii) develop educational materials for producers and processors regarding safe use of chemicals associated with production and processing, iv) develop a user-friendly computer software recordkeeping program, v) determine fate of residues from farm to processing plant to final retail product, vi) conduct any additional sampling to improve database on residues.

The other project on microbials addresses the following objectives: i) collect available data to define aquacultured food safety problems and design a control program, ii) conduct a forum to assess all relevant data on safety of aquacultured foods, iii) develop a bibliography of available information relative to food safety and sanitation in the aquaculture industry, iv) evaluate data on microbial quality of catfish, crawfish and rainbow trout processing and distribution operations, v) investigate various methods to reduce and detect significant pathogenic and spoilage microorganisms on processed catfish, crawfish and rainbow trout, vi) conduct a food safety HACCP audit to determine if this approach would be cost-effective and result in increased product safety, and vii) produce new publications to complement existing materials on food safety and sanitation.

One outcome of these projects was the convening of an Aquaculture Products Safety Forum in February 1993 that had objectives to: i) determine present status of microbes and residues related to the safety of Southeastern U. S. aquacultured products, ii) propose what the status of microbes and residues related to the safety of these products could be or should be within 3 to 5 years, and iii) develop an action plan to bridge the gap between present status and proposed future status. A proceedings was developed that included presented papers and recommendations (14). The highlights of this Forum were also communicated via a live national satellite videoconference on Aquaculture Product Safety in June 1993.

The Northeastern Regional Aquaculture Center (NRAC) recently approved a project to develop a model quality assurance program for the aquaculture industry in the Northeast. Project objectives include the following: i) develop two introductory quality assurance programs designed to provide finfish and shellfish growers with best management practices for continued production of safe, quality products, ii) develop a brochure for producer use to promote public awareness of the quality assurance programs. This project is pending final approval by USDA.

The North-Central Regional Aquaculture Center (NCRAC) has conducted surveys related to seafood product quality and published their findings (15). The Center for Tropical and Subtropical Aquaculture (CTSA) has also supported projects concerning marketing and shellfish sanitation programs with findings also published in available reports (16, 17).

Joint Subcommittee on Aquaculture

The Joint Subcommittee on Aquaculture (JSA) is a national federal agency coordinating body chaired by the USDA's Secretary of Agriculture (designate) that addresses various issues of national scope and importance. Associated with the JSA are numerous national task forces or working groups that address specific topics. One is the Working Group on Quality Assurance in Aquaculture Production that was formed in 1990 and includes representatives from Federal agencies, aquaculture industries, national trade organizations, academia and others interested in aquaculture quality assurance matters. This Working Group is serving as a national Forum to identify issues and develop action strategies to coordinate efforts primarily directed at preventing the occurrence of violative drug and pesticide residues in aquacultural food products.

The Working Group has been addressing several objectives and has developed several products for national distribution to the aquaculture industry and persons associated with it. The objectives include the following.

- i) develop a comprehensive publication summarizing Federal regulation of drugs, biologicals and chemicals used in aquaculture production (18),
- ii) develop a central databank of drug and chemical information for compounds used in aquaculture production through the FARAD program,
- iii) develop a publication on guide to the proper use of drugs, pesticides and vaccines used in aquaculture to include a listing of Federally approved, registered and licensed regulated products expected to be completed in 1994 (G. L. Jensen, 1993, ES-USDA, personal communication),

- iv) encourage and coordinate the development of producer quality assurance programs,
- v) seek clarification of regulatory status of compounds used in aquaculture production and,
- vi) encourage education of Federal/state agency personnel with regulatory jurisdiction of drugs, chemicals and biologicals on aquaculture practices and regulatory needs.

The JSA Working Group has also supported the need for and creation of a part-time position of National INAD Coordinator whose function is to work with INAD holders and applicants, FDA, the USDA Interregional Project No. 4 (IR-4) Animal Drug Program, private industries and other Federal agencies to coordinate the development and collection of data required for FDA approval of new animal drugs for aquaculture.

Conclusion

The U. S. Department of Agriculture is recognizing more the importance of the seafood sector in U. S. and global markets and the issue that aquaculture products are farm-raised products and thus regarded as being agricultural products. Pending Federal legislation can provide further clarification on this issue within USDA. With an increased awareness of and expanding involvement in aquaculture, USDA can be expected to continue supporting food safety and quality research and educational programs nationwide that are relevant to enhancing our Nation's commercial aquatic food sector and providing consumers with high quality, wholesome aquatic food products.

USDA has various programming structures that facilitate the coordination of research and educational outreach efforts at the national and regional levels. Other networks including the State Cooperative Extension Services and companion Sea Grant Marine Advisory Services offer educational program capabilities that reach a vast nationwide constituency that is involved with aquatic foods production, processing, handling, distribution, marketing and consumption.

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ABSTRACTS

FDA CERTIFICATION PROGRAM FOR SEAFOOD EXPORTED TO THE EC

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STATUS OF THE U.S. DEPARTMENT OF COMMERCE INSPECTION PROGRAM

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MANDATORY NUTRITION LABELING UPDATE

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The new nutrition labeling regulations are a result of the Nutrition Labeling and Education Act of 1990. The primary objectives are; 1) to clear up consumer confusion about food labels, 2) aid consumers in making healthy food choices, and 3) to encourage manufacturers to produce innovative food products that improve the quality of the food supply and that make healthy choices available to the consumer.

The four major effects are 1) the list of nutrients whose content levels are required were revised; 2) a new format made up of three major sections, a) title, serving size and servings per container, b) macronutrients and calories, and c) micronutrients and footnotes; 3) nutrition labeling is mandatory for most packaged foods; and 4) nutrients are to be declared based upon standardized serving sizes.

The regulation include 1) mandatory declaration of nutrient content, definitions of nutrient content claims (descriptors) that can be used, and 3) what health claims can and can not be declared.

OVERVIEW OF PHOSPHATE USE IN SEA SCALLOP PROCESSING

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Phosphates are used within the sea scallop industry for the primary purpose to control moisture content. Sodium tripolyphosphate (STP) is the most widely used phosphate product in scallop processing. Effectiveness of STP in controlling scallop moisture can be highly variable, depending on factors such as reproductive state of scallops at time of harvesting; on-board handling and stowage practices; STP concentrations used in processing solution; method of phosphate application (was, dip, soak, vacuum tumbling); and, the use of sodium chloride (NaCl) in STP solutions. Scallop nutritional equivalency is effected by resulting moisture content due to these variables. Increasing scallop moisture content decreases scallop nutrient levels. Moisture loss due to freeze/thawing and cooking contributes to the loss of water-soluble nutrients. The use of STP reduced scallop drip loss, which in turn, reduced scallop nutrient loss.

Phosphates have also been used to remove crystalline precipitates (white spots) from imported bay scallops from China and domestic sea scallops. Sodium hexametaphosphate (SHMP pH 7.0) and a commercial blend of STP, SHMP, and acid pyrophosphate (pH 7.2) was demonstrated to dissociate these precipitates.

REGULATORY PERSPECTIVE ON THE USE OF PHOSPHATES IN THE SEAFOOD INDUSTRY WITH EMPHASIS ON SCALLOPS

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In July of 1992 the Food and Drug Administration issued an interim policy that required labeling sea scallops with over 80% moisture as X% water added Scallop Product. This interim policy also required that the phosphates used in processing be declared on the label and set a maximum limit of 84% moisture of 25% moisture added for marketing of scallop product.

A field assignment was issued in November of 1992 for districts to inspect domestic scallop processors and to sample their product and check for labeling compliance. Thirteen domestic processors were inspected and samples were collected and analyzed. Samples were collected from 21 consignees of these firms. The moisture content of domestic scallops analyzed ranged from 78.4 to 86.6%. In addition, 32 shipments of imported scallops were sampled and analyzed for moisture content. The moisture range measured in imported scallops was from 75.4 to 89.6%. The results of these findings will be discussed in detail.

DETECTION OF ADDED PHOSPHATES IN SEAFOODS

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The use of thin layer chromatography techniques to distinguish between naturally occurring mono phosphates present in all seafood and added phosphates in the form of tripolyphosphate salts will be discussed. The combined TLC methods of Gibson and Murray, 1973; and Redde and Finne, 1985; were used to show the capabilities of the technique for inspection purposed. Tripolyphosphate usage was clearly detectable after 3 months of uninterrupted frozen storage. The drip from the product is excellent sampling material making the TLC method quick and easy.

This method is only qualitative at this time and has several limitations. Additional work-up is necessary to detect abusive practice. Tripolyphosphates hydrolyse over time to naturally occurring phosphates. Therefore, with prolonged storage, detection of initial usage decreases.

FOOD SAFETY AND SANITATION IN THE SEAFOOD INDUSTRY

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The production of safe and quality foods in the primary objective of all food processors. Microorganisms can cause both a quality defect (spoilage) and a safety defect (foodborne illness). Control of microbiological contamination requires an understanding of microorganisms and the factors that influence their growth, survival and the factors that contribute to the microbiological contamination of food. The costs of a foodborne disease outbreak; product recall, adverse publicity, lawsuits, etc., make prevention of contamination the primary goal of the food processing industry.

Contamination of seafood products by microorganisms or other contaminants, can be minimized by an effective sanitation program. This paper will examine the causes of food borne disease, the objectives of a sound sanitation program and discuss types of products used to clean and sanitize food processing equipment. New sanitation concepts also will be presented.

A NEW LOOK AT REGULATORY MICROBIOLOGICAL STANDARDS, SPECIFICATIONS AND GUIDELINES FOR SEAFOOD PRODUCTS

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The paper describes the difficulty in establishing Microbiological Standards, Guidelines, and Specifications for Foods. The background on the subject is offered and the confusion relative to the subject is detailed. The microbiological criteria established for seafoods in the United States are discussed. The international aspects of establishing microbiological criteria through the Codex Alimentarius Commission is also elaborated. Future actions relative to the subject are predicted. The future actions include the need for increased awareness of alternative approaches such as HACCP, as well as the need for increased understanding of the statistical reality of Acceptance Sampling Plans. The author points out how a lack of statistical understanding of sampling plans often leads regulators to a false sense of security. The author also predicts how in the United States, microbiological standards for raw foods will be elaborated incorporating the use of 3-class sampling plans instead of the 2-class sampling approach currently employed for pathogens in cooked products.

HACCP FOR MICROBIOLOGICAL HAZARD CONTROL IN SEAFOOD Processing Plants

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Over the next few years, many seafood processors will be implementing new programs designed to increase product safety. One such program, HACCP, has received much attention as a means of monitoring and controlling the hazards that exist in food processing plants. In addressing these hazards, detection and containment of bacterial pathogens in plants is a key safety issue targeted in many HACCP programs.

Application of HACCP to monitoring and control of microbiological hazards is not difficult. The options facing a processor who wishes to implement microbiological HACCP will be discussed. Properties of different microbiological tests will be compared, with particular focus on testing *Listeria*. A 24 hour quantitative method for *Listeria* testing, called Listertest, will be among the tests presented. Finally, examples which illustrate microbiological testing and clean-up of contaminated environments will be analyzed.

THE REVISED FISH LIST

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In recent years, there has been an increase in seafood consumption in the United States. Increased consumption, along with increase importation of unfamiliar seafood and use of different names for the same seafood in different regions of the country, led the Food and Drug Administration (FDA) and the National Marine Fisheries Service to recognize a need for a single source for recommended or required market names for seafood sold in interstate commerce in the United States.

In 1988 "The Fish List" was published by FDA to provide a source of names which would facilitate order in the marketplace and reduce confusion among consumers. This list established some order in the marketplace, however, the invertebrate species (mollusks and crustaceans) were not included. The FDA established an updated list, entitled "The Seafood List", which includes the invertebrate species sold as seafood in the United States. The list is located in the Center for Food Safety and Applied Nutrition Thesaurus for easy access to update and locate information.

The "Seafood List" represents an extensive, although not complete, listing of seafood commonly sold in the United States. This list is divided into two main parts: Part I-Vertebrate and Part II-Invertebrate. Each part is divided into two sections. Section I lists the species alphabetically by market names, followed by the common, scientific, and vernacular names. Section II lists the species alphabetically by the scientific names, followed by the market names. These sections are placed in "The Seafood List" to aid in locating the species of interest. However, the listed market name or the common name should be used to label seafood products entering interstate commerce. The agency discourages the use of vernacular names. Vernacular names used outside the region where the name is commonly known may mislead consumers and could result in regulatory actions by the agency. These names have been included on the list for information purposes only and to reference the vernacular names to the appropriate common and market names. Use of the common and market names supplied in "The Seafood List" will promote labeling consistency in the various areas of the United States. It will enhance the ability of consumers to make informed choices among seafood products. In addition, the list will provide the seafood industry with uniform nomenclature and assurance that the use of the listed common or market name to identify seafood will be in compliance with food labeling requirements.

HACCP CURRICULUM IN A COLLEGE PROGRAM

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The development and pilot study of a HACCP curriculum for a two-year focused seafood program at the college level will be explained. The course, "HACCP and Quality Control Program" was designed as a four credit elective course for Kingsborough Community College's Seafood Business Management associate degree program. The draft course consists of both lecture and analytical laboratory exercises. The content of the course material and the evaluation of the student's learning process will be discussed. The importance of such education and training will be examined. This work was funded by NOAA Grant No. NA26FK0400-01.

TEMPERATURE MEDIATED MULTIPLICATION OF *VIBRIO VULNIFICUS* In Shellstock Oysters

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Cook and Ruple (J. Food Prot. 52:343-349,1989) reported that naturally occurring V. vulnificus multiplied in post-harvest Gulf coast oysters between harvest and arrival at the processing plant and in oysters held at 22 and 30°C. Murphy and Oliver (Appl. Environ. Microbiol. 58:2771-2775, 1992) failed to verify the multiplication of V. vulnificus in shellstock oysters using a transposon-containing strain of the organism and a direct plating technique. The present research attempts to resolve the differences in these findings.

V. vulnificus was enumerated in oysters immediately after harvest and in oysters from the same lot of shellstock after storage at various temperatures. Enumeration techniques used most-probable-number alkaline peptone broth enrichment combined with isolation on CPC agar and confirmation with an EIA technique (Tamplin et al., Appl. Environ. Microbiol. 57:1235-1240, 1991). At 10°C, no multiplication of V. vulnificus was observed. However, at temperatures above 13°C, V. vulnificus multiplication was confirmed and found to be temperature dependent. V. vulnificus numbers increased by 1 to 2 log units in shellstock oysters held at summer ambient air temperatures for 12 h.

IMPROVED ENRICHMENT BROTH FOR RECOVERY OF *VIBRIO VULNIFICUS*

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Optimum bacteriological media are needed to detect *V. vulnificus* in shellfish products. Plate culture, monoclonal antibody and DNA probes, as well as polymerase chain reactions, all require enrichment broths to increase *V. vulnificus* numbers to detectable levels. Currently, alkaline peptone water (APW), composed of 1% peptone and 1% NaCl at pH 8.4, is the standard enrichment broth for *V. vulnificus*, as well as many other *Vibrio* spp. Although APW can support growth of *V. vulnificus*, it must be incubated for up to 16 h, delaying results, and many times producing false-negative reactions due to overgrowth by other bacteria. Consequently, recovery of *V. vulnificus* can be enhanced by increasing the selectivity and enrichment properties of broth, thereby elevating *V. vulnificus* numbers in shorter time intervals. Following a thorough evaluation of various enrichment broths under laboratory conditions, selected media were evaluated with field samples of oysters containing natural populations of *V. vulnificus*. Modified APW (5% peptone, 1% NaCl, 0.08% cellobiose) yielded higher *V. vulnificus* MPN values within 8 h incubation, compared to standard APW and modified APW with antibodies. Modified APW containing 2 or 4 U/ml colistin methanesulfonate slightly inhibited *V. vulnificus* growth within 8 h, however, *V. vulnificus* constituted a greater proportion of total bacteria/ml of broth at both 8 and 16 h, compared to standard APW. APW containing colistin also resulted in less overgrowth by endogenous bacteria at 16 h, compared to standard APW. APW containing colistin also resulted in less overgrowth by endogenous bacteria at 16 h incubation. A one-year field test is in progress to determine if these new enrichment broths enhance detection of *V. vulnificus* using culture, monoclonal antibody and DNA probe methods.

PREPARATION OF MONOCLONAL ANTIBODIES TO SANITOXIN USING RECOMBIANT PHAGE ANTIBODY SYSTEM

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No abstract submitted

BIOCHEMICAL, SEROLOGICAL, AND MOLECULAR CHARACTERISTICS OF ENVIRONMENTAL AND CLINICAL *VIBRIO VULNIFICUS* STRAINS

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Defining discriminating characteristics of *Vibrio vulnificus* can be important in epidemiological and ecological studies. In the present study, biochemical, serological and molecular techniques were used to compare 41 isolates of *V. vulnificus* from clinical and environmental sources. Biochemical characteristics were determined using BIOLOG™ microwell cluster plates, testing variations in utilization of 95 different carbon sources. Analysis of interstrain homology showed that isolates could be clustered at specific similarity coefficients. Antibodies prepared to nine clinical strains showed reactions with clinical isolates, but with none from environmental sources. Restriction-fragment-length-polymorphism of *V. vulnificus* DNA was measured by ribotyping and pulsed-field gel electrophoresis (PFGE). Restriction endonuclease digestion of DNA followed by Southern analysis using ³²P-labeled *E. coli* ribosomal RNA showed that RFLP patterns did not adequately discriminate isolates. In contrast, PFGE RFLP patterns showed that each isolate was unique. These results indicate that various typing methods may be useful for investigating sources of *V. vulnificus*, its mode of transmission and virulence.

DETECTION OF CIGUATERA TOXINS IN FINFISH USING CIGUATECT TEST KITS

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No abstract submitted

AN INTERMEDIATE MOISTURE PRODUCT FROM MACKEREL USING SALTING, FERMENTATION AND DRYING

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Intermediate moisture products were prepared from mackerel (Rastrelliger kanagurta) using salt curing in 20% or saturated brine for 20 hr and fermented with an inoculum level of 10^8 cells/mL of Pediococcus cerevisiae and dried in the sun (28-33C) for 23 hr or in an electric oven (40-45C) for 14 hr to a minimum desired moisture level of 18%. The chemical and microbiological studies correlated well with the organoleptical results which indicated a shelf-life of 4 months for salted and dried products and 7 months for fermented products.

RHEOLOGY OF FISH GELS

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Rheological studies of fish protein gelation commonly focus on either development of the gel during heating (isothermal or ramp heating), or on properties of the finished gel measured at room temperature. Torsion gelometry is ideally suited to measuring textural properties of foods gels over a range of temperatures, and was recently applied in our laboratory to measuring textural changes in surimi gels as a function of serving temperature. Interesting trends in this data led us to exploring the use of such data, augmented by small amplitude oscillatory strain and stress relaxation measurements also measured over a range of preliminary results in studying surimi gels by this approach, with particular emphasis on the low temperature setting or "surwari" gelling phenomenon of surimi.

EFFECT OF EXPOSURE TO LOW PH ON THE SOLUBILITY OF FISH MYOFIBRILLAR PROTEINS IN WATER

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Ten species of fish were surveyed relative to the solubility of their proteins in water. The proteins of 6 species were soluble; the initial pH values of these species ranged from 6.5 to 7.3. The proteins of 4 species had poor solubility and the initial pH of their muscle ranged from 5.9 to 6.5. The question investigated was whether the poor solubility of the latter 4 species was due to a low initial pH.

To test this, the pH of cod muscle, a species in which the myofibrilla proteins are soluble in water, was adjusted to lower pH values and held for 20 min. Some decrease in solubilization was observed, but it was not as great as that of the 4 species that had initial low proteins to a value similar to that of species with low initial pH. The effect of low pH treatment could be reversed by re-adjusting the pH to a higher value before washing. Improvement in solubilization of mackerel muscle, a species whose proteins are poorly soluble in water, was obtained by exposing the mackerel muscle tissue to a higher pH before washing with water. It appears that the inhibitory effect of a low pH in the initial stages was more likely related to removal of specific protein(s) which limit the solubility of other proteins rather than to a denaturing effect of low pH.

PHYSICAL PROPERTIES OF ATLANTIC MACKEREL SURIMI

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No abstract submittted

IMPORTANT CONSIDERATIONS IN DEVELOPMENT OF VALUE-ADDED FISH MINCE-BASED PRODUCTS

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Fish mince is produced from intact or defected fillet, frame, or H&G, and then converted into washed mince such as surimi or formed products without washing. Currently, white fish species are primary source of mince. The focus of the talk is on the mince-based products which are produced from fish mince after appropriate formulation with a variety of functional ingredients. Important elementss to be considered are species characteristics (source of fish mince), type and form of products, mincing method, ingredient selection, formulation and preparation methods, texture and flavor modification, batter and breading, and forming method. Successful product development requires identification of marketable product, proper selection of ingredients, favor and batter/breading with correct formulation. Optimization of flavor, texture and physical stability (thermal and freeze-thaw stability) will also be discussed.

PACIFIC WHITING PRODUCT DEVELOPMENT

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No abstract submitted

THE EFFECTS OF FROZEN STORAGE TEMPERATURE ON THE HIGH QUALITY SHELF LIFE OF SELECTED SEAFOODS

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Results of studies on the frozen storage shelflife of pristine quality products held at 18°C, -23°C, and -29°C are presented. Cod fillet blocks, whole glazed sockeye salmon, glazed headless white shrimp, and Alaska pollock surimi were removed every 5 weeks from each storage temperature, and assessed for quality by sensory and physiscal/chemical methods in comparison to a control sample held at -62°C. Sensoric testing remains the most reliable and discriminating tool for quality assessment. For all products, there were substantial gains in keeping quality when temperatures were maintained at or below -23°C. Packaging practices are also discussed as they pertain to enhanced product quality over frozen storage.

USE OF ELECTRICAL STIMULATION TO CONTROL SEAFOOD QUALITY

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The need of an inexpensive, portable, rapid, but reliable and nondestructive method of determining the liveliness of shellfish, prior to it being processed, has existed for many years. Such a method, based upon electrical stimulation, has been developed. In addition to being a very reliable method of determining if crab, lobster, and mussels are alive it has other useful applications. Use of such a method allows crab, lobster and mussels to be processed long after each would have to be discarded, when evaluated using traditional methods. Since the development of the prototype, an improved model has become available, commercially.

HANDLING FRESH TUNA IN DISTRIBUTION

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FDA's Office of Seafood has completed the preliminary analysis of data from two assignments issued in response to the reported histamine illnesses from eating imported fresh tuna in early May, 1992. The purpose of the first assignment was to evaluate imported fresh tuna and mahimahi for temperature abuse, histamine, organoleptic decomposition analysis. The second assignment evaluated the handling of fresh scombrotogenic fish in domestic distribution channels.

During the Import Assignment, 49% of the 1,118 fish tested for temperature were also analyzed for histamine and decomposition. Of the fish subjected to analysis, 4.8% showed evidence of decomposition and 2% exceeded 5 mg histamine/100g. Three percent of the fish tested on entry to the U.S. for temperature were 45°F or higher. None of these fish that were analyzed were positive for histamine however, two showed evidence of decomposition. From this data it appears that the temperature of the fish upon receipt cannot be the sole indicator of potentially violative product.

The results of the Domestic Assignment show that the cumulative time to deplete product at the supplier, wholesale and retail levels of the distribution chain can be as long as 14 days. 38 samples were collected from retail operations. Five of these samples showed evidence of decomposition and five samples exceeded 5mg histamine/100g. Thermometers in refrigerators, display cases and other storage areas need to be calibrated. Sufficient wet ice should be used to withstand transport temperatures. Product codes and employees training should be utilized.

BIOLOGICAL PROCESSING OF SARDINE PLANT WASTEWATER AND APPLICATION FOR AGRICULTURE

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The most important industry in the zone of Guaymas is the sardine industry, which is also the principal contaminant source to the bay. Approximately 80% of the weight of sardine processed is converted into effluents containing protein and ammonia compounds which are dumped into the bay. Methods for avoiding the dumping of the effluents of the sardine process have been proposed, like evaporation or flocculation of the protein. Both methods have high costs of installation and operation, and the final products have not gotten good acceptance in the market. A new method has been attempted using enzymes and bacteria to convert the protein and ammonia content of the effluents into nitrates. The final product of such biological process is a naturally enriched water. The results show that this water has a high nitrate content, a pH close to neutral and almost no turbidity. Such water has been used for cultivating microalgae, which are widely used in aquaculture. The water has applications also in agriculture

FATTY ACID AND PROXIMATE COMPOSITIONS OF WILD AND CULTURED GULF OF MEXICO STURGEON, *ACIPENSER OXYTHYNCHUS DESTOTOI*

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Cultured and wild samples of Gulf of Mexico sturgeon (*Acipenser oxythynchus destotoi*) were obtained. Proximate composition was determined for muscle using official AOAC methods except lipid, which was measured using Bligh and Dyer extraction. Fatty acid methyl esters were prepared using BF₃/Methanol catalyzed transesterification and were analyzed by GC using a 30m Omegawax 250 column. Fatty acids were identified using FAME standards and cod liver oil. They were verified using AgNO₃-TLC and GC-MS.

The meat of wild Gulf sturgeon ranged from 1.5 to 24.1% lipid and 66.5 to 76.6% moisture, while that of cultured fish were leaner (0.83-5.3% lipid and 77.1-88.2% moisture). Since the cultured fish were younger and exposed to similar rearing conditions, their lipid contents were quite uniform. However, the variations in the lipid contents of wild sturgeon did not affect the fatty acid composition of the oils. In saturated acids, the average for 16:0 was 26.1% in wild fish and 18.3% in cultured, whereas 18:0 was 3.1% less in wild fish (2.4 vs. 5.5%). The contents of 18:1 ω 7 and 20:1 ω 6, were found in cultured fish oils. No significant difference was found in the fatty acid compositions of other dienes, trienes and tetraenes. For polyunsaturated fatty acids, 22:6 ω 3 was about 11.4% in cultured fish and half this value in wild fish. The 22:4 ω 6 contents were 4 to 5 times higher in wild fish compared to cultured.

ANTICMICROBIAL ACTIVITY OF CHITOSAN

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Chitosan is a natural product used in cosmetics, pharmaceuticals, agriculture, food and waste treatment. It is a deacetylated derivative of chitin which is found in many crustacean shells. The molecular structure of chitosan is similar to cellulose where an hydroxy group is substituted by an amino acetyl group.

There are various kinds and grades of chitosan commercially available. These are mainly related to the type of raw materials and the specific process used to extract the chitin, and finally to the varying degree of deacetylation. Our approach to characterize these chitosans is to evaluate their antimicrobial activity and some chemical properties. This report related to the microbiological characteristics.

The minimum inhibition concentration (MIC) has been tested with *Pseudomonas aeruginosa* and *Aspergillus niger* on one type of chitosan. Two microbiological culture media were tested: a broth and a agar. The MIC for *A. niger* in the liquid medium was not found because no differences were observed between the control and the concentrations of chitosan tested. Wit *P. aeruginosa* a 95% inhibition was observed after fifteen minutes between the control and the tested concentrations but there was no evidence of significant differences between any of the concentrations tested.

In the solid medium, the MIC test of chitosan against *P. aeruginosa* between 0.25% and 0.5%. With 0.25% of chitosan 36.7 organisms were inhibited. However, 99.9% were inhibited with 0.5% of chitosan. The results obtained indicated that this microbiological test will be a useful tool to characterize different types of chitosan.

SENSORY ANALYSIS APPLICATIONS TO HARMONIZE EXPERT ASSESSORS OF FISH PRODUCTS

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No abstract submitted

DEVELOPMENT OF OFF-FLAVOR IN HYBRID STRIPED BASS GROWN IN RECIRCULATING AQUACULTURE SYSTEMS

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Hybrid striped bass (*Morone saxatilis* x *Morone chrysops*) were grown under commercial conditions in two different recirculating aquaculture systems. The system 1 configuration consisted of 42 concrete tanks containing 65,000 gallons of water each with its separate rotating biological filter. The system 2 configuration consisted of three concrete tanks in series with a common water source and one biological tower. The fish were grown to market size (1.5 approximate average size) within 18 months. Fish from both systems were found to contain a musty unacceptable flavor when subjected to sensory evaluation. Analysis by gas liquid chromatography (GLC), with confirmation by mass spectroscopy, confirmed the presence of 2-methylisoborneol. Microbiological analysis of scrapings from the two filter matrices revealed the presence of *Actinomyces* spp. A depuration process was able to remove the 2-methylisoborneol in the fish tissue within one-week period when evaluated by sensory analysis with GLC confirmation.

MODELLED INTERACTIONS WITH FISH MUSCLE

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No abstract submitted

USE OF GRAS COMPOUNDS TO ELIMINATE *VIBRIO VULNIFICUS* FROM OYSTERS

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No abstract submitted

PILOT PLANT PRODUCTION OF MACKEREL SURIMI

Herbert O. Hultin
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No abstract submitted

