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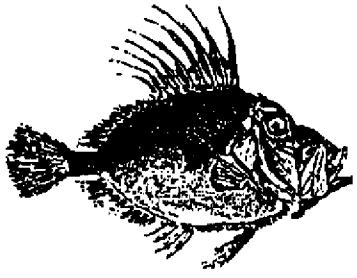
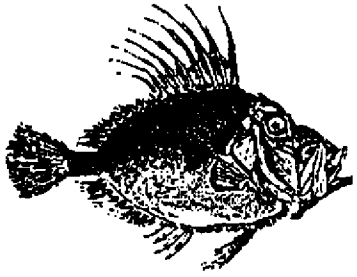
Tropical and Subtropical Fisheries Technological Conference of the Americas

in second joint meeting with

Atlantic Fisheries Technology Conference



Conference Proceedings
Fifteenth Annual Conference
December 2-5, 1990
Orlando, Florida



Florida Sea Grant Program
Building 803
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Gainesville, FL 32611

FLORIDA SEA GRANT COLLEGE PROGRAM

FIFTEENTH ANNUAL CONFERENCE

TROPICAL AND SUBTROPICAL FISHERIES TECHNOLOGICAL CONFERENCE OF THE AMERICAS

in second joint meeting with

ATLANTIC FISHERIES TECHNOLOGY CONFERENCE

December 2-5, 1990
Orlando, Florida

Conference Chairman,
W. Steven Otwell, Ph.D.
Food Science and Human Nutrition Department
Florida Sea Grant College Program
University of Florida
Gainesville, Florida

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

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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ABSTRACTS

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

CHALLENGES IN SEAFOOD TECHNOLOGY IN 1990's

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Good morning! I would like to welcome you all to Orlando and hope that your meetings in the next few days are productive, informative and a lot of new ideas and discussions take place. The entire seafood industry looks to you to help meet the challenges that the next decade will bring to us.

Today, I would like to talk to you about some of the trends and issues I think will significantly impact the way do business in the future and the opportunities they hold for all of us. I truly believe we are working in a rapidly changing business environment and the environment is going to change faster and get even more challenging and more competitive in the future.

Unit expansion will continue to outpace sales growth so that industry average sales per unit will continue to remain flat, or may even decline slightly. Most of the increases in total sales will come from off-premises, take-out dining. This trend of off-premise dining will dramatically impact the way all of us view the seafood business in the future.

Seafood is versatile and has shown that as the dining-out public continues to change it's eating trends and profiles, seafood preparations have easily kept pace with these sometimes dramatic swings. Through the 1990's, most food-service operators expanded their menus to offer more seafood selections. They have found seafood to be a great vehicle to use in communicating variety, value and quality to the dining public.

Many experts agree by the year 2000 the typical consumer will be:

- older than the current profile guest (from 38 to 48 years of age);
- not only older, but wiser. He or she will be more health conscious. They will know more about nutrition, cholesterol, triglycerides, in other words what's good or bad for them;
- better educated, more widely traveled and more sophisticated than in preceding generations;
- wanting quicker, friendlier, and better service than ever before.

There will be increased emphasis on higher flavor levels. We will see more ethnic food blended into american cuisine, with italian and oriental being the most popular. By the year 2000, oriental preparations will be a mainstream item in most american diets.

Seafood is and will continue to be a tremendous growth vehicle for the food service industry through the 1990's and because of its link to health and nutrition, seafood will continue to increase in popularity. We feel increased attention will be given to previous underutilized species. Improved harvesting technologies will allow us to utilize larger areas of our world's oceans, especially deep-sea species; and continued improvement of the technologies of aquaculture and the expansion of farming for new species, will continue to keep supply at the levels needed to meet demand.

As we all know, all seafood products are coming under stricter scrutiny by both the dining public and the government. Quite frankly, due to in 1989 seafood was the whipping boy of the food industry negative publicity over the safety and edibility of our products. Even though most of us believe that this publicity is bordering on sensationalistic journalism, it has forced the foodservice industry into rethinking its marketing strategies for seafood and has caused many operators to change their menus and eliminate certain species from use. The seafood industry and organizations like yours will have to work closer to ensure that the real, factual, story reaches the public.

Because of the increased government regulations at both the state and federal levels, and the increased fear of a damaging lawsuit, both the buyer and sellers of seafood products will need to be more selective of where and from whom they source their raw material. Many ask where will supply come from? To meet the increasing demand for seafood products, more and more of our supply will come from imports. Not only will we receive more raw materials from other countries, but more value-added processing will occur overseas. The U.S. will continue to face a difficult labor shortage at least through the mid-1990's when the children of the baby boom generation begin to come of age and move into the workplace.

Factors such as rising real estate, energy and labor costs, and a shortage of skilled labor will have an effect on kitchen design, equipment, and products we use. Computerization and advances in the food preparation and packaging techniques will offer new solutions to old design problems. Kitchen designers and engineers will find ways to make work areas and equipment more versatile and automated. The kitchen areas will be more productive and shrink as a percentage of the entire restaurant area. This will leave more space for the income-generating front of the house and change the type of products we demand.

The availability of' engineered' foods will increase. These will include products that are low in salt, low in fat, high in fiber, and purer. The advantages of these new methods for food production include; 1) creating new and satisfying taste sensations, 2) limiting the effect of raw ingredient shortages 3) convenience, and 4) assisting in diet control.

The impact of the microwave oven has not reached it peak. Today, nearly 50% of american homes have a microwave. By the year 2000 nearly 90% will have one since speed and convenience will be so important.

However, even with these positive changes, we feel government will become more involved in sampling and pathogen assessment. Today, government is in our kitchens, in our dining rooms, and in our businesses. They will continue to get us to pay for the programs they cannot fund both indirectly through taxes and increasingly through mandated programs financed directly by the industry. This mandated government intrusion into our business will be one of the most damaging and burdensome pressures facing operators in the years ahead.

As you can see, the 1990's will be a fast paced, rapidly changing environment. There will be many challenges ahead for all of us, but I am an optimist, and truly believe that for every challenge, there are two opportunities which will arise. At this time I would like to discuss how we can work together to meet these challenges and turn them into opportunities.

The seafood industry, along with the National Fisheries Institute, and the National Marine Fisheries Service, are working on the Mandatory Seafood Inspection Program, which when completed and adopted by Congress, will help restore public confidence in seafoods and give both the foodservice and seafood industries better guidelines and information to become better operators.

I am not here today to discuss which agency should be responsible for such an inspection program, but that a HACCP based program is needed and that you as seafood technicians need to help ensure that this program is well thought out, that the science being used is fair and the technologies and techniques are state of the art. We need your input to ensure that this HACCP inspection program insures that american public a safer and more wholesome product. But at the same time we need to review all food regulations to ensure that seafood is being fairly judged by the same standards that regulate the beef and poultry industries in this country and the world.

It is time for our government, organizations like yours, and the seafood industry to expand their thinking from a regional perspective and national perspective, to a world perspective. As we have all read, the U.S. industry will have to think globally to be competitive and successful. It is truly becoming a work market faster than most people realize. We have to remember as we develop our HACCP program that it will effect every nation that will export to our shores. In the next few years, as you complete your projects which will make seafood safer and improve quality, remember that whatever you develop will impact the world market. We will need to improve information flow such that as technologies are developed around the world, that we have systems in place which will quickly and fairly determine their positive and negative merits. In the 1990's we will have to develop a networking mind set which will tie in with all the world governments, academia, and regulatory agencies together to set world standards for food safety and quality.

As food safety and quality become more of an issue with the public, they will be looking to you for solutions which will help determine public policy here in our country and abroad. As more public pressure is placed on the government health agencies, we will be looking to you to provide the research and solutions which will help shape food policy in the 1990's.

The seafood industry will be viewing you as the problem solvers of the 1990's. We all know that their is too much energy being spent on pointing out problems and far to little being spent on coming up with solutions. In the late 1980's, we became reactionaries, but in the 1990's you will need to become visionaries. You will have to anticipate problems and spend more time developing solutions.

With the demand for a safer food supply, this can only lead to more samples being taken. We will need for you to develop faster testing methods to isolate specific pathogens of concern, levels of chemical additives, and tests for decomposition.

New research will have to be done to determine the proper acceptable levels of bacteria in all foods and seafood needs to be judged fairly against the other protein resources. With the accelerated development of aquaculture we are seeing a whole new flora of bacteria on seafood products, salmonella being just one of them. At what level is salmonella safe on any food?

We need to continue making our coastal shellfish resource safe and restore consumer confidence in this industry. Is depuration the answer and what further technologies do we need to develop. A faster test to determine Vibrio sp. is needed.

Is irradiation the answer to all our problems in the seafood industry? Has enough research been done to determine if there are side effects that have not surfaced as yet. If irradiation can be used on other protein resources, why not seafood?

As demand for seafood grows, we will look more and more to aquaculture products. Can the biologists give us better species through genetic engineering. Can we improve feeds to speed up growth rates to make farming more profitable to encourage more investment to meet the increasing demand for seafood worldwide.

We could talk for hours about the challenges that we face in the 1990's and all the opportunities that will arise for all of us. I wish to conclude with three important statements:

1. We need to remember that it is a world market and developing quickly.

2. Seafood is consumed in larger quantities in the world than in the U.S. and that our regulations need to work in coordination with the other nations of the world, and we need to share technologies with each other.

3. There is a need to set up standardization of international regulations which will guarantee a safe food supply to all, while setting fair standards for all the different food groups.

What I am proposing is no easy task. We will have to be proactive and anticipate the needs of the seafood industry. Things will happen faster in the 1990's than at any other time period in history. But, ladies and gentlemen, I feel we are up the challenges of our industry and look forward to the 90's and the year 2000.

EVALUATION OF FISHERY FOOD PRODUCT SAFETY

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Fish consumption in the United States has increased remarkably in recent years partly at least on the basis of a public perception that fish is a "healthy" food. It is paradoxical therefore that there is also widespread concern over the safety of seafoods and this is fueled by often ill considered and sometimes exagerated claims by protagonists of both sides of the safety argument. The facts in the issue are not easy to establish because of the nature of our disease reporting system in the U.S. and the dispersed and small scale of seafood-borne incidents. Nevertheless some data are available through CDC compilations, F.D.A. sources, State Health statistics and the general scientific literature. These sources were the basis of the following discussion.

Data from CDC files for the period 1978 to 1987 indicate that in that period seafood were responsible for 9 - 15% of all foodborne outbreaks each year (1). This is somewhat greater than for all other animal products during the same period and seems disproportionally high in view of the much smaller consumption of seafoods. However when the numbers of people actually made sick are considered a somewhat different picture emerges (table 1). While seafood were involved in 10.5% of reported outbreaks they only caused 3.6% of cases; about half of either of the other animal food categories. Nevertheless a significant number of people were reported as getting sick from seafood. A more detailed breakdown of the CDC data for seafood-borne illness reveals that most outbreaks were due to toxins while most cases were caused by unidentified agents (10). Identified microbiological incidents were third in number both for cases and outbreaks (table 2). Fish were implicated in most outbreaks and shellfish, dominantly molluscs, caused most cases.

Table 1. Fish and Shellfish Outbreaks and Cases in Relation to all Animal Food Sources and Total Food Borne Disease (from CDC Data for 1978-1987 including all cases

	OUTBREAKS	CASES
Meats (Beef & Pork)	298 (5.6%)	11,517 (6.9%)
Poultry (Chicken & Turkeys)	164 (3.1%)	10,563 (6.3%)
Seafoods (Fish & Shellfish)	558 (10.5%)	5,980 (3.6%)
All Foods	5,317	166,579

Table 2. Causes of Seafood-borne Illness (CDC 1978-1987).

	Toxins	Microorganisms	Chemical & Parasites	Unknown	(Total)
Shellfish					
Outbreaks	13	40	1	118	(172)
Cases	137	476	57	3271	(3941)
Fish					
Outbreaks	336	29	3	16	(386)
Cases	1548	227	61	203	(2039)
Total					
Outbreaks	349	69	4	134	(558)
Cases	1685	703	118	3474	(5980)

Source: Liston, 1990.

The toxin related incidents were mostly due almost equally to ciguatera and scombroid fish poisoning with a small number caused by paralytic shellfish poisoning, PSP, (table 3). The relatively small number of PSP incidents and the absence of other shellfish intoxications stands in sharp contrast to the dominance of shellfish as a reported cause of individual cases. Categorization of disease outbreaks by the major type of finfish involved (table 4) confirms the dominance of the two natural intoxications, ciguatera and scombroid. The fish most commonly identified as a cause of foodborne illness are, with the exception of tuna, relatively minor components of the seafood consumed by Americans (13). Why then do fish intoxications loom so large in the foodborne disease statistics? There appear to be at least three factors involved. One is the unusual pattern of symptoms which makes incidents highly visible and likely to be reported,another is the strong regionality of occurence, particularly of ciguatera, which emphasises the importance of incidents locally and third is the fact that intoxications are reported as outbreaks regardless of the number of cases. Looking quickly at tables 5 & 6 we can see that nearly all the ciguatera occurs in a few States and territories in which it is an important cause of illness among the local population (including Guam which was omitted from this table but is second only to Hawaii).Scombroid poisoning is more widely reported but shows concentration in a few states and is highly species related with mahimahi the single most reported species. Incidently,this seems to be mostly imported mahimahi as is the second most important species, tuna. Other reported causes of fishborne illness are all microbial and except for botulism appear to be related to errors in handling at the service level. Botulism occurs at a rather consistent low level and is mainly due to consumption of marine animal foods of ethnic origin by native Alaskans (table 7).

Table 3. Distribution of Toxic Outbreaks and Cases. (CDC 1978-1987)

	Outbreaks	Cases
Total Toxins	349 (62.5%)	1685 (28.0%)
Ciguatera	179 (32.0%)	791 (13.0%)
Scombroid	157 (28.0%)	757 (13.0%)
PSP	13 (2.5%)	137 (2.0%)

Table 4. Associated with Food-Borne Disease 1978-86 (compiled from CDC and NETSU Reports)

FINFISH	OUTBREAKS	ETIOLOGY
Reef Fish	126	Ciguartera
Mahi Mahi	55	Scombriod
Tuna	52	Scombroid Few Enterics
Bluefish	15	Scombroid
Salmon	3	Scombroid Botulism
Mackerel, Redfish, Marlin	1 (ea)	Scombroid
Sole	1	Salmonella
"Other Fish"	57	Ciguatera Scombroid Botulism (3)
Kapchunka, Marinated Fish, Fermented Fish	1 (ea)	Botulism
Gefelte Fish, Fish Combo	1 (ea)	Salmonella
Fish Salad	1	Hepatitis
"Eskimo" Ethnic Food	15	Botulism

Source: (4-8)

Table 5. Outbreaks and Cases of Ciguatera in USA, 1978-1987

State	Outbreaks	Cases	Percent Outbreaks	Percent Cases
California	1	2	0.6	0.3
Florida	9	35	5.0	3.0
Hawaii	144	560	80.0	71.0
Louisiana	1	6	0.6	0.8
Puerto Rico	13	73	7.0	9.0
Vermont	1	3	0.6	0.4
Virgin Islands	9	110	5.0	14.0
Washington	1	2	0.6	0.3
TOTAL	179	791		

Source: (9, 10)

Table 6. Scombroid Fish Poisoning in the USA, 1978 - 1987

State	Outbreaks		Cases	
Alaska	3		17	
Arizona	3		7	
California	18	(12%)	69	(9%)
Connecticut	8		47	
D.C.	1		3	
Florida	1		20	
Hawaii	45	(29%)	171	(23%)
Idaho	1		4	
Illinois	3		35	
Indiana	1		4	
Kentucky	1		7	
Maine	3		54	
Maryland	1		10	
Michigan	3		25	
Minnesota	1		24	
Nebraska	1		10	
New Jersey	4		42	
New Mexico	1		2	
New York	30	(19%)	122	(16%)
North Carolina	1		10	
Pennsylvania	2		4	
Texas	2		11	
Vermont	3		6	
Virginia	2		13	
Virgin Islands	1		5	
Washington	16		35	
Wisconsin	1		1	
TOTAL	157		758	

Source: (9)

Table. 7 Fish-Borne Botulism, 1978-87

State	Seafood Product	Outbreaks	Cases
Alaska	Fish Eggs	7	8
	Seal/Meat or Oil	5	7
	Fish Heads	2	11
	"Other Fish"	2	2
	Walrus Blubber	1	1
	Unknown	1	1
New Jersey	Kapchunka	3	7
California	"Other Fish"	1	1
Hawaii	"Other Fish"	1	1
Idaho	"Other Fish"	1	1
Washington	Home Canned Salmon	1	1
Puerto Rico	Home Marinated Fish	1	3

Source: (4-9)

A general breakdown of cases due to bacteria for finfish and shellfish is shown in Table 8. It is interesting that the only naturally occuring bacteria reported by CDC to cause disease from finfish were C. botulinum (type E) and V. cholerae (8). However the situation with shellfish is quite different. This distinction is even clearer in table 9,in which outbreaks are shown (8, 14). Naturally occuring vibrios were most commonly implicated among bacteria and toxins are represented only by a few PSP incidents. Mostly after the fact analyses of a large number of cases ascribed to unknown etiological agents very strongly implicates Norwalk or Norwalk-like viruses. Incidentally analysis of limited data from States indicates V. parahaemolyticus as a major cause of sporadic single person incidents adding even more to the total in table 9. But of course the vibrio inducing greatest concern is V. vulnificus because of the frequently fatal disease it causes in susceptible people almost always from consuming raw oysters (12). There is an interesting association between geographic origin of clams and oysters and the incidence of seafoodborne disease. Most clam related outbreaks are reported from northern states and are dominantly due to viruses while most oyster outbreaks occur in southern states and are due to vibrios (14). This clearly bears a relationship to water temperature in the growing areas which in colder areas supports survival of viruses but inhibits vibrio growth while the converse is true in warm water areas. Of course it is also important to note that vibrios are part of the normal microflora of molluscs while the viruses are derived in most cases from sewage (sometimes treated sewage).

Table 8. Bacteria Reported As Cause of Seafood-borne Disease Confirmed Cases 1973-1987 (CDC)

C. botulinum	—	38
C. perfringens	28	46
Salmonella	80	67
Shigella	77	60
S. aureus	9	3
B. cereus	6	4
V. cholerae 01	14	2
V. cholerae non-01	11	—
V. parahaemolyticus	176	—

The hazard to consumers from chemical contaminants in fish is quite difficult to assess because there is still considerable disagreement among knowledgeable scientists concerning effects of the low levels of contaminants found in fish (10). Moreover there are no public health statistics on "chronic" illness identifiable as caused by seafood. Clearly there is undesirable and probably dangerous contamination of certain species of fish in limited geographic areas including certain parts of the Great Lakes system and other rivers and inland lakes subject to industrial contamination (9). This is also true for a few inshore marine locations most of which have been quite widely publicised (16). However public authorities have in most cases been alerted to such local areas of contamination and have taken steps to warn recreational and subsistence fishers and where appropriate to embargo commercial fishing on the basis of likely hazard. There does not seem to be any convincing evidence of widespread contamination of the U.S. fish supply with chemical residues which present a large and immediate danger to consumers (table 10). Some scientists are concerned with long range and sometimes subtle effects of present levels of contamination by such substances as PCBs and mercury particularly for sensitive groups such as young children,pregnant women and individuals with underlying chronic disease (10). All agree on the necessity of strong laws and effective enforcement action to greatly reduce and perhaps eventually eliminate the dumping of chemical wastes into oceans and streams.

Table 9. Seafoods Associated with Food-Borne Disease, 1978-86 (Compiled from CDC and NETSU Reports)

Shellfish	Outbreaks	Etilogy
Hard Clams	111	Most unknown virus
Other Clams	20	Most unknown, virus or vibrio
Oysters	136	Vibrios, few virus
Mussels	8	Paralytic shellfish poison, few vibrios
Scallops	2	Paralytic shellfish poison
Limpets	2	"Chemical"
Shrimp	11	Vibrio and other bacteria
Crab	2	Staphylococcus aureus
"Shellfish"	18	Vibrio, PSP, enterics, hepatitis

Source: (4-9; 14)

Table 10. Dietary Exposures Estimated from Selected FDA Surveillance Data, 1984-1988 (Source: 10).

Limit Chemical	Estimated Aggregate Exposure (mg/kg/day) U.S. Finfish	U.S. Shellfish	All Imported	Total	EPA Cancer Potency (mg/kg/day)$^{-1}$ [a]	Indicated Upper-Confidence-Cancer Risk
Organics						
Benzene hexachloride	2.9×10^{-8}	1.2×10^{-8}	1.0×10^{-8}	5.1×10^{-8}	6.3	3.2×10^{-7}
Chlordane	2.3×10^{-7}		5.1×10^{-8}	2.8×10^{-7}	1.3	3.6×10^{-7}
Dacthal (DCPA)	4.0×10^{-8}			4.0×10^{-8}		
tDDT	8.8×10^{-6}	2.1×10^{-8}	2.5×10^{-7}	9.0×10^{-6}	0.34	3.1×10^{-6}
Dieldrin	4.9×10^{-7}		2.5×10^{-8}	5.1×10^{-7}	16	8.2×10^{-6}
Endrin	1.7×10^{-8}			1.7×10^{-8}		
Heptachlor	4.3×10^{-8}		3.1×10^{-8}	7.4×10^{-8}	4.5	3.3×10^{-7}
Lindane			1.5×10^{-9}	1.5×10^{-9}	1.3	2.0×10^{-9}
Mirex	8.7×10^{-8}			8.7×10^{-8}		
Nonachlor	2.4×10^{-7}		1.5×10^{-8}	2.6×10^{-7}		
Octachlor	1.0×10^{-7}			1.0×10^{-7}		
Omethoate	1.2×10^{-8}			1.2×10^{-8}		
Pentachlorophenol	1.5×10^{-9}			1.5×10^{-9}		
Pentachloroaniline		2.0×10^{-9}		2.0×10^{-9}		
PCBs	6.9×10^{-6}	6.3×10^{-7}	3.7×10^{-7}	7.9×10^{-6}	7.7	6.0×10^{-5}
2,3,7,8-TCDD	1.5×10^{-11}			1.5×10^{-11}	1.6×10^{5}	2.3×10^{-6}
Tecnazene			1.5×10^{-9}	1.5×10^{-9}		
Total						7.5×10^{-5} [b]
Metals					Total in µg/day	
Aluminum		5.3×10^{-4}		5.3×10^{-4}	37	
Arsenic	5.3×10^{-7}	1.1×10^{-5}	1.1×10^{-7}	5.4×10^{-4}	38	
Beryllium		1.1×10^{-7}		1.1×10^{-7}	0.0	
Cadmium	9.6×10^{-7}	1.4×10^{-5}	9.5×10^{-6}	2.4×10^{-5}	1.7	
Chromium		8.2×10^{-6}		9.0×10^{-6}	0.6	
Lead	2.6×10^{-6}	2.8×10^{-6}	5.9×10^{-6}	1.1×10^{-5}	0.8	
Mercury	1.1×10^{-5}	1.2×10^{-5}	1.2×10^{-5}	3.5×10^{-5}	2.5	
Selenium		1.5×10^{-6}		1.5×10^{-6}	0.1	

[a] This level is an upper estimate or the actual risk may be as low as zero.

[b] Because these are 95% upper-confidence-limit estimates it is not strictly correct to add them. However, the statistical error in this case is not large compared to the other uncertainties of the analysis.

Figure 1. Orgin of Seafood-Borne Disease (Source: Liston, 1990)

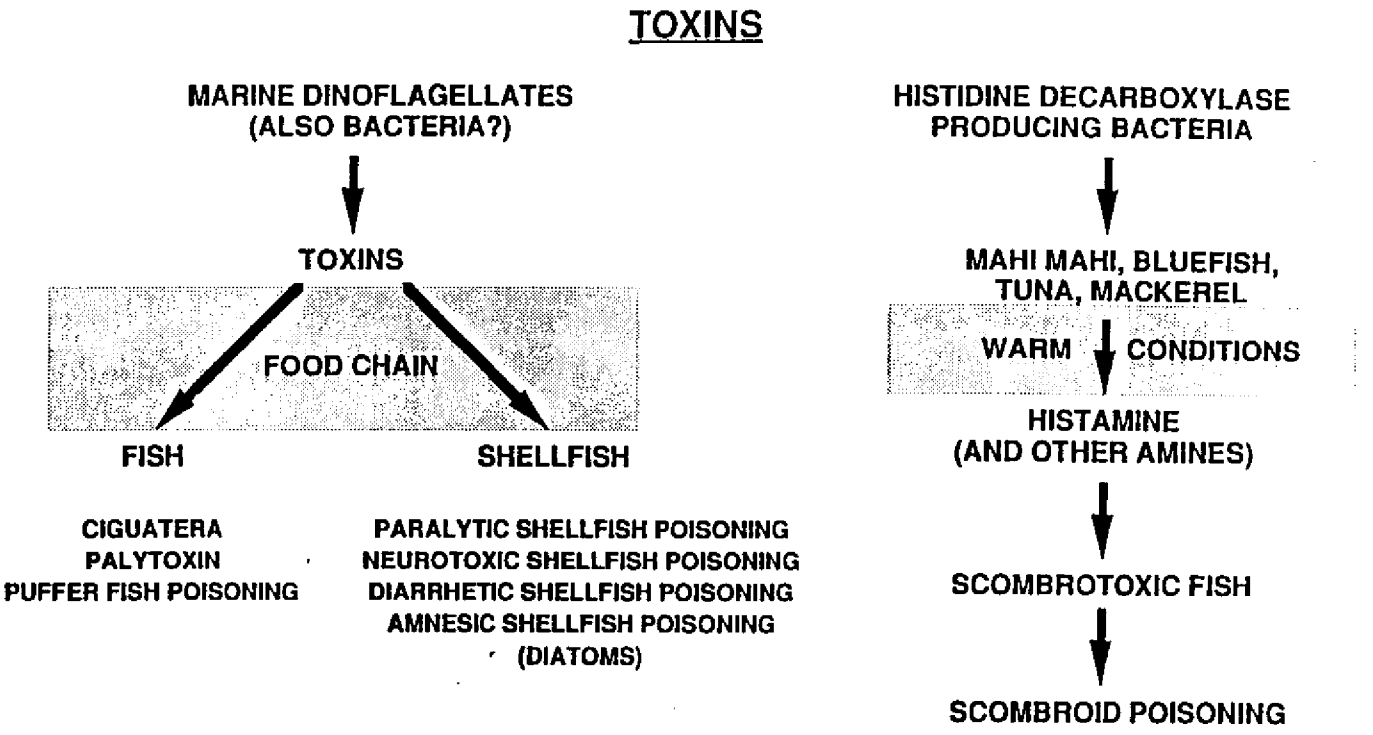
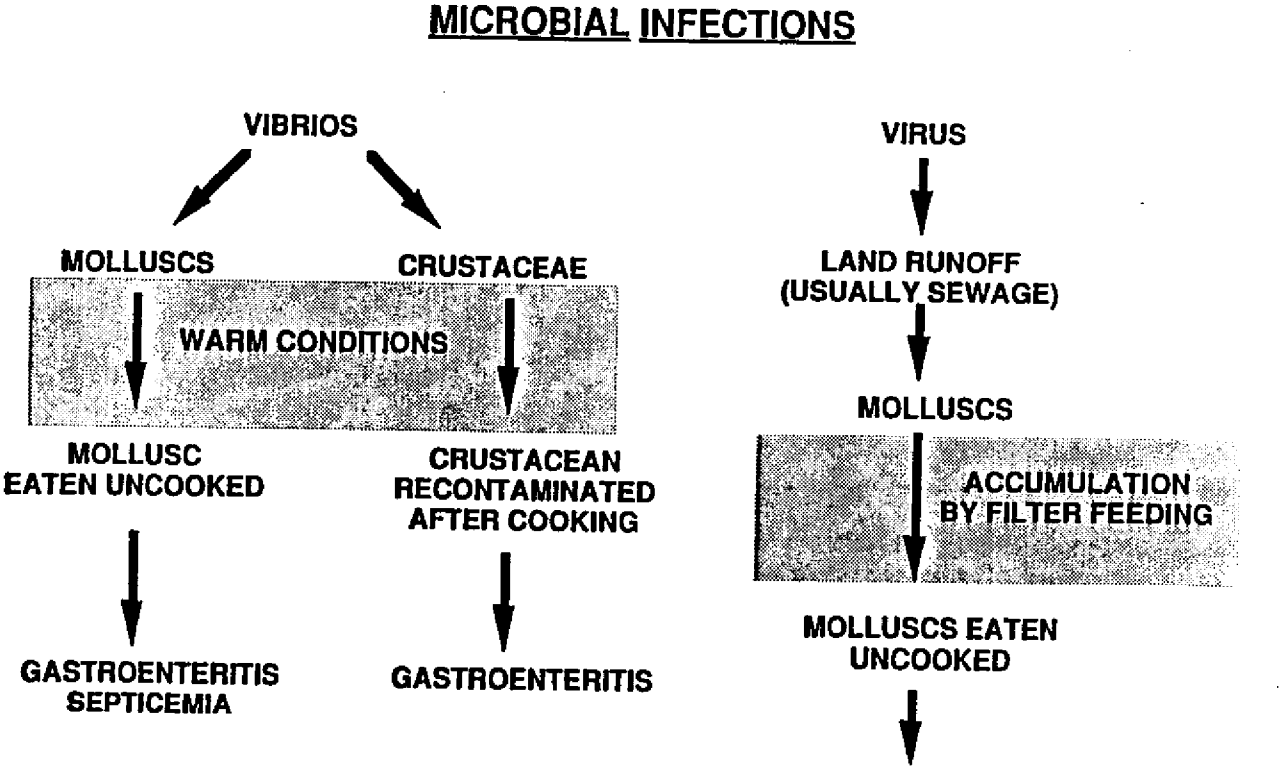


Figure 2. Orgin of Seafood-Borne Disease (Source: Liston, 1990)



A general view of seafood safety based on the available evidence indicates that hazards mostly originate in the environment either through natural processes as in the case of toxins and vibrios or by contamination of growing waters as for viruses and chemical contaminants. Scombroid poisoning may be considered an exception to this concept since toxin development in that case is due to failures in handling procedures that permit mesophilic bacteria to grow and produce histamine but even here the elements are originally present in the living fish (15). The other major factor that jumps out from the data is the role of consumers in facilitating their own illness. Obviously the most critical factor here is consumption of raw molluscan shellfish, but errors in food preparation and storage also contribute significantly to bacterial illness (2). The major pathways of seafood related human disease are shown in figs 1 & 2.

With the exception of the natural toxins and perhaps some chemical contaminants in certain species of fish from particular areas,fish and shellfish available to the American public are safe when properly handled and adequately cooked. We need to provide better information to the public in a balanced but realistic way of both the benefits and, where they exist, the potential hazards of eating seafood so that they can make informed choices in purchases and preparation of fish and shellfish.

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FOOD AND DRUG ADMINISTRATION AND NATIONAL MARINE FISHERIES SERVICE JOINT SEAFOOD INITIATIVE: STATUS

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INTRODUCTION

Seafood safety is regulated at the Federal level primarily by the U.S. Food and Drug Administration (FDA) which operates a comprehensive, mandatory program under the authorities of the Federal Food Drug and Cosmetic Act and the Public Health Service Act. In addition to this mandatory program, the states operate mandatory seafood regulatory programs designed to meet their regional needs. The National Marine Fisheries Service (NMFS) operates a voluntary, fee-for-service inspection program under the authorities of the Agricultural Marketing Act of 1946 and the Fish and Wildlife Act of 1956. A Memorandum of Understanding (MOU) exists between FDA and NMFS relating to this program. Under that MOU, NMFS ensures that industry participant's operations and products meet the requirements of the FD & C Act, as well as NMFS own quality and identity requirements.

It is well recognized that these programs differ in approach from the current continuous in-plant visual inspection provided by the U.S. Department of Agriculture for the red meat and poultry industries. These differences have led some individuals and groups to call for a major upheaval in the legislative mandates governing food safety in this country. The 101st Congress responded to these groups by drafting some 10 bills dealing with seafood inspection. At the close of Congress the Senate had approved a bill which would transfer authority for seafood from FDA to the USDA. That bill was introduced to and rejected by the House which passed a bill differing in fundamental respects from the Senate Bill, principally by retaining seafood safety authority in FDA. The Congress adjourned without attempting to reach agreement on a seafood bill.

While the current administration disagreed that redeligation of authority for seafood safety should occur, the FDA and NMFS agreed that they could enhance their respective seafood programs independently of any new legislation by jointly designing and administering a new seafood inspection program. Such an undertaking would have the benefits of responding to the immediate calls for changes in seafood regulation while not weakening current programs, and would pave the way for rational changes in the future.

The generic HACCP models developed during the three year Model Seafood Surveillance Project conducted by NMFS and firms representative of the industry were becoming available for use throughout the seafood industry to design operation specific HACCP plans. The National Academy of Sciences report on Seafood Safety and regulation would be completed by the beginning of 1991, thus providing additional guidance to the two agencies on any beneficial modification of current seafood enforcement and research work. The two agencies recognized that they already had the tools, expertise, and concepts to proceed with a new seafood inspection program using these resources.

FDA and NOAA/NMFS began the design of a new, jointly operated inspection program and enlisted the cooperation of the seafood industry to the extent that industry trade groups and individuals would participate. This joint program is based on the principles of the Hazard Analysis Critical Control Point system of food inspection. This system requires the industry to develop and maintain records of control points in their process that have been determined critical for the control of product safety, plant/food hygiene, and economic fraud hazards associated with the particular product. Industry and government then become partners in the business of food inspection.

This program will be entered by the firm on a voluntary basis. A contract will be signed between the firm and FDA and NOAA as to the conditions and details each party will provide. Although every firm is currently responsible to provide a product which complies to the laws and regulations of food production, the signing of the contract by the firm will state boldly the firm's commitment to the consumer by not only providing complying product but requesting the government to monitor its progress to ensure this commitment. For this commitment the producer will be able to place a mark of inspection indicative of the program on those products which meet the requirements, if he so chooses. Fees to recover the cost of the program will be assessed to the participating firms.

This program is unique in that it will be jointly operated by both agencies each with a particular role. NOAA will be responsible, in general, for the routine monitoring of the firms activities. The FDA's role is to verify the effectiveness of the program itself. In addition, in keeping with the principles of HACCP, this program will eventually cover all points in the distribution chain--from water to consumer. Sectors of the program are under development for domestic processors, foreign processors and imports, retail, and molluscan shellfish. Other sectors will be developed as needed.

PROGRAM DESIGN

Each sector program is expected to have these elements: Plan submission, plan review, plan acceptance, validation, monitoring, and verification.

- Plan submission, review, and acceptance are jointly operated and maintained by FDA and NOAA.

- Validation is the process of ensuring that the plan as written conveys an accurate picture of how the facility is operating as well as whether the facility is following the written HACCP-based plan. The validation is performed jointly by both agencies.

- Monitoring - NOAA will monitor the facility's operations for adherence to its HACCP-based plan(s), through inspections of the facility and record review. In addition, sample collection and analysis may be used to determine compliance with the HACCP plan(s)

- Verification - FDA will notify the Regional NOAA inspection office, then visit the facility to conduct an inspection to evaluate the validity of the current HACCP plan(s), the facility's adherence to its plan, NOAA monitoring procedures, adherence to the FD & C Act, and also where applicable, to inspect the contract laboratory to ensure that samples received for analysis are being examined correctly.

STATUS AND SUMMARY

The ANPR and invitation to participate in the first processor level pilot have been issued. The results of the pilot studies and responses to the ANPR questions will serve as guidance on the final structure of the program. The pilot studies will test the success of industry in designing and operating under HACCP plans and of government in adapting inspection procedures to be able to more efficiently and effectively inspect firms so organized and with appropriate records available.

We quickly recognized that everything cannot begin at once. The smoothest way to start up what we expect to become a very large and comprehensive program appeared to be to work with the major segments of industry in an organized fashion. To do this we began a pilot study at the processing phase of seafood production. This pilot will be followed by the other previously listed sectors. It is not yet clear whether a pilot will be needed for the harvest phase of seafood production.

Initially sixteen firms expressed interest in the first pilot and sent representatives to the FDA/NOAA training course offered in August 1990 to familiarize them with details of the program and how to apply. To date ten firms have agreed to participate in the domestic processors pilot study. Of these nine have submitted HACCP plans. These plans have been reviewed and comments made back to the firms. Once the plans have completed the review process, we will begin to schedule the on-site validation visits. The domestic processors pilot is underway.

In order to initiate pilots for the retail and shellfish sectors a different approach is being used than the one utilized for processors. Both agencies wished to take advantage of the existing state and local shellfish and retail inspection programs, with which FDA has had a long association through the FDA Retail Foods Protection Program and the Shellfish Branch in the Division of Cooperative Programs. This was accomplished by enlisting the participation of the National Conference on Food Protection and the Interstate Shellfish Sanitation Conference. It is expected that the monitoring inspection functions will be carried out with the state and local inspectors normally responsible for the retail and shellfish firms which will join our new joint program. These inspectors will have undergone the same training as the FDA investigators and NOAA inspectors.

SEAFOOD SAFETY CONCERNS AND FREQUENCIES OF SEAFOOD CONSUMPTION

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Safety of the nation's food supply has received significant attention in recent years from researchers, policy makers, food producers, consumers, and the mass media (7). Growing interest in food safety stems from greater environmental and health awareness and growing fears about food contamination. Seafood products have not been immune to safety concerns that characterize most food product categories. Media reports of marine and fresh water pollution, contaminants in fish and shellfish, and seafood-borne illnesses have raised questions about seafood safety and adequacy of federal efforts to monitor seafood.

There is no general consensus on the safety of seafood in the U.S. On one hand, the U.S. General Accounting Office, after a review of Center for Disease Control and Food and Drug Administration statistics on seafood-borne illnesses, seafood samples, and processing plant inspections, concluded that U.S. seafood supply is essentially safe, and a mandatory federal inspection program is not warranted. On the other hand, several public interest groups, notably Public Voice for Food and Health Policy, claim federal efforts to monitor seafood are inadequate and permit serious threats to public health (4,5,8). A comprehensive study on seafood safety conducted under the auspices of the Institute of Medicine affirms that the seafood supply is essentially safe. However, it concludes that there are some areas of risk and recommends improvements in federal, state and local inspection programs (6).

Given the nutritional and health merits of seafood, expansion of national seafood consumption is a desirable public policy objective. Rising per capita seafood consumption (from 12.8 pounds in 1980 to 15.9 pounds in 1989) suggests that this objective is being met. The growth is attributable to a variety of factors including media reports in the early 1980s of medical findings that increased seafood consumption reduces the risk of heart disease. Partly because of health concerns, American consumers also reduced red meat consumption, substituting seafood, poultry, and other foods that are low in total fat, saturated fat, and cholesterol. Increased availability of fresh seafood in retail stores and restaurants also encouraged increased seafood consumption. However, health concern is an argument in consumers' utility function (3). Consequently, greater concern about seafood safety should slow down growth in seafood consumption and encourage substitution of safer food forms. This raises several questions. Are consumers particularly concerned about seafood safety? Does their concern have the potential to reduce seafood consumption? Will a mandatory seafood inspection program allay their concern and foster growth in seafood consumption?

It has been shown that willingness to pay a premium price for food decreases with an increase in hazard content (2). Hence, willingness to pay a premium price for federally inspected seafood should be directly related to the amount of safety enhancement such inspection is expected to entail. A mandatory federal inspection program is likely to raise costs to consumers

both in higher taxes to support governmental administrative costs and in higher seafood prices to cover program implementation costs to the U.S. seafood industry. Whether or not such a program enhances seafood consumption depends on the level of consumer confidence it engenders and the direct costs to consumers. Information on the potential impact of a mandatory inspection program on seafood consumption patterns, and on the willingness of seafood consumers to pay a premium for inspected seafood will contribute to assessments of merits of such a program.

Empirical attempts to investigate the relationships among seafood safety concerns, seafood consumption, and willingness to pay for inspected seafood are few. Based on a group of New Jersey consumers, this study investigates several issues related to seafood safety. Specifically, an attempt is made to provide answers to the following questions.

(i) What concerns do consumers have about seafood safety and which types of seafood are they most concerned about?
(ii) Are consumers more cautious today about the seafood they consume than they were five years ago?
(iii) Do consumer concerns about seafood safety affect their seafood consumption patterns?
(iv) Are consumers willing to pay more for federally inspected seafood?
(v) Will mandatory federal inspection reduce consumer concerns about seafood safety and result in increased seafood consumption?

METHODS

The data needed to answer the above questions came from an intercept survey of 450 individuals attending the 1990 New Jersey Seafood Festival in Belmar, New Jersey. The event was attended by about 12,000 people. For responses to be representative of household food purchase decision makers, the survey was administered only to persons 18 years or older. The questionnaire contained questions regarding socio-economic and demographic characteristics, opinions about seafood, consumption patterns, types of concerns respondents have about seafood, and whether or not respondents are willing to pay a premium price for inspected seafood.

To generate answers to questions (i) and (ii), means of relevant variables were constructed and analyzed. To generate answers to questions (iii), (iv), and (v), the correlation analysis procedure in FOCUS was used to evaluate the relationship between relevant variables. A zero value for the correlation coefficient implies no correlation while a +1 (-1) value implies perfect positive (negative) correlation. The extent to which a coefficient deviates from zero reflects the extent of positive or negative correlation. Since most variables have limited range and many are binary choice variables, correlation coefficients between them are expected to not deviate markedly from zero. However, the relative magnitudes of even low correlation coefficients still indicate relative degrees of correlation among the variables considered.

RESULTS AND DISCUSSION

Results of Mean Analysis

Mean values of socio-economic and demographic variables and consumer opinions about seafood are summarized in Table 1. Mean age of survey respondents is 45 years. More females than males participated in the survey. Mean household size is 2.8. Survey respondents are generally well-educated; most have post-high school training. With a mean household income

of about $50,000, respondents are generally well-off. The average respondent dines out once per week. Almost all respondents (98 percent) believe that seafood is good for your health.

When asked their reasons for eating seafood, over half indicated that it "tastes good" and "is healthy and nutritious", about a third indicated that it "is easy to prepare," and about a quarter indicated that seafood "adds variety to meals." In spite of the importance of health factors as a reason for consuming seafood, very few respondents indicated that they eat seafood because of their doctor's advice. Consumers also do not perceive seafood as "being a good buy". Promotion of the merits of seafood, attempts to make seafood more affordable, and more frequent advice from physicians about the health advantages of seafood may be vehicles to future increases in seafood consumption.

Table 1. Socio-Economic Demographic Factors, Opinions About Seafood, and Frequency of Consumption: Mean Values and Correlation Coefficients.

	Mean[a]	Correlation Coefficients Frequency of Consumption Relative to		
		5 Years Ago[h]	Current[h]	Socio-
Economic Demographic Factors:				
Age	44.60[b]	0.14		0.29
Frequency of Dining Out	2.96[c]	0.06		0.20
Household Income	2.55[d]	0.03		0.02
Sex 0.37[e]		0.01		0.07
Education Completed	3.32[f]	0.01		0.02
Household Size	2.77[g]	-0.08		-0.09
Is Seafood Good for Your Health?	0.98	0.07		0.20
Why Do You Eat Seafood?				
Tastes Good	0.61	-0.01		0.05
Is Healthy and Nutritious	0.54	0.15		0.27
Is Easy to Prepare	0.34	0.06		0.13
Adds Variety to Meals	0.24	0.02		-0.07
Good Quality is Available	0.12	0.05		0.12
Is a Good Buy	0.06	0.00		0.07
Was Suggested by Doctor	0.06	0.04		0.02

Range of responses:

[a] 0 = No, 1 = Yes, unless otherwise specified.

[b] 18-72 years. Survey was administered to persons 18 years of age or older.

[c] 0 = never, 1 = several times/year, 2 = once or twice/month, 3 = once/week, and 4 = two or more times/week.

[d] 1 = less than $25,000, 2 = $25,000-$49,999, 3 = $50,000-$74,999, 4 = $75,000-$99,999, and 5 = $100,000 or more.

[e] 0 = female and 1 = male.

[f] 1 = grade school, 2 = high school, 3 = technical school. 4 = college, and 5 = graduate or professional school.

[g] 1 - 9+ persons.

[h] 1 = perfect positive correlation, -1 = perfect negative correlation.

Mean values of responses to questions on current consumption patterns and reasons for not consuming more seafood appear in Table 2. The average respondent consumes seafood once per week, consumes seafood at-home slightly less than once per week, and orders seafood 50 to 75 percent of the time when dining out. The average consumer (71 percent) increased his/her consumption of seafood over the last 5 years. This is consistent with the national trend. Compared to a 1985 survey by Better Homes and Gardens, this survey's respondents consume seafood more often, consume seafood more often at home, dine out less, and order seafood more often when dining out (1).

The primary reason cited for not eating seafood more often at home and for not ordering seafood more often when dining out is "it's too expensive". Although "am concerned about contaminants" and "can't get freshness and quality I like" were among the other important reasons for not consuming more seafood, price appears to be the major deterrent to more frequent seafood consumption. Although the proposed mandatory federal seafood inspection program could increase consumer confidence in seafood safety, it could also result in decreased seafood consumption if it raises seafood prices significantly.

Table 2. Frequency of Current Consumption and Reasons for Not Consuming More Seafood: Mean Values.

	Mean[a]
Frequency of Current Seafood Consumption	
In general	3.26[b]
At-home	2.92[b]
Order when dining away-from-home	3.04[c]
Relative to 5 years ago	0.74[d]
Why Don't You Eat Seafood More Often At Home?	
It's Too Expense	0.30
Family Doesn't Like It	0.10
Don't Know How To Cook It	0.09
Don't Like Smell	0.08
Am Concerned About Contaminants	0.08
Can't Get Freshness and Quality I Like	0.07
Type Preferred Not Available	0.07
Don't Like Handling It	0.04
Don't Like Taste/Flavor	0.02
Why Don't You Order Seafood More Often When Dining Out?	
It's Too Expense	0.21
Type Preferred Not Available	0.11
Portions Served are Insufficient	0.10
Can't Get Freshness and Quality I Like	0.10
Not Familiar With Menu Items	0.08
Am Concerned About Contaminants	0.07
Don't Like Taste/Flavor	0.02

Range of responses:

[a] 0 = no, 1 = yes, unless otherwise indicated.

[b] 0 = never, 1 = several times/year, 2 = once or twice/month, 3 = once/week, and 4 = two or more times/week.

[c] 0 = never, 1 = less than 25%, 2 = 25-49%, 3 = 50-74%, and 4 = 75% or more of the time when dining out.

[d] -1 = less, 0 = the same, 1 = more.

The second most important reason cited for not eating seafood more often at home is that "family members don't like it". This factor is likely to be increasingly important as a deterrent to at home consumption as family size increases. Other reasons cited for not eating seafood more often at home include "don't know how to cook it," "don't like smell," and "type preferred is not available". "Type preferred not available," "insufficient portions served," and "am not familiar with menu items" are other reasons cited for not ordering more seafood when dining out. "Don't like taste/flavor" is not an important deterrent to increased seafood consumption either at home or when dining out. Educational programs on seafood preparation and how to minimize off odors when preparing seafood, and new products which are appealing to children, minimize odors, and are easy to prepare may be useful avenues to increasing at home consumption.

Mean values of responses to the questions about type of concerns about seafood and means by which caution is exercised in consuming seafood appear in Table 3. In response to the question "What concerns you about seafood?", 63 percent chose contaminants, 31 percent chose "concerned about uninspected seafood", and 29 percent chose "bacteria from poor handling". Only 10-14 percent expressed concern about parasites, additives, and viruses and toxins. With the major concern of respondents being contaminants, uninspected seafood, and bacteria from poor handling, it appears that for federal inspection to allay the fears of consumers it should at least address these issues.

In response to the question "Are you more cautious when eating seafood today compared to five years ago?", a little over half (56 percent) indicated that they are. The fact that fewer respondents are more cautious (56 percent) than are concerned (63 percent) about seafood safety indicates that concern about seafood safety does not automatically result in greater caution, that is, behavioral change. When asked "How do you exercise caution when eating seafood?", the majority of respondents chose by eating less "raw shellfish" and "raw finfish". Greater reduction in raw versus fresh cooked shellfish and finfish indicates that consumers understand that cooking seafood reduces many health risks. Only 6 percent reduced consumption of all seafood as a result of concern about seafood safety.

The heavy media coverage of marine pollution and seafood contaminants in New Jersey in recent years motivated specific survey questions about whether consumers had exercised caution by eating New Jersey seafood. In spite of considerable negative media, relatively few of the survey respondents (11 percent) reported decreasing their consumption of New Jersey finfish and shellfish. It is possible that the festival did not attract people with serious concerns about New Jersey seafood quality. Furthermore, low frequency seafood consumers are more likely to have reduced seafood consumption than respondents of this survey who are clearly seafood enthusiasts. It has been argued that federal inspection of seafood has the capacity to encourage seafood consumption (9). The fact that a strong majority (71 percent) of survey respondents expressed a willingness to pay more for federally inspected seafood indicates that consumer satisfaction is likely to increase under such a program. This issue is pursued further below.

Results of Correlation Analysis

Correlation coefficients indicating relationships between frequency of seafood consumption (current and relative to five years ago) and hypothesized related factors are reported in Tables 1 and 3. Related factors of particular interest are those indicating: socio-demographic characteristics, opinions about seafood, types of concerns about seafood, and means by which caution is exercised in consuming seafood.

Table 3. Concern and Caution Regarding Seafood Consumption and Frequency of Consumption: Mean Values and Correlation Coefficients.

Mean[a]	Frequency of Consumption[b] Relative to 5 Years Ago	Current	What Concerns
You About Seafood?			
Contaminants	0.63	0.02	-0.06
Uninspected Seafood	0.31	0.01	0.04
Bacteria from Poor Handling	0.29	-0.01	-0.01
Parasites	0.14	0.09	-0.05
Additives	0.13	0.05	-0.02
Viruses & Toxins	0.10	0.05	-0.03
Are You More Cautious When Eating Seafood Compared to 5 Years Ago?	0.56	-0.10	-0.06
How Do You Exercise Caution When Eating Seafood? By Eating Less:			
Raw Shellfish	0.28	0.01	0.04
Raw Finfish	0.21	0.10	0.04
Fresh (cooked) Shellfish	0.16	-0.06	0.04
Processed Seafood	0.11	-0.04	0.00
New Jersey Finfish and Shellfish	0.11	-0.09	-0.05
Fresh (cooked) Finfish	0.07	-0.12	-0.05
All Seafood	0.06	-0.13	-0.08
Are You Willing to Pay More for Federally Inspected Seafood?	0.71	-	-

Range of responses:
[a] 0 = No, 1 = Yes
[b] 1 = perfect positive correlation, -1 = perfect negative correlation

Among the socio-economic and demographic variables, age of consumer is most positively correlated with both the growth in seafood consumption over the past five years and frequency of current seafood consumption. Increased seafood consumption among older consumers may be driven by greater health concerns. Frequency of current seafood consumption is strongly correlated with frequency of dining out, reflecting the importance of away from home consumption in overall seafood consumption. Household size is inversely correlated with both growth in seafood consumption over the past five years and frequency of current seafood consumption. This is attributable to lower per capita disposable incomes of larger families. However, recalling that "family members don't like it" was the second most important reason cited for not consuming seafood more often at home, suggests that the taste preferences of children also is a contributor.

The opinion that seafood is "good for one's health" is positively correlated with both growth in and current seafood consumption consumption. However, the latter is more strongly correlated. Among the reasons given for eating seafood, the strongest degree of correlation with growth in and current seafood consumption is found with the opinion that seafood is "healthy and nutritious". Consumers who believe that seafood is "easy to prepare" and that "good quality seafood is available" tend to have increased seafood consumption over time and currently to eat

more seafood. Those who eat seafood based on their doctor's advice tend to have increased consumption over time. Current seafood consumption is positively correlated also with the opinion that seafood "tastes good" and "is a good buy". However, growth in seafood consumption is not. Current seafood consumption is negatively correlated with the opinion that seafood adds variety to meals. Hence, consumers who look at seafood as a source of variety may be low frequency consumers; for seafood enthusiasts, variety may not be an important reason for seafood consumption.

Frequency of current consumption is inversely correlated with concern about each potential source of seafood safety problem (i.e., contaminants, parasites, viruses and toxins, additives, and bacteria from poor handling) except uninspected seafood. That is, people with these concerns consume less than those not concerned about these issues. This is consistent with the arguments of Falconi and Roe (2) that perceptions of greater health hazards result in lower levels of consumption, all other factors held constant. However, growth in seafood consumption is not inversely correlated with concern about any seafood safety issue, except bacteria from poor handling. Hence, in spite of reduced consumption as a result of concern about seafood safety, total consumption for the aggregate sample has increased over time. Apparently, for the sample, greater awareness of health benefits of seafood and other factors have exerted a greater positive influence on seafood consumption. The argument can also be made that growth in consumption among persons that have increased consumption has been greater than the decline in consumption among those that have reduced consumption.

Recall that fifty-six percent of respondents indicated that they are actually more cautious today when eating seafood than they were five years ago. The inverse correlation between the expression of caution and both current and growth in seafood consumption indicates that those consumers that become cautious as a result of their concerns about seafood safety actually reduce seafood consumption. Given that overall consumption increased over time and concern about seafood is not inversely correlated with seafood consumption, reduced consumption by consumers that have been more cautious is outweighed by the enthusiasm of those who are not more cautious. Incentives to increase seafood consumption (i.e., awareness of the health benefits and increased income) apparently have exerted a stronger influence on seafood consumers than negative factors.

Those who exercised their caution by decreasing their consumption of all seafood, fresh (cooked) finfish, New Jersey finfish and shellfish, fresh (cooked) shellfish, and processed seafood reduced their consumption of seafood over time. On the other hand, those who exercised their caution by consuming less raw finfish and raw shellfish over time increased their overall consumption of seafood over time. These results suggest that concern about fresh (cooked) finfish and shellfish and processed seafood, items which comprise the bulk of seafood consumed, is what reduces total seafood consumption, and that concern about raw fish (sushi, seviche, etc.) and shellfish, which is a small percentage of total seafood consumed, may actually increase total seafood consumption. This indicates substitution among seafood types in response to concern. Greater concern about raw seafood seems to encourage the substitution of fresh (cooked) and processed seafood for raw seafood in the diet with the result of increased seafood consumption over time.

Correlation coefficients showing relationships between the willingness to pay more for federally inspected seafood and hypothesized causal and related factors are reported in Table 4. With regard to socio-economic and demographic factors, survey results indicate that willingness to pay is positively correlated with the level of education, household income, and age. This is not surprising because level of education is expected to be directly related to awareness of health risks, household income with ability to pay, and age with increased health concerns. Males and larger families are less willing to pay more for federally inspected seafood. The latter may be explained by lower per capita discretionary income.

Table 4. Willingness to Pay More For Federally Inspected Seafood Versus Related Factors: Correlation Coefficients.

	Willingness to Pay More For Federally Inspected Seafood[a]
Socio-Economic and Demographic Factors	
Level of Education	0.04
Household Income	0.03
Age	0.02
Household Size	-0.05
Sex	-0.09
Frequency of Seafood Consumption	
Curent Total	0.09
Relative to 5 Years Ago	0.05
Current, At-Home	0.04
Current, Away-From-Home	0.00
Reasons for Not Eating Seafood More Often At Home	
Am Concerned About Contaminants	0.11
Don't Like Taste/Flavor	0.08
Don't Like Smell	0.05
Can't Get Freshness and Quality I Like	0.05
It's Too Expensive	-0.20
Reasons for Not Ordering Seafood More Often When Dining Out	
Can't Get Freshness and Quality I Like	0.01
Am Concerned About Contaminants	-0.01
It's Too Expense	-0.16
Concerned About	
Uninspected Seafood	0.11
Additives	0.05
Contaminants	0.03
Parasites	0.03
Bacteria From Poor Handling	0.01
Viruses & Toxins	0.00
More Cautious When Eating Seafood Today Compared to 5 Years Ago	0.15
Exercise Caution by Eating Less	
Raw Finfish	0.09
New Jersey Fish and Shellfish	0.09
All Seafood	0.05
Processed Seafood	0.03
Fresh (Cooked) Shellfish	0.02
Fresh (Cooked) Finfish	0.01
Raw Shellfish	0.00

Range of Responses:

[a] 1 = perfect positive correlation, -1 = perfect negative correlation

Expressed willingness to pay more for federally inspected seafood is positively correlated with all of the following: frequencies of current seafood consumption, seafood consumption relative to five years ago, and current seafood consumption at home. Frequency of away from home consumption is not directly correlated with willingness to pay, perhaps because consumers already pay premium prices for seafood purchased away from home. Hence, frequent seafood consumers tend to be more willing than less frequent ones.

With respect to reasons for not eating more seafood at home, concern about contaminants, taste/flavor, smell, and freshness and quality are positively correlated with willingness to pay. The more consumers are concerned about the expense of seafood both at home and away from home, the less willing they are to pay a premium for federally inspected seafood. None of the other factors affecting decisions to order seafood while dining out, including the quality issues (freshness and quality, and contaminants), are correlated with willingness to pay. Apparently, consumers are less concerned about seafood safety when dining out and federal inspection is less likely to encourage seafood consumption away from home.

As one would suspect, consumers concerned about uninspected seafood are the ones most willing to pay more for federally inspected seafood. Concern about additives, contaminants, and parasites are also positively correlated with willingness to pay although not as strongly. A surprising finding is that consumers concerned about bacteria from poor handling and viruses and toxins are least willing to pay more.

Increased caution in consuming seafood relative to five years ago is positively correlated with willingness to pay more for federally inspected seafood. With respect to the means by which caution is exercised, the following are apparent: reduced consumption of raw finfish and New Jersey fish and shellfish are most positively correlated with willingness to pay. Although reduced consumption of all seafood, processed seafood, fresh (cooked) shellfish, and fresh (cooked) finfish also are positively correlated with willingness to pay, they are not as strongly so. Apparently, New Jersey consumers want to feel more confident about locally caught fresh fish and shellfish, and all raw finfish. Surprisingly, there is no correlation between reduced consumption of raw shellfish and willingness to pay more for federally inspected seafood.

CONCLUSIONS

Results suggest that the major source of consumer seafood safety concern is contaminants followed by, in order of importance, uninspected seafood, bacteria from poor handling, parasites, additives, and viruses and toxins. A little more than half of consumers reported that they are more cautious in the seafood they consume today compared with five years ago. Consumers are most cautious with respect to raw seafood than cooked (fresh) and processed seafood; roughly one-quarter of consumers have reduced their consumption of raw shellfish and finfish. Consistent with the thesis of Falconi and Roe (3), consumers concerned about seafood safety consume less than those who are not. However, overall consumption of seafood increased during the past five years because factors enhancing seafood consumption apparently outweighed factors that tended to reduce consumption. Perceptions that seafood is healthy and nutritious and is good for one's health are important factors encouraging greater seafood consumption. Furthermore, caution with respect to raw shellfish and finfish actually increases total seafood consumption as consumers substitute safer types of seafood such as fresh (cooked) finfish and processed seafood.

Even though 71 percent of respondents indicate a willingness to pay more for federally inspected seafood, survey results indicate a resistance to further price increases. The foremost reason given for not consuming more seafood both at home and when dining out is that seafood is already too expensive. Among the reasons given for not consuming more seafood both at home and away from home, concern about expense is the most inversely correlated with

willingness to pay. Apparently, although mandatory seafood inspection is likely to allay consumer concern about seafood safety, particularly the general concern that it is not an inspected commodity, such a program may not result in increased seafood consumption to the extent that it raises prices significantly.

ACKNOWLEDGEMENTS

This project was supported by a grant from the New Jersey Commission on Science and Technology and by funds appropriated under the Hatch and McIntire-Stennis Acts. Survey implementation by Pat O'Keefe, President, and members of the Belmar Tourism Association, statistical assistance by Jim McDonald, and manuscript preparation by Susan Howard are gratefully acknowledged.

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A STUDY OF CONSUMERS' PERCEPTIONS OF SHELLFISH HEALTH RISKS

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INTRODUCTION

Food safety is a concern for food processors and food system regulators because of the multiple health risks from food contaminants. These health risks include long-term mortality risk due to carcinogens and both mortality and morbidity risks from bacterial or viral contaminants. Economists have documented numerous examples of the negative effects of short-term contamination scares on market demand for food (4, 5, 8, 12, 21, 26, and 29). But relatively little is known about consumers' impressions of the safety of specific foods. Nor have the factors that determine individual consumer's safety perceptions of specific foods been investigated.

Consumers may have different views of a food's safety. Psychologists have shown that individuals selectively form risk perceptions based on prior experiences, personal characteristics and cognitive skills. It is important to understand the factors that influence these differences in consumers' subjective perceptions so that food processors and food safety regulators can effectively respond to food safety concerns.

In this paper we investigate the determinants of safety perceptions for oysters and shrimp by consumers in the southeastern U.S. Oysters are especially relevant because spokespersons for the seafood industry have cited health risks from shellfish as a serious impediment to growth in seafood consumption (3, 14). The U.S. Food and Drug Administration estimates that raw or partially cooked molluscan shellfish are 83 to 122 times more likely to cause illnesses than chicken on a pound for pound consumed basis. On the other hand, shrimp are relatively safe to consume (28).

Illnesses attributed to contaminants in shellfish range from minor stomach distress and diarrhea to severe intestinal disorders that can be fatal (9). Currently, shellfish safety is regulated under the voluntary National Shellfish Sanitation Program. But, shellfish are not subject to the same type of continuous, on-site inspection used for other flesh products such as beef and chicken. Congressional legislation has been proposed to overhaul and expand the shellfish inspection program, but no action has been taken because of differing opinions about the appropriate regulatory agency, the effects on consumer demand, and administrative costs.

In the next section we review some of the psychological and economic literature on risk perceptions to identify possible determinants of subjective food safety perceptions. We focus on risk characteristics and individual attitudes and background as the primary determinants. In Section 3 we describe our sample of individuals in the southeastern U.S. and explain the structure of the survey instrument. Section 4 presents empirical results from a predictive model of consumers' safety perceptions of oysters and shrimp. As we discuss in Section 5, our results

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indicate that consumer attitudes, knowledge, and the characteristics of the risk have a significant effect on consumers' safety perceptions of the two shellfish products.

THE BASIS OF HEALTH RISK PERCEPTIONS

There is a distinction between objective risk and subjective risk (11). Objective risk is a measure of risk based on experimental evidence. Subjective risk or risk perceptions, on the other hand, rely on the perceptions of those individuals who assess the risk. Hence, the most relevant risk to individual consumers is their subjective perceptions of a risk, and the following review focuses on determinants of subjective risk.

Risk is an abstract concept that is not easily understood by most individuals. Simon (18) was the first to observe that human information processing abilities limit individuals' response to risks. He introduced the notion of "bounded rationality" that suggests cognitive limitations force people to construct simplified models of complicated mental tasks. One consequence of bounded rationality is that individuals may ignore uncertainty (10). But, typically people make risk judgments based on very general inferential rules known as heuristics to reduce difficult mental tasks to simpler ones.

One heuristic that has special relevance to risk perception is "availability" - an event is perceived likely or frequent if instances of it are easy to imagine or recall (27). This heuristic suggests that personal knowledge and experience are important determinants of perceived risk (20). Availability is also affected by factors unrelated to frequency of occurrence. For example, a recent disaster or a vivid film could strongly influence risk perceptions (19). Past studies of consumers' response to food contamination events have introduced availability of risk information by measuring the extent of newspaper coverage (e.g., 21, 26, 29). But, since these studies were based on market data, they did not explore the effects of individual heterogeneity in risk knowledge and experience on the formation of risk perceptions.

Crider et al. (6) and Hogarth (10) also suggest that the interpretation of available safety information may be influenced by unique personal characteristics. What a person expects to see (or hear, touch, smell, or taste) can greatly affect perceptions. Motivations can cause one to perceive what one needs or wants. Also, sensory experiences during childhood can alter the way (s)he perceives risky events. Thus, it is likely that individuals will respond differently to publicized food contamination incidents depending on their expectations, motivations and past experience with the food.

In addition to the availability heuristic and personal interpretation, other potential determinants of risk perceptions have been proposed. Starr (24) reviewed data on risk exposure and levels of participation in different activities and observed that "... individuals appear willing to accept risks from voluntary activities roughly 1,000 times greater than they would tolerate from involuntary activities" (p. 1237). Later, Starr et al. (25) speculated that this phenomenon was due to the perceived controllability of the risk. If people believe they can manage the risk situation, they may take the chance. If the risk is not considered controllable, they become fearful and risk-averse, and will accept only a much smaller risk.

Another factor in the formation of risk perceptions cited by Starr (24) was the probable severity of a negative outcome. For example, commercial air transportation is perceived to be more risky than auto travel because the results of an aviation accident are far more severe than the consequences of an auto accident. This suggests that individuals may have different safety perceptions if they believe an activity could be fatal than if they expect only temporary disability.

An additional determinant of safety perceptions is people's preferences for certain beliefs. According to the theory of cognitive dissonance, a habitual behavior can lead individuals to adjust

their beliefs to agree with the behavioral pattern (1). For instance, a person who continues to work in a dangerous job tends to believe the job is not dangerous.

The conceptual aspects of risk perceptions provide a framework to analyze food safety perceptions. But, most of the findings mentioned above were based on studies of environmental hazards, occupational hazards, technological risks, chronic diseases, etc. The generality of these propositions to food health risks has yet been investigated.

To operationalize this analysis, perceived risk was measured empirically by a unidimensional rating scheme that puts the safety of oysters or shrimp on a single scale, e.g., from "very safe" to "very unsafe." This rating approach has been used to elicit safety perceptions for a variety of hazards (e.g., Spence et al., 23, 30, 7 and 22) but it has not been used for specific food products. This approach has the advantage of simplicity and the rating task can be easily understood by most individuals.

These conceptual and practical concerns led us to posit the following model of individual's safety perceptions of oysters or shrimp:

> SAFETY RATING = f (AWARENESS, PRIOR ILLNESS, FREQUENCY OF USE, HEALTH STATUS, CONTROLLABILITY, SOURCE OF RISK, AGE, EDUCATION, LOCATION OF RESIDENCE, CHILDREN, RELIGION, LIKELIHOOD OF ILLNESS, SEVERITY OF ILLNESS).

Drawing on the availability heuristic, individuals' safety rating of a food is influenced by their awareness of health problems due to the food. This awareness may come from media reports or from friends and family. Prior illness from eating a specific food should influence the safety rating since these consumers have direct experience with the health risk. Also, the frequency of eating a food can influence safety perceptions. Individuals who regularly consume a food and have had no adverse health effects may prefer to ignore information about product risks according to cognitive dissonance.

If the risk is considered controllable by a person, (s)he may simply discount the risk level. The level of controllability may depend on the expected source of the health risk. If the health risk is due to improper food preparation in the home or restaurant, the risk is more manageable by a consumer. But, if the health risk enters in harvesting and processing practices, the consumer may have little control. Also, past studies have found that young and middle age consumers are probably more aware of microbial contaminants in foods than older consumers (17). But, people's ability to control a risk may also increase with age. Similarly, if a consumer considers him/herself in good health, (s)he may perceive a smaller risk. Earlier studies also suggest consumers with more education are less likely to expect bacterial contamination in meat products purchased from stores (17).

Residents in coastal areas may be more knowledgeable of shellfish safety problems because of economic importance of the industry locally, media coverage, product availability, and consumption opportunities. On the other hand, they may also perceive smaller risk in these foods because their consumption habits reinforced the belief that shellfish are not unsafe. The presence of children in the household may lead a consumer to be more adverse to illnesses from foods. A consumer's religious affiliation can influence food safety perceptions. For example, orthodox Judaism prohibits shellfish consumption due to a fear of contamination. It would be expected that Jewish consumers would have lower safety ratings of shellfish products. Finally, the two characteristics of a risk, likelihood and severity, can be used to predict the safety rating since they represent individual components of the risk. They can be evaluated to determine whether the two components have a distinct effect on safety perceptions.

SURVEY DESIGN AND STATISTICAL PROCEDURE

A random digit telephone survey of adults (18 years or older) in the Mid-Atlantic and Southeastern states constitutes the source of data for this analysis. These states were Delaware, Maryland, Virginia, North and South Carolinas, Georgia, Florida, Alabama, Mississippi, Louisiana, and Texas. The region was selected because most oysters harvested in the Southeast are marketed in these states. The survey was administered by a private market research firm in two waves: January 8-19, 1990 and April 10-June 27, 1990. The scope and framing of questions in the survey were based on focus group interviews with shellfish consumers and nonconsumers during the summer and fall of 1989 (13). The phone survey sample was stratified to provide proportional representation for each state in the region.

The interviews typically lasted about 10-15 minutes. Respondents reported their frequency of oyster consumption during the one-year period prior to the survey, their frequency of shrimp consumption in the two-month period prior to the survey, their safety ratings of oysters and shrimp on a 1 ("Not safe at all") to 7 ("Perfectly safe") scale, knowledge of and experiences with unsafe oysters or shrimp, and demographic profiles. No specific contaminant was cited as the cause of safety problems so the ratings could reflect both short-term morbidity risks and long-term mortality risks. No distinction was made between at-home and restaurant consumption of the shellfish because of the many non-food characteristics of restaurant meals that could influence perceptions. Also, all product forms (raw, canned and cooked) were considered the same since contaminants could be present in each form.

Descriptions and sample statistics for the variables used in this analysis are reported in Table 1. Interviews that had missing values for any of the variables were deleted. Consequently, 606 and 650 complete interviews were available for oysters and shrimp models, respectively. Both the mean and median safety rating of oysters (3.90 and 4.00) were lower than that of shrimp (4.80 and 5.00).

The sample mean of the chance of getting sick from oysters (2.30) shows the average respondent thought it was fairly likely to become ill from eating unsafe oysters. The mean value of the severity of illness variable for oysters (1.78) indicates that, on average, the respondents expected a relatively severe illness if they ate bacterially contaminated oysters. In contrast, the corresponding sample values for shrimp suggest the average respondent believed shrimp were less likely to cause illness and the severity of illness is lower than oysters.

An additional variable reported in Table 1 that has not been discussed previously is a dummy variable for a respondent's viewing the American Broadcasting Company's report on raw shellfish safety during their "20/20" television news show on February 9, 1990. This report focused on the Food and Drug Administration risk estimates cited earlier in this article, problems in the current shellfish inspection program, and examples of the health problems caused by contaminated shellfish. Since this report occurred after the first wave of telephone interviews, it provided a unique opportunity to identify the influence of national media attention on individual consumer's safety perceptions of oysters.

Table 1. Characteristics of the Samples

Variable[a]	Mean (Standard Deviation) / Median	
	Oysters	Shrimp
Safety Rating	3.90 (1.62) / 4	4.80 (1.43) / 5
Awareness	0.87 (-) / 1	0.54 (-) / 1
Prior Illness	0.07 (-) / 0	0.04 (-) / 0
Frequency of Use	0.74 (0.67) / 1	1.18 (0.94) / 1
Health Status	1.64 (0.72) / 2	1.69 (0.73) / 2
Control	2.52 (0.99) / 2	2.50 (0.99) / 3
Water	0.49 (-) / 0	-
Process	0.39 (-) / 0	-
Store/Restaurant	0.10 (-) / 0	-
Age	1.78 (0.66) / 2	1.75 (0.68) / 2
Education	3.93 (1.20) / 4	3.83 (1.19) / 4
Location of Residence	0.60 (-) / 1	0.63 (-) / 1
Child	0.33 (-) / 0	0.34 (-) / 0
Religion	0.02 (-) / 0	0.02 (-) / 0
Chance of Illness[b]	2.30 (0.83) / 2	2.73 (0.80) / 3
Severity of Illness[b]	1.78 (0.80) / 2	2.06 (0.84) / 2
20/20	0.15 (-) / 0	0.14 (-) / 0

[a] The variable definitions are: Safety Rating is a respondent's assessment of oyster/shrimp safety on a 1-to-7 semantic differential scale, 1 = not safe at all, 7 = perfectly safe; Awareness is 1 if (s)he has read or heard about illnesses or diseases caused by unsafe oysters/shrimp, 0 otherwise; Prior Illness is 1 if (s)he has gotten sick from eating bad or unsafe oysters/shrimp, 0 otherwise; Frequency of oysters/shrimp use in the past year/two months = 0 for a non-user, 1 if the respondent ate less than or equal to once per month, 2 if (s)he ate from 2 to 4 times per month, = 3 if more than 4 times per month; self-rated Health Status for the respondent's age, 1 = excellent, 4 = poor; perceived Controllability of shellfish safety problems by individuals = 1 if not controllable, 4 if very controllable; Water = 1 if the respondent thought the primary source of food safety problems with oysters was in the water where oysters grow, = 0 otherwise; Process =1 if the respondent thought the primary source of food safety problems was the processing and transportation of oysters, 0 otherwise; Store/Restaurant = 1 if the respondent thought the primary source of food safety problems was in the stores or restaurants that sell oysters; Age of the respondent, 1 = 18 - 34 years, 2 = 35 - 64 years, 3 = over 65; Education of the respondent, 1 = grade school, 6 = post-graduate; Location of Residence = 1 if the respondent's residence is more than 100 miles from the nearest coast, 0 otherwise; Child = 1 if there are children under 12 years of age in the respondent's household, 0 otherwise; Religion = 1 if Jewish, 0 otherwise; perceived Chance of getting sick from oysters/shrimp = 1 if very likely, = 4 if not at all likely; perceived Severity of such illness = 1 if very severe, = 4 if not at all severe; 20/20 = 1 if the respondent saw the ABC 20/20 program on raw shellfish safety, 0 otherwise.

[b] The introduction to these questions is "sometimes foods are not safe because of bacterial contamination."

It is known that certain categories of individuals are more vulnerable to oyster safety problems than others. For example, people who eat raw oysters have higher chances of getting sick from the food. In addition, individuals with a history of diabetes or liver diseases are susceptible to Vibrio vulnificus, a natural bacterium that can cause severe illness or even death.

A comparison of oyster risk perceptions and awareness of oyster safety problems between various categories of respondents in the oyster sample is shown in Table 2. Generally speaking, the median rating and proportion of individuals who had heard or read about unsafe oysters appear to be similar between each of the three pairs of respondents. However, median and proportion tests indicate no significant differences in either the median or proportion, except that more individuals in the with-liver-diseases group were aware of oyster safety problems (100%) than those in the without-liver-diseases group (87%).

Table 2. Comparisons of Group Median and Proportion for Various Categories of Respondents

Response	Interviewee: Without a history of	Interviewee: With a history of	Test Statistic[a]
	Diabetes		
Oyster Safety Rating (Median)	4.00	4.00	0.03
	Liver Diseases		
	2.00	4.00	0.57
	Raw Oysters[b]		
	4.00	4.00	0.96
	Diabetes		
Awareness of Oyster Safety Problems (Proportion)	0.89	0.86	0.33
	Liver Diseases		
	1.00	0.87	9.41
	Raw Oysters		
	0.93	0.91	0.72

[a] The null hypothesis for safety rating is the two medians in each pair of groups are same. The null hypothesis for the awareness is that the two proportions in each pair of groups are the same. The test statistics are approximated by standardized normal distribution.

[b] Interviewees with a history of eating raw oysters are those who ate raw oysters during the one-year period prior to the survey. Interviewees without such a history are those who ate oysters during the same period but did not have any raw oysters.

EMPIRICAL RESULTS

Respondents' ratings of oyster and shrimp safety were used in an ordered probit statistical model to examine the effects of each variable on safety perceptions. The ordered probit model (15) provides statistical estimates of the relationship between a set of independent

variables and an ordinal ranking of individual preferences. A coefficient indicates the change in probability of a higher ranking attributable to an independent variable. Test statistics for individual coefficients and the model can be interpreted in the same way as results from multiple regression analysis.

Maximum likelihood estimates for the ordered probit model of oyster safety ratings are presented in Table 3. The first column lists the estimates and their asymptotic standard errors for the oyster sample, and the second for the shrimp sample. Most of the variables related to the availability heuristic had the expected sign and were statistically significant. Individuals who indicated they had heard or read about illnesses caused by contaminated oysters or shrimp had a lower safety rating than individuals who had not. Respondents who had a prior illness attributable to oysters also gave a lower safety rating but the coefficient was not statistically significant. Meanwhile, respondents who reported sickness experience with unsafe shrimp tended to give a lower safety rating for shrimp. The average individual, whether an oyster consumer or not, gave a significantly lower safety rating to oysters if they had seen the 20/20 program. This program, which presented many facts that probably were not known to the general public, appears to have influenced the information available to these viewers and had a negative impact on oyster safety perceptions. But the effect of this program does not seem to carry over to respondents' beliefs of shrimp safety as the corresponding coefficient in the shrimp model is not statistically significantly.

Individuals who consumed oysters or shrimp more frequently generally gave oysters or shrimp a higher safety rating. It suggests a form of cognitive dissonance in that frequent consumers may adjust their safety perceptions to agree with current eating habits.

The perceived controllability of the health risk was also important. Individuals who disagreed with the statement, "... there isn't much the average person can do to avoid safety problems from shellfish products," were more likely to believe they could control the health risks from oysters or shrimp. Therefore, they were more likely to give a higher safety rating to these products. Individuals who agreed were more likely to give a lower rating. Also, individuals who believed that the health risks originated from the waters where oysters grew were more likely to give a lower safety rating than others who attributed the risks to themselves.

The age and education of the respondent had an impact on the safety rating. Consistent with the results in Penner et al. (17), older respondents were more likely to give a higher safety rating than younger respondents. Whether the difference in perceptions can be attributed to better understanding of food contaminants by younger people or the perceived controllability of the risks increase with age cannot be determined from the data. Respondents who had more education were more likely to give oysters or shrimp a lower safety rating. This suggests that more educated consumers may be better informed about the health risks of oysters or shrimp.

Other demographic variables also influenced the safety rating. Consumers who lived more than 100 miles from the coast gave lower safety ratings to oysters than coastal residents.Since specialty seafood stores are more common in coastal areas, consumers may feel that these stores are discriminating in the selection of suppliers as well as in product handling and augment their ability to control risks (13). This result may also reflect greater familiarity of seafood products by consumers in coastal areas. However, location of residence did not significantly influence respondents' beliefs about the safety of shrimp. The other demographic variables, presence of a young child in the respondent's household and the respondent's religious affiliation, did not have a significant effect on the safety rating.

Table 3. Maximum Likelihood Estimates from Ordered Probit Model of Oysters and Shrimp Safety Ratings

Variable	Coefficient (Standard error) Oysters[a]	Shrimp[b]
Intercept	1.15 (0.50)	1.64 (0.30)
Awareness	-0.68 (0.15)**[c]	-0.46 (0.09)**
Prior Illness	-0.23 (0.18)	-0.54 (0.27)**
Frequency of Use	0.37 (0.07)**	0.17 (0.05)**
Health Status	0.08 (0.06)	-0.05 (0.06)
Control	0.11 (0.04)**	0.07 (0.04)*
Water	-0.68 (0.40)*[d]	-
Process	-0.49 (0.41)	-
Store/Restaurant	-0.53 (0.42)	-
Age	0.21 (0.07)**	0.17 (0.07)**
Education	-0.11 (0.04)**	-0.11 (0.04)**
Location of Residence	-0.17 (0.09)*	-0.14 (0.09)
Child	0.01 (0.10)	-0.04 (0.09)
Religion	-0.47 (0.33)	-0.56 (0.41)
Chance of Illness	0.37 (0.06)**	0.19 (0.05)**
Severity of Illness	0.14 (0.06)**	0.14 (0.05)**
20/20	-0.35 (0.12)**	-0.20 (0.12)
N	606	650
-Log L	1029.20	1061.90
-Log L(Slopes = 0)	1119.10	1121.50
McFadden's R^2	0.07	0.06

[a] Estimated underlying thresholds (for the dependent variable) and their standard errors are: μ_1=0.57(0.07), μ_2=1.25(0.08), μ_3=1.94(0.09), μ_4=2.69(0.10), and μ_5=3.31(0.13).

[b] Estimated underlying thresholds (for the dependent variable) and their standard errors are: μ_1=0.49(0.10), μ_2=1.18(0.12), μ_3=1.80(1.12), μ_4=2.67(0.13), and μ_5=3.46(0.14).

[c] ** denotes the coefficient is significant at 0.05 level.

[d] * denotes the coefficient is significant at 0.10 level.

Finally, the characteristics of the risk are important. Individuals who felt that an illness from eating oysters or shrimp was not very likely gave higher safety ratings than individuals who believed an illness was likely. In addition, individuals who did not expect a severe illness even if an illness from oysters or shrimp did occur gave a higher safety rating than individuals who expected a severe illness. Both of these variables were highly significant but the chance of illness variable had a larger effect on the safety rating than the severity variable. This suggests that consumers may be more concerned about the likelihood of getting sick from one of the shellfish than the severity of the illness they would experience.

IMPLICATIONS FOR SHELLFISH INSPECTION PROGRAMS AND MARKETING PROMOTIONS

The results from our survey of consumers in the southeastern U.S. confirm that many of the determinants that influence individuals' perceptions of risks in everyday activities also influence their perceptions of oyster or shrimp safety. These subjective perceptions are influenced by media information and many indirect types of information. It is clear, however, that each individual interprets this information in a different way according to his or her personal characteristics, attitudes toward risk controllability, and behavioral habits.

The empirical analysis shows that the feeling of control over health risks is positively associated with respondents' safety perceptions of the two shellfish products. Thus, the effectiveness of a comprehensive shellfish safety regulation system in improving the safety perceptions of oysters would be enhanced by a program that not only regulates harvesting and processing practices but also improves consumers' ability to control the health risks from oysters. This type of consumer education program would make it clear that consumers can take an active role in assuring the safety of the shellfish they eat. The emphasis on controllability is particularly relevant since oysters are often eaten raw, illegal or recreational harvest of oysters is widespread (16), and people with certain health problems should avoid raw shellfish (2).

One part of such a program would be an education campaign by the seafood industry or government or both to improve consumers' knowledge of and ability to purchase, handle and prepare shellfish. The campaign could also include information about the susceptibility of high-risk categories of individuals as they may have underestimated the risk of eating oysters while they can least afford to do so. To the extent that the probability of becoming ill from eating unsafe oysters can be minimized by consumer discretion, information on cooking methods and presence of bacterial or viral contaminants in oysters would help reduce the number of shellfish-borne illnesses and promote better product image among consumers. While this type of effort may have some short-term negative impacts on product demand because consumers will be made more aware of safety problems, the long-term effects would probably be better product image.

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A SURVEY TO DETERMINE THE PREVALENCE OF RAW OYSTER CONSUMPTION IN HIGH RISK INDIVIDUALS FOR VIBRIO VULNIFICUS INFECTION

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INTRODUCTION

Vibrio vulnificus is a toxin-forming, lactose fermenting, halophilic bacteria found in coastal and brackish waters of the United States. Vibrio vulnificus infection can lead to morbidity, amputation, and even mortality in high-risk patient groups. This includes individuals with cancer, hepatic disease, renal disease, Diabetes Mellitus, HIV-infection, and the elderly (1,2,3,4,5). The elderly are included in this list because of compromised health and weakened immunity from the aging process (6).

Vibrio vulnificus produces extracellular proteins that have proteolytic or elastase and hemolytic activities, toxic to tissue culture cells (2). These products work together causing severe disease that is observed in infected people (2). Symptoms reported with V. vulnificus primary septicemia include: fever, chills, skin lesions, nausea, vomiting, diarrhea, hypotension, and shock. Warm water greater than 20 degrees celsius and salinity between 0.7 and 1.6 percent is optimal for Vibrio vulnificus proliferation (2). During the summer months when ocean water temperatures increase, the numbers and incidence of infection dramatically rise.

The death rate from Vibrio vulnificus primary septicemia has been reported as 45-60% in several studies (4). Berg et al (1) reports average mortality to be 46-75 percent. Mortality can approach 100 percent if the individual has hypotension (7). There is a strong relationship between eating raw oysters and primary septicemia among infected individuals (4). Most patients with septicemia report eating raw oysters 24 to 48 hours before the onset of symptoms (4) and raw oysters are now thought to be the main vehicle for entrance of Vibrio vulnificus via the gastrointestinal tract (8). Johnston et al (9) found that 89 percent of patients who developed primary septicemia had eaten raw oysters within two weeks before the onset of illness.

Klontz et al (10) reported 66 percent of persons with Vibrio vulnificus primary septicemia had liver disease, and 95 percent had both liver and chronic disease. Eight-eight percent of persons with wound infections due to Vibrio vulnificus had exposed that wound to seawater (10).

In 1988, the State of Florida reported 57 Vibrio infections. Of these, fourteen were Vibrio vulnificus infections. The median age of patients was 37 years, and the majority of cases were reported in July. Fifty-three percent were associated with raw oyster consumption and 23 percent with wound exposure.

Because organisms that cause food poisoning and infection occur naturally, they are difficult to control. In 1988, FDA funded research to develop a gene probe to detect Vibrio vulnificus in seafood (6). Unfortunately, there are no required sanitation or other public health controls to test for Vibrio vulnificus.

For people in high-risk categories, knowledge of the dangers of raw seafood ingestion due to Vibrio vulnificus can mean the difference between life and death (11). Educational efforts

have targeted the medical profession for recognition and treatment of the disease. Less efffort has been given to prevention of infection. Educational workshops revealing good manufacturing principles for seafood processors is one educational effort. Depuration using ultraviolet light and an added bacteriocide helps decrease bacterial count and increase the shelf life of oysters that are contaminated (12). However, the area of prevention that has been neglected is the need for increased "at risk" consumer awareness of the dangers of raw oyster consumption.

Because high mortality rates have been associated with Vibrio vulnificus infections, patients must be warned about the life threatening infections and complications associated with consumption of raw oysters and other seafoods. The purpose of this study was to survey a sub-group of high-risk individuals and determine the percentage of individuals advised against raw oyster consumption, the source of information, and prevalence of raw oyster consumption. A comparison to a study in 1988 by Johnson et al (13) was used to determine whether awareness has increased in two years. This study was developed to help determine a need for patient education and the appropriate sources of education.

METHODS

A. SAMPLE:

Subjects in this survey (n=164) were volunteers recruited from seven different Florida locations encompassing the cities of Gainesville, Dunedin, West Palm Beach, Palatka, and Naples. Data were collected on scheduled dates from August - November, 1990, and all patients entering the clinics on these dates were asked to participate in the survey. No attempts were made to randomize the sample. Six groups at risk for Vibrio vulnificus infection were identified. These included hepatic disease, renal disease, Diabetes Mellitus, oncology, HIV infection, and the elderly. Elderly volunteers (n=29) were recruited from the Geriatric Research Center in Dunedin, Florida. These subjects were included because of increased susceptibility to Vibrio vulnificus infection. Subjects from Dunedin were surveyed at their places of residence. None were currently being treated for an illness which made them susceptible to Vibrio vulnificus infection. Subjects with hepatic disease (n=30) and renal disease (n=18) were recruited from outpatient clinics at Shands Hospital in Gainesville, Florida. Oncology patients (n=21) were surveyed from North Florida Regional Medical Center in Gainesville, Florida. Finally, HIV positive subjects (n=62) were recruited from outpatient clinics in Alachua County, Palm Beach County, and Collier County. Four patients with diabetes were surveyed at Putnam Community Hospital in Palatka, Florida.

B. QUESTIONNAIRE DEVELOPMENT:

Validity and reliability of the questionnaire used in this survey were determined by Johnson et al (13) in 1988 during an initial study of the awareness of hazards related to raw seafood consumption. The original questionnaire was revised and illustrated to make it more esthetically pleasing, but the questions were not altered. Therefore, the results from Johnson et al (13) can be statistically compared to this current study. Survey information was gathered from medical records and individual interviews. The subjects were asked to provide information about current food habits and nutrition knowledge.

C. DATA COLLECTION PROCEDURES:

This study was reviewed and approved by the Health Center Institutional Review Board at the University of Florida. Individuals meeting the criteria for one of the high-risk groups were asked to participate in this study. All received and signed an Informed Consent. The questionnaire took from three to seven minutes to complete and were mostly self-completed. After being surveyed, participants received a pamphlet on Vibrio vulnificus and basic principles of avoiding infection were discussed. Any questions or problems that participants had were also addressed.

Ages, diagnoses, and medications were validated through medical records, except for the elderly population, where medical records were not available. Data were collected for 164 individuals from August until November, 1990.

RESULTS AND DISCUSSION

Participants in this study were categorized into six high-risk groups for Vibrio vulnificus infection. Table 1 summarizes each high-risk group, the mean age, and standard deviation of participants.

Table 1: Mean Age of Participants for Each High Risk Group

HIGH RISK GROUP	N	MEAN AGE	STD. DEVIATION
Hepatic Disease	30	40	15.6
Renal Disease	18	39	18.8
HIV positive	62	36	9.4
Oncology	21	56	14.4
Elderly	29	84	3.5
Diabetes	04	55	20.0

There is a statistical difference ($p < .05$) when comparing high risk groups for the ratio of males to females, which can mostly be attributed to the HIV positive population. Table 2 summarizes the frequency and percentage of males and females in the high risk groups for Vibrio vulnificus infection.

Table 2: Frequency and Percentage of Male and Female Participants in High Risk Groups

HIGH RISK GROUP	MALE		FEMALE	
	Frequency	%	Frequency	%
Hepatic Disease	17	10.4	13	7.9
Renal Disease	6	3.7	12	7.3
HIV positive	53	32.3	9	5.5
Oncology 7	4.3	14	8.5	
Elderly 9	5.5	20	12.2	
Diabetes 2	1.2	2	1.2	
Total Male		57.3	Total Female	42.7
Total				100.0

In this study, it was determined that 29 participants were taking medication that would make them more susceptible to Vibrio vulnificus infection. Twelve of the 21 participants in the Oncology group were receiving chemotherapy and 17 of the 18 renal participants were on corticosteroid therapy. Both medications are known to increase susceptibility to infection due to their immunosuppressive effects. Sixty-three percent of the participants in this study (n=104) were not instructed in any "special diet". Forty-one percent were females (n=43) and 59 percent

(n=61) were males. Thirty-seven percent (n=60) of the participants were instructed on a special diet. Forty-five percent were female (n=27) and 55 percent (n=33) were males. There was no statistical difference between males and females at $p < .10$.

Of 164 participants only 25.6 percent (n=42) remember being told to avoid eating raw oysters. Table 3 summarizes the frequency and percentage of participants told to avoid raw oysters. Of the 42 participants who remember being told to avoid raw oysters, 28.6 percent (n=12) were females and 71.4 percent (n=30) were males, which was statistically significant at $p < .05$. Only 19.5 percent (n=32) participants remember being told to cook raw oysters before eating them. Of the 32 participants who knew to cook raw oysters before eating them, 21.9 percent (n=7) were females and 78 percent (n=25) were males. This, again was statistically significant at $p < .05$. Overall, men were more aware of the advisability of avoiding eating raw oysters or of cooking before eating them than women. This significance again may be attributed to the HIV positive population. The HIV positive group is mostly males and 34 percent (n=21) of the patients were aware of the advisability of avoiding raw oyster consumption, the highest percentage out of all the high risk groups. The other percentages are summarized in Table 3 and are as follows: Hepatic Disease (30%), Diabetes (25%), Renal Disease (22%), Oncology (19%), and Elderly (10%). Accurate statistical significance using Chi-Square could not be determined, since the number of subjects was < 5 in four of the six high risk groups.

The overall treatment of an HIV positive individual in South Florida commonly involves counseling on food safety practices. This may explain the higher level of knowledge of the dangers of raw oyster consumption. Elderly in this study are not under the supervision of health professionals for an illness that makes them more susceptible to Vibrio vulnificus. Therefore, they have less opportunity to learn of the dangers of raw oyster consumption. This level of awareness is similar to the healthy general public. The results of this study show that only 10 percent of the elderly surveyed are aware of the hazards of raw seafood consumption, the smallest percentage of all groups. The source of education for this group is principally mass media.

Sources of nutrition information about the dangers of raw oyster consumption varied from health professionals to mass media. Table 4 summarizes where participants received their information.

Table 3: Frequency and Percentage of Participants Told to Avoid Raw Oysters in Each High Risk Group.

HIGH RISK GROUP	N	FREQUENCY	% IN GROUP	%
Hepatic Disease	30	9	30	5.5
Renal Disease	18	4	22	2.4
HIV positive	62	21	34	12.8
Oncology	21	4	19	2.4
Elderly	29	4	10	1.8
Diabetes	4	1	*25	*.6
Total				25.6%

*Because of small population size numbers might not be indicative of sub-group of Diabetics at risk for Vibrio vulnificus.

Most learned to avoid raw oysters through more than one source, 37.8 percent (n=17). Television was a source of information alone for eight people. This was attributed to a "20/20" special on the hazards of raw oysters.

Overall, 36 percent of participants (n=59) eat raw oysters. The largest percentage of participants eating raw oysters in a particular group is Oncology, with 61.9 percent surveyed. Fifty-eight percent of the elderly admit to raw oyster consumption; both of these groups rated lowest in knowledge of the hazards of raw oyster consumption. The results for the remaining groups are as follows: HIV positive (29%, n=18), Diabetes Mellitus (25%, n=1), Hepatic Disease (23, n=7), and Renal Disease (17%, n=3). HIV positive individuals have been known to increase raw oyster consumption because of the high zinc content in oysters. Zinc is an important part of immune function. However, the belief that increased zinc intake above the RDA improves immune function is inaccurate and may lead to dangerous food practices.

The largest reason for not eating raw oysters was not liking them, (63%, n=67). The second largest reason was being told to avoid them, (20.8%, n=22). Two reported under "comments" that they no longer like raw oysters because they became ill after eating them.

Only 9.1 percent (n=15) of total participants reported eating raw oysters in the past six months. This might be attributed to the time of the survey. Oyster season occurs during the winter months, and the previous six months before the survey included spring and summer months, when oyster consumption is lowest.

Knowledge and information does not necessarily ensure compliance. Of the 42 participants that were told to avoid raw oysters, 21 percent (n=9) still eat them. Knowledge must be present for compliance to occur, and compliance is seen in 33 out of the 42 participants who were told to avoid them.

A chi-square test for probability was used to help determine if there is a significant difference between the knowledge of participants surveyed in 1988 by Johnson et al (13), and this current study. It was reported in 1988, that out of 57 participants, eight (14%) knew to avoid eating raw oysters. This current study shows that out of 164 participants, 42 (25.6%) know to avoid raw oysters. This is significant at $p < .10$. In other words, there has been a significant increase in knowledge of the dangers of raw oyster consumption. The two factors most likely to account for the improvement, is the inclusion of the HIV positive group and the recent "20/20" television special on hazards of raw oysters.

Table 4: Where Knowledge of the Hazards of Raw Oysters was Learned

SOURCE OF INFORMATION	FREQUENCY	%
Doctor	3	6.7
Dietitian	4	8.9
Nurse	2	4.4
Friend	2	4.4
Relative	1	2.2
Television	8	17.8
Magazine	3	6.7
Newspaper	3	6.7
Bible	1	2.2
Personal Beliefs	1	2.2
More Than One Answer	17	37.8
Total		100.0

CONCLUSIONS

The subjects surveyed in this study were not randomly selected and in some groups, the numbers surveyed were small. Therefore, they are not representative of the entire population of individuals who fall into one of the high-risk groups for Vibrio vulnificus infection. These results, however, do indicate that among clinics surveyed, there appears to be a significant increase in awareness about the microbiological hazards associated with raw oyster consumption.

Because Vibrio vulnificus is such a virulent organism, and mortality rates for infection range from 45-60% (4), education efforts must target prevention. In the past ten years, Vibrio vulnificus infections continue to occur despite attempts to educate the medical profession and oyster processors. Suggested ways to further knowledge of hazards of raw oyster and seafood consumption may be through: 1. PSAs (Public Service Announcements) during the summer months especially, 2. Increased distribution of educational materials, such as pamphlets through the Department of Natural Resources, 3. Issuing of warning labels in restaurant menus or marquis about hazards of raw seafood ingestion for high-risk groups. Louisiana, as of August 1, 1990 will require warning labels on all raw shellfish produced or sold in the state as a form of prevention (12).

In conclusion, these data can show that there is still a lack of awareness of the dangers of raw seafood consumption in high risk individuals. Knowledge, however, appears to have increased in the past two years due to health professionals and mass media. Future efforts should be aimed at the consumer and targeted towards prevention since available treatments for Vibrio vulnificus infections are ineffective in a majority of the cases.

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RELATIVE PREVALENCE OF ECONOMIC FRAUD IN THE SEAFOOD INDUSTRY

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With the recent focus on improved seafood inspection much has come to light on public health considerations in consuming fish and fishery products. However, the occurrence of fraudulent practices in the trade of seafood has been largely overlooked as a hazard to the consumer. Economic fraud data was scrutinized as part of the Model Seafood Surveillance Project (MSSP) to design a new mandatory seafood inspection system based upon the Hazard Analysis Critical Control Point (HACCP) concept. The prevalence of fraud, along with the cost to the consumer, was estimated and placed in proper perspective with other hazards to be controlled in the new system.

INTRODUCTION

Fraudulent practices in the seafood industry represent a specific set of hazards to the consumer. These economic fraud hazards are overshadowed by product safety concerns. In this era of tightened resources, regulatory effort is focused toward what makes people sick. Databases such as those maintained at the Centers for Disease Control (CDC) help us understand the prevalence and significance of public health problems in consuming seafood, but there is little corresponding data to aid in understanding the magnitude and impact of economic fraud.

Not all economic fraud is intentional. It can be perpetuated because of a lack of information or misunderstanding. No matter what the reason, however, industry's desire for a "level playing field" to combat fraud is strong and consumers want full value.

One of the first documented indications of this industry concern was a 1985 survey conducted by the National Fisheries Institute (NFI) (7). The survey concluded that economic fraud issues demanded attention. Quotes such as, "I know a packer that right out and says, I've got some beautiful turbot; we'll relabel it any way you want" and "There's no problem more serious than non-enforcement of labeling laws", were dominant throughout this report. There was general agreement among the industries (processing, distributing, and importing firms) as well as retailers and restauranteurs that there is widespread abuse of overglazing and overbreading of fishery products, inaccurate net weights, and species substitution. However, the same survey participants were in general agreement that fishery products were labeled according to regulations (Tables 1 and 2).

A more recent survey conducted by the Southeastern Fisheries Association (SFA) within their "Industry and Regulatory Interface Project" (5) also revealed concern over economic fraud issues. A scale of one to seven was used to weight problem areas where seafood product quality is violated or lost. Included in these areas were economic fraud issues such as species substitution, use of glaze, and use of phosphates, along with non-economic fraud issues such as on-board vessel handling practices, processing, frozen storage, and use of sulfites. Only one issue, related to

vessels, received a greater overall weight than the three economic fraud issues (Figure 1).

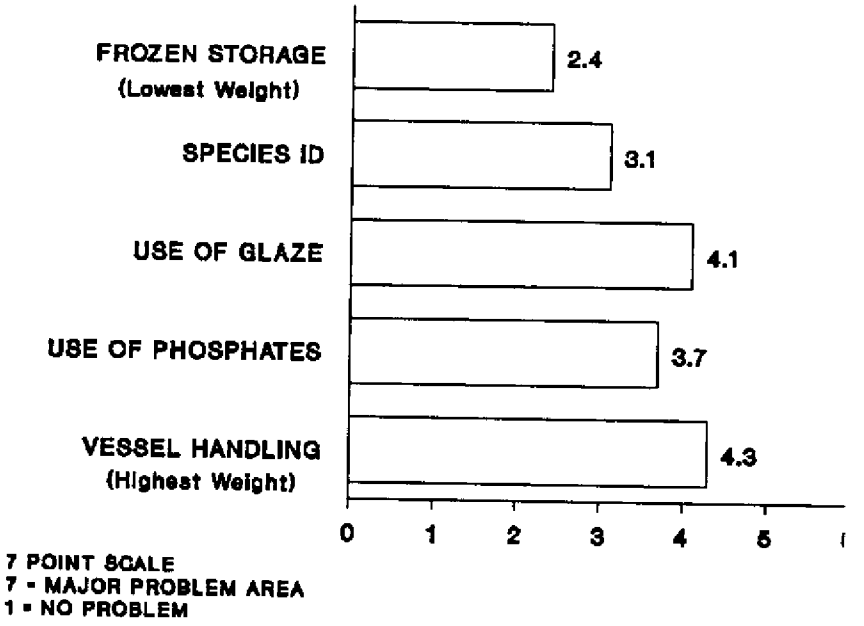
Table 1. 1985 NFI MEMBERSHIP SURVEY RESULTS
Processing/Distributing/Importing Firms

		AGREE %	PART. AGREE %	TOTAL %
A.	Overglazing of fishery products is a widespread abuse.	37	35	72
B.	Overbreading of fishery products is a widespread abuse.	34	22	56
C.	Inaccurate net weights are a widespread abuse.	30	28	60
D.	Improper substitution of fishery products is a widespread abuse.	30	39	69
E.	Fishery products are labeled according to regulations.	23	45	68

Table 2. 1985 NFI MEMBERSHIP SURVEY RESULTS
Retailers and Restauranteurs

		AGREE %	PART. AGREE %	TOTAL %
A.	Overglazing of fishery products is a widespread abuse.	27	19	46
B.	Overbreading of fishery products is a widespread abuse.	37	26	63
C.	Inaccurate net weights are a widespread abuse.	33	30	63
D.	Improper substitution of fishery products is a widespread abuse.	33	30	63
E.	Fishery products are labeled according to regulations.	8	42	50

Figure 1. SFA INDUSTRY AND REGULATORY INTERFACE PROJECT RESULTS



Except for these documented industry concerns, economic fraud information or data is most difficult to obtain. We have classified the available information into three categories: databases (specific and incidental), published reports, and observations and interviews.

DATABASES

There are few databases designed specifically to collect economic fraud information. In most cases, when fraud data are reported, they are incidental discoveries.

Specific economic fraud databases include data from the National Seafood Inspection Laboratory (NSIL), state directed surveys, and weights and measures programs.

The NSIL analyzes samples upon specific request (i.e., for suspect products). The most comprehensive data in this database relate to species substitution. Over a three year period (fiscal years 88-90) 59 percent of samples labeled cod, 57 percent of the product labeled haddock, 56 percent of the product labeled flounder or sole, and 51 percent of the product labeled red snapper were found not to be cod, haddock, flounder or sole, and red snapper, respectively. Figures like these are confirmed by some states. For example, Florida conducted a survey in Fiscal Year (FY) 88-89 which indicated that 38 percent of the fish fillets sampled for species identification were misbranded.

Incorrect weights and measures also can be indicators of economic fraud and may be identified from specific databases. For example, states have bureaus that deal with the collection of weights and measures data. Further, the U.S. Department of Commerce (USDC), in its voluntary inspection

program, analyzes product lots for specific criteria, including weights and measures.

We contacted six states' Weights and Measures Divisions. Only California and Michigan had product specific data. Alabama, Florida, and New York could not break out seafood related data. Washington's data was not computerized; they could not supply data to us without a labor intensive activity.

In 1989 seafood labeled by retail markets in California was surveyed. Twenty-eight percent of the packages sampled for net weight contained less than labeled; however, the average error for all of the retailers surveyed was +3 percent. In other words, the retailers, on the whole, were overpacking. Similarly, packages of seafood labeled by the processor were sampled. In this survey, 12.5 percent of the packages were less than labeled. Again, however, the overall tendency was to overpack. For processors' labels the average error was +2 percent.

The sampling of seafood packages for weights in Michigan offered a different insight. While general sampling of seafood products again gave a two percent overpack, specific shortweight problems were evident for Individually Quick Frozen (IQF) shrimp (Table 3). State officials in Michigan as well as Florida confirmed that inaccurate net weight problems occur more frequently with IQF products.

An examination of USDC lot inspection certificates for an average three month period confirms our findings from state data (Figure 2). When "proper net weight" is defined by specification, i.e., what the buyer will accept, the percent average error is +1.9. If "proper net weight" is defined in exact units of measure (0.01), then the average error is +1.5 percent.

The economic impact of underpacking IQF shrimp was examined. An analysis using the Michigan data's average error of 8.3 percent underpacking of IQF shrimp is exemplified in the following: Let a processor produce 917,000 pounds of IQF shrimp (any product type). He sells this lot as 1,000,000 pounds. Assuming a wholesale price of $4.00 per pound, the processor receives $332,000 in fraudulent profit. The consumer, on average, purchasing one pound of IQF shrimp receives 0.92 pound. At a retail selling price of $6.36 (1) per pound, the consumer overpaid by $0.51. Frozen Food Age (1) reports that retailers sold 9.8 million pounds of frozen peeled shrimp, cooked and raw, from October, 1989 to October, 1990. A large grocery chain has estimated that 80 percent of their frozen shrimp is IQF. Thus in this example, U.S. consumers were losing $4 million on underpacking of IQF shrimp.

Non-specific (incidental) databases are more numerous than specific databases. Examples of these databases include Food and Drug Administration (FDA) consumer complaints, FDA adverse samples and import detentions, and state adverse samples.

In FY 90, FDA's consumer complaint database indicated that 16 percent of complaints in which no illnesses were reported were related to economic fraud. When all complaints including those related to illness were considered a ten percent economic fraud complaint ratio was obtained.

In regard to FDA's adverse samples, a 1988 Government Accounting Office (GAO) study (2) on seafood safety reported that eight FDA district offices had 1,514 adverse samples in 1986. Of these, 220 were misrepresented. This is a 14.5 percent violative rate. Again, these FDA data were not specific to economic fraud. In most cases, economic fraud data were discovered incidentally. Hence, in actuality, the data probably are underestimated.

Similarly, import detentions indicate economic fraud violations in seafood products. In 1989 FDA's detentions of seafood products for economic violations related to 0.2 percent of the weight, but 13 percent of the total number of detentions.

In 1989-90 the state of Florida analyzed over 800 samples of seafood products and found that, of those checked for economic fraud, 14 percent were misbranded. Additionally, 12 percent were adulterated and 21 percent in non-compliance, both of which may include some economic fraud violations. Again, it must be cautioned that the 800 samples were not all examined for economic fraud.

Thus while these data represent scanty information not arrived in a systematic way, the numbers are such that it is clear that substantial economic fraud exists and is a consumer hazard. It also appears that economic fraud is underreported since the datasets were not designed specifically to obtain economic fraud information (Table 4).

In order to calculate the potential costs of economic fraud to consumers, for illustrative purposes assume ten percent economic fraud in the U.S. seafood supply. In 1989 there were 12.3 billion pounds of round weight fishery products (8). If one assumes a 50 percent loss in processing these products, the supply becomes 6.15 billion pounds. If there is ten percent economic fraud in the industry, then 615 million pounds of fishery products on the market in 1989 would have been fraudulent. Since consumers spent $28.1 billion in 1989 (8), then the average price paid, assuming 6.15 billion pounds of after processing supply, was $4.57 per pound. Making the assumption that minimally ten percent of the price of the product equates to an economic fraud, this price differential relates to $0.46 per pound due to economic loss and total economic fraud of $283 million (based upon 615 million pounds of fraudulent product).

Even if we assume that 60, 40, or 30 percent is lost in processing, the result is still $283 million per year because of the principle of mathematical equivalency. Consider further that short weights or substitutions are more likely found on high value products rather than the average $4.57 per pound estimate. This implies that the ten percent fraud value and final national fraud level may be larger.

Table 3. MICHIGAN TEST RESULTS FROM SEAFOOD SAMPLING FOR WEIGHTS (9/87-11/90)

PRODUCT	RESULTS	PERCENT RANGE
IQF SHRIMP	SHORT	-20.9 to -5.9
IQF SHRIMP	SHORT	-7.4 to +5.2
IQF SHRIMP	SHORT	-13.8 to -12.8
IQF SHRIMP	SHORT	-5.3 to -2.7
IQF SHRIMP	SHORT	-15.3 to -1.8
IQF SHRIMP	SHORT	-18.6 to -11.3
IQF SHRIMP	SHORT	-4.2 to +3.0
IQF SHRIMP	SHORT	-12.8 *
IQF SHRIMP	SHORT	-7.5 to -5.2
IQF SHRIMP	SHORT	-18.2 to -1.8

*RANGE NOT PROVIDED FOR THIS SAMPLE

Figure 2. USDC LOT INSPECTION CERTIFICATES

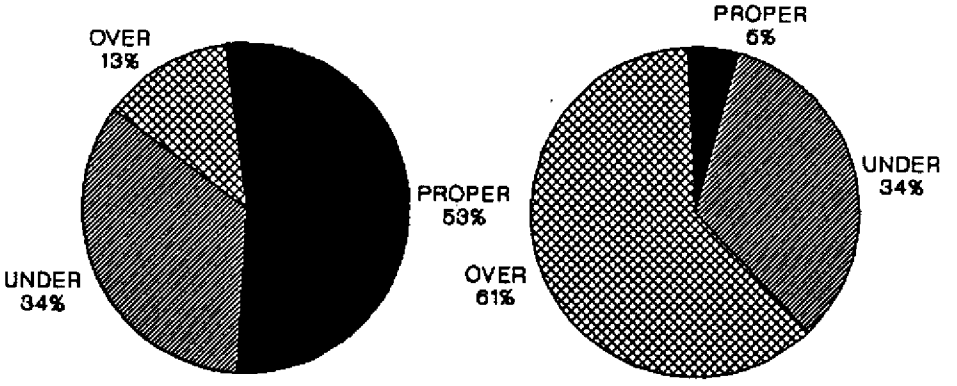


Table 4. SUMMARY NON-SPECIFIC ECONOMIC FRAUD DATABASES

PERCENT	DATABASES
13	of 1989 IMPORT DETENTIONS
14	of FY 89-90 SEAFOOD SAMPLES ANALYZED IN FLORIDA
14.5	of 8 FDA DISTRICT OFFICES' (1986) SEAFOOD SAMPLES
10-16	of FY 90 FDA CONSUMER COMPLAINTS

Let's take an example outside of the seafood industry for clarification. Suppose a consumer purchases 20 pounds of ground sirloin at $5.00 per pound, for a total of $100.00. The consumer unwraps the meat at home and finds that ten percent (2 pounds) is, in fact, ground chuck. The price of ground chuck was ten percent less than ground sirloin, i.e., $4.50 per pound. The ground chuck still can be used, but the consumer overpaid for it by $1.00 (total).

Another way to estimate the magnitude of economic fraud is to use per capita consumption figures. In this example, calculating the cost of ten percent economic fraud again leads to over $280 million. Since the per capita consumption in 1989 was 15.9 pounds and the civilian resident population was 246.6 million persons (8), a total of 3.92 billion pounds was consumed in the U.S. With an expenditure of $28.1 billion, the average price paid was $7.20 per pound. If ten percent of the price of the product was related to economic fraud, then $0.72 per pound was economic loss. This equates to only $1.14 per capita but a total of $281 million (390 million pounds of fraudulent product) of economic fraud in 1989.

Thus a $280 million figure, based upon the types of databases, assumptions made and round-off error in computations in the examples given, would be a reasonable estimate of economic fraud. In reality, the loss to the consumer might exceed this figure.

PUBLISHED REPORTS

Published reports usually center on the conduct of a specific survey. For example, species identification of retail red snapper fillets in Florida indicated that 64 percent of the samples labeled red snapper were misbranded (4). Newspapers have run similar studies. One from the Sun-Sentinel in Orlando, FL in December, 1988 concluded that 90 percent of red snapper was misbranded (3). The Asbury Park Press in Asbury Park, NJ in May of 1989 found 78 percent misbranded red snapper (6). If these three surveys were random, then the average indicates that 77 percent of red snapper on the market is in fact some other species.

Consumer costs and processing profit of red snapper substitution has been determined. In 1980 a study performed under contract for National Oceanic and Atmospheric Administration (NOAA) indicated that there were 12 million pounds of red snapper purchased at the point of sale. With the implication that 77 percent of red snapper is mislabeled, this figure indicates that 9.24 million pounds was not red snapper. Based on the ex-vessel prices, the price differential between red snapper and other snappers such as gray, lane, vermillion, etc., is approximately $1.00 per pound. This price differential would be carried through the market place resulting in $9.24 million fraud.

From a processing/distributing viewpoint, such fraudulent practices can be very lucrative. In 1990, if a firm purchased whole pacific rock fish, which is often substituted for red snapper, the firm would pay about $0.32 per pound. Once the purchase of this rock fish was relabeled as red snapper, it could be sold at $4.00 per pound instead of the $1.10 average selling price for a whole rock fish. A firm engaging in such fraudulent practice would have a selling margin of $3.68 per pound, $2.90 of which is fraudulent. Thus, the firm would receive almost five times as much as it should.

OBSERVATIONS AND INTERVIEWS

During the conduct of the MSSP testing, team evaluators made observations related to economic fraud: 1) Deliberate underpacking; 2) Shrimp soaked in a phosphate solution (to enhance moisture absorption) more than 12 hours; 3) Shrimp double soaked, i.e., soaked in phosphate solution, removed, and soaked again; and 4) Scallops soaked in a phosphate solution more than 24 hours, resulting in not only water absorption but an increase in count size as well. When those practices were observed, it was brought to the attention of the firm's management that the practice was questionable. Since the evaluators were there under industry invitation and not as regulators, it was left to the firm's management to correct (or not) the practice.

States were queried on economic fraud investigations. As a rule, the states were aware of the problems but were unable to take action because of limited resources. The exceptions to this was Florida; it has conducted specific surveys on species substitution, as earlier described.

Over 40 large chain grocery (supermarkets) stores also were queried. Only one store responded; it had no information or data, but expressed its concern over the occurrence of economic fraud practices in the seafood industry.

CONCLUSIONS AND RECOMMENDATIONS

As a result of these investigations the following conclusions were drawn:

1) In general, databases contain economic fraud information because of incidental discovery.

2) Not all fraud is intentional.

3) Economic fraud is not sufficiently addressed by regulators because of lack of resources.

4) As with seafood product safety issues, economic fraud issues appear to be product specific.

5) From the consumer viewpoint, short weight does not appear to be as big a problem as species substitution.

6) Economic fraud in the seafood industry is a "shrouded hazard", which could approach $280 million per year.

It is recommended that if a mandatory HACCP-based inspection system is instituted, it should focus resources to control economic fraud hazards.

Additionally, reliable baseline data on economic fraud is needed. Specific surveys on the prevalence of species substitution and problematic species for this practice, the excessive use of food additives such as phosphates that promote water pick-up, and short net weights or counts (again with the definition of problematic species or packaging types) should be performed. The collection of such data can be done within or external to any new mandatory seafood inspection program.

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PROCESSING STRATEGIES TO REDUCE THE NUMBERS OF *Vibrio vulnificus* IN OYSTERS

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Vibrio vulnificus has been documented to cause primary septicemia in individuals with certain types of underlying disease (1, 6). More recently, *V. vulnificus* has been linked with gastroenteritis in individuals with no underlying disease (4). These illnesses occur primarily during the summer months when oysters are known to contain high levels of *V. vulnificus* (4, 7).

The numbers of *V. vulnificus* cells which must be ingested to produce primary septicemia or gastroenteritis in humans is unknown. However, it is apparent that any steps which can be taken to minimize the level of *V. vulnificus* in shellfish will improve the safety of the shellfish with respect to the organism.

Vibrios are known to be cold sensitive and Oliver (5) demonstrated that *V. vulnificus* died quickly in homogenized oyster meats when held in the cold. Ruple et al.(7) observed a marked reduction of *V. vulnificus* in whole oyster meats when stored on ice. These findings have lead us to explore the use of cold treatment as a potential processing step which would reduce the level of *V. vulnificus* in oysters without destroying the raw characteristic of the oysters. This paper also reports findings on the use of mild heat treatments to destroy *V. vulnificus* in raw oysters.

MATERIALS AND METHODS

Source of Oysters

Oysters used in these studies were harvested in Louisiana and transported to Mississippi through normal commercial routes. Oysters were shucked in seafood processing plants as part of normal plant operation. Tests with oysters were conducted on the same day the oysters were shucked.

Pure cultures of *V. vulnificus*

These cultures were isolated from raw oysters and have been maintained in the culture collection at the Gulf Coast Research Laboratory.

Cold temperature studies with pure cultures

Cultures were grown on a shaker overnight in T_1N_1 broth (1% tryptone, 1% NaCl). Cultures were diluted in phosphate buffered saline (PBS) and inoculated into the suspension medium at a final concentration of approximately 1,000,000 colony forming units (CFU) per mL. Suspension media were tryptic soy broth (TSB), PBS and PBS+2% NaCl. Tubes of the inoculated suspension media were held at 4°C, 0°C, and -20°C. At desired intervals, the tubes were warmed or thawed in cold running tap water, diluted in PBS and plated on tryptic soy agar using a spread plate technique. Plates were counted after 48 hrs incubation at room temperature.

Thermal death studies with pure cultures

Cultures were grown as stated above and diluted to a concentration of approximately 1 billion CFU per mL. Ten mL of PBS+2% NaCl was placed into a 22 X 175 mm tube which contained a spin bar. The tubes were held in a water bath while mixing to equilibrate the

temperature. Tubes were inoculate with 0.1 mL of the diluted culture to achieve a final concentration of 1,000,000 CFU per mL. At predetermined time intervals, 1 mL samples were removed from the tubes, quickly cooled to 10°C, diluted as necessary and plated as described above. D-values were determined from semi-log plots of the data (2).

Procedure for V. vulnificus counts in oysters

Analyses were performed on duplicate 100 g samples of oyster meats. Meats were homogenized in phosphate buffered saline and enriched for V. vulnificus in alkaline peptone water using a 3 tube MPN. Following 12 to 16 hours incubation at 35°C, all tubes showing growth were streaked onto CPC agar plates and incubated for 24 hours at 40°C (3). Typical and atypical colonies were picked from CPC plates for confirmation as V. vulnificus using the ELISA procedure described by Tamplin et al. (8). Results were reported as an average of the duplicate samples, rounded to two significant figures, and expressed as MPN of V. vulnificus per gram of oyster tissue.

Cold temperature studies with oysters

In each study, oyster meats or shell stock oysters were obtained from a single shipment of oysters. Shucked oyster meats were divided into 100 g portions and placed in sterile wide-mouth glass bottles. Oyster meats to be stored at 0°C were held in melting crushed ice. Those stored at -1.9°C were held in a circulating bath of refrigerated 50% ethylene glycol. Ice crystal formation was not observed in oyster meats held at this temperature.

Shellstock oysters were held in a refrigerator at 4°C, placed in plastic bags and stored between layers of melting ice (0°C) or held at -1.9°C. The latter temperature was achieved by placing the oysters in an insulated chest equipped with cooling coils. Refrigerant chilled to -1.9°C was pumped through the coil. A thermocouple placed in the center of one oyster in the chest was used to verify the temperature.

Studies with frozen oysters

Oysters meats were frozen by three different procedures. A commercial liquid carbon dioxide freezer set for -30°C was used to produce individually quick frozen (IQF) oysters. After freezing, the oysters were divided into portions of approximately 100 g and placed in zip-seal freezer grade plastic bags for storage. A commercial blast freezer adjusted to -23°C was used to freeze 100 g portions of oyster meats in zip-seal freezer grade plastic bags. Oysters remained in the freezer for 24 hrs before they were transferred to the storage freezer. A third freezing technique similar to what may be used in the home consisted of placing the oysters in plastic bags in 100 g amounts and freezing them in a laboratory freezer at -20°C. All frozen oysters were stored in the laboratory freezer at -20°C. On a predetermined schedule, bags of frozen oysters were removed from the freezer and placed at 4°C overnight to thaw before testing for V. vulnificus.

Heat treatment of oyster meats

Oyster meats were heat treated in a circulating water bath constructed from a insulated plastic container equipped with a heater, thermoregulator and submersible pump. The water was heated to the desired temperature before introduction of the oyster meats. Exposure times were either 5 or 10 minutes. During the heating period, the meats remained suspended due to the action of the moving water. At the end of the heating period, the meats were immersed in ice water for five minutes to promote rapid cooling. In some experiments, the control oysters were subjected to the same exposure routine except the water was kept at 5°C.

RESULTS AND DISCUSSION

Cold Studies

When pure cultures of V. vulnificus were placed in suspension media and held at temperatures of 4°C and 0°C, all cultures experienced a rapid time dependent decrease in numbers (data not shown). However, V. vulnificus could be cultured from 0.1 mL portions of all media after 14 days of storage at both temperatures. A similar pattern of decreasing numbers was observed with naturally occurring V. vulnificus in cold stored shucked oyster meats (table 1) and in cold stored shellstock oysters (table 2).

Table 1. Effect of cold storage on the numbers of V. vulnificus in oyster meats.

DAYS IN STORAGE	STORAGE TEMPERATURE: IN MELTING ICE (0°C)	STORAGE TEMPERATURE: REFRIGERATED (-1.9°C)
DAY 0	>110,000*	>110,000
DAY 2	15,000	43,000
DAY 4	3,900	1,500
DAY 6	430	21

* MPN of V. vulnificus per gram of oyster meat.

Table 2. Effect of storage time on the numbers of V. vulnificus in shell oysters held at different storage temperatures.

DAYS IN STORAGE	SHELLSTOCK STORAGE CONDITIONS: REFRIGERATED (4°C)	IN MELTING ICE (0°C)	REFRIGERATED (-1.9°C)
DAY 0	2300*	2300	2300
DAY 2	2300	2300	510
DAY 7	590	430	580
DAY 14	930	160	5

* MPN of V. vulnificus per gram of oyster meats.

Freezing pure cultures of V. vulnificus in suspension media at -20°C reduced the number of culturable cells more quickly than did holding the cultures at 0°C. However, cells could be easily recovered after 19 days of frozen storage (data not presented). The freezing and storage of oyster meats containing naturally occurring V. vulnificus resulted in a decrease in the numbers of but not the elimination of V. vulnificus (table 3).

Table 3. The effect of freezing by different techniques and frozen storage time on the numbers of V. vulnificus in oyster meats. Frozen oysters were stored in zip-seal plastic freezer storage bags at -20°C

STORAGE TIME AFTER FREEZING	FREEZING TECHNIQUE		
	IQF FROZEN -30°C	BLAST FROZEN -23°C	LAB FROZEN -20°C
BEFORE FREEZING	150,000*	150,000	150,000
AFTER FREEZING	930	93	430
WEEK 1	93	750	430
WEEK 2	7.5	93	230
WEEK 4	0.4	93	4.3
WEEK 8	0.9	1.5	15
WEEK 12	0.9	43	23

* MPN of V. vulnificus per gram oyster meat.

These findings document that cold treatment will bring about a significant reduction in the numbers of V. vulnificus in oysters, but the reductions are time dependent. Oysters have a defined shelf life whether in the shell, as shucked meats stored on ice or frozen. Therefore, it would be impractical to hold oysters for the time period necessary to insure that the V. vulnificus had been eliminated.

Heat studies

Preliminary studies with pure cultures indicated that temperatures above 45°C were necessary to bring about the rapid death of V. vulnificus. D-values were measured at 47°C on 52 pure cultures of V. vulnificus. The average D_{47}-value was 78 sec. (s.d.± 30 sec.). Eighteen of these cultures which had the longest survival time were tested at 50°C and the resulting D_{50}-value was 38 sec. (s.d.± 12 sec.). The low D-values indicate that V. vulnificus is very heat sensitive.

The effect of heat treatment on the numbers of V. vulnificus in oyster meats is shown in table 4. A 10 min. treatment at 50°C proved adequate to reduce V. vulnificus to a non-detectable level.

Table 4. Effect of heating time and temperature on the numbers of V. vulnificus in oyster meats.

HEATING TIME	HEATING TEMPERATURE		
	45°C	47°C	50°C
0 MIN.	59,000*	59,000	59,000
5 MIN.	29,000	14,000	220
10 MIN.	870	4	<0.3

* MPN V. vulnificus per gram oyster meat.

Oysters which had been heated at 50°C for 10 min. were stored on ice for a 14 day period and examined periodically for V. vulnificus (table 5). A comparison was made with oysters that had been exposed to the same treatment time but at a water temperature of 5°C. The 50°C reduced the levels of V. vulnificus in the oyster meats to a non-detectable level immediately. Further, there was no evidence of resuscitation of any V. vulnificus cells during storage on ice for 14 days.

Table 5. The effect of heat treatment and storage time on the numbers of V. vulnificus in oyster meats. Meats were packed in containers and held in melting ice.

STORAGE AFTER TREATMENT	TREATMENT	
	NO HEAT TREATMENT	OYSTERS HEATED AT 50°C FOR 10 MIN.
BEFORE TREATMENT	4300*	4300
AFTER TREATMENT	2300	<0.3
DAY 2	450	<0.3
DAY 6	4	<0.3
DAY 9	<0.3	<0.3
DAY 12	4	<0.3
DAY 14	<O.3	<0.3

* MPN of V. vulnificus per gram of oyster meat.

SUMMARY

The fact that V. vulnificus is a significant public health problem for a portion of the population justifies the study of processing steps that may result in the reduction or elimination of this organism from raw molluscan shellfish. Oysters are the molluscan shellfish most frequently associated with cases of primary septicemia caused by V. vulnificus. This study has verified the sensitivity of V. vulnificus to the cold by showing that their levels are reduced with time when held at temperatures below 4°C. These authors are aware of the research concerning "the viable but not culturable cells" that result from cold treatment of pure cultures of V. vulnificus. At present, techniques are not available to allow us to assess the significance of this phenomenon, if it exists, in oysters during cold storage.

Mild heat treatment of oysters for short periods of time have proven successful to reduce the numbers of V. vulnificus in shucked oyster meats to a level where they can no longer be detected. Following refinement, techniques using mild heat treatments may prove an acceptable addition to oyster processing to insure the safety of the product with respect to V. vulnificus.

ACKNOWLEDGEMENT

This research was supported by the National Marine Fisheries Service under grant NA90AA-H-SK051 and by the State of Mississippi. The authors wish to acknowledge the technical assistance of Vontrell Tapp, Kathy Dawson, and Gina Capley.

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SURVIVAL AND CULTURABILITY OF VIBRIO VULNIFICUS IN ARTIFICIAL SEAWATER MICROCOSMS

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INTRODUCTION

Vibrio vulnificus is one of several types of Vibrio bacteria occurring world-wide. It occurs naturally along United States coastal waters, including the Atlantic and Pacific Coasts, and the Gulf of Mexico. It has also been isolated from seawater, sediment, plankton, and animals; mainly oysters (10).

Systemic infection involving this organism can result in fever, chills, nausea, and abdominal pain. V. vulnificus infections can be sometimes life threatening and can progress rapidly due to its highly invasive nature. In individuals with preexisting liver disease who reported consumption of raw seafood, greater than 50 % mortality has been reported (4). In addition to being foodborne, V. vulnificus can cause severe infections by exposing a skin lesion, an open wound with seawater. Skin lesions are characterized by redness, swelling, and intense pain and can result in amputations (9).

Another interesting aspect of the pathogenesis of V. vulnificus is the discovery that two colony morphotypes exist, the "translucent" and the "opaque". The presence of an acidic polysaccharide layer at the cell surface, which has antiphagocytic properties, accounts for the opacity of the colonies and the virulence of V. vulnificus (7,11).

Infections with V. vulnificus occur mainly in the summer months, and it is only during these months that the organism can be recovered from seawater or oysters. This marked seasonality was attributed to the die-off of the V. vulnificus in seawater during the cold months. It now appears, however, that this apparent decrease in cell culturability may not be due entirely to cell death, but to a situation where the organism enters into a viable but non-culturable (VBNC) state. This may be the result of sublethal injury to the cell or a strategy of the cell to conserve energy and survive. A viable but non-culturable stage of bacteria has been documented (1,2), allowing for a possible persistence of human pathogens in the aquatic environment (5).

MATERIALS AND METHODS

1- Preparation of artificial seawater (ASW):

Artificial seawater was prepared as follows: Artificial seasalt (Forty Fathoms) was dissolved in distilled water to a specific gravity of 1.022 (30 parts per thousand, ppt). The solution was then passed through a 0.22 um filter and autoclaved at 121oC for 15 min.

2- Preparation of V. vulnificus microcosms:

Stock cultures of strain C7184, opaque (O) and translucent (T), V. vulnificus were grown on heart infusion (HI) (100 ml) broth and grown overnight at room temperature. The cells were then harvested by centrifugation at 10,000 x g for 20 min. and washed twice with 50 ml of ASW and centrifuged (20 min. at 10,000 x g). The cells were then suspended in ca. 10 ml of ASW and aseptically transferred to a 1 l flask, prewashed with 6N HCl, containing 750 ml of ASW (8). The V. vulnificus microcosms were incubated at various temperatures and the survival of the organisms was monitored over time.

3- Incubation of microcosms at various temperatures:

Opaque and translucent morphotype microcosms of V. vulnificus were incubated at 37o, 25o, and 4oC. Aliquots from the microcosms were examined at time 0 and weekly thereafter. Total bacterial numbers were determined by the acridine orange direct count (AODC) (3) whereas the actively metabolizing cells were counted using the direct viable count (DVC) method of Kogure et al. (6). Ten microscopic fields were counted per sample. Culturable cell counts were determined by plating on the non-selective HI agar and the vibrio-selective thiosulfate-citrate-bile salts-sucrose (TCBS) and cellobiose polymyxin colistin (CPC) agar media in duplicates. ASW was used for making dilutions throughout the experiment. Conversions from one morphotype to the other were monitored on HI agar plates.

After the organisms became non-recoverable on agar media plates, 10 ml samples from the microcosms were inoculated into 3 tubes of double-strength alkaline peptone broth (APB) (10 ml) pH 8.4, and incubated at 37oC for 24 hr. When these alkaline peptone tubes were all negative, the microcosms were presumed to be in the non-culturable state.

4- Resuscitation of the VBNC cells of V. vulnificus:

After 5 weeks of incubation at 4oC, aliquots from the VBNC V. vulnificus microcosms were drawn and incubated at room temperature for 48 hr. Samples for total cell counts (AODC), direct viable counts (DVC), and media plate counts were examined.

RESULTS AND DISCUSSION

1- Microcosms incubated at 37oC:

V. vulnificus cells went partially into the VBNC but remained culturable for the duration of the experiment (11 weeks). The direct viable counts (DVC) rather stabilized in the fourth week of incubation. The encapsulated and the non-encapsulated forms (T & O) had similar profiles (Graph 1), and conversions between morphotype were not observed.

2- Microcosms incubated at 25oC:

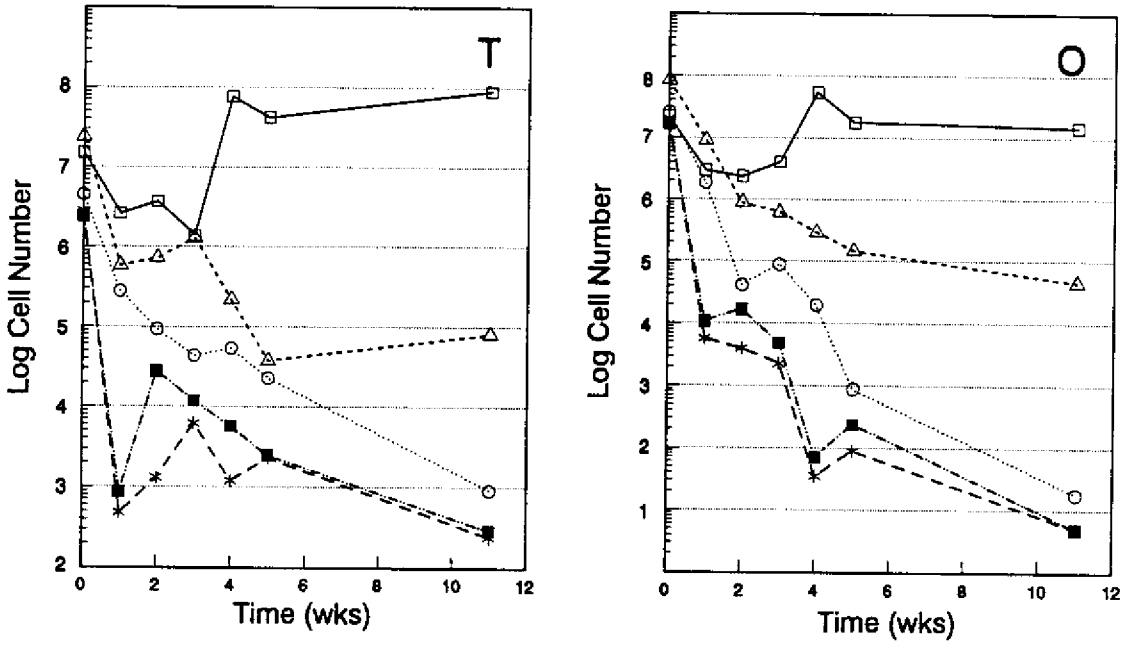
Similar results were observed with microcosms incubated at 25oC where the agar media counts decreased steadily but did not reach 0 after 11 weeks. DVC also decreased at least 3 logs which indicates that some of the V. vulnificus cells went into the VBNC state (Graph 2). In contrast to incubation at 37oC, at 25oC a conversion from the avirulent (T) to the virulent (O) form was observed, but the converse (virulent to avirulent) was not observed. This conversion started on the fourth week of incubation and at the end of the experiment (week 11) all the translucent cells had transformed to the opaque form. This might be explained by the fact that the polysaccharide capsule helps in the adsoption of nutrients to the cell. As the organisms were incubated in a nutrient deprived environment, this could have triggered the formation of the capsule. It should be noted that this effect might be temperature related also, because it did not occur at 37oC.

3- Microcosms incubated at 4oC:

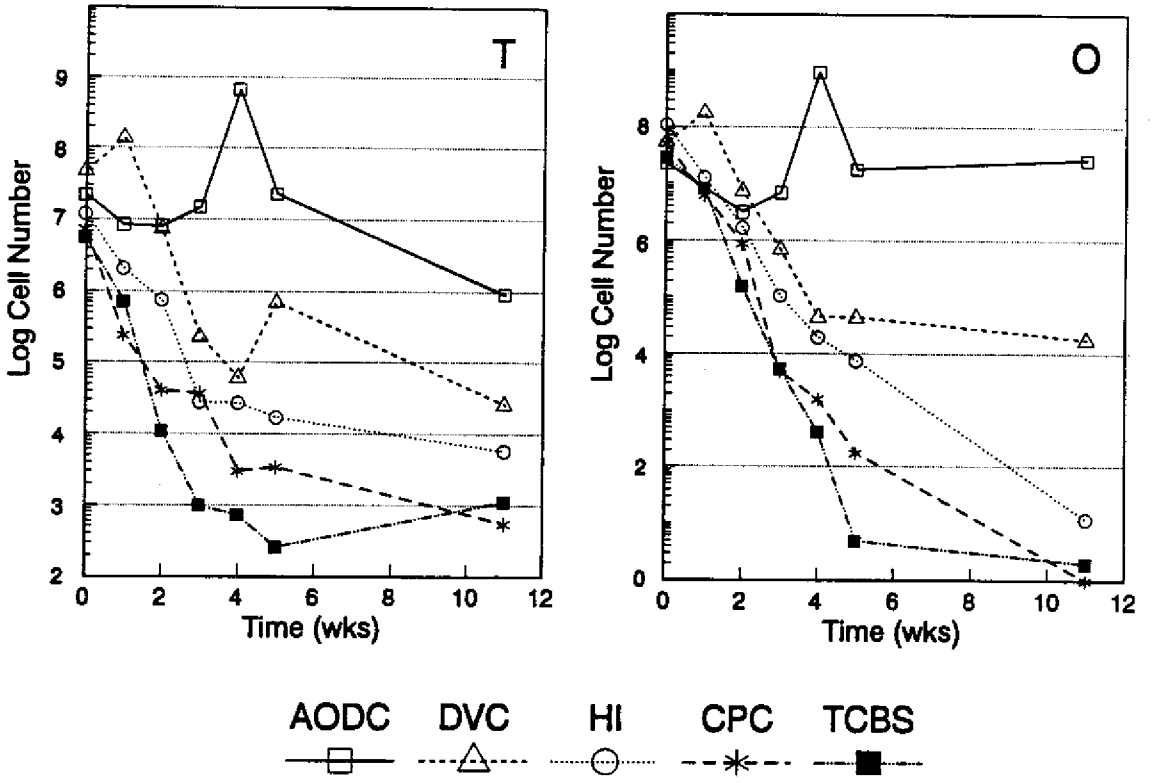
V. vulnificus cells incubated in microcosms at 4oC went into the VBNC state in 4-5 weeks. The total cell count (AODC) remained rather constant and the organisms changed from rods to round cells. The direct viable count (DVC) has decreased by 3 logs on week 4 and the cells changed from being very long and filamentous in fresh cultures to slightly enlarged (double the size of a normal rod) upon incubation in yeast extract and nalidixic acid. The culturability of the cells on agar media decreased quickly and was <1 CFU/ml microcosm on the fourth week.

Culturability on TCBS and CPC selective media decreased quicker than on the non-selective heart infusion media. This indicated that as cells get stressed due to starvation, they are unable to grow on selective or inhibitory media and later they fail to be recovered on non-selective media.

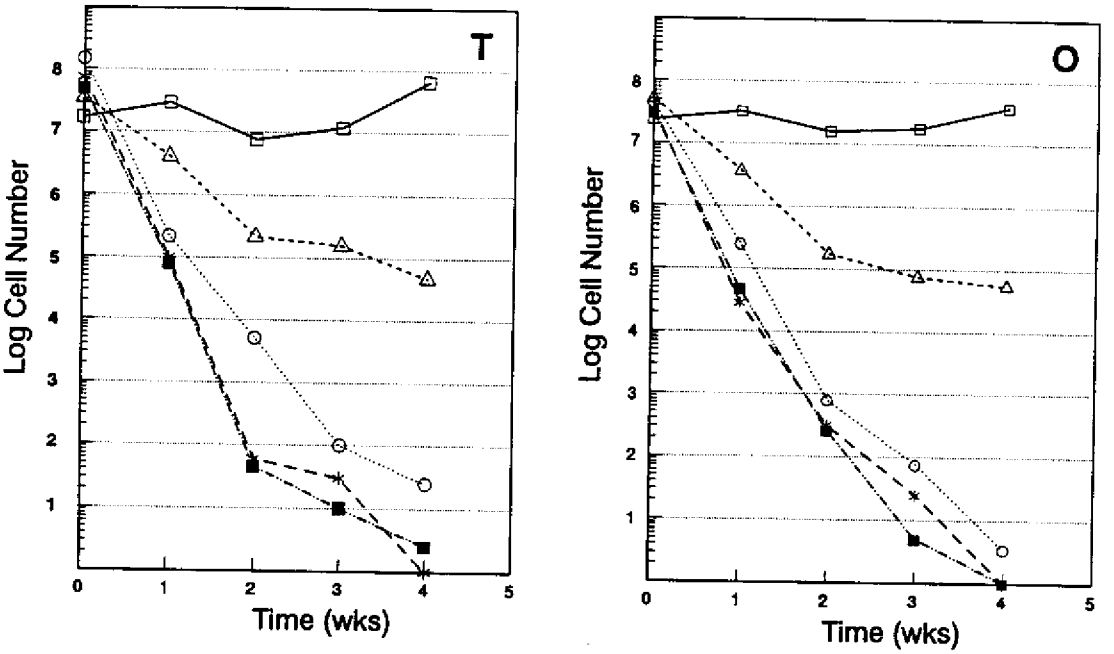
Graph 1. Survival of V. vulnificus in Artificial Seawater Microcosms upon Incubation at 25°C.



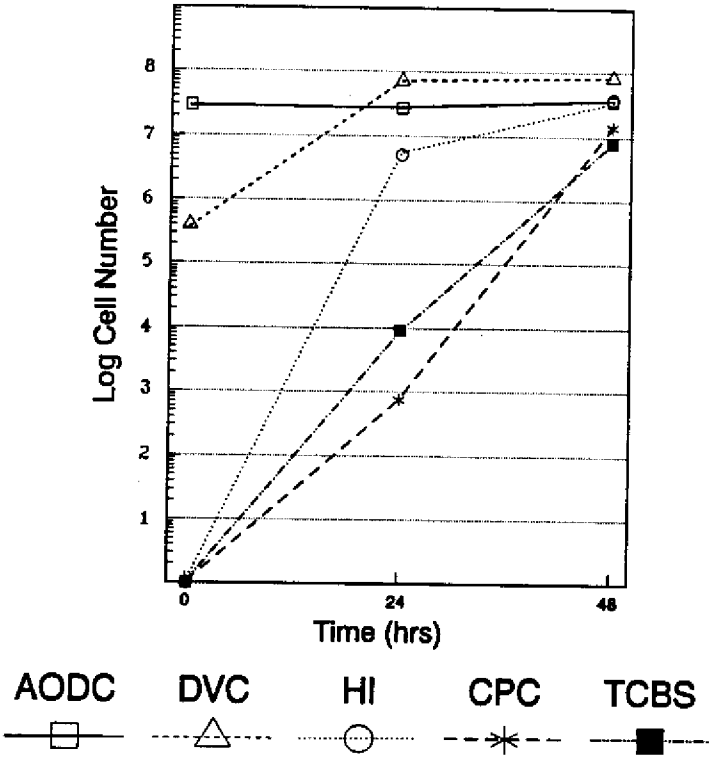
Graph 2. Survival of V. vulnificus in Artificial Seawater Microcosms upon Incubation at 37°C.



Graph 3. Survival of V. vulnificus in Artificial Seawater Microcosms upon Incubation at 4°C.



Graph 4. Resuscitation of V. vulnificus VBNC artificial seawater microcosms after incubation at RT



Both morphotypes had similar profiles (Graph 3) as they went into the VBNC state. No conversions between the two forms were observed.

4- Resuscitation of VBNC V. vulnificus:

As mentioned above, microcosms were considered to be in the VBNC state when they were negative on 3/3 double strength APB tubes after incubation at 37oC for 24 hr. When aliquots of these microcosms were incubated at room temperature in the same ASW, they became culturable again after 24 hr, and increasing to reach the original number of total cell count (Graph 4). When the DVC were examined the resuscitated cells changed from being slightly enlarged, which is typical of a VBNC cell, to being very long which characterizes fresh culture cells. It is worth mentioning that when the cells are in a normal metabolizing state, they increase in number upon the addition of yeast extract even in the presence of nalidixic acid which inhibits cell division of gram negative bacteria. This might be due to incomplete inhibition by nalidixic acid or due to breaking of the very long cells during the addition of stain and filtration (DVC procedure). This would explain the increase in DVC above the total cell count (AODC) at 24 hr. This was a problem with fresh cultures and it was solved by decreasing the incubation time suggested by Kogure to 2-3 hr for actively growing cells.

It should also be noted that the total cell count (AODC) was stable throughout the experiment. This could rule out any cell division upon incubation. These cells, which were round when in the VBNC state, became rod shaped similar to fresh cultures. After 48 hr the plate counts increased reaching the total cell count. This raises the question whether the DVC was a true enumeration of VBNC cells or if all the V. vulnificus cells went into the VBNC state including those that were presumed dead (AODC - DVC).

CONCLUSION

Vibrio vulnificus was able to survive a nutrient deprived environment (ASW) when incubated at room temperature and 37oC and remained culturable on laboratory media for 11 weeks. Conversion from the avirulent type (T) to the virulent type (O) was observed only when the bacteria were incubated at room temperature. However, the organism went into the viable but non-culturable state in 5 weeks, when incubated at 4oC in the same medium. The V. vulnificus were able to resuscitate and became culturable again in 24 hr when the temperature of incubation was changed from 4oC to room temperature (ca 25oC).

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GAMMA IRRADIATION OF VIBRIO PARAHAEMOLYTICUS IN THE BLUE CRAB (CALLANECTES SAPIDUS)

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INTRODUCTION

Vibrio parahemolyticus is a naturally occurring pathogen found in the Gulf of Mexico year round due to warmer temperatures (4) and as a result, V. parahemolyticus has been responsible for several food poisoning incidents in recent years (1).

V. parahemolyticus is also a known pathogen in blue crabs (2,3) and could easily be introduced into the processing plant as part of its natural flora. It is possible for cross contamination to occur while simultaneously processing crayfish (Procambaris clarkii) with blue crab (Callinectes sapidus). In addition to contamination of seafood, it could also pose a health problem to the consuming public and processing employees. In order to protect consumer safety and provide an economically viable product, it is necessary to control the presence of this pathogen.

Low dose gamma irradiation has been shown to be both safe and effective in the pasteurization of foods. Irradiation of fruits, vegetables and spices is already wide-spread and approved. Irradiation has also been shown to be highly effective in preventing sprouting, molds and larva maturation in fruits and vegetables (7).

The longer shelf life of fresh foods resulting from low dose gamma irradiation would allow greater distribution and thus provide a greater economic impact in marketing fresh products (5,6). Therefore, the purpose of this study was to determine the effect of pasteurization levels of low dose gamma irradiation on V. parahemolyticus in crab and the effect of refrigeration storage (4°C) on the survival of the V. parahemolyticus in Louisiana blue crab.

MATERIALS AND METHODS

Organisms

Vibrio parahemolyticus (serotype 05:17) strain 116 was obtained from the microbial collection of the Department of Food Science at Louisiana State University. This culture was donated to the department by R. Sakazaki, National Institute of Health, Tokyo, Japan.

The organism was maintained on trypticase soy agar with 3% NaCl on slants. The organism was transferred to fresh media on a weekly basis.

Preparation of Crab Homogenate

Freshly processed crab meat were donated by a local Baton Rouge seafood processor. The nonsterile crab meat was prepared by blending one part crab with one part sterile saline (3% NaCl) solution in a Waring blendor to form a smooth paste. The sterile crab meat was prepared in the same manner, except that the crab meat was autoclaved at 121°C for 15 minutes before blending with 3% sterile saline solution.

Preparation of Inoculum

Stationary log phase cells of V. parahemolyticus (serotype 05:17) were grown for 18 hours at 37°C and transferred for 3 successive days to insure log phase growth. A loopfull of the organism was innoculated into 50 ml of tryptic soy broth (3% NaCl), placed on a rotary water bath shaker at 125 rpm, and incubated for 12 horus at 37°C and served as the starter culture.

One ml of the starter culture was placed into 99 ml of tryptic soy broth and grown until a reading of 0.6 absorbance (600 nm) was obtained on the Bausch & Lomb Spectronic 70 (Bausch & Lomb Inc., Rochester, NY). The media suspension was then representative of a population of 1.0×10^7 CFU (colony forming units) Vibrio parahemolyticus/ml.

Ten ml of the broth was added to 10 gram samples of crab homogenates to achieve a final concentration of 1.0×10^7 V. parahemolyticus/g sample.

Irradiation of the Samples

The whirl packs containing the inoculated sterile and nonsterile samples were transported to the LSU Nuclear Science Center and placed in a water tight diving bell, which was filled with ice and sealed. The diving bell was then lowered into the irradiation pit to expose the samples to the cobalt-60 source which emitted 0.68 Gy/minute.

The samples were exposed to 0.10, 0.20, and 0.35 kGy, respectively. The packs were then removed, placed in ice and transferred back to the laboratory at the Department of Food Science for analysis and storage. Control samples were treated in the same manner, except the low dose gamma irradiation treatments were omitted.

Enumeration of Vibrio parahemolyticus

The crab meat were examined for the presence of V. parahemolyticus at 0, 7, 14, and 21 days, respecstively, after cold storage at refrigeration (4°C) temperature. Both sterile and nonsterile samples were enumerated by the most probable number method (MPN) on tryptic soy agar (3% NaCl) and on thiosulfate-citrate-bile salts- sucrose agar (TCBS) media. The sterile and nonsterile plating and counting of V. parahemolyticus were performed in triplicate.

The plates were incubated for 24 hours at 35°C after streaking. At that time, the plates were removed and counted employing the MPN methodology. Further biochemical tests were run on TCBS positive plates to confirm the presence of V. parahemolyticus.

The number of surviving organisms were calculated by determining the average number of the triplicate samples of V. parahemolyticus recovered from samples that were exposed to the same low dose gamma irradiation dosages, and the same storage time and refrigeration temperature treatments.

RESULTS AND DISCUSSION

Effects of Radiation

In the sterile homogenates an original innculation 1.2×10^7 CFU (colony forming units)/g V. parahemolyticus were reduced 4 log cycles at 0.10 kGy, 6 log cycles at 0.20

kGy, and no V. parahemolyticus were recovered following irradiation with 0.35 kGy as seen in Figure 1.

In nonsterile crab meat the original innoculum 4.4 X 10^7 CFU (colony forming units)/g V. parahemolyticus were reduced 4 log cycles at 0.10 kGy, 5 log cycles at 0.20 kGy, and 6 log cycles at 0.35 kGy of gamma irradiation as seen in Figure 2.

The reduction of V. parahemolyticus in both the sterile and nosterile samples with low dose gamma irradiation can be easily seen in Figures 1 and 2. Therefore, low dose gamma pasteurization dosages can be used to reduce the number of V. parahemolyticus in blue crab meat.

Effect of Time

There was a general reduction in numbers of V. parahemolyticus during the three-week trial period at refrigeration temperature (4°C) in the nonirradiated, sterile and nonsterile crab meat samples. In the sterile crab meat, the V. parahemolyticus declined from 1.2 X 10^7 CFU/g at 0 kGy on day 0 to 9.0 X 10^1 CFU/g at day 21. At 0.10 kGy the number of colonies in the sterile homogenate was reduced from 1.2 X 10^7 CFU/g to no growth evident on day 21. With a pasteurization dose of 0.35 kGy there was no growth of V. parahemolyticus detected on any of the testing days (days 0, 7, 14, and 21) thus complete elimination and destruction.

With a low gamma irradiation dose of 0.1 kGy, the population in the nonsterile crab meat was reduced from the original innoculation of 4.7 X 10^7 CFU/g to 3.0 X 10^1 on day 21. At a 0.2 kGy dose the V. parahemolyticus in the nonsterile crab meat homogenate was reduced from the original innoculation of 4.7 X 10^7 CFU/g to no growth evident on day 21. At a 0.35 kGy dose the V. parahemolyticus in the nonsterile crab meat was reduced from 4.7 X 10^7 CFU/g to no growth evident on days 7, 14 and 21 thus complete elimination and destruction.

CONCLUSIONS

The results indicate that at a low gamma irradiation dosage of 0.35 kGy, V. parahemolyticus can be reduced approximately 4 log cycles during the first 7 days of storage on ice at refrigeration temperature (4°C) in nonsterile crab meat and reduced an additional 3 logs by day 21. In sterile crab meat, at 0.35 kGy irradiation, V. parahemolyticus was reduced immediately to 0 after irradiation and remained at 0 during the entire 21-day storage period at refrigeration temperature (4°C). It appears that some protection is offered to V. parahemolyticus by the presence of other microorganisms to V. parahemolyticus by low dose gamma irradiation, but only up to 14 days of storage at refrigeration temperatures (4°C).

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FIG 1: Survival of V. parahaemolyticus in STERILE blue crab meat after irradiation

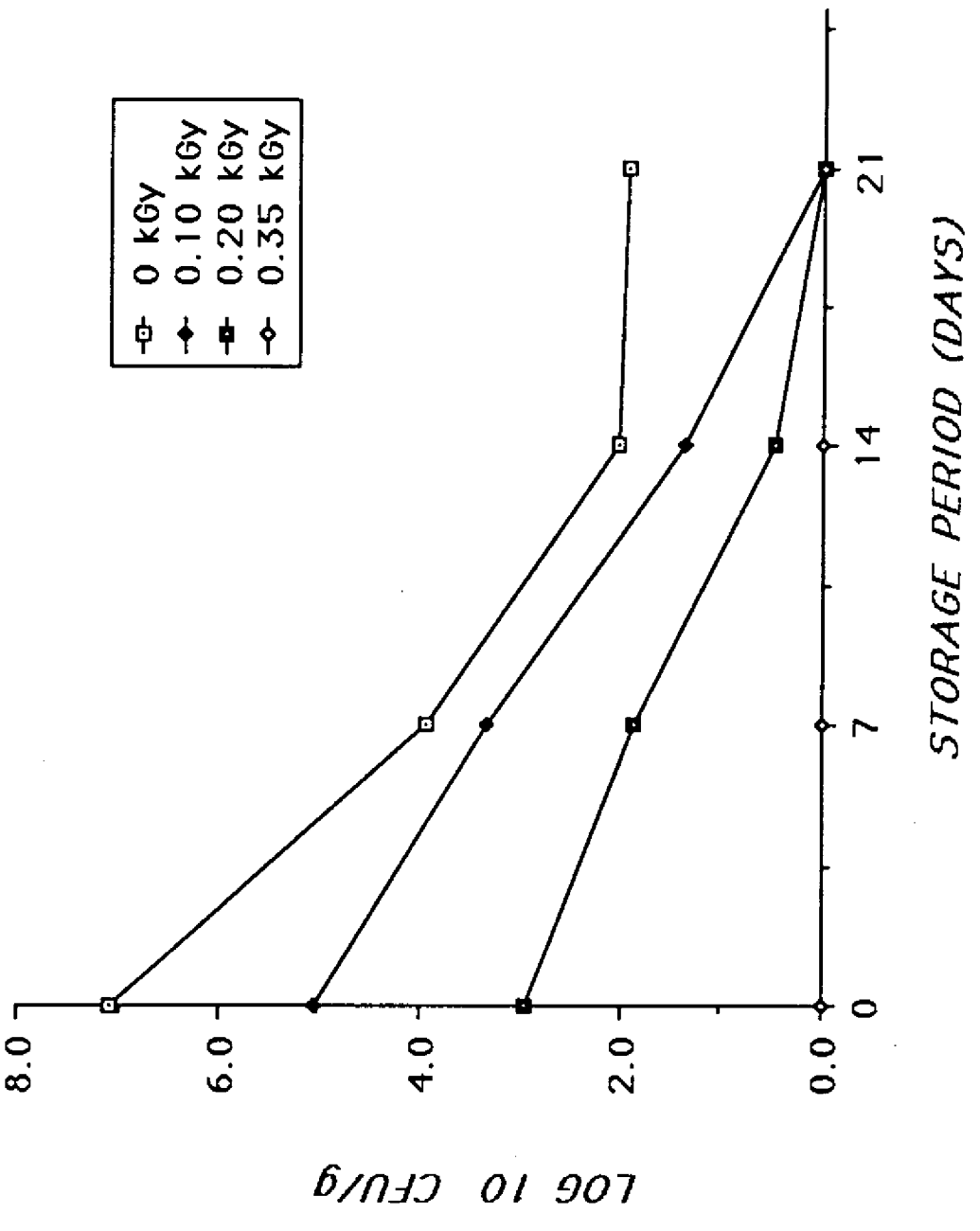
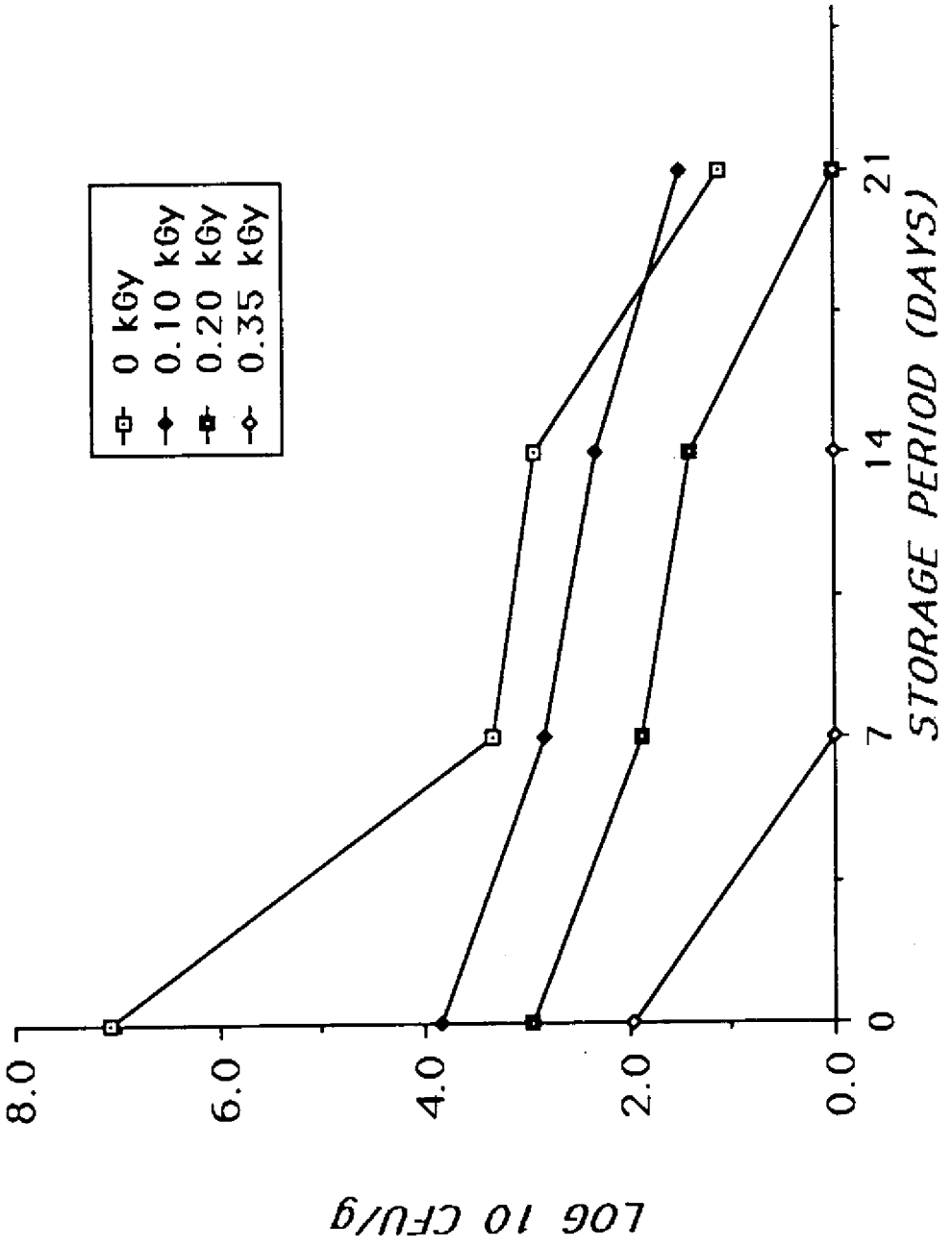


FIG 2: Survival of V. parahaemolyticus in NONSTERILE blue crab meat after irradiation



COMPARATIVE EFFECTS OF IONIZING RADIATION AND HIGH ENERGY ELECTRON BEAMS ON MOLLUSCAN SHELLFISH

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There exists a public health risk associated with the consumption of raw or inadequately cooked shellfish. Several bacterial agents have been isolated from shellfish like the American oyster (Crassostrea virginica), perhaps most importantly being the Vibrio species. Vibrio vulnificus is a halophilic bacterium that is located in warm estuarine waters, and has been recently implicated as a human pathogen (6).

V. vulnificus is the causative agent of a primary septicemia that is rapidly induced, and often fatal in certain individuals who have a history of liver disease or have some other immunocompromised condition (2). V. vulnificus demonstrates a seasonal variation, and seems to pose the most significant health risk in the warm summer months (7). Because of this potential health risk, several methods have been proposed to reduce bacterial numbers and increase the shelf-life of molluscan shellfish.

The proposed methods include the use of gamma irradiation, high energy electron beams and depuration as means of reducing bacterial numbers and also increasing product shelf-life. Shellstock oysters were exposed to varying doses of gamma irradiation, and were subsequently tested for microbiological and shelf-life consequences. Similar tests were performed using a linear accelerator, and depuration. The linear accelerator data shows the results in reducing bacterial numbers, while the depuration data shows the effects on increasing shelf-life.

MATERIALS AND METHODS

Source of Oysters

Oysters were provided by two Florida shellfish processors. One source was Leavin's Seafood, located in Apalachicola, FL., and the other source was Calvert's Seafood, located in Cedar Key, FL. Oysters were harvested during the morning hours, and then transported to the University of Florida, Gainesville, in the evening, where they were stored at 34-36oF, for next day irradiation, or depuration. Gardner and Watts (3) showed that oysters which received 3.0 kGy or more of gamma radiation, did not die until 7 hours post irradiation.

Source of Gamma Radiation

The source of gamma radiation used was a 60Co unit called a Gamma-Cell 220. This was a 30 kilocurie source that is located on the campus of the University of Florida at the USDA Entomology Research Center. The unit was equipped with a 6" by 8" opening in which oysters could be placed for irradiation. The subsequent flow chart details how the shellstock oysters were irradiated and monitored.

Source of a Linear Accelerator

The preliminary data that is presented, was obtained by Dr. Rodrick from a linear accelerator located at the Mevex Corporation, in Ontario, Canada. The University of Florida is obtaining its own linear accelerator from France, which should be operational March 1, 1990, so that the experiments may be repeated. Shellstock oysters were exposed to varying doses from the linear accelerator, and bacterial numbers were expressed in colony forming units per gram of meat

Depuration Source

Depuration tanks are generally 55 gallons or larger, and are filled with a 2.5% salt. The tanks are equipped with a recirculating pump and filter system, as well as disinfecting ultraviolet light. The oysters are placed in the tanks for 48 hours @ 70-72oF, and allowed to filter feed. At the end of 48 hours, oysters are removed, stored at 34-36oF, and monitored for death.

Methodology Flow Chart

The flow chart of Figure 1. demonstrates how the oysters were handled during the gamma irradiation experiments. Approximately 12-15 oysters were placed in a 6" by 8" plexiglass container packed with ice above and below. Oysters were irradiated at 1, 2, and 5 kGy. Separate experiments were performed for the microbiological and shelf-life studies. The microbiological analysis consisted of carrying out a serial dilution (10-1 to 10-8) using 50 grams of irradiated oyster meat homogenized in peptone water, and then preparation of Most Probable Number (MPN) tubes in alkaline peptone water @ 2.5% NaCl. Positive MPN tubes were transferred to TCBS (thiosulfate citrate bile salts) agar for selection of "vibrio-like" organisms, and to CPC (cellobiose polymixin colistin) agar for selection of V. vulnificus (5). V. vulnificus was enumerated by the MPN technique (1). All bacteriological media were obtained from Difco Corp.

RESULTS AND DISCUSSION

The MPN results of control and irradiated oysters, obtained from Cedar Key and Apalachicola, are found in Tables 1 and 2. These tables consist of data obtained using oysters . A 3-4 log reduction in bacterial numbers was observed at every exposure dose, with the exception of the 2 kGy exposure of the Apalachicola oysters, as compared to the non-irradiated controls. Tables 3 and 4 show the results of the MPN'S, as well as the numbers of bacteria observed on TCBS and CPC agar for both the Apalachicola and Cedar Key oysters. There were no V. vulnificus bacteria found on any of the CPC agar plates streaked with irradiated oyster homogenate. This would indicate that V. vulnificus is in fact radiosensitive, and this correlates well with the work of Grodner (4).

Tables 5 and 7 show the effect on shelflife of irradiating oysters at 1 and 5 kGy. At the 1 kGy exposure, 50% of the oysters were dead within 12 days, whereas at the 5 kGy exposure, 50% of the oysters were dead within 7 days. Table 6 shows the effects on shelflife of 2 kGy exposure, as well as some depuration data. Fifty percent of the oysters were dead within 10 days after 2 kGy exposure, however even after twenty days post depuration, 50% of the oysters were not dead. The data in Table 8 shows depuration results obtained by Leavin's Seafood. One-half of the oysters were dead within 31 days.

Table 9 shows the results obtained using the linear accelerator in Ontario, Canada. The cfu/g of bacteria were reduced as exposure dose was increased. Furthermore, there was also a marked reduction in bacterial numbers in oysters that were exposed on both sides.

Irradiation proved to have potential in reducing the numbers of V. vulnificus in shellstock oysters. V. vulnificus appears to be radiosensitive, as it was not detected in any irradiated sample (negative CPC results). Bacterial numbers are in fact reduced 3-4 log cycles, however the product shelf-life is compromised.

CONCLUSIONS AND REFERENCES

The use of gamma irradiation, high energy electrons and depuration could prove to be valuable mechanisms for reducing the risk associated with the ingestion of raw shellfish. Gamma irradiation and high energy electrons appear to be very succesful for reduction of bacterial numbers, however shelf-life is compromised. For irradiation to be successful, both enhanced food safety and extended product shelf-life are required, however both of these criteria do not

appear to be met. Depuration and the linear accelerator may be the answer for increasing the safety of raw shellfish ingestion. Further research is being performed before the plausibility of high energy electrons beams can be addressed.

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Figure 1. Methodology Flow Chart

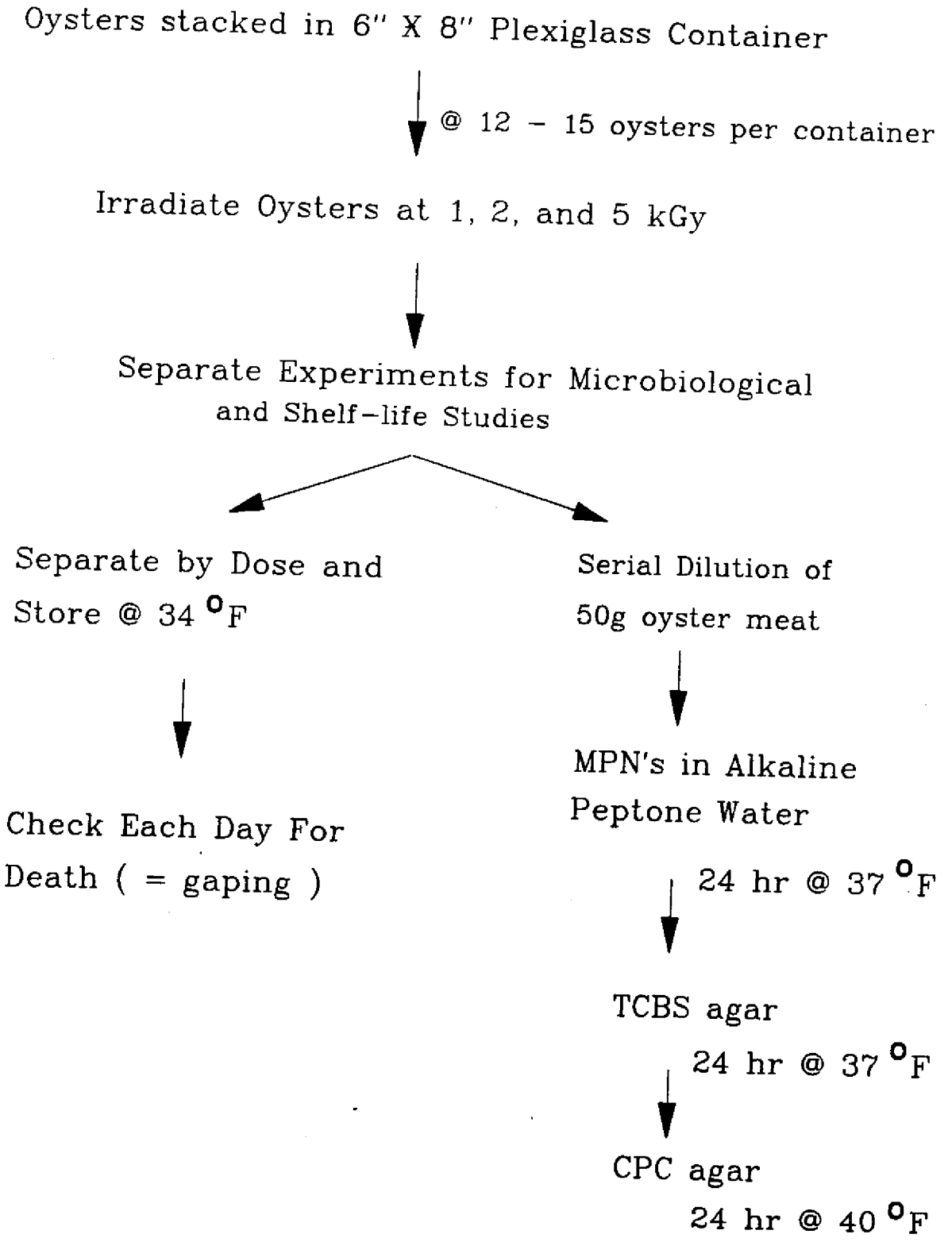


Table 1. Crassostrea virginica Irradiation MPN's in Alkaline Peptone

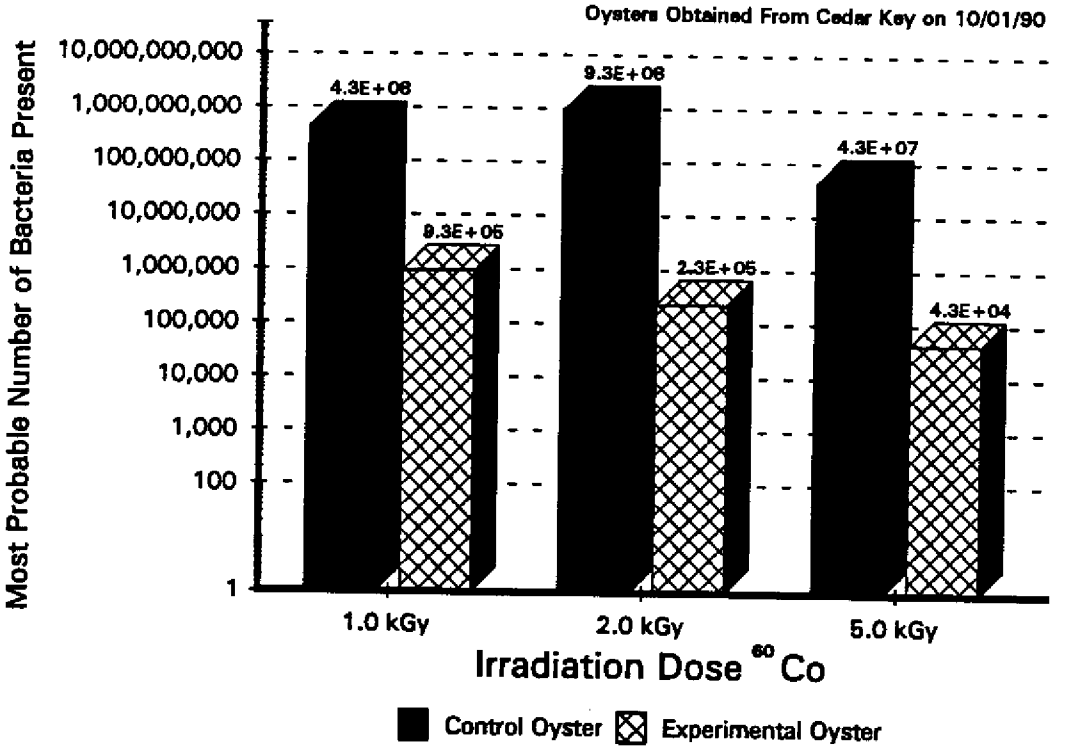


Table 2. Crassostrea virginica Irradiation MPN's in Alkaline Peptone

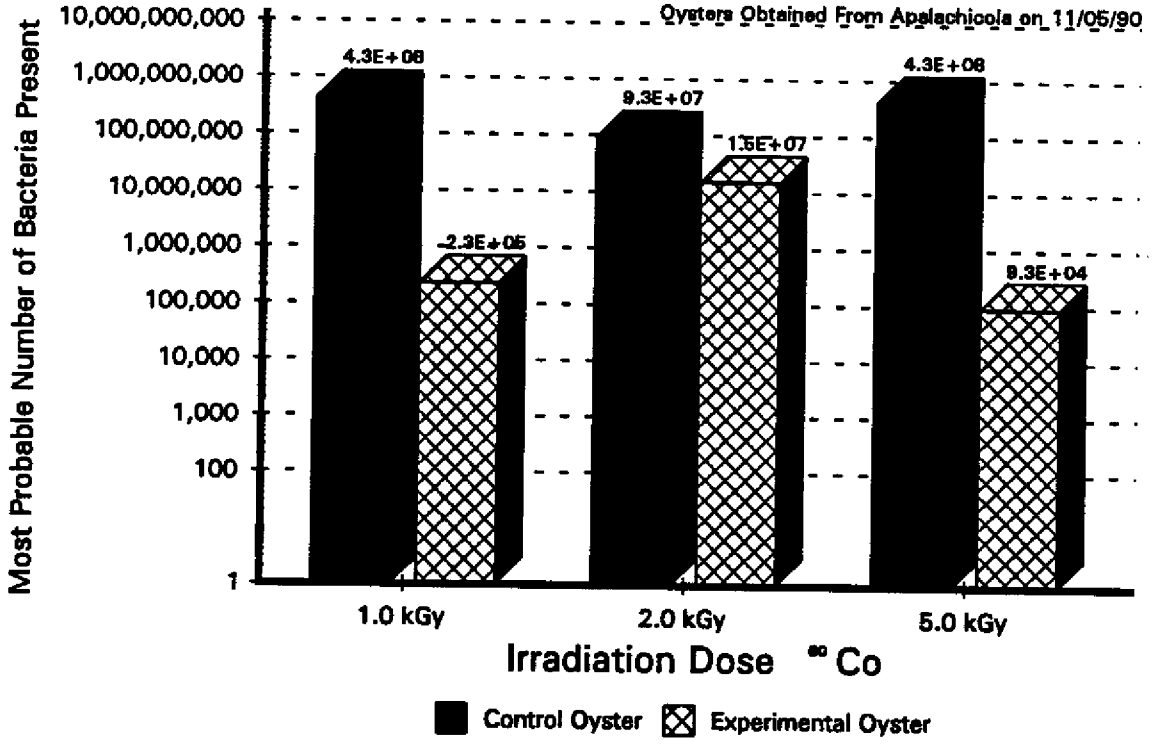


Table 3. Post-Irradiation Microbiological Consequences 10/01/90

	1 kGy		2 kGy		5 kGy	
	con	exp	con	exp	con	exp
MPN's Alk. Peptone	4.3E8	9.3E5	9.3E8	2.3E5	4.3E7	4.3E4
TCBS confirmed	2.3E8	9.0E4	4.3E8	---	2.3E7	---
CPC agar	9.0E7	---	2.3E8	---	9.0E6	---

Table 4. Post-Irradiation Microbiological Consequences 11/06/90

	1 kGy		2 kGy		5 kGy	
	con	exp	con	exp	con	exp
MPN's Alk. Peptone	4.3E8	2.3E5	9.3E7	1.5E7	4.3E8	9.3E4
TCBS confirmed	4.3E8	---	4.3E7	2.3E6	2.3E8	9.0E3
CPC agar	4.0E7	---	9.0E6	---	2.3E8	---

Table 5. Shelf-life Data of Irradiated Oysters
(Avg. observed @ 1.0 kGy)

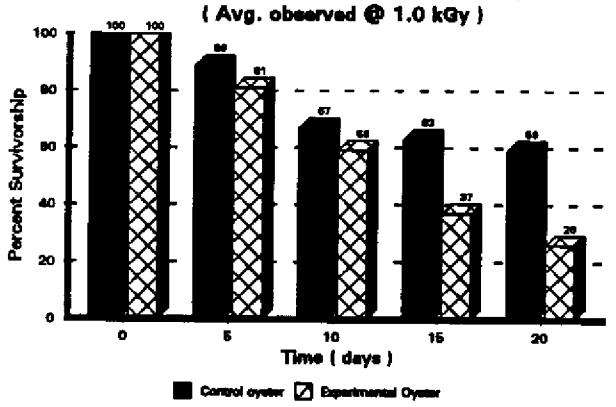


Table 6. Shelf-life Data of Irradiated and Depurated Oysters
(Avg. observed @ 2.0 kGy)

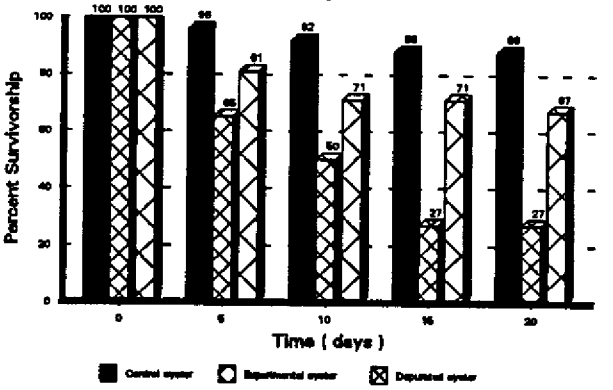


Table 7. Shelf-life Data of Irradiated Oysters
(Avg. observed @ 5.0 kGy)

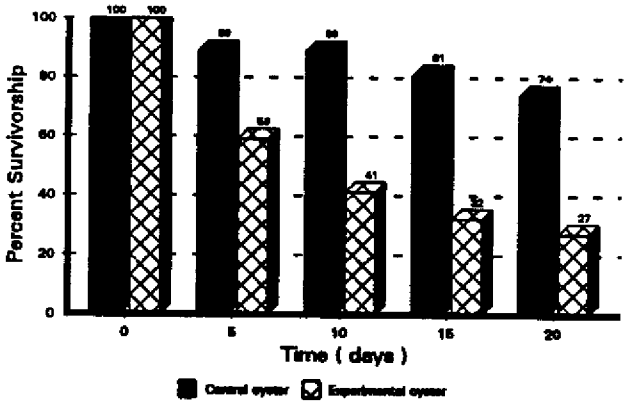


Table 8. Oyster Depuration Data (Summer 1990)

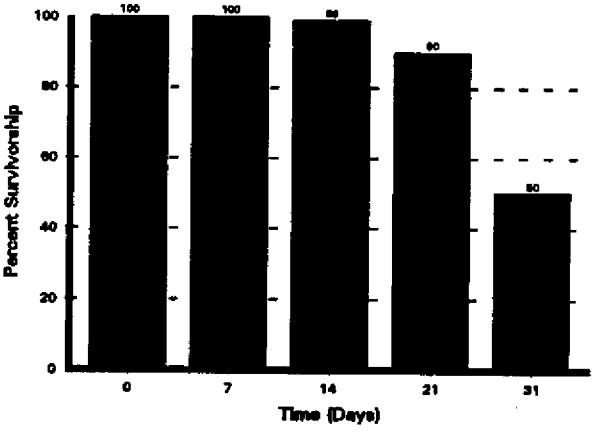
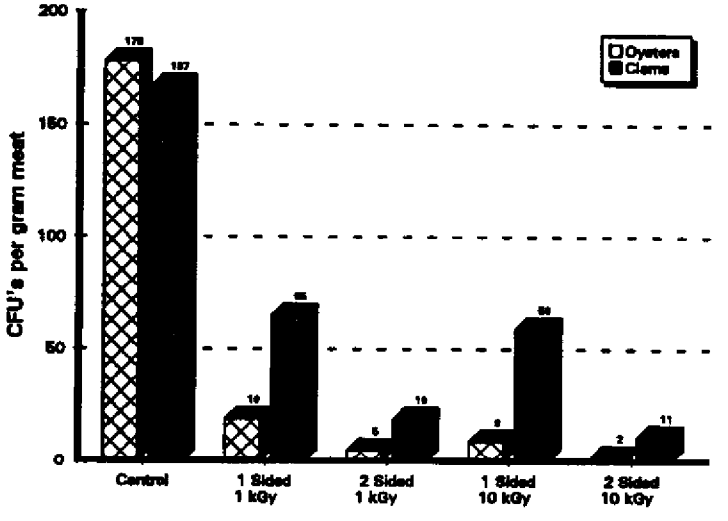


Table 9. Linear Accelerator Data on Oysters and Clams



INTERNAL DOSIMETER FOR IRRADIATED SEAFOODS

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INTRODUCTION

There is an increasing application worldwide of ionizing radiation as a method for prolonging the shelflife and enhancing the safety of various foodstuffs, including seafood products (7,12). Associated with this, there is a need to develop a method which is reliable, and relatively easy to use, whereby the amount of ionizing radiation dose received by the foodstuffs can be determined. Dr. Mark Desrosiers, of the National Institute of Standards and Technology (NIST) has recently been cited as saying that "the NIST is seeking screening methods for irradiated foods which also may measure the dose of irradiation. Such a method would be used for post-irradiation detection, he indicated." (3). This will lead to increased consumer acceptance of irradiated foods, and provide a means by which sovereign governmental regulatory authorities can enforce their respective laws governing the use of ionizing radiation in food processing. These laws vary considerably among the nations of the world (12). In order for such a method to be useful, certain criteria should be fulfilled, including: (1) inexpensive, (2) easy to perform, (3) sensitive detection of radiation damage, (4) stability of the radiation damage being detected, and (5) reproducible.

Previous efforts in the development of a dosimeter for irradiated foodstuffs have focused on detection of unique radiolytic products or chemical changes in the food. Techniques have included electron spin resonance spectroscopy, high resolution nuclear magnetic resonance spectroscopy, mass spectrometry, direct epifluorescent filter technique, and gas chromotography (9,10,14).

This paper presents the technique of horizontal agarose slab gel electrophoresis for detection of DNA single-strand and double-strand breaks as a means of measuring ionizing radiation damage in cell tissue of irradiated foodstuffs. The technique has been previously used for detection of ionizing radiation damage in DNA of cultured insect cells (15), and has been applied for quantitation of single- and double-strand breaks in DNA irradiated in vitro (6). The technique has been demonstrated to distinguish shrimp and oysters which are not irradiated from those which have been exposed to ionizing radiation doses of 1, 2, 3, and 5 kGy. These preliminary results suggest that this technique will be useful in the estimation of the amount of ionizing radiation received by these seafoods. Data not reported in this paper indicate that the technique can be applied to chicken, turkey, and mullet. It is reasonable to expect that, indeed, this technique can be used as an ionizing radiation dosimeter in a variety of foodstuffs, providing that DNA can be extracted from the foodstuffs with sufficiently large molecular weight to allow detection of the strand breaks above a background level of strand breaks that are introduced as a result of the tissue processing for DNA extraction and analysis.

MATERIALS AND METHODS

Overview

Figure 1 presents a flow chart of the technique.

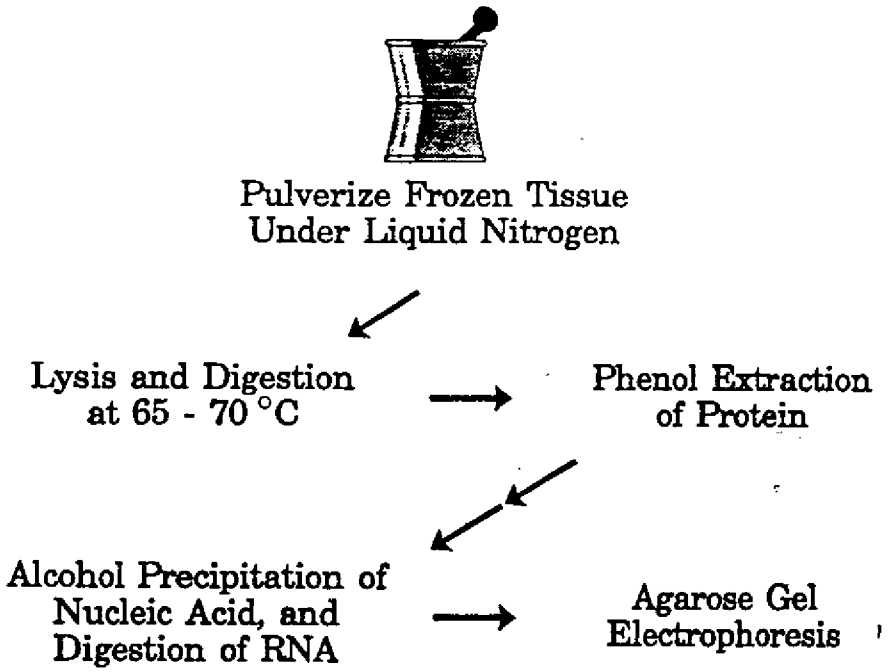


Figure 1. Diagram outlining the steps of the procedure used in obtaining crude DNA extracts for measuring DNA strand breaks by agarose gel electrophoresis.

Figures 2 and 3 outlinethe use of agarose gel electrophoresis for the detection of radiation-induced strand breaks in DNA. An important, and reasonable assumption, isthat the number of strand breaks increases as the radiation exposure dose increases.

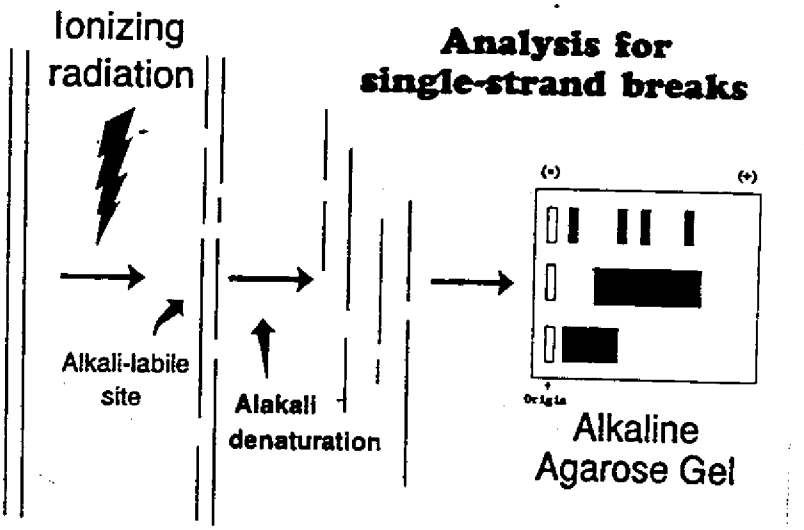


Figure 2. Measurement of DNA single-strand breaks by alkaline agarose gel electrophoresis. Molecular weight marker DNA in lane 1, DNA from irradiated sample in lane 2, and DNA from control (unirradiated) sample in lane 3.

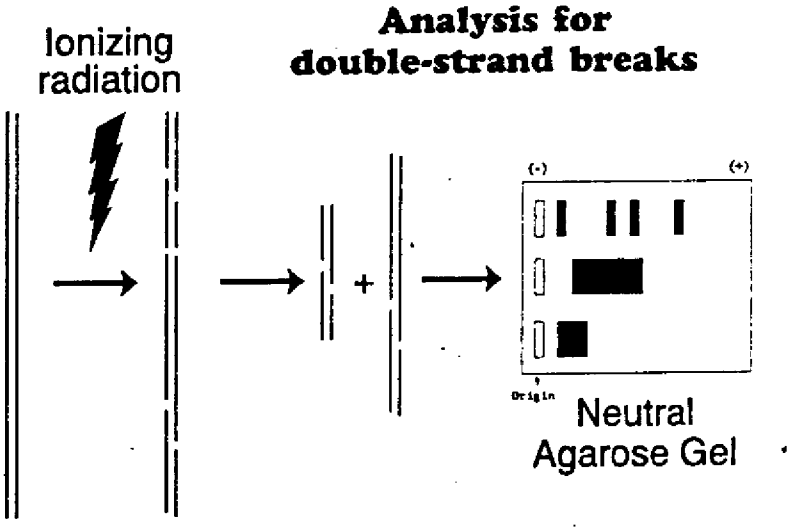


Figure 3. Measurement of DNA double-strand breaks by neutral agarose gel electrophoresis. Molecular weight marker DNA in lane 1, DNA from irradiated sample in lane 2, and DNA from control sample in lane 3.

Approximately 0.5 g of tissue has been found to be sufficient to carry out the analysis. The dark bands of the agarose gels depicted indicate the location of the DNA, as detected by the presence of ethidium bromide stain which intercalates in the DNA and fluoresces orange when excited with near-UV radiation. Samples are applied into wells of the agarose gel, indicated by the open rectangular boxes, and DNA migration in the gel is toward the positive electrode of the electrophoresis apparatus (left to right in the diagram). The rate of migration is directly dependent on the size of the DNA fragments. Smaller DNA fragments are characterized as having faster rates of migration. The gel electrophoresis profiles of the DNA from irradiated samples is broader (more smeared) than that of control DNA because of the wider range of DNA sizes from irradiated samples, as expected from the random distribution of strand breaks in the DNA from radiation damage. Molecular weight marker DNA has sharp bands, representing the collection of homogeneous size classes of DNA in the sample. The details of the technique are now discussed.

Extraction of DNA from foodstuffs

This procedure is modified from the method described in Current Protocols in Molecular Biology (4). Finely dice 0.5 g of tissue sample from the foodstuff with a scissors or scapel, and transfer to a mortar and pestle. Add liquid nitrogen to the pestle to cover the tissue, and grind the frozen tissue under liquid nitrogen. Transfer the frozen, powdered tissue, using a pre-chilled spatula into a 30 ml Corex centrifuge tube, containing 4 ml digestion buffer, which has been equilibrated to 65-70 °C, and incubate
20 min. at this temperature. Digestion buffer contains 100 mM NaCl, 50 mM tris-HCl, pH 8, 25 mM EDTA and 0.5% SDS. The solution is not allowed to age more than two weeks before use.

Remove the sample from the 70 °C water bath, allow to stand several minutes at room temperature, and then hold on ice for 20 - 30 min. Centrifuge at 10 kRPM in a Sorvall SS-34 anglehead rotor (12,000 X G), at 2 °C for 10 min. Carefully remove the tubes from the rotor, and hold in an ice-water slush while decanting the supernatant with a pipete (recording the volume of the supernatant), and transferring to a 15 ml Corex centrifuge tube. Add an equal volume of redistilled phenol which is buffered according to the procedure described in Current Protocols in Molecular Biology (pp. 2.2.1 - 2.2.3) to the samples, vortex at moderate speed for thorough mixing, and let stand 15 min. at room temperature. Redistilled phenol is obtained from VWR Scientific, Inc., Marietta, GA, and was manufactured by IBI (a Kodak Company).

Centrifuge the sample at 5 kRPM in a Sorvall HB-4 swinging bucket rotor (4,000 X G) at room temperature for 5 min. Carefully decant the top aqueous phas, and transfer to a fresh 15 ml Corex centrifuge tube, noting the volume. Add 0.1 volume 3 M sodium acetate, and 3 volumes cold absolute ethanol to the sample, transfer to an ice bath for 10 min., then hold in a -20 °C freezer for one to two hours.

Centrifuge the alcohol precipitate at 9 kRPM in a Sorvall HB-4 rotor (13,000 X G) at 2 °C for 20 min. Pour off the supernatant, drain the pellet well, and resuspend the pellet in 0.2 ml 0.1X TNE buffer. Full strength TNE contains 10 mM tris-HCl, pH 8, 100 mM NaCl, and 1 mM EDTA. Store the sample at 4 °C until ready to carry out agarose gel electrophoresis.

Alkaline agarose gel electrophoresis

Detection of radiation damage in DNA by horizontal agarose gel electrophoresis was first introduced by Achey et. al. to measure cyclobutane pyrimidine dimers in DNA extracted from cells exposed to ultraviolet (UV) radiation (2). The principal of the method, specifically the detection of DNA single-strand breaks, was extended to include DNA damage arising from ionizing radiation damage (6,15), in the form of strand breaks in the DNA strands.

All gel electrophoresis is performed using a DNA Sub Cell™ Electrophoresis System (Bio-Rad Laboratories, Richmond, California). A 60 ml volume of molten agarose gel is poured into the 15 x 10 cm gel tray, resulting in a 4 mm thick slab gel. Sample wells are made using a 15 well comb, to form rectangular wells that are 5 x 1.5 mm. Sample volumes between 8 and 16 ul are applied to the wells, with loading buffer which contains a tracking dye and glycerol. The actual amount of sample loaded into the well is adjusted so that approximately equal quantities of DNA are loaded in each well. Heat-treated RNaseA is added to a final concentration of 50 ug/ml, and RNA is digested for 30 minutes at 37 °C. The reaction is stopped by adding 1 volume loading buffer to 5 volumes of sample, and the entire volume is loaded into the well.

The loading buffer consists of 1 part 500 mM Na_3EDTA to 9 parts glycerol, to which is added 20 mg bromocresol green per 10 ml of the mix. Immediately before use, 3 parts of 1 N NaOH is added to 10 parts of the mix.

Electrophoresis is carried out for 4 hours at 28 volts (1.65 V/cm), 5 hours at 24 volts (1.4 V/cm), or 16 hours at 12 volts (0.75 V/cm). The running buffer contains 0.03 N NaOH, and 2 mM trisodium EDTA. After electrophoresis, the slab gel is transferred to a tray to soak 30 minutes in 250 ml neutralizing buffer, which is removed and replaced with 250 ml neutralizing buffer containing 100 ng/ml ethidium bromide. Soak the gel for 30 minutes, remove and safely discard the ethidium bromide solution. The gel is transferred to a near-UV transillumination table, and photographed.

Neutral agarose gel electrophoresis

The procedures are the same as for alkaline agarose gel electrophoresis, except that the running buffer is TAE or TPE buffer (4,13). The loading buffer contains 0.25% bromophenol blue as a tracking dye, and 50% glycerol.

Electrophoresis is carried out for 16 hours at 12 volts (0.75 V/cm). The staining procedure is the same as used for alkaline gels, with omission of the neutralization step.

DNA Molecular weight markers

Eco RI restriction endonuclease digest of lambda-DNA (called DNA molecular weight marker I) and Hind III restriction endonuclease digest of lambda-DNA (called DNA moleclar weight marker II) were obtained from Boehringer Mannheim. The base pair lengths of the DNA fragment mixtures (in kilobase pair units - kbp) are: 21.2, 7.4, 5.8, 5.6, 4.9, and 3.5 for DNA MW I marker DNA, and 23.1, 9.4, 6.6, 4.4, 2.3, 2.0, 0.56, and 0.12 for DNA MW II marker DNA.

The 1 Kb DNA ladder consists of 1014 bp fragments that are linked together, providing a mixture of fragments which differ from one another by 1014 bp lengths, up to a maximum of 12.2 kbp, and is a product of Bethesda Research Laboratories, Inc., Gaithersburg, Maryland.

Lambda DNA (48.5 kbp length) is obtained from Sigma Chemical Co., St. Louis, Missouri.

Marker DNA is diluted with TNE buffer to final concentrations of 0.05 ug/ul, except lambda DNA is diluted to 0.033 ug/ul. 15 ul volumes of the diluted marker DNA are applied to the wells being used to calibrate the migration distance of DNA with known size, except the lambda DNA volume applied is 5 ul.

Densitometer scanning analysis of photographs

A video densitometer is used to scan the photographs, and to plot the intensity of the bands as a function of distance of travel from the origin. The camera 1-D scanning program software is used as provided by Biomed Instruments (Fullerton, CA). The video camera, which is attached to an IBM PC computer, is a product of Biomed Instruments.

Irradiation of food samples

A custom-made cobalt-60 irradiator was used for radiation exposure of shrimp samples. The irradiator is a cave-type, with a centrally positioned rod containing 600 Ci of cobalt-60 pellets sealed in a 5/8" diameter stainless steel rod, which can be raised and lowered into the irradiation chamber for radiation exposures. The design of the irradiator has been described (11).

Whole shrimp were exposed to cobalt-60 gamma radiation at a distance of 2" from the cobalt-60 rod, providing an exposure dose rate of 13.3 Gy/min. The exposure dose rate was determined by Fricke ferrous sulfate dosimetry. Radiation exposures were performed at room temperature, in air.

Irradiation of oysters is described elsewhere in these Proceedings (8).

RESULTS AND DISCUSSION

Alkaline agarose gel electrophoresis of shrimp DNA

DNA extracts from control and irradiated shrimp were subjected to alkaline agarose gel electrophoresis. Figure 4 is a photograph of this gel. DNA molecular weight markers were run in lanes flanking the DNA samples from shrimp.

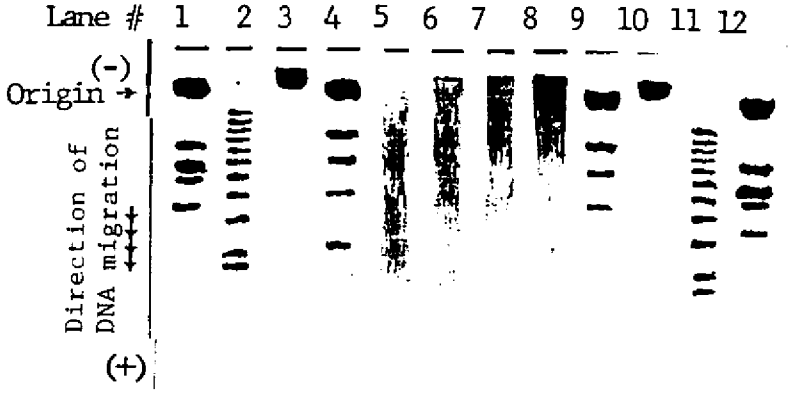
DNA extracted from shrimp exposed to increasing doses of radiation migrates further in the gel, reflecting the accumulation of single-strand breaks in the DNA during irradiation, accompanied by smaller single-strand DNA fragments, which migrate further in the gel during electrophoresis. This salient aspect provides a means to determine radiation exposure of food products.

Smearing of the shrimp DNA bands results from the random distribution of the single-strand breaks. Control DNA also shows a distribution of fragment sizes, caused by the physical shearing of the DNA during extraction. All radiation doses result in additional strand breaks beyond those contained in DNA from the control sample, as observed by the migration profiles of the DNA samples.

Figure 5 is a video densitometer scan of lanes containing DNA MW marker II, and DNA from control shrimp. The relative migration rates provide an estimated molecular weight for the DNA samples. The sharp spike to the far right is the end of the gel at the positive electrode. Comparison of the peak associated with the largest fragment of Hind III-digested DNA with that of the control shrimp DNA gives an estimated average length for the control DNA of 40 kb.

Video densitometer scans for control and irradiated shrimp DNA are in Fig. 6. DNA extracted from shrimp decreases in length as the radiation dose is increased, and the size distributions of DNA from irradiated samples are significanlty greater. There is a relatively constant fraction of DNA with a size less than 1 kb for all doses of radiation, which may represent a portion of shrimp DNA that is sensitive to strand break damage from ionizing radiation.

Figure 4. Rendition of alkaline agarose gel. Whole shrimp were exposed to cobalt-60 gamma irradiation as described in the text. The radiation doses are indicated. Electropohoresis was in 0.5% agarose gel for 5 hrs. at 1.4 V/cm. Lane 1: DNA MW I; lane 2: 1 kb ladder; lane 3: lambda DNA; lane 4: DNA MW II; lane 5: 3 kGy; lane 6: 2 kGy; lane 7: 1 kGy; lane 8: control; lane 9: DNA MW II; lane 10: lambda DNA; lane 11: 1 kb ladder; lane 12: DNA MW I.



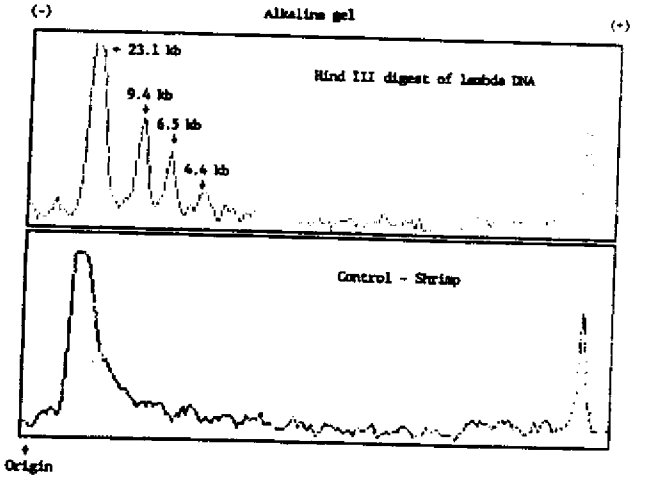
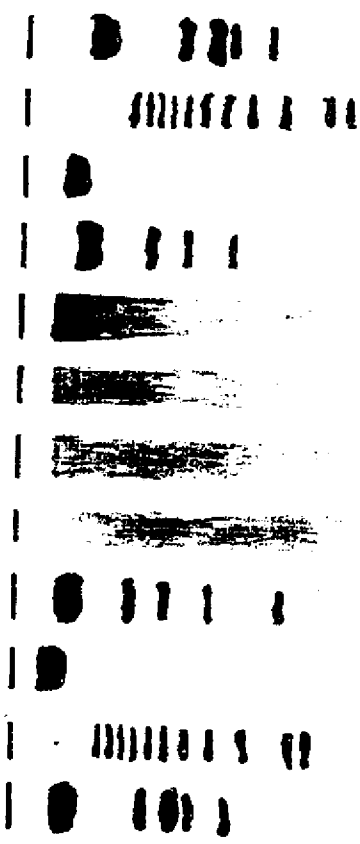
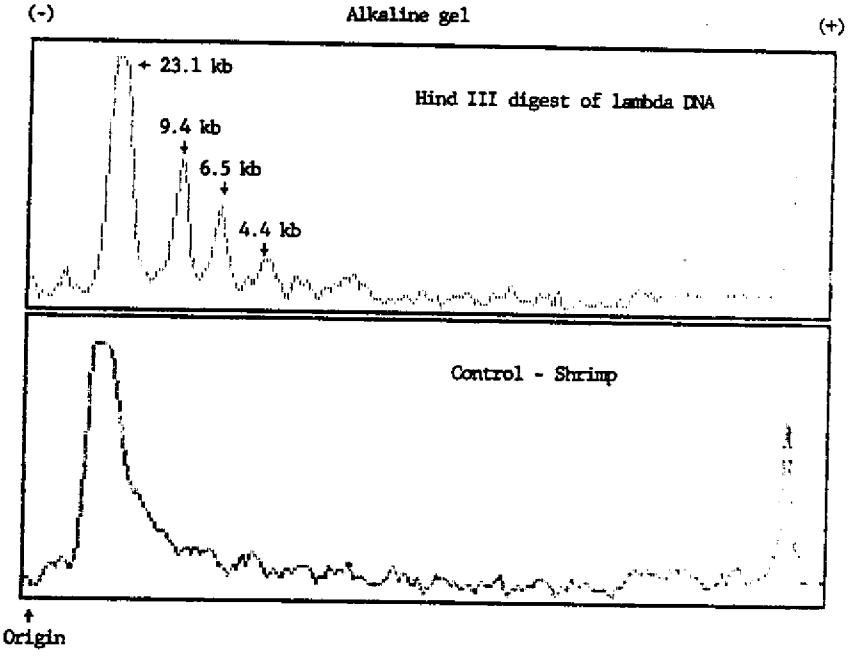


Figure 5. Panel (a). Video densitometer scan of lane 9, Fig. 4.
Panel (b). Video densitometer scan of lane 8, Fig. 4.

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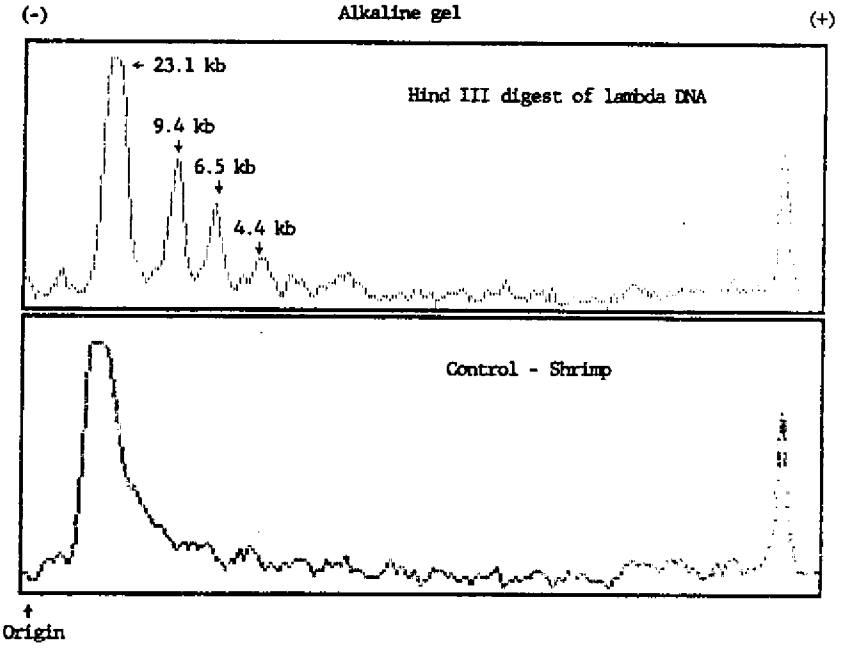
23.1 kb
9.4 kb
6.5 kb
4.4 kb
Hind III digest of lambda DNA
Control - Shrimp
Origin


(-)
Alkaline gel
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← 23.1 kb
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4.4 kb
Hind III digest of lambda DNA
Control - Shrimp
Origin

The most important feature of Fig. 6 is the measureable decrease in DNA size with increasing radiation dose, resulting from accumulation of single-strand DNA breaks as the radiation dose is increased. Because of the quantitative relationship between radiation dose and DNA strand breaks, a measure of the radiation dose in the food sample can be obtained by measuring the number of strand breaks.

Neutral and alkaline agarose gel electrophoresis of oyster DNA

Adductor muscle tissue and hemolymph fluid were obtained from oysters which had been irradiated by Mr. Dusty Dixon, as described in these Proceedings (8). Grinding of the sample under liquid nitrogen was not required for the hemolymph samples. Control DNA samples from hemolymph and adductor muscle tissue were run on alkaline and neutral agarose gels, and video densitometer scans of photographs of the gels are in Figs. 7 and 8. There is a greater difference in the profiles for the neutral gels than for the alkaline gels, which is expected for DNA samples with larger differences in double-strand breaks, arising from the ommission of the grinding step during processing of the hemolymph.

Video densitometer scans for photographs of neutral agarose gels on DNA samples from control and irradiated oyster hemolymph are plotted in Fig. 9. Fig. 10 presents the profiles for scans of photographs of neutral agarose gels of the adductor tissue DNA. Visual inspection of results from both hemolymph and adductor muscle samples shows that the DNA is progressively smaller in size for samples exposed to increasing doses of radiation.

Alkaline agarose gel electrophoresis of the hemolymph and adductor muscle samples qualitatively gave similar results to those observed using neutral gels, with regard to the comparative migration of the DNA samples extracted from control and irradiated samples. However, the samples did not have as clear a distinction of the profiles among the control and irradiated samples. It is possible that at the higher radiation doses of this experiment, there is a decrease in the resolution due to excessive numbers of single-strand breaks, whereas double-strand breaks, known to have a yield one-twentieth that of single-strand breaks, are more appropriate for dose analysis at high radiation levels.

Another possibility is that there were some problems with the configuration of the oyster samples during irradiation, and that the radiation field was nonuniform, resulting in different oysters being exposed to different exposure dose rates.

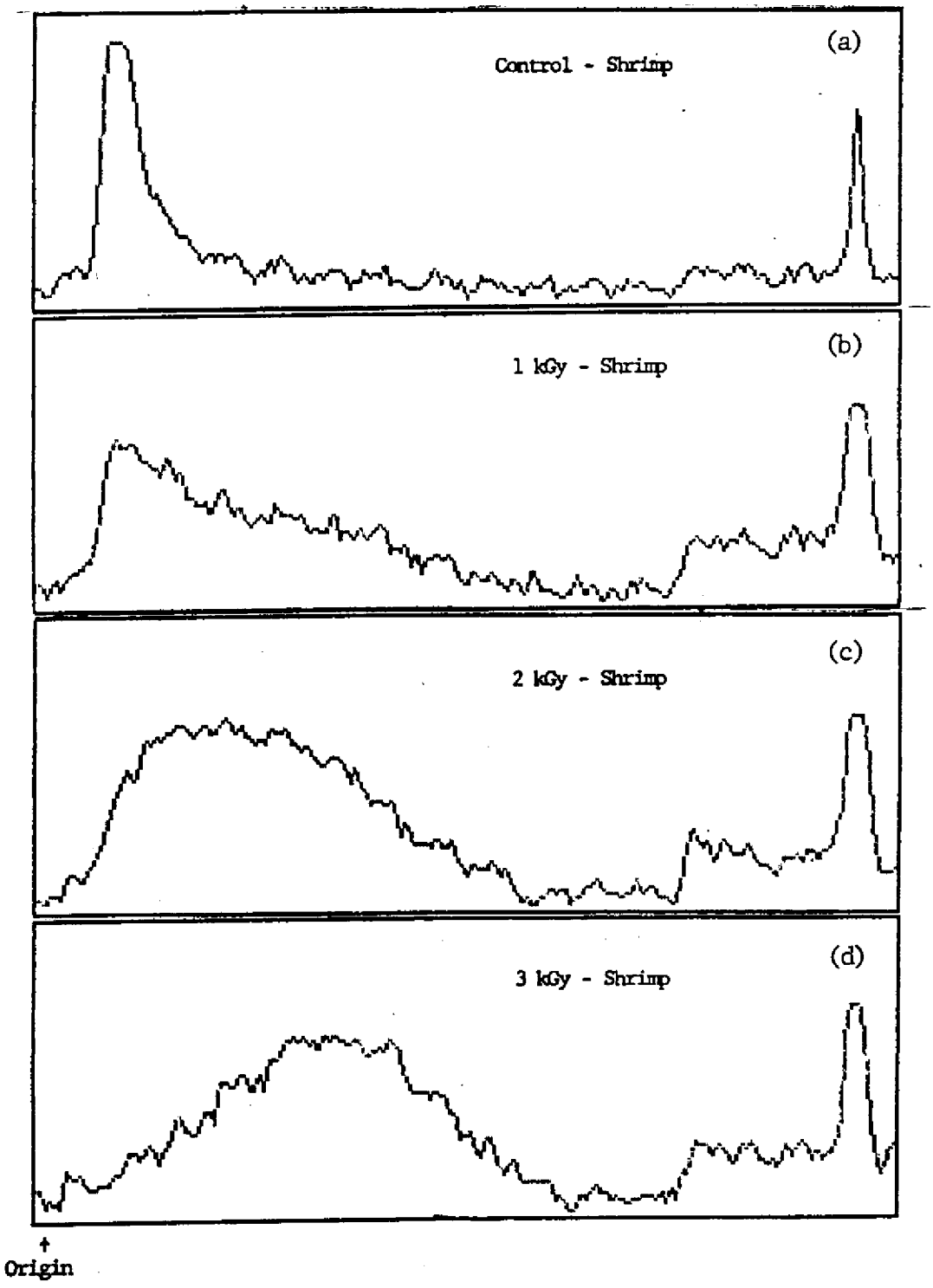

Figure 6. Video densitometer scans of lanes containing shrimp DNA samples from Fig. 4. Panel (a) = lane 8; panel (b) = lane 7; panel (c) = lane 6; panel (d) = lane 5.

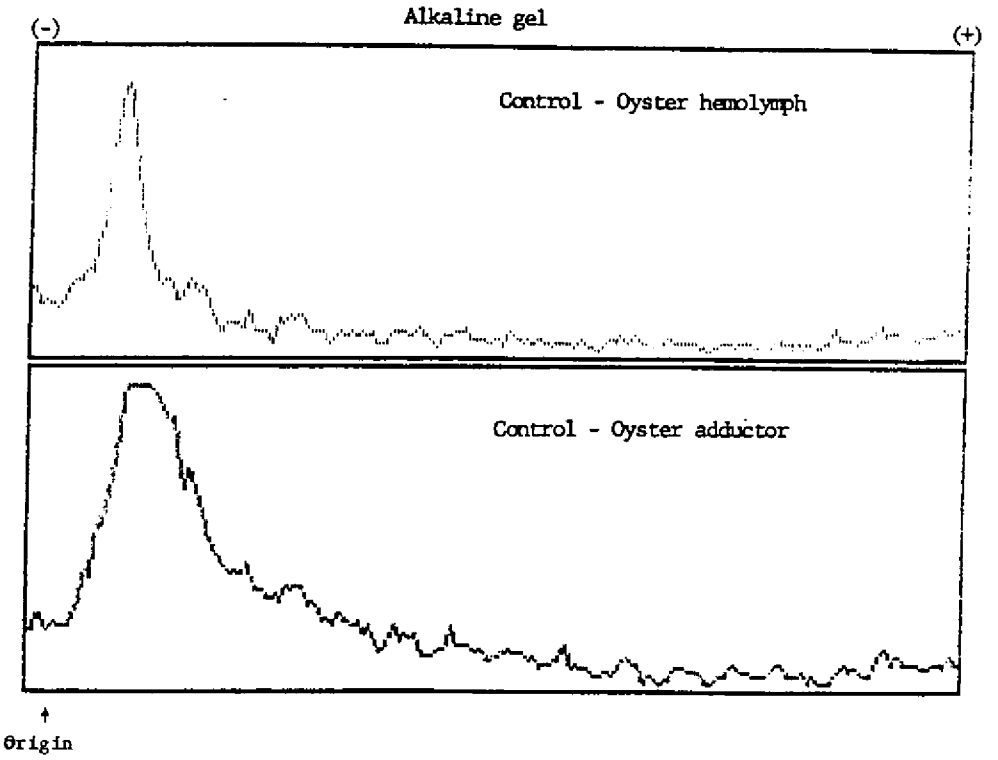

Figure 7. Video densitometer scans of photograph of alkaline agarose gel electrophoresis analysis of control oyster hemolymph DNA (panel a) and control oyster adductor muscle tissue DNA (panel b). Electorphoresis was carried out in 0.6% agarose gel for 16 hours at 0.75 V/cm

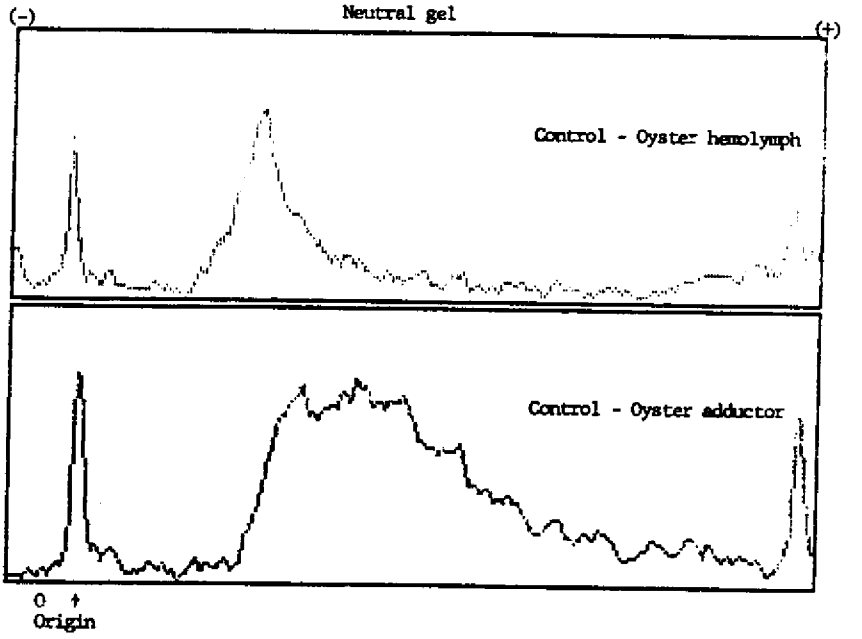

Figure 8. Video densitometer scans of photograph of neutral agarose gel electrophoresis analysis of control oyster hemolymph DNA (panel a) and control oyster adductor muscle tissue DNA (panel b). Electrophoresis was carried out in 0.5% agarose gel for 16 hours at 1 V/cm using TAE buffer as the running buffer.

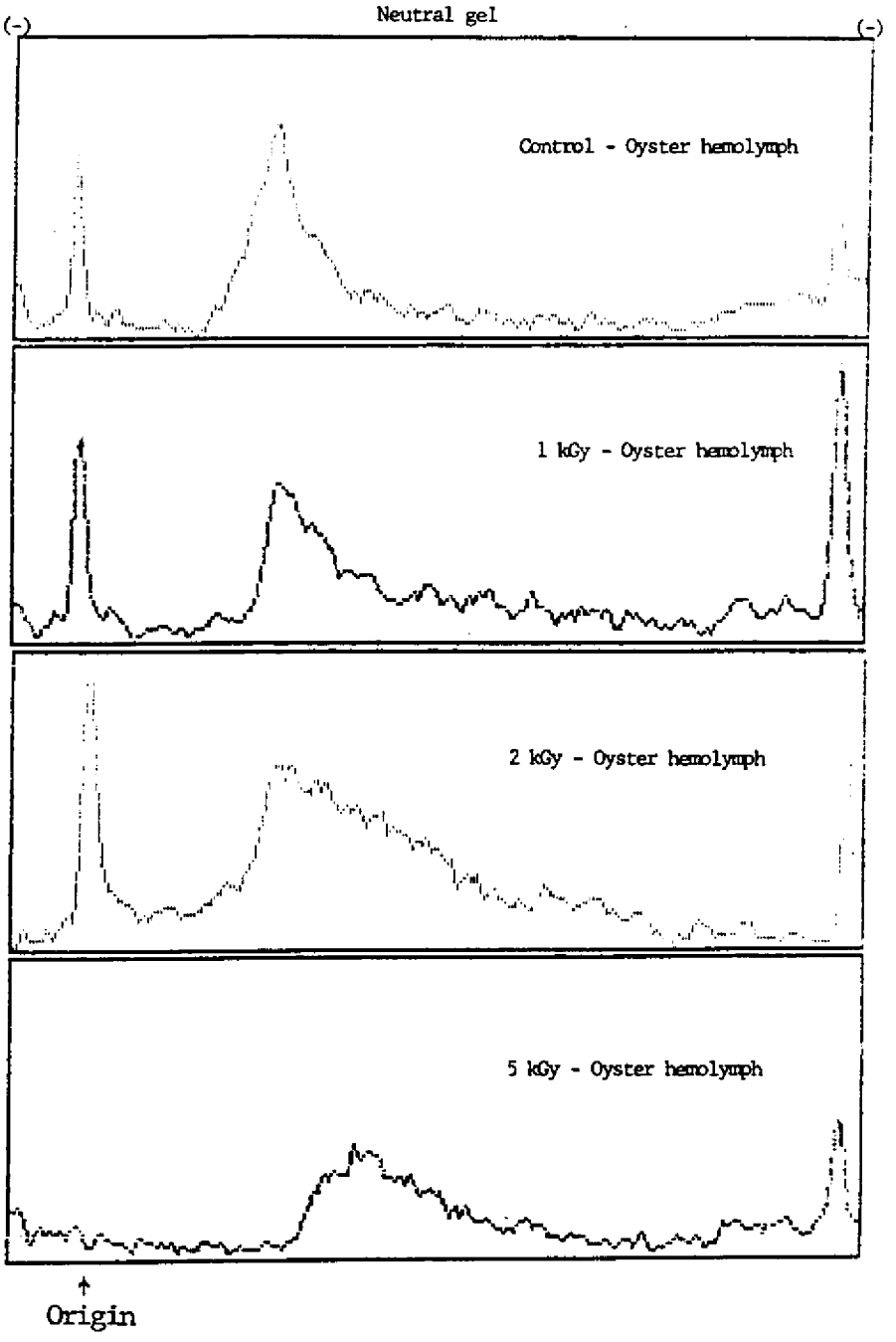

Figure 9. Video densitometer scan of photograph of neutral agarose gel electrophoresis analysis of oyster hemolymph DNA. Panel a = control; panel b = 1 kGy; panel c = 2 kGy; panel d = 5 kGy.
Electrophoresis was as in Fig. 8.

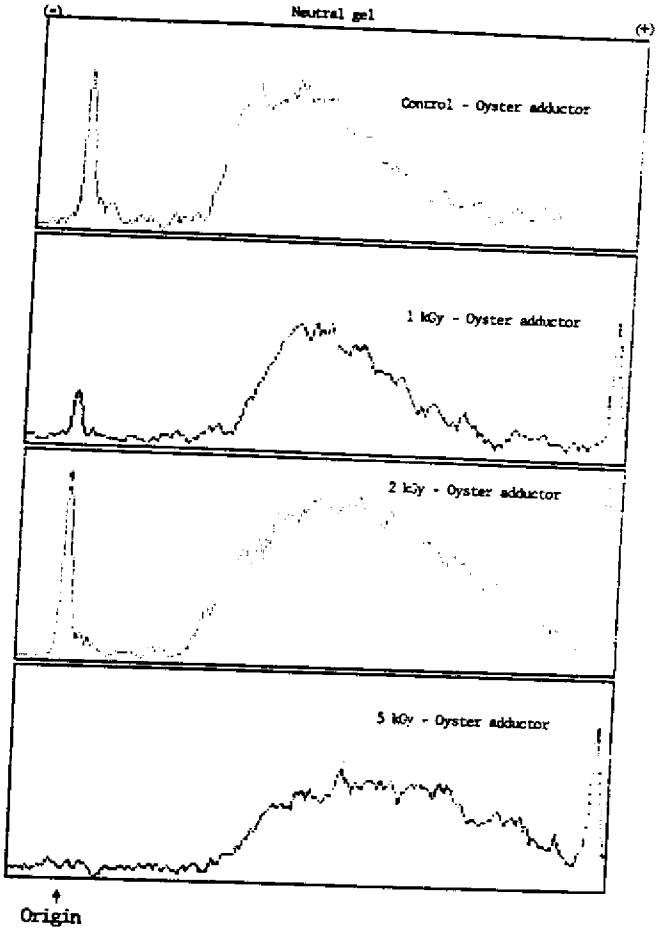

Figure 10. Video densitometer scan ofphotograph of neutral agarose gel electrophoresis analysis of oyster adductor muscle tissue DNA.
Panel a = control; panel b = 1 kGy; panel c = 2 kGy; panel c = 5 kGy. Electrophoresis was as in Fig. 8.

CONCLUSIONS

Analysis of DNA strand breaks in DNA has been shown to provide a method for determining the amount of radiation exposure in several seafood samples. The technique presented here will likely be useful for a variety of foodstuffs. Data from our laboratory indicates that the technique can be applied to mullet and poultry (chicken and turkey).

There are a number of questions which must be answered before this technique can be used with confidence for measuring radiation doses in foods. Firstly, the time elapsed between radiation exposure of the food and analysis for strand breaks may influence the number of strand breaks observed, because of the action of enzymatic repair systems for this type of damage which has been shown to be active in prokaryotes and eukaryotes (5). Previous data indicate that a constant fraction of single- and double-strand breaks is repaired in the dose range from

0.25 kGy to 1 kGy in cultured mammalian cells (5). If this is true for food tissue, then analysis for strand breaks will still provide a measure of exposure dose, even in the presence of active repair of the damage. Experiments must be designed to answer this question.

Another requirement for validation of this dosimeter method is to ascertain the influence of food handling, especially the temperature and length of storage of the food product. Strand breaks may be introduced into DNA by physical, chemical, and enzymatic mechanisms. Chemical induction of DNA strand breaks is not expected to be a problem under the normal accepted procedures for food processing. Enzymatically-produced strand breaks could result from the release of digestive enzymes from lysozomes, when organellar membranes are disrupted, for example by freezing the tissue. However, as described in this paper, strand break analysis of seafoods which had been frozen is able to distinguish irradiated from control samples. A direct comparison of strand breaks in frozen and unfrozen tissue for unirradiated samples of foods to which this technique is to be applied will be required to determine whether freezing alone can give rise to strand breaks. Preliminary results do not indicate that a detectable number of strand breaks are introduced by feezing.

Physical agents that are relevant as possible sources for strand breaks are heat and microwaves. Neither of these agents are reported to introduce any significant number of strand breaks. Direct tests will be performed on food products for which this technique is proposed as a dosimeter for ionizing radiation exposure in order to determine whether these physical agents might produce detectable amounts of DNA strand breaks.

The preliminary results of this paper show that agarose gel electrophoresis of DNA samples from seafoods can be used as an indication of radiation exposure of the seafoods. Only two varieties of seafood are the subject of this paper, however, it is reasonable to expect that the technique will be useful for a broad variety of seafood products as a dosimeter for radiation exposure. This technique will provide a means to enhance consumer confidence in irradiated foods, and a method by which regulatory agencies can enforce the regulation of this activity.

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COMPARISON OF DIFFERENT EXTRACTION METHODS ON THE PURIFICATION OF SHRIMP POLYPHENOLOXIDASE

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INTRODUCTION

Polyphenoloxidase (PPO), also known as polyphenolase, phenolase, tyrosinase and catechol oxidase, has been implicated in the discoloration of fruits, vegetables, and crustaceans (3, 4). The formation of melanin or black spot on the surface of crustaceans is of concern to the seafood industry because the unappealing discoloration reduces marketability (5).

Affinity chromatography was shown by Simpson et al. (5, 6) to be superior to ion-exchange chromatography on the recovery and purification of shrimp PPO. However, the former method is tedious and the use of ammonium sulfate precipitation, if not performed cautiously, adversely affects PPO recovery.

Stelzig et al. (7) described a method using butanol for apple PPO purification which gave high enzyme yield and little phenol oxidation. Since the browning in shrimp and other crustaceans has not been extensively studied as fruits and vegetables, and crustacean PPO employed in most studies was semi-purified which causes difficulty in relating the findings exclusively to PPO (5), it becomes necessary to develop new procedures or modify the established methods for better extraction of crustacean PPO. Thus the objective of this study was two-fold: (a) to compare the effect of various stirring (extraction) times on the recovery of shrimp PPO following the procedures of Simpson et al. (6), and (b) to compare the effectiveness of the two extraction methods, ammonium sulfate fractionation and butanol extraction, on shrimp PPO yield, enzyme specific activity, and purification fold.

MATERIALS AND METHODS

Cephalothorax of non-sulfited white shrimp (*Penaeus setiferus*) and pink shrimp (*P. duorarum*) were frozen in liquid nitrogen and ground into a fine powder using a Waring blender at high speed. Shrimp powder was stored at -20°C until needed.

Effect of various stirring (extraction) time on PPO recovery

Shrimp PPO was extracted according to the method of Simpson et al. (6) with slight modifications. Shrimp powder was added to three volumes of a 0.05M sodium phosphate buffer (pH 7.2) containing 1M NaCl (extraction buffer); the suspension was made up to 0.2% with Brij 35. For each shrimp species, 4 samples were prepared and stirred at 4°C for 0.5, 1, 2, and 3 hr, respectively. After centrifugation at 23,000*g* for 30 min at 4°C, each supernatant was checked for total volume, protein content, and PPO activity.

Purification of shrimp PPO using ammonium sulfate fractionation method

The supernatant prepared from the samples stirred for 0.5 hr was fractionated with ammonium sulfate [$(NH_4)_2SO_4$], and the precipitate between 0-40% saturation was collected by centrifugation at 23,500*g* for 30 min (4°C). The pellet was resuspended in extraction buffer containing 40% $(NH_4)_2SO_4$, homogenized with a Dounce manual tissue grinder, and then centrifuged at 23,500*g* for 20 min (4°C) (1). The final precipitate was resuspended in extraction

buffer, homogenized again and then subjected to hydrophobic interaction chromatography using a phenylsepharose CL-4B column.

Purification of shrimp PPO using butanol extraction method

The method of Stelzig et al. (7) was followed with slight modifications. Shrimp powder was added to 3 volumes of 0.1M sodium phosphate buffer (pH 6.0) containing 0.3M sucrose. The suspension was stirred at 4°C for 30 min, then centrifuged at 20,000*g* for 20 min (4°C). The pellet was washed and suspended with the same buffer and the suspension was made up to 2% Triton X-100. Following incubation at 25°C for 15 min, the suspension was centrifuged at 40,000*g* for 30 min (4°C). The supernatant was extracted with -20°C n-butanol and the aqueous phase collected was dialyzed at 4°C overnight against three changes of water using dialysis tubing with exclusion limit of molecular weight 6,000 - 8,000. The dialysate was concentrated utilizing an Omega stirred cel fitted with YM 10 filter and resuspended in extraction buffer before being loaded onto a phenylsepharose CL-4B column.

Phenylsepharose CL-4B chromatography

The partially purified PPO preparation loaded onto the phenylsepharose CL-4B column pre-equilibrated with extraction buffer was eluted with a stepwise gradient of elution buffer [100% extraction buffer (9 mL), 50% extraction buffer in water (24 mL), and 10% extraction buffer in water (24 mL)], followed by 50% ethylene glycol (12 mL), and distilled water (150 mL) at a flow rate of 0.2 mL/min (4°C) (1). Three-mL fractions were collected and the protein profile was determined spectrophotometrically at 280 nm. Fractions showing PPO activity were pooled and concentrated utilizing an Omega stirred cel fitted with YM 10 filter.

Protein quantitation and PPO activity determination

The protein content of all enzyme preparations were determined using the Bio-Rad protein assay kit with bovine serum albumin as standard. PPO activity was assayed at 25°C for 5 min by mixing 80 μL enzyme preparation with 1.12 mL 10mM L-DOPA in 0.05M sodium phosphate buffer (pH 6.5). The enzyme activity was determined by monitoring the rate of dopachrome formation at 475 nm using a Beckman DU-7 spectrophotometer. One unit of enzyme activity was defined as an increase in absorbance per minute at 475 nm.

Statistical analysis

Statistical analysis was performed using a PC SAS package (2). Duncan's Multiple Range Test was performed to determine any significant difference among various treatments.

RESULTS AND DISCUSSION

Effect of stirring (extraction) time on PPO recovery

Stirring of shrimp powder in extraction buffer affected the recovery of PPO. For both pink and white shrimp, the increase in stirring time from 0.5 to 1, 2 or 3 hr significantly ($P < 0.05$) increased the total protein content of the extractants (Table 1). The total PPO activity and the specific activity were, however, significantly reduced with increasing stirring time. Thus a 0.5 hr stirring time is adequate to produce high PPO recovery and specific activity.

Table 1. Effect of different extraction times on the recovery of pink and white shrimp polyphenoloxidase in terms of total activity, protein content, and specific activity

Extraction time (hr)	Total protein content (mg)	Total enzyme activity (units)	Specific activity (units/mg)
Pink shrimp			
0.5	166.01[a]	2.31[a]	0.0141[a]
1.0	178.67[b]	1.75[b]	0.0097[b]
2.0	208.85[c]	1.65[b]	0.0079[c]
3.0	240.89[d]	1.29[c]	0.0052[c]
White shrimp			
0.5	171.25[a]	8.56[a]	0.0495[a]
1.0	174.41[a]	7.16[b]	0.0420[b]
2.0	189.55[b]	6.05[c]	0.0335[c]
3.0	212.95[c]	7.33[b]	0.0350[c]

Values with different superscript in the same column are significantly different ($P < 0.05$) from each other.

Phenylsepharose CL-4B chromatography

The chromatographic profiles of pink shrimp PPO following butanol extraction and ammonium sulfate fractionation were similar (Fig. 1). They both showed enzyme activity at fraction number 39. However, the PPO prepared from butanol extraction had a higher activity.

Contrast to pink shrimp, the elution profiles of white shrimp PPO following butanol extraction and ammonium sulfate fractionation were different (Fig. 2). Though both extraction methods showed peak enzyme activity at fraction 46, the PPO prepared from butanol extraction showed a greater enzymatic activity than the ammonium sulfate treatment.

An excessive amount of proteins other than PPO was eluted erratically from the phenylsepharose CL-4B column for white shrimp subjected to ammonium sulfate fractionation when compared to that subjected to butanol extraction. The erratic nature of the elution profile could be due to the presence of pigments which were not thoroughly removed from white shrimp PPO during the serial fractionation steps using ammonium sulfate. These pigments tend to impede the elution of the PPO. When butanol extraction was used, the pigment and lipid micelles were solubilized and retained in the organic phase; and the proteins were present in the aqueous phase.

Fig. 1 Chromatographic profile of pink shrimp polyphenoloxidase using phenylsepharose CL-4B column

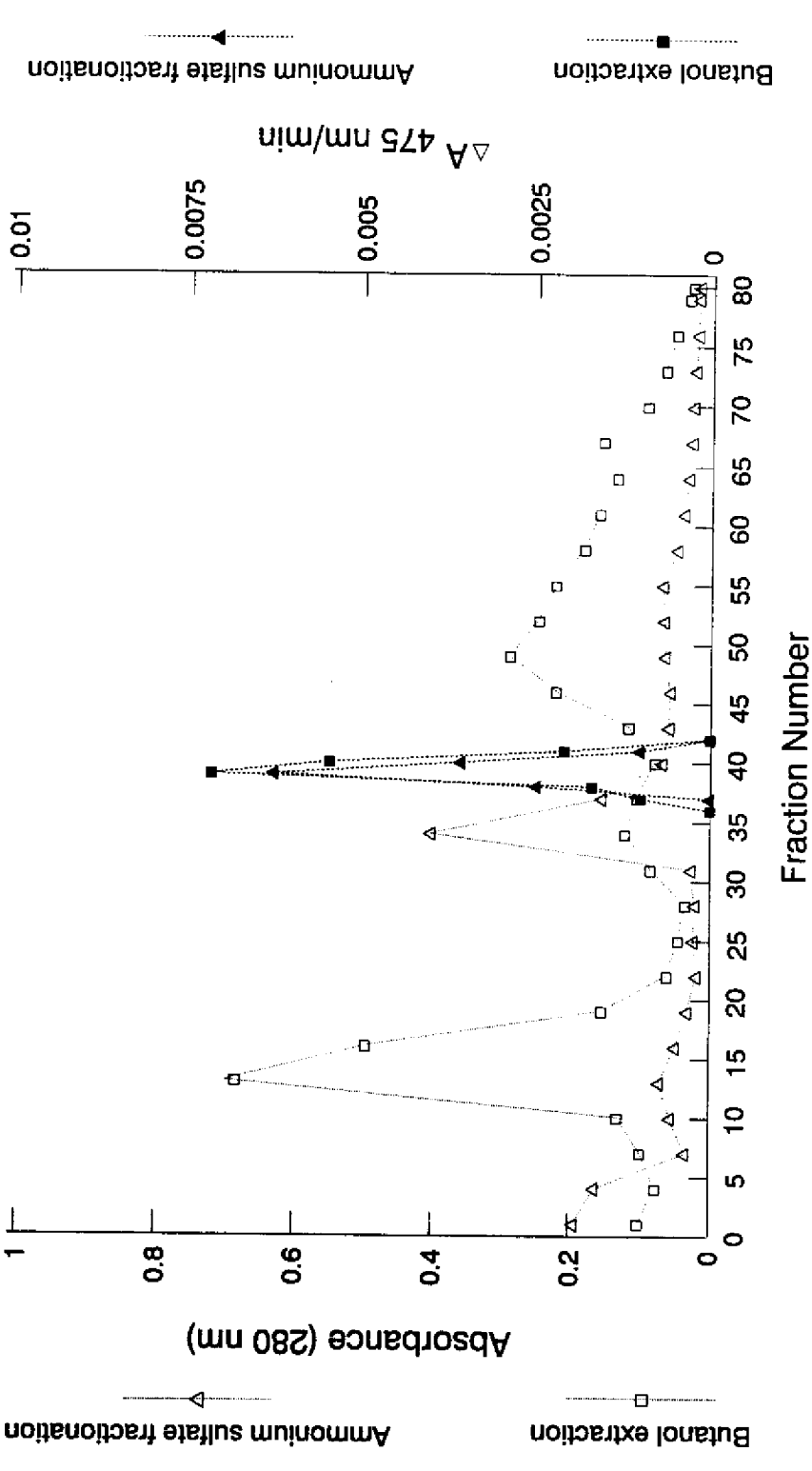
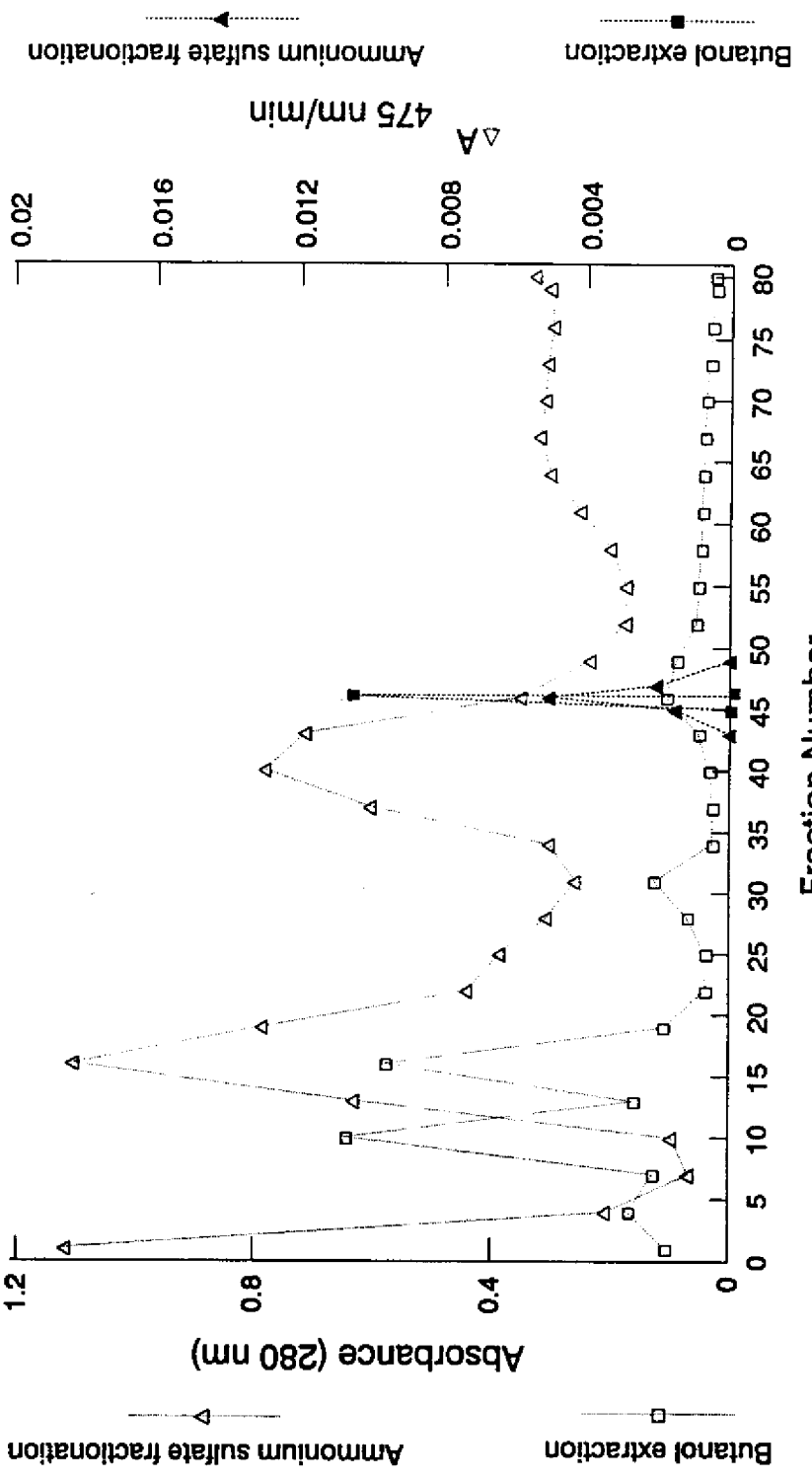


Fig. 2 Chromatographic profile of white shrimp polyphenoloxidase using phenylsepharose CL-4B column



Purification of PPO from shrimp

Compared to butanol extraction method, the ammonium sulfate fractionation of pink shrimp PPO yielded higher total protein content, but lower specific activity and purification fold after elution from the CL-4B column (Table 2). Ultimately, the percent yield and the purification fold of PPO prepared by butanol extraction were 3.7 and 3.6 times, respectively, greater than those by ammonium sulfate treatment.

Though the total PPO activity of the crude pink shrimp supernatant following butanol extraction was lower than that of ammonium sulfate fractionation, higher PPO yield was observed for butanol-extracted suspension sample (Table 2). Such abrupt increase in PPO yield was probably due to the removal of sucrose from the supernatant after the second centrifugation. The presence of sucrose in the initial supernatant may inactivate PPO and thus lower PPO yield.

The purification scheme of white shrimp PPO is shown in Table 3. For crude supernatant, the use of ammonium sulfate fractionation resulted in greater total protein content and enzyme activity than the butanol extraction method. However, butanol extraction samples eventually had greater PPO yield and purification fold and PPO having higher specific activity. It was speculated that the ammonium sulfate method did not function as effectively as the butanol method in removing pigments or pigment-bound proteins from the PPO. It was also noted that white shrimp PPO was more susceptible to inactivation than pink shrimp PPO using either ammonium sulfate fractionation or butanol extraction. Such characteristics was also confirmed by Simpson et al. (5, 6).

CONCLUSION

The study indicates that a 0.5 hour stirring time is adequate for the recovery of PPO. The extraction of PPO from white and pink shrimp using butanol was superior to ammonium sulfate fractionation with respect to specific activity, PPO yield, and purification fold.

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Table 2. Purification scheme of polyphenoloxidase from pink shrimp

Step	Total volume (mL)	Total protein (mg)	Total activity* (units)	Specific activity (units/mg protein)	Yield (%)	Purification fold
Ammonium sulfate fractionation						
Crude supernat.	34.5	169.7	3.67	0.022	100	1
before HIC elution**	10	8.1	0.79	0.097	21.5	4.4
HIC eluant	9	0.3	0.38	1.42	10.4	64.0
Butanol extraction						
Crude supernat.	16	58.7	0.83	0.014	100	1
before HIC elution	7	4.3	0.60	0.14	72.3	18.8
HIC eluant	6	0.1	0.32	3.20	38.6	229

* One unit = $\Delta A_{475\ nm}$/min

** Hydrophobic interaction chromatography using phenylsepharose CL-4B column.

Table 3. Purification scheme of polyphenoloxidase from white shrimp

Step	Total volume (mL)	Total protein (mg)	Total activity* (units)	Specific activity (units/mg protein)	Yield (%)	Purification fold
Ammonium sulfate fractionation						
Crude supernat.	34	193.8	2.38	0.012	100	1
before HIC elution**	16	27.1	0.80	0.030	33.6	2.5
HIC eluant	7	0.56	0.31	0.55	12.9	46.1
Butanol extraction						
Crude supernat.	16	68.3	1.10	0.016	100	1
before HIC elution	8	5.0	0.45	0.089	40.9	5.6
HIC eluant	6	0.06	0.21	3.50	19.1	218

* One unit = $\Delta A_{475\ nm}$/min

** Hydrophobic interaction chromatography using phenylsepharose CL-4B column.

CHARACTERIZATION OF ALKALINE METALLOPROTEINASES WITH COLLAGENASE ACTIVITY FROM THE MUSCLE OF PACIFIC ROCKFISH (*SEBASTES SP.*)

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INTRODUCTION

The twelve known types of collagen share a general structure consisting of three polypeptide units (α chains) which together form a long triple-helical structure and shorter non-triple helical domains(41). Intramolecular aldol cross-links and intermolecular aldimine and oxo-imine cross-links between a chains result in formation of dimer(β) and trimer (γ) units as well as higher molecular weight aggregates. Type I collagen, a fiber forming collagen, is distributed in all structural levels of muscle tissue. Types III, IV and V collagen have also been identified in muscle tissue (2). Fiber forming collagen self-assembles to form fibrils where these rod-like molecules stack over one another staggered by one-fourth of their length (Fig. 1). The mechanical strength, solubility and susceptibility of collagen to enzyme catalyzed hydrolysis is influenced by the organization of collagen molecules as fibrous structures. These same properties of collagen may also be influenced by the association of fibrils with non-collagen connective tissue constituents, particularly the proteoglycans (17).

Enzyme catalyzed degradation of collagen has been implicated in quality deterioration of seafood products by several investigators (5,14,23,46,47,52). However, enzyme(s) responsible for collagen degradation in muscle from postharvest fish have thus far not been identified. The collagen triple helix is highly resistant to the action of most proteinases, but it is known that connective tissue cells from mammals and reptiles synthesize and secrete a family of proteinases that act on collagen (1,2). It has been suggested that collagen in fish muscle has a relatively high turnover rate compared to land animals. For example, collagen turnover in Atlantic cod is coupled to heavy feeding- starvation cycles during the year (33,34).

Collagenase is universally distributed in extracellular connective tissue of all organs of rat examined by immunohistochemical methods (42). Mammalian collagenase (matrix metalloproteinase 1) is a Zn^{+2}-metalloproteinase capable of cleaving fibrous collagen across the three chains(Fig. 1A). The locus of cleavage is three-quarters the length of the molecule from the amino terminal end, thus forming two triple helical products called TC^A and TC^B. These products are less stable than the intact molecules and denature at physiological temperature. The denatured chains are susceptible to rapid and extensive cleavage by gelatinases and other proteases like cathepsins B, L and N in phagolysosomes (Fig. 1). Other mammalian collagenases specifically hydrolyze native non-fibrous collagens (matrix metalloproteinase 2, Type IV collagenase, gelatinase) associated with basement membranes by an analogous mechanism and have specific gelatinase activity (51). Stromelysin (matrix metalloproteinase 3) has broad activity on a variety of extracellular matrix macromolecules including proteoglycans, fibronectin, laminin, types IV and IX collagen and is responsible for full activation of procollagenase (44). The catalytic activity of collagenases are latent and can be activated by enzymes such as plasmin and trypsin, by organomercurials, by heat treatment, and by removal of inhibitors or addition of Ca^{+2} (Fig. 2). Activation of procollagenases by different mechanisms may result in intermediate sized forms of collagenase with only partial activity (Fig. 3; 44).

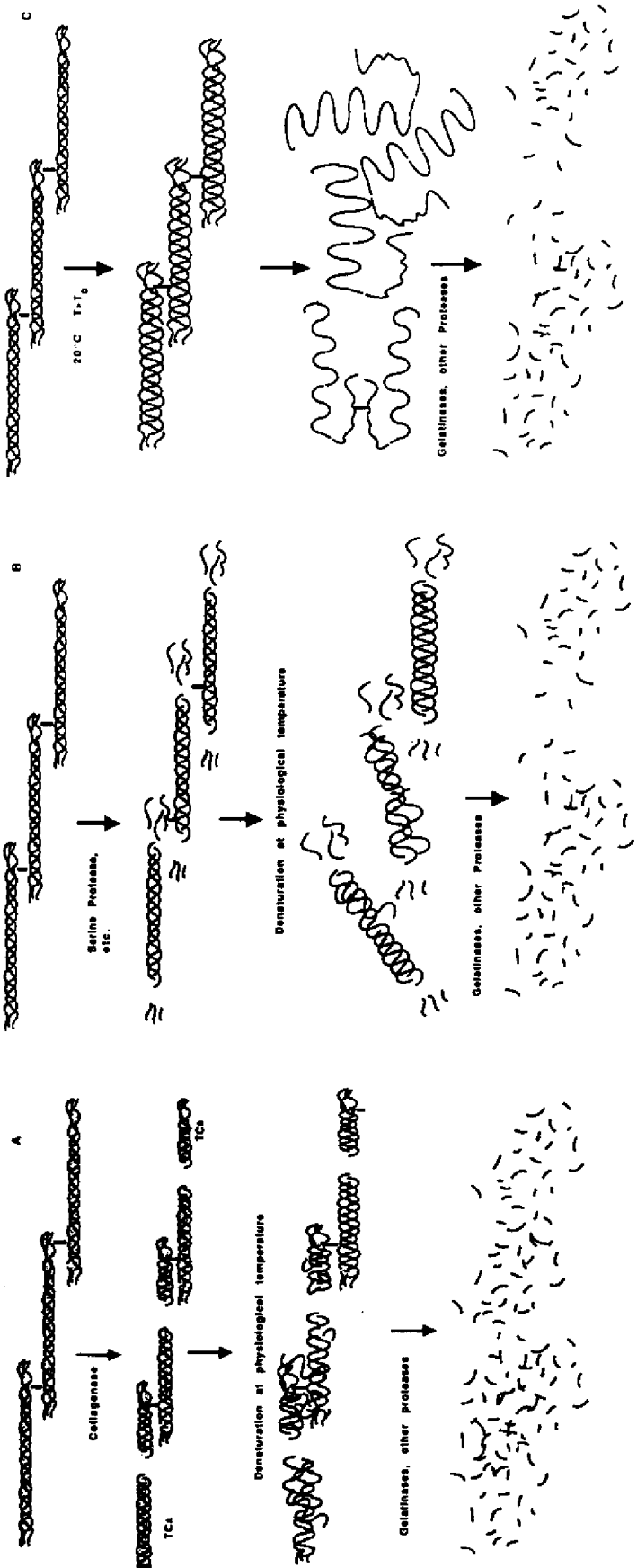

Figure 1. Diagram showing native collagen degradation in tissue initiated by A. tissue collagenase, B. neutral proteases, and C. thermal denaturation of the collagen. Adapted from (2).

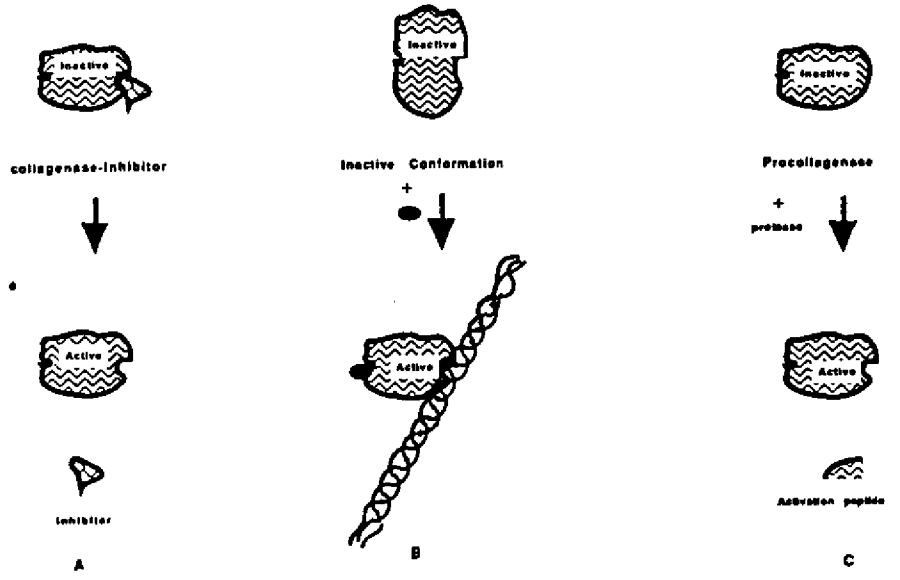

Figure 2. Diagram showing possible explanations for latent collagenase activity. A. Inhibitors, e.g. plasma a2 macroglobulin, B. Conformational changes in collagenase, e.g.by binding to the surface of the collagen fibril, and C. Proteolysis of the extension peptide of procollagenase, e.g. by trypsin.

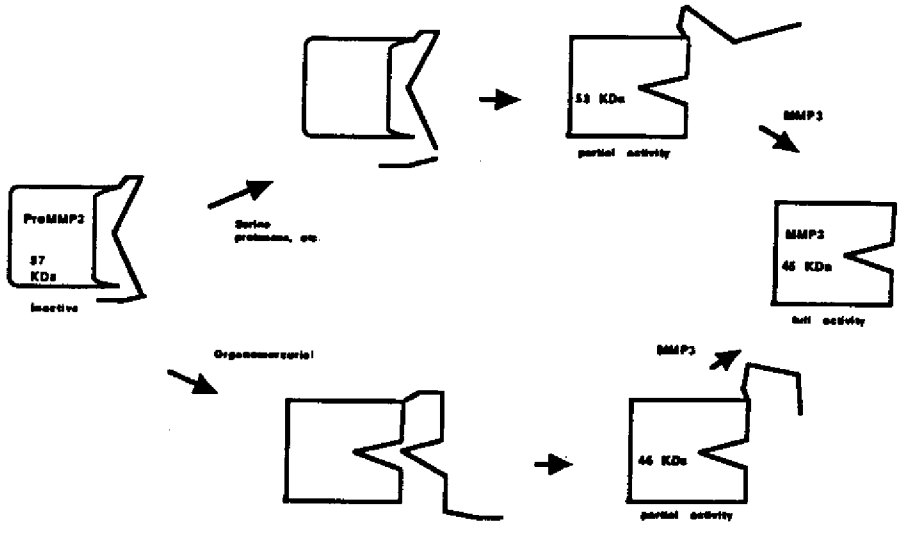

Figure 3. Stepwise activation of procollagenase by proteases or by self-activation promoted by organomercurials. ProMMP3 is pro-matrix metalloproteinase 3(pro-subtilysin). Adapted from (44).

It is also known that neutral proteinases and serine proteases secreted by leukocytes, e.g. cathepsin G and elastin, hydrolyze the non-helical end regions of collagen between the cross-link and the triple helix thereby initiating a degradative cascade similar to that started by collagenase (Fig. 1B). When the collagen triple helix is denatured, e.g. by raising the temperature to initiate thermal denaturation (Fig. 1C) the collagen chains are readily susceptible to hydrolysis by a wide range of proteases.

Earlier we reported the relationship between collagen degradation and texture softening of Pacific rockfish (8,9). The solubility of collagen in postharvest Pacific rockfish muscle increased coincidental with texture softening during storage of sterile fillets at 0°C. Immuno-reactive collagen degradation products did not form and loss of α, ß and γ collagen chains did not occur during storage of rockfish on ice. On the other hand, incubation of sterile rockfish muscle at 20-30°C resulted in rapid disappearance of all collagen chains and was accompanied by a nearly complete loss of fillet integrity. Using an immunological method (8) we did not detect the collagen fragments(TC^A and TC^B) which are characteristic of collagen degradation by collagenase(s). These studies were carried out *in situ* and therefore it is possible that other proteases present in the fish muscle rapidly hydrolyzed initial products of chain degradation.

Here we report studies designed to identify and isolate collagenolytic enzymes from the muscle of Pacific rockfish. Collagen isolated from rockfish muscle contains enzyme(s) which solubilize native collagen fibrils at neutral pH. However, the amount of collagenase activity present was extremely low and attempts to purify and characterize the activity were not successful. Acetone powder from rockfish muscle was found to contain two metalloproteinases which hydrolyze collagen and gelatin but not casein on sodium dodecylsulfate polyacrylamide gel (SDS-PAGE) zymograms. The enzymes also exhibited low activity in solubilizing native collagen fibrils and did not hydrolyze hide powder azure, azo-casein and synthetic substrates for trypsin and chymotrypsin and collagenase when assayed in solution.

MATERIALS AND METHODS

Isolation of enzymes

Collagenase

Collagenase was extracted from rockfish skin and muscle tissue by the procedure of Weeks *et al.*(56). The principle of this method is that bound collagenase is dissociated from collagen fibers in the presence of 0.10 M Ca^{+2}. Activity was recovered from rockfish muscle either by heating for 10-15 min at 55-65°C or by incubating 24 h at 37°C in the presence of calcium. The activity was recovered in the 30-60% ammonium sulfate fraction.

Metalloproteinase(gelatinase)

Acetone powder was prepared from rockfish muscle with chilled acetone and butanol (15). Approximately 10 g of acetone powder were homogenized (1 min, low speed, Waring Blender) with 200 ml Tris-Cl buffer (0.025M, pH 7.5) containing 10 mM $CaCl_2$, 0.05% Brij 35, and 0.05% NaN_3. The homogenate was incubated with shaking at 37°C for 1 h, and then centrifuged at 20,000 x g for 30 min. The supernatant was saved and the pellet was re-suspended in 200 ml of buffer and the procedure repeated. The supernatants were combined and held at 4°C for 48 h after which the precipitate formed was removed by centrifugation. The resulting supernatant was brought to 30% saturation with $(NH_4)_2SO_4$ and stored overnight at 4°C. The precipitate was removed by centrifugation and the supernatant was brought to 80% saturation with ammonium sulfate and stored overnight at 4°C. The precipitate was collected by centrifugation and suspended in 50 ml of extraction buffer. The suspension was stirred for 2 h at 4°C and the supernatant collected by centrifugation. Gel filtration of this supernatant was performed on a 1.5

x 115 cm column packed with Bio-Gel A-0.5 m equilibrated with extraction buffer containing 0.2 M NaCl. Two peaks with collagenolytic activity were obtained. The active fractions were pooled, dialyzed against water and lyophilized.

Electrophoresis

Sodium dodecylsulfate, polyacrylamide gel electrophoresis (SDS-PAGE) was done by the method of Laemmli (31) using 7.5% polyacrylamide. Gels were stained with Coomassie Blue R such that collagen stained metachromically(pink) while non-collagen protein stained orthochromically(blue)(40).

Enzyme assay methods

Collagenase

Assay of "true collagenase" activity was based on breakdown of radioactive collagen fibrils. Acid soluble collagen was extracted and purified from lingcod skin by the method described by Cawston and Murphy (7). The final product was shown to be pure by SDS-PAGE and amino acid analysis. Lingcod collagen forms fibrils at lower temperatures (15°C) than mammalian collagen. Collagenase substrate was prepared by radiolabeling of pure lingcod skin collagen with pyridoxal phosphate and NaB^3H_4 as described by Birkedal-Hansen and Dano (3). The radiolabeled substrate contained about 2.5 μCi/mg of collagen. The substrate had an optimum temperature for fibril formation of 15°C and melting of fibrils occurred at about 20°C and higher temperatures. The assay used (3) is based on solubilization of gelled-radiolabeled collagen.

SDS-PAGE zymograms

Metalloproteinases isolated from acetone powder were also assayed by substrate SDS-PAGE (18,24,25) using either 0.1% gelatin , 0.1% lingcod skin collagen or 0.1% b casein as substrate in polyacrylamide gel. After electrophoresis, gels were incubated for 24 h at 20-24°C in Tris-Cl, pH 8.0 normally containing 10 mM $CaCl_2$ and 1 mM NEM. After staining with Coomassie Blue and destaining clear (unstained) zones indicated enzyme activity. Metalloproteinases were also assayed with a synthetic substrate for collagenase (57).

RESULTS AND DISCUSSION

Collagenase activity based on fibril solubilization

Purified lingcod skin collagen had an optimum temperature to form fibrils at pH 7.5 of 15°C. Melting of the fibrils occurred, on the other hand, at 20°C and higher temperatures. Reduction of the purified collagen with NaB^3H_4 in the presence of pyridoxyl phosphate resulted in tritium incorporation of about 2.5 μCi per mg collagen. The radiolabeling did not change the fibril formation properties and the melting temperature of the collagen. The radiolabeled collagen formed fibrils with low blanks (< 10% of total hydroxproline or radioactivity) and resistance to trypsin degradation since less than 20% of the total collagen was solubilized by treatment with this enzyme. The collagenase activities of the acetone powder extract, 30-80% saturation ammonium sulfate fraction, and gel filtration fractions containing the 98 and 47 Kdalton proteases are shown in Table 1. The amount of fibril collagen which was solubilized by the rockfish enzyme(s) was low and did not increase with longer assay times. The specific activity of *Clostridium histolyticum* collagenase (type 1A) was about 5-10 times greater than the rockfish muscle proteases purified by gel filtration. SDS-PAGE of the solubilized collagen did not reveal products (TC_A and TC_B; Fig. 1) characteristic of mammalian tissue collagenase nor other products with a molecular weight lower than the collagen a chains. MMP-3 is able to remove the

Table 1. Solubilization of Tritiated Collagen by Rockfish Proteases at 15°C, pH 7.5.

FRACTION	VOLUME	PROTEIN	U	PROTEIN	U	SPECIFIC ACIVITY
	ml	mg/ml	cpm/100 µl	mg	cpm(x1000)	U/mg
Crude Extract	700	4.45	400	3115	2,800	899
A.S. fraction	50	20.28	42,000	1014	2,100	2071
A0.5(98 Kd)	35	0.42	17,100	14.7	595	40,476
A0.5(47 Kd)	32	0.35	22,100	11.2	707	63,143

U= cpm ^{3}H/12 h at 15°C.
Clostridium histolyticum collagenase, type 1A(3510 U/10 µg); Bovine trypsin (540U/10 µg)

NH_2-terminal propeptides from type I procollagen and a small amount of procollagen may have been present in the substrate. It is of interest that the observed results are indicative of a procollagenase, like matrix metalloproteinase 3 (MMP-3), since it was dependent on activating the enzyme with the organomercurial, 4-aminophenyl mercuric acetate (44) and was inactivated by metalloproteinase inhibitors, EDTA (20 mM) or 1,10-phenanthroline (20 mM). The enzyme required $CaCl_2$ for activity and was stimulated by N-ethylmaleimide (NEM, 1 mM) as well as APMA (1 mM). The low activity observed and difficulties encountered with this assay because of interference with fibril formation and fibril stability led us to employ other methods to assay this enzyme fraction.

SDS-PAGE substrate zymograms

Initial studies revealed that the rockfish muscle enzyme fraction, which had limited activity on collagen fibrils, did not hydrolyze azocasein, hide powder azure, TAME or BAEE. However, assay of this enzyme fraction using SDS-PAGE substrate gels for gelatinase or collagenase activity (24,25) revealed regions of proteases corresponding to major activity at molecular weights of 47 and 98 Kdaltons (Fig.4A). Both enzymes readily hydrolyzed soluble collagen as well as gelatin but were inactive in zymograms containing ß-casein. For this reason, additional experiments with SDS-PAGE zymograms were used to characterize the enzyme fraction.

Influence of inhibitors and activators

The influence of various compounds on the activity of rockfish muscle proteases is summarized in Table 2. Activity of the two proteases on SDS-PAGE zymograms, as well as on solubilization of fibril collagen, was dependent on the presence of $CaCl_2$ (Fig. 4D & E; Fig. 6 A & B) and was stimulated by NEM (Fig. 6F), or APMA (Fig. 6E) in the incubation buffer. The protease activity was also stimulated when 0.5% SDS was included in the incubation medium (Fig. 6J). No activity was observed when $CaCl_2$ was not in the assay medium. NaCl (20 mM) was not effective as a replacement for $CaCl_2$ (Fig, 4E) and no stimulation of activity was observed when cysteine (10 mM) or dithiothreitrol (Fig. 6 I) was included in an assay buffer. The activation of rockfish gelatinase by the organomercurial APMA is a characteristic in common with mammalian tissue procollagenases (Fig.3). Mammalian tissue collagenases are Zn^{+2} containing enzymes, and as expected, activity of both rockfish enzymes were inhibited by the metalloproteinase inhibitors, EDTA and 1, 10-phenanthroline (Fig. 6C & D). The response of other muscle proteases to inhibitors is summarized in Table 3. Heat stable alkaline proteases are common in fish muscle but are normally classified as thiol or serine proteases rather than metalloproteinases. Collagenases isolated from digestive organs of fish are classified as serine proteases. Other proteases from muscle tissue which are active on collagen, such as cathepsins or elastase, are not metalloproteinases. These results indicate that the two rockfish proteases are different from heat stable alkaline proteases and other muscle proteases and similar to tissue collagenases with respect to their classification as metalloproteinases (24, 25, 44, 53). Also, rockfish proteases, like mammalian tissue collagenases, require Ca^{+2} for activity (Table 4). On the other hand, collagenases from digestive organs and alkaline proteases from muscle tissue are not appreciably influenced by this cation (58).

Influence of temperature and pH on rockfish proteases

The 47 and 98 Kdalton proteases retained activity when assayed on SDS-PAGE substrate gels after 1 h incubation at 50°C in Tris-Cl buffer, pH 8.0, containing 10 mM $CaCl_2$ and 1 mM NEM (Fig. 4B). After 1 h at 60°C in the same buffer the 47 Kdalton protease was inactive, however, the 98 Kdalton protease retained activity when assayed on SDS-PAGE zymograms(Fig. 4C). The stability of the rockfish proteases at relatively high temperatures is similar to that reported for heat stable alkaline proteases isolated from muscle (Table 5). There do not appear to be reports describing the temperature optimum of tissue collagenases since these are normally assayed at near the physiological temperature of the animal.

Table 2. Inhibitors and Activators of Rockfish Muscle Proteases.

COMPOUND	CONDITIONS OF ASSAY
Required	
$CaCl_2$ (10 mM)	Collagen, Gelatin (SDS-PAGE zymograms); collagen fibrils (radio-assay); TNBS assay of gelatinase.
Activators	
N-ethylmaleimide (1mM)	Collagen, Gelatin (SDS-PAGE zymograms); collagen fibrils (radio-assay); TNBS assay of gelatinase.
4-Aminophenyl mercuric acetate (1 mM)	" "
Sodium dodecylsulfate (0.5%)	Collagen, Gelatin (SDS-PAGE)
Inhibitors	
Ethylenediaminetetraacetic acid (20 mM)	Collagen, Gelatin (SDS-PAGE zymograms); collagen fibrils (radio-assay); pH-stat assay
1,10 -Phenanthroline (20 mM)	Collagen, Gelatin (SDS-PAGE zymograms); collagen fibrils (radio-assay)
No Effect on Activity	
Dithiothreitol (1mM)	Collagen, Gelatin (SDS-PAGE zymograms); collagen fibrils (radio-assay).
Cysteine (10 mM)	pH-stat
NaCl (20 mM)	Collagen, Gelatin (SDS-PAGE zymograms); collagen fibrils (radio-assay)
Phenylmethylsulfonyl fluoride (1 mM)	Collagen, Gelatin (SDS-PAGE zymograms); collagen fibrils (radio-assay)

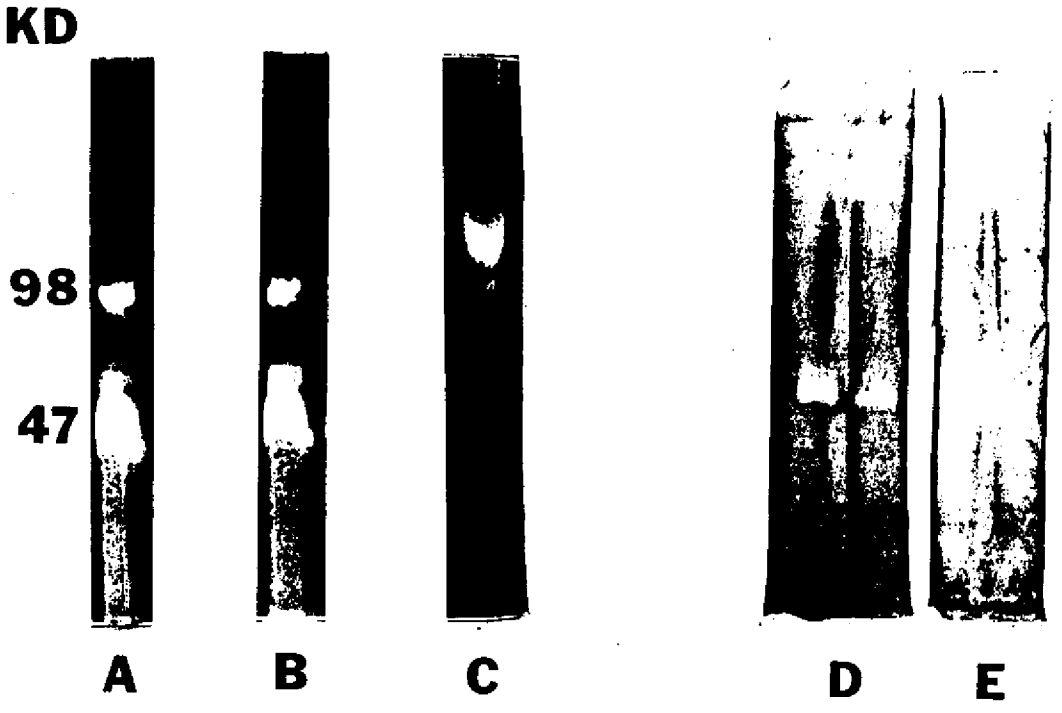

Figure 4. SDS-PAGE substrate gel of Blue rockfish muscle metalloproteinases.incubated in Tris-HCl, pH 7.5 containing 10 mM $CaCl_2$.for 24 h at room temperature A. enzyme recovered from gel filtration chromatography(pooled 50 and 100 Kdalton fractions), B.enzyme (same as in A) was incubated at 50°C for 1 h prior to electrophoresis, C.enzyme (same as in A) was incubated at 60°C for 1 h prior to electrophoresis, D. enzyme fraction (50 Kdalton) from gel permeation chromatography, E. same as D but $CaCl_2$ was replaced by NaCl (20 mM). Substrate in gel was lingcod skin collagen (0.1%). Molecular weight corresponding to 98 and 47 Kdalton is labeled adjacent to gel track A.

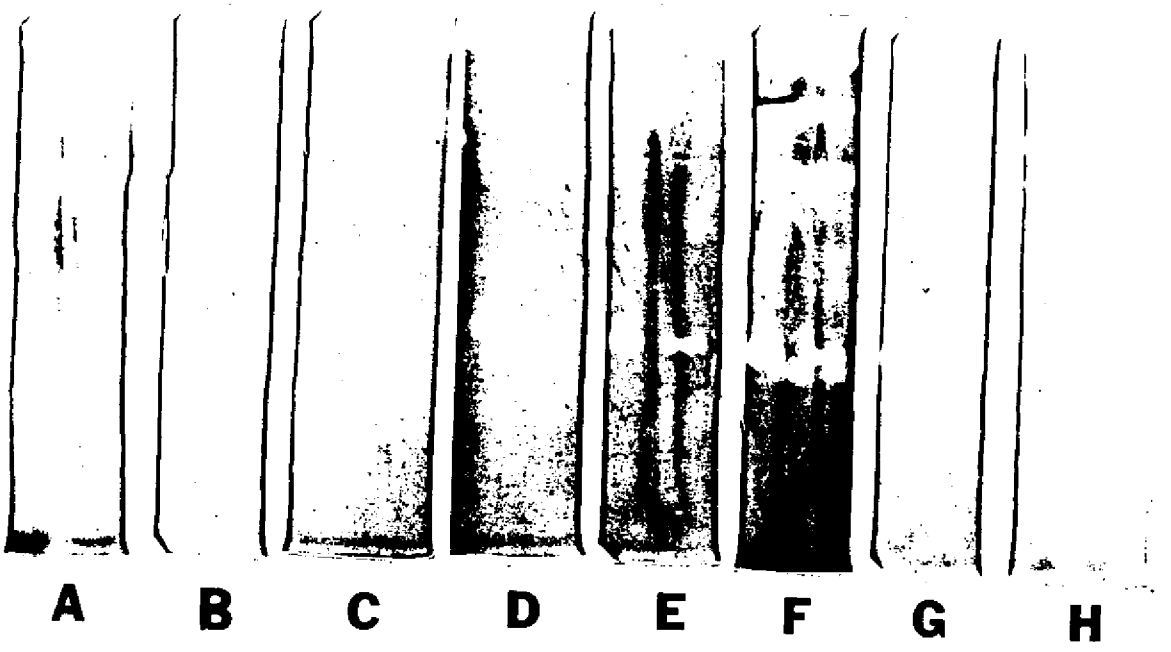

Figure 5. SDS-PAGE substrate gel of rockfish proteases recovered from gel permeation chromatography (pooled 50 and 100 Kdaltons). Incubation buffer after electrophoresis was buffer containing 10 mM $CaCl_2$ and 1 mM NEM at different pH values. A. pH 3.0, B. pH 4.0, C. pH 5.0, D. pH 6.0, E. pH 7.0, F. pH 7.5, G. pH 8.0, G. pH 8.0, H. pH 9.0. The 47 and 98 Kdalton zones showed increasing activity from pH 6.0 to 9.0.

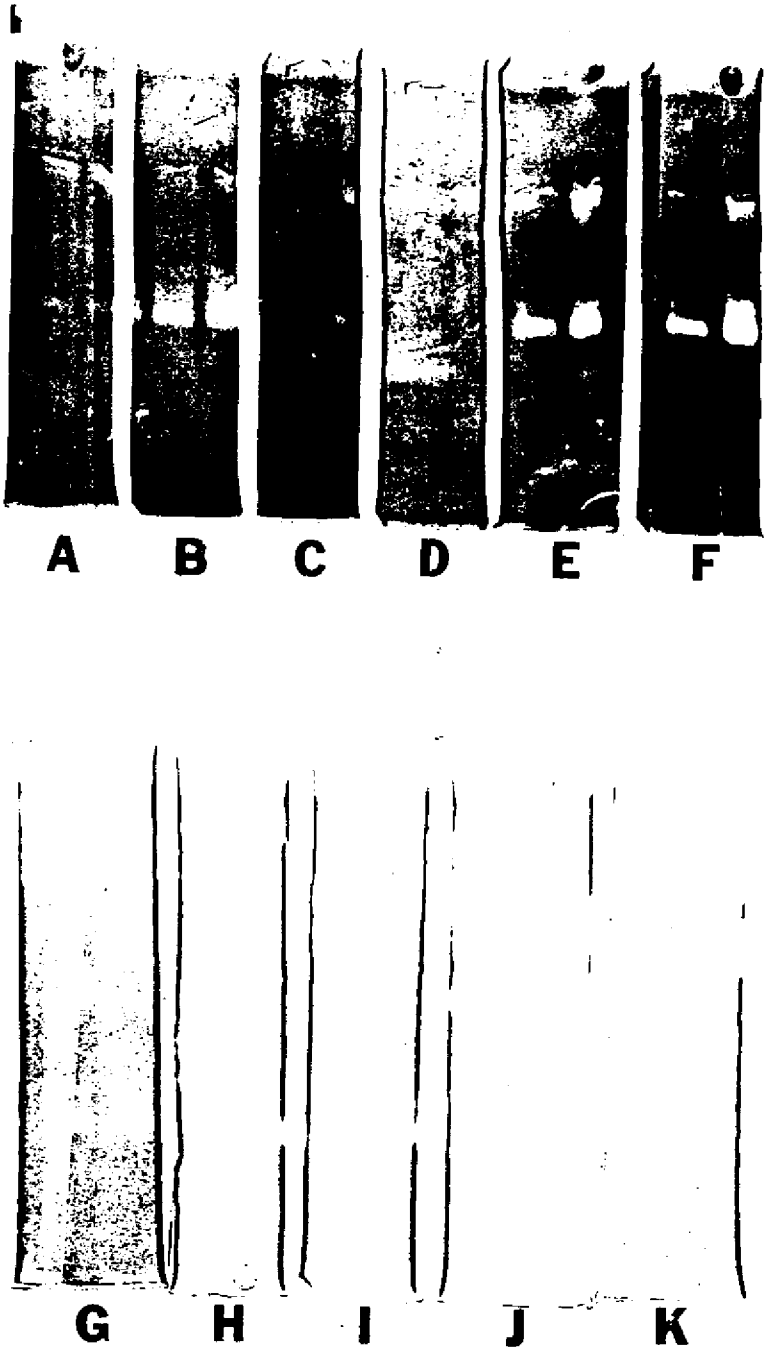

Figure 6. SDS-PAGE substrate gel of rockfish proteases recovered from gel filtration chromatography (pooled 50 and 100 Kdalton). Substrate gel was incubated in Tris-Cl, pH 7.5 containing A. no $CaCl_2$, B &G. 10 mM $CaCl_2$, C. 10 mM $CaCl_2$ and 20 mM EDTA, D. 10 mM $CaCl_2$ and 20 mM 1,10 phenanthroline, E. 10 mM $CaCl_2$ and 1 mM APMA, F. 10 mM $CaCl_2$ and 1 mM NEM, H. 10 mM $CaCl_2$ and 1 mM DTT, I. 10 mM $CaCl_2$ and 0.5% SDS, J. 10 mM $CaCl_2$ and 1:10 dilution of rockfish muscle extract.

Table 3. Classification of Various Muscle Proteases and Collagenases Based on Inhibitor Response.

SOURCE	CLASSIFICATION	REFERENCE
Muscle Alkaline Proteinases		
Barracuda	Thiol protease	28
Carp	Thiol protease	28
Croaker,Atlantic	Thiol protease	37
Croaker, White I	Serine protease	6
Croaker, White II	Thiol protease	19
Croaker, White	Thiol protease	37
Rat	Thiol protease	38
Rat myofibrillar	Serine protease	11
Collagenases		
Trout ceca	Serine protease	58
Crab hepatopancreas	Serine protease	15,50
C. histolyticum	Metalloproteinase	
Bovine aortic endothelium	Metalloproteinase	10
Human skin	Metalloproteinase	16
Rat skin	Metalloproteinase	54
Tadpole tail	Metalloproteinase	43
Other Proteinases Active on Collagen		
Cathepsin G	Serine protease	1
Elastase	Serine protease	2
Cathepsins B,L,N,S	Aspartic	1

Table 4. Influence of Ca^{+2} on Alkaline Proteases and Collagenases.

SOURCE	INFLUENCE OF CA^{+2}	REFERENCE
Muscle Alkaline Proteases		
Carp	slight stimulation	28
Croaker, Atlantic	none	32
Croaker, White	slight inhibition	37
Rat	none	11
Shrimp	none	13
Collagenases		
Digestive(serine)	none	58
Tissue(metallo-)	required	1,54
Rockfish Enzymes		
Rockfish I	required	
Rockfish II	required	

Table 5. Temperature and pH Optima of Alkaline Proteinases and Collagenolytic Enzymes.

SOURCE	TEMP. OPT. (°C)	PH OPT.	REFERENCE
	Alkaline Proteinase		
Barracuda	62	8.0	30
Carp	65	8.0	37
Croaker(Atlantic)	60	8.0	32
Croaker(White)	60	8.0	37
Filefish	60-65	7.8-8.3	45
Mackerel	60	8.0	47
Menhaden	60	7.5-8.5	4
Mullet[2]	65	8.0	12
Rat cytosolic	--	9.4	11
Rat myofibrillar	--	9.6	11
Salmon(Chum)	65	8.0	55
Shrimp	60	8.0	13
25 species	60-65	7.9-8.1	29
	Collagenase		
Crab(Cunchatka)	--	7.9	50
Crab(Fidler)	--	8.0	15
Human leucocyte	--	7.8	2
Human skin	--	8.0	16
Mackerel ceca	25	--	47
Prawn hp	--	7.5	46
Tadpole fin	--	8-9	43
	Rockfish Enzymes		
Rockfish I	60+	9	
Rockfish II	50-60	9	

Table 6. Molecular Weights of Muscle Alkaline Proteinases and their Subunits.

SOURCE	ENZYME	SUBUNIT(S)	REFERENCE
	Kdaltons		
Barracuda	780	----	29
Carp	780	----	27
Carp	600	150	36
Croaker(Atlantic)	80	----	32
Croaker(White) I	132	----	6
Croaker(White) II	1363	18-60	19
Croaker(White)	470	45-57	37
Croaker(White)	920	----	35
Lobster	740	----	29
Shrimp	250	96	13
Rat	31	----	11
Rat	450	----	38
	Rockfish Enzymes		
Rockfish I	98	98	
Rockfish II	47	47	

The pH optimum of rockfish muscle proteinases assayed with lingcod skin collagen on SDS-PAGE zymograms appears to be near pH 9 (Fig.5). Mammalian tissue collagenase, digestive collagenases and heat stable alkaline proteases from muscle all have optimum activity at alkaline pH values (Table 5).

Molecular weights of rockfish proteases

Gel permeation chromatography of the ammonium sulfate fraction of rockfish proteases resulted in recovery of about 90% of the activity in two fractions corresponding in molecular weight to approximately 100 and 50 Kdaltons (Table 1). The specific activity of the two fractions on radioisotope labeled collagen fibrils was increased by about 20 and 30 fold respectively (Table 1). SDS-PAGE substrate zymograms revealed the 100 and 50 Kdalton fractions corresponded to the 98 and 47 Kdalton proteases (Fig. 4A & D). The results of gel permeation chromatography and SDS-PAGE reveal that the rockfish muscle proteases described in this paper differ from heat stable alkaline proteases which have been reported to be high molecular weight proteins composed of subunits (Table 6). The molecular weights of digestive collagenases are reported to be around 25 Kdaltons (Table 7). Although molecular weights ranging from about 40 to 100 Kdaltons have been reported for mammalian tissue collagenases, most collagenolytic enzymes have a molecular weight around 50 Kdaltons (Table 7). It is also known that stepwise activation of procollagenase can result in multiple forms of collagenase with similar molecular weights (Fig. 3; 44). It is therefore interesting that zymograms of rockfish proteases frequently show doublet and triplet zones of clearing around the R_f corresponding to 98 and 47 Kdaltons.

Substrate specificity

The ability of rockfish metalloproteinases to hydrolyze different substrates is summarized in Table 8. The metalloproteinases have low but measureable activity on collagen fibrils and rapidly hydrolyze gelatin and soluble collagen on SDS-PAGE zymograms. Incubation of metalloproteinases with soluble collagen resulted in initial formation of fragments similar to TC^A and TC^B and eventually to complete degradation of a, b and g chains to low molecular weight products (Fig. 7).

On the other hand, the metalloproteinases did not hydrolyze two general protease substrates, azocasein and hide powder azure, TAME and BAEE in solution and did not hydrolyze b-casein in SDS-PAGE zymograms.

CONCLUSIONS

Previous studies in our laboratory showed that rockfish muscle contains endogenous factors that rapidly and extensively degrade tissue collagen when the muscle is subjected to a temperature of 20°C or higher (8). Here we show that rockfish muscle contains two alkaline, heat stable proteases. The enzymes were identified in acetone powder extracts from several species of rockfish including Speckled (*Sebastes maliger*), Blue (*S. mystinus*), Bocaccio (*S. paucispinus*), Widow (*S. entomelas*), and Yellowtail (*S. flavidus*). The enzymes are metalloproteinases and are similar in many ways to matrix metalloproteinases (gelatinases) identified in mammalian cell culture exudates. Similarities of the rockfish enzymes with matrix metalloproteinases include molecular weight (especially the 47 Kdalton enzyme), requirement of Ca^{+2} for activity, activation by the organomercurial APMA, limited ability to solubilize collagen fibrils, and apparently high substrate specificity for soluble collagen and gelatin. Further study of these enzymes is recommended because they appear to be responsible for the loss in fillet integrity of fish which are temperature abused prior to chill storage.

Table 7. Molecular Weights of Collagenases and Related Metalloproteinases.

SOURCE (TISSUE)	MOLECULAR WEIGHT	REFERENCE
	Kdaltons	
C. histolyticum	24, 26	48
Crab, Cumchatka (hepatopancreas)	27	50
Crab, Fidler (hepatopancreas)	25	15
Human (bone marrow)	75, 57, 22	22
Human (fibroblasts)	42, 46	3
Human (fibroblasts)	41-52	53
Human (smooth muscle)	42	49
Human (leukocytes)	50-53	2
Mouse (tumor)	62, 68	2
Rabbit (brain)	47	24
Rabbit (brain)	92, 67, 55	25
Rabbit (fibroblasts)	57	2
Rabbit (fibroblasts)	92, 68	10
Rabbit (bone)	51, 65	2
Tadpole (fin, skin)	50	43
Rockfish	98, 47	

Table 8. Substrate Specificity of Rockfish Muscle Metalloproteinases I and II.

SUBSTRATE	CONDITIONS
Substrates Hydrolyzed	
Collagen fibrils	pH 7, 15°C, ^{3}H-lingcod skin collagen
Soluble collagen	pH 8, 37°C, lingcod skin collagen
Soluble collagen	pH 7-9, 20°C, SDS-PAGE zymogram, product analysis
Gelatin	pH 7-9, 20°C, SDS-PAGE zymogram, product analysis
Substrates Not Hydrolyzed	
Azocasein	pH 8, 20°, 37°, 50°C
Hide Azure	pH 7.5-8, 20°, 37°, 50°C
TAME	pH 8, 20°C
BAEE	pH 8, 20°C
β-casein	pH 8, 20°C, SDS-PAGE zymogram

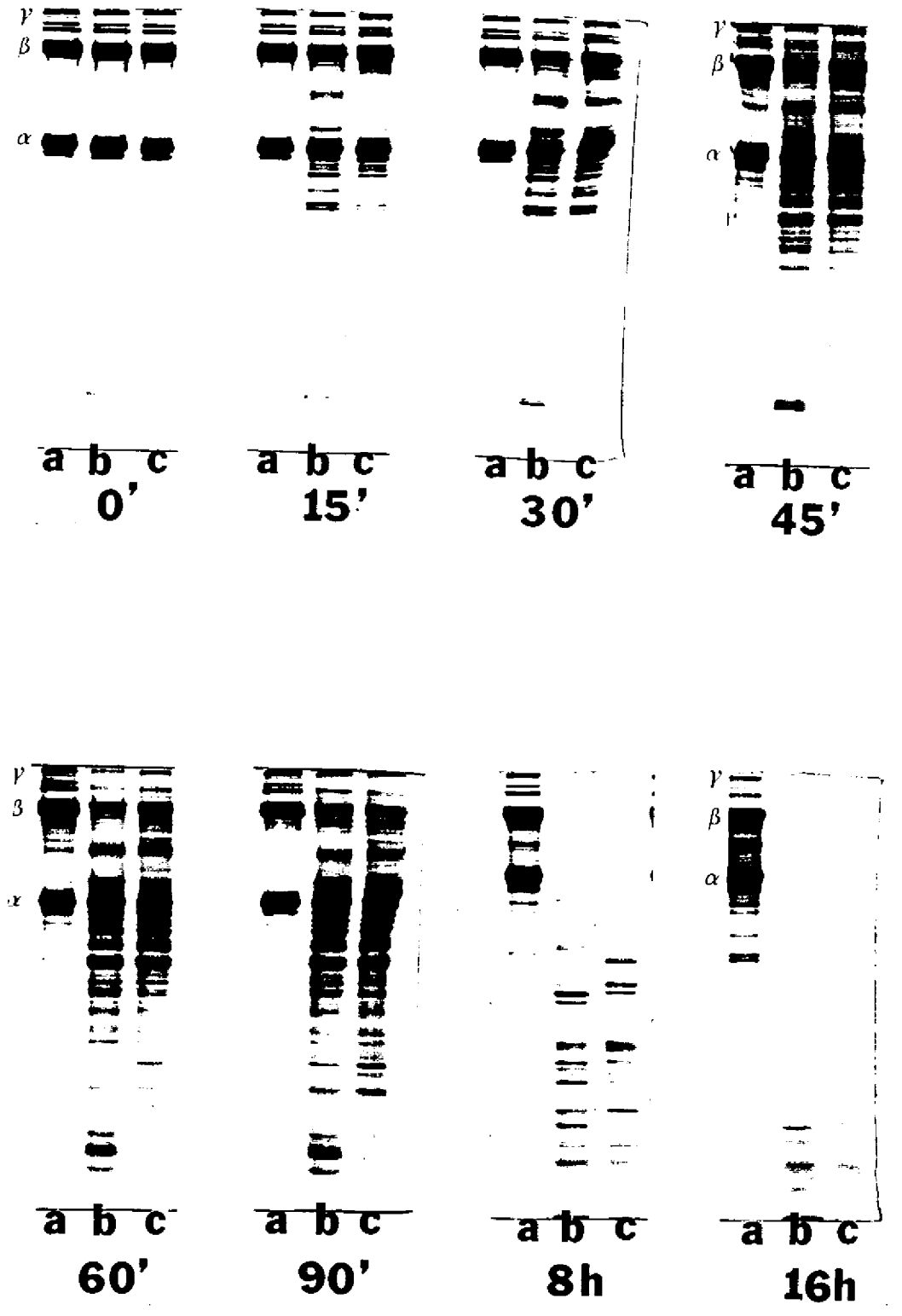

Figure 7. SDS-PAGE electrophoresis of lingcod skin collagen incubated with rockfish metalloproteinases. Collagen (600 mg) was incubated in 0.75 ml 0.017 M Tris-Cl, pH 8.5 , 37°C (a) no enzyme added, (b) 98 Kdalton protease, (c) 47 Kdalton protease. An aliquot equivalent to about 40 mg collagen substrate was applied to the gel after different times of reaction. After 90 minutes a, b and g collagen chains have completely disappeared.

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USE OF ALUMINA RODS FOR QUANTITATIVE ANALYSIS OF CHOLESTEROL IN MARINE PRODUCTS WITH THE TLC/FID IATROSCAN SYSTEM

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Consumers are very concerned about the cholesterol content of their foods because of the alleged role of dietary cholesterol in the development and progression of cardiovascular diseases. Seafoods provide low calorie protein as well as beneficial omega-3 fatty acids. The cholesterol content of some seafoods including shrimp and mollusks is reasonably high, ranging from about 100-200 mg/100g wet weight (1). This has resulted in all seafoods often being mistaken as having high cholesterol levels. The development of analytical methods of cholesterol determination that provide rapid and accurate results is of interest and is the subject of this work.

Measurement of total sterols can be effected by using a number of different techniques. Chromatographic techniques (TLC, HPLC, GLC) have largely superseded older colorimetric methods. Kovacs et al (5) examined the use of gas chromatography on packed columns and different sample preparation techniques for the analysis of cholesterol in fishery-based foods. They found that a simple, single-tube saponification procedure was effective and compared well with official AOCS saponification but was 20 times more economical in time and solvent usage. Walton et al. (14) compared this method with a TLC-FID technique and found no difference in the quantitative accuracy between the two methods for marine samples. An examination of their internal standard calibration curve indicates that the response of cholesterol was 3-4 times greater than cholestane for any given mass. This is unsurprising given the effect of Rf on response (11) and the large Rf of cholestane compared to cholesterol. However, large differences in response between compound analyzed and internal standard are generally undesirable. The results from internal standard TLC-FID (IS-TLC), area TLC-FID (A-TLC) and GLC agreed reasonably well but there was no clear precision improvement with use of cholestane internal standard (average coefficient of variations were 11.3, 9.8 and 11.0 for IS-TLC, A-TLC and GLC respectively).

The purpose of this work was to examine the use of alumina rods for the quantitative determination of cholesterol in marine-based food product, caviar, and to compare accuracy and precision of results from TLC/FID using alumina rods, silica S-III rods and GLC.

MATERIALS AND METHODS

TLC/FID

An Iatroscan TLC-FID Mk-IV apparatus was obtained from RSS Inc. (Costa Mesa, CA). Silica S-III and Alumina A rods were used. Data handling was effected with an analog-digital card, 80386-based IBM clone computer and TSCAN software (RSS Inc.). This allowed storage, retrieval, reintegration and statistical treatment of the data.

Gas Liquid Chromatography

The gas chromatography conditions were: GC-14A gas chromatograph and autosampler (Shimadzu), column 15 M x .32 mm i.d. 0.25 μM film methyl silicone (Hewlett Packard), oven temperature 265 °C, injector and detector 295 °C, helium carrier pressure 0.5-1.0 kg/cm^2. A split splitless injector was used in split mode with a 60:1 split ratio. Make up gas pressure was 0.5 kg/cm^2.

Solvents and reagents

All solvents were HPLC grade and were obtained from Fisher (Orlando, FL). The chloroform was stabilized with 0.6% ethanol. All reagents were ACS grade or better.

Samples

Caviar samples (beluga, osetra, lumpfish) were obtained from Continental Gourmet Imports (New York). The morphological characteristics of the eggs were consistent with their species (F. Chapman, personal communication).

Lipid was extracted by using a modified Bligh and Dyer chloroform-methanol extraction as described by Christie (3). Saponification was done as suggested by Kovacs et al. (5) method B. Briefly, 50 mg of lipid was saponified with 2 ml 95% ethanol and 0.5 ml 50% w/v KOH at 100 °C for 1 hour. After cooling and addition of 1.5 ml water, the unsaponifiable matter was extracted with 4 x 2 ml hexane. The hexane extracts were pooled, the solvent removed, and the residue dissolved in 2 ml chloroform.

Cholesterol determination

The cholesterol content was determined both on whole lipid and after saponification by using TLC-FID using both S-III and A rods and by using gas liquid chromatography. The TLC-FID solvent systems used were hexane-ethyl ether-formic acid (96:3:1 v/v) and chloroform. Each of these solvents were used for A and S-III rods. The elution time varied from 5 to 40 minutes.

Response factors

Equal weights of cholesterol and cholestane were used to produce standard solutions containing equivalent amounts of each compound to determine response factors. Standard calibration curves from 1-30 μg sterol were prepared for both rod types developed in both solvents.

RESULTS AND DISCUSSION

Response factors

The response of cholesterol and cholestane on the GLC column was dependent on the carrier gas pressure. The mean (standard deviation) response factor for cholesterol/cholestane was 0.838 (0.01) at a carrier gas pressure of 1.0 compared to 0.474 (0.001) at 0.5 kg/cc. The peak shape (height/area) for cholesterol was better at the higher pressure. This is in spite of the linear flow velocity being closer to optimum at 0.5 kg/cc for the 15 M column with helium carrier gas.

The standard curves for cholesterol on the different rods are shown in Figure 1. The curves illustrate that the effect of solvent system was not as strong for A compared to S-III rods. The areas for cholesterol on A developed in either chloroform or 96:3:1 were higher than S-III especially at low load level.

The response of cholesterol and cholestane on the TLC/FID rods depended on the time of development, the rod type and the solvent system. Figures 2 and 3 illustrate 5 and 20 minute developments of equal weights of cholesterol and cholestane (5 μg) on alumina and silica S-III rods developed for 20 minutes in chloroform. The response of cholesterol and cholestane decreased with increasing Rf. This decreasing response with increasing Rf is well known (11,12).

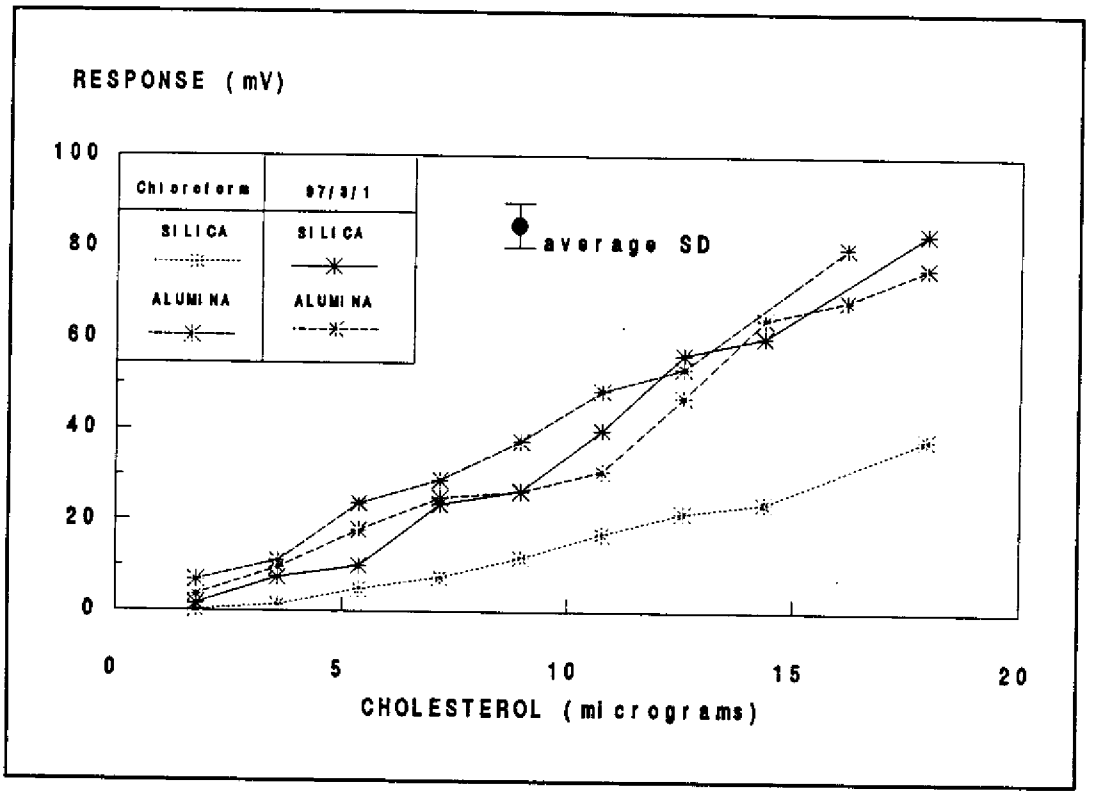

Figure 1. Standard calibration curves for cholesterol on alumina and silica S-III rods

The S-III rods appeared to give similar response for cholestane at low Rf compared to alumina but at increasing Rf the relative response of cholestane (response for S-III/response for A) decreased. Part of this effect could be attributable to the higher Rf for cholestane on S-III compared to the more retentive A rods. However, in some cases where the Rf on A and S-III rods were similar, the area on the S-III rods were still lower. The response on non-developed rods were not significantly different ($p>0.05$, data not shown) so the differences must be partly due to Rf but also to differences in phase thickness, particle size as well as to the differences in the adsorptive behavior of alumina and silica.

Cholesterol in marine samples

Figures 4 and 5 illustrate typical chromatograms from spotting ca. 250 μg of whole lipid extract on silica and alumina rods. The ability to quantitate free cholesterol in total lipids is slightly better for alumina rods because of the greater response at these low cholesterol levels. The triglyceride peak on the silica S-III rods shows partial separation on the basis of saturation. The use of catalytic hydrogenation of samples has been shown to improve peak shape by reducing saturation of different lipid classes to be closer to the saturated standards commonly used (2,10,13).

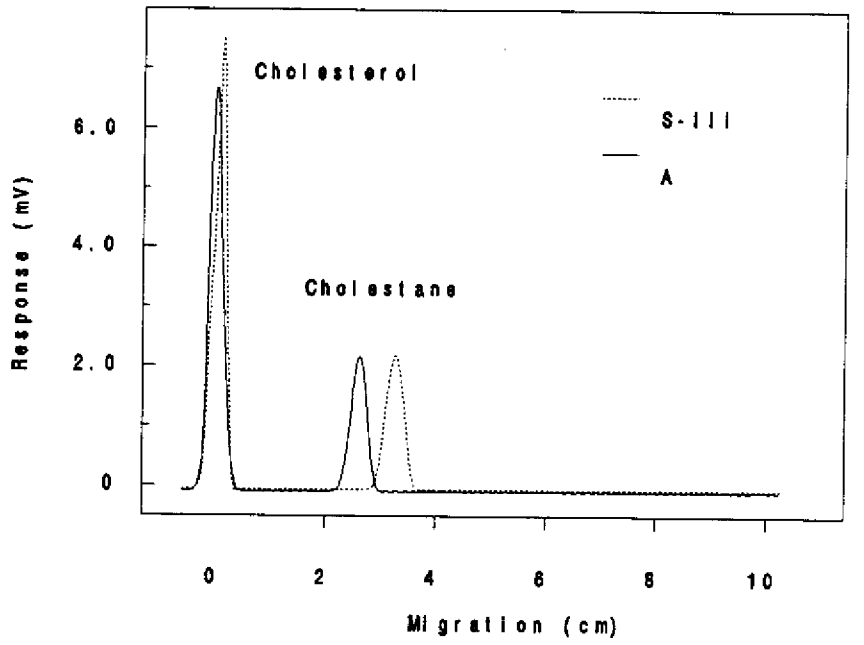

Figure 2. Five minute development

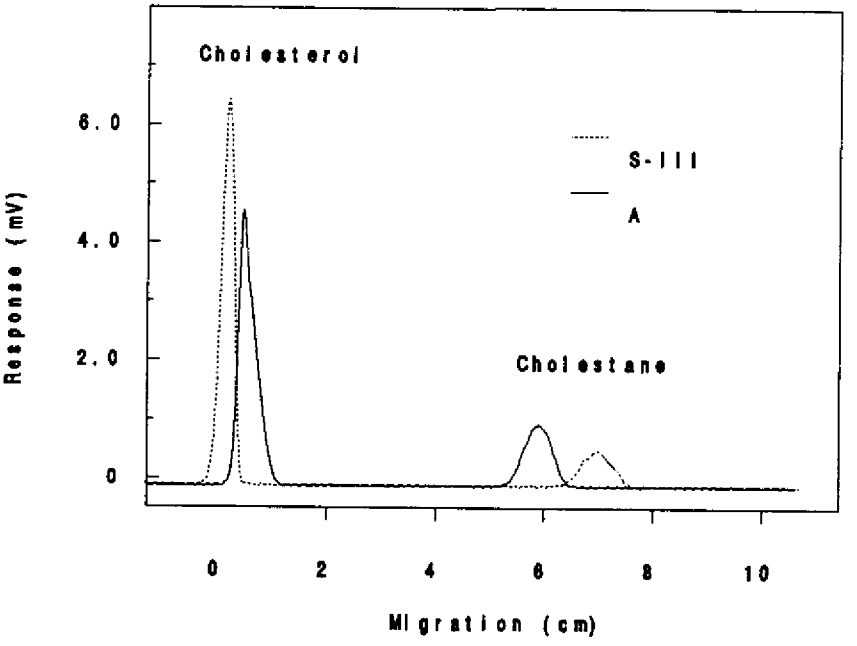

Figure 3. Twenty minute development

There was no partial separation of triglycerides on the alumina rods. Alumina TLC has been reported to produce separation of methyl esters on the basis of unsaturation without added silver (9) but these authors found no separation for triglycerides. The alumina rods did not separate methyl esters of cod liver oil (data not shown) and thus it appears likely that there are large differences in the chromatographic behavior of alumina on plates and the sintered glass-alumina on the chromarods.

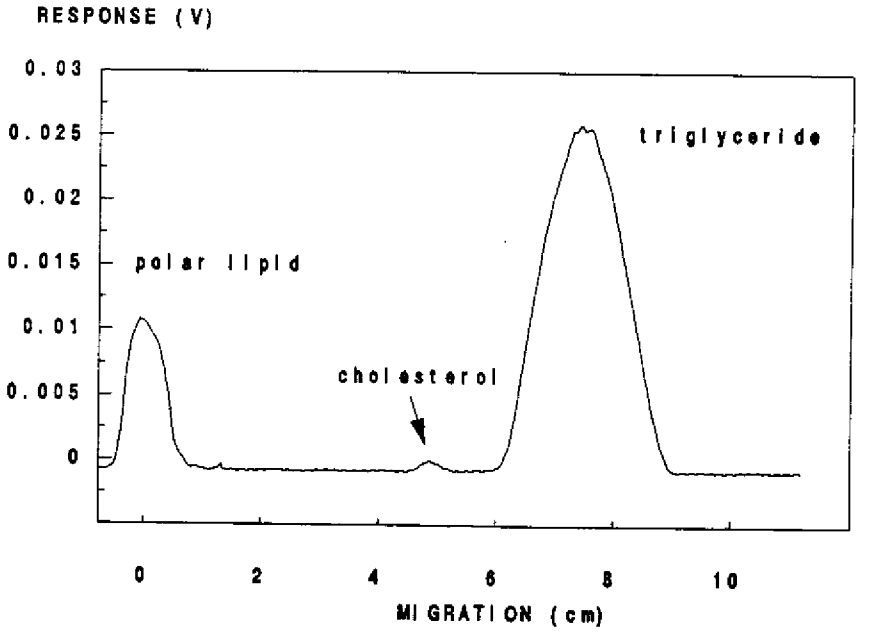

Figure 4. Beluga caviar whole lipid on alumina rod. Solvent chloroform: 30 minute development

A chromatogram of the unsaponifiable matter from osetra caviar is shown in Figure 6. It can be seen that the relative area of cholesterol and internal standard are quite different for A and S-III rods, as was seen with standards. Thus, it appears necessary to create a calibration curve of area cholesterol/area cholestane vs weight cholesterol/weight cholestane as was done by Walton et al. (14). Due to the dependence of area of both cholesterol and cholestane on Rf, and the effect of solvent strength, degree of hydration, development time and tank saturation on the velocity of migration and the Rf, it is apparent that the methodology must be rigidly standardized to achieve reproducible results.

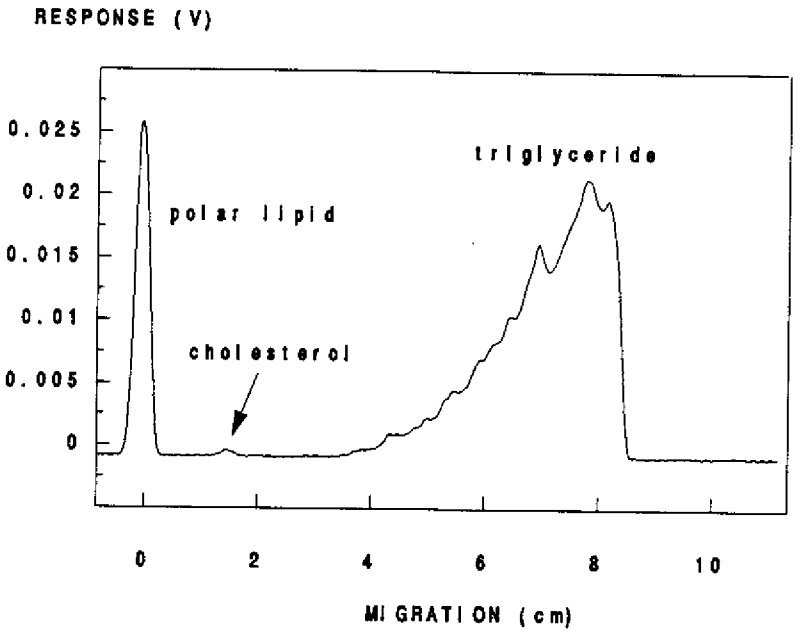

Figure 5. Beluga caviar whole lipid on silica rod. Solvent 96:3:1 (hexane-ethyl ether-formic acid): 30 minute development.

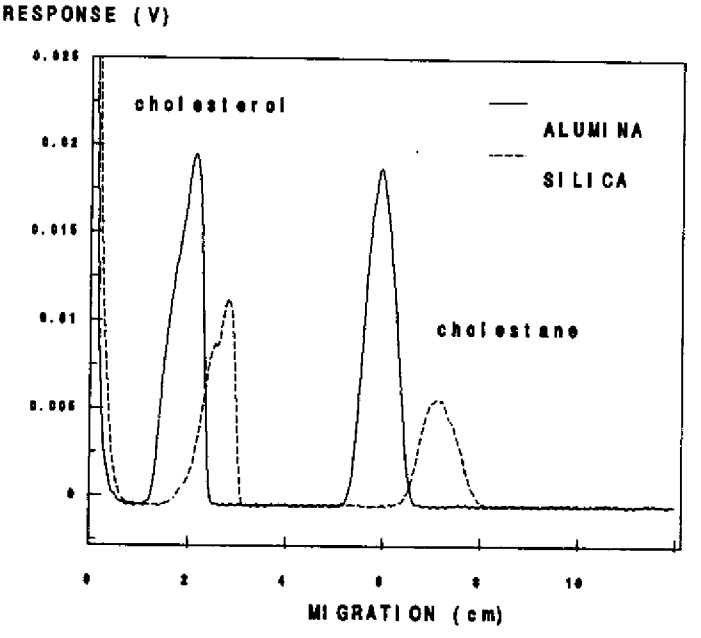

Figure 6. Chromatograms of osetra unsaponifiables on alumina and silica rods. Solvent: chloroform 20 minutes.

Table 1 illustrates results obtained from GLC compared to silica and alumina rods for both whole lipid and unsaponifiable matter. The results obtained for GLC were not significantly different from A for beluga and osetra but the A rods produced significantly higher numbers for lumpfish. Although Walton et al. (14) suggest a 40 minute development time in 97:3:1 (hexane-ether-formic acid), under the conditions of this study the cholestane standard was overdeveloped in 35 minutes and had run off the top of the rod.

It is important to realize that there is little or no separation of plant sterols from cholesterol on chromarods. This appears to result from the importance of the C-3 hydroxyl compared to the C-17 substitution (12). Most marine foods contain cholesterol predominantly (95-99%) but mollusks contain appreciable amounts of phytosterols (4,6,7,8). Thus, if marine samples containing appreciable amounts of sterols other than cholesterol are to be analyzed, GLC or other chromatographic techniques must be used.

Table 1. Comparison of Different Methods of Determining Cholesterol

Method	Caviar Sample		
	Beluga	Osetra	Lumpfish
Whole lipid			
Alumina	151 (37)[a*]	164 (28)[a]	171 (15)[a]
Unsaponifiables			
S-III	422 (43)[c]	579 (29)[c]	516 (67)[d]
Alumina	378 (13)[b]	455 (20)[b]	469 (10)[c]
GLC	342 (47)[b]	404 (36)[b]	303 (28)[b]

* mean (standard deviation), 10 rods in duplicate cholesterol mg/100g sample wet weight
different letters within column indicate $p<0.05$

CONCLUSIONS

The difference in polarity between cholestane and cholesterol causes problems when using cholestane as an internal standard because of the vastly different response factors. This problem is more severe with S-III rods compared to alumina. Results from GLC compared better with alumina rods compared to silica when used without correction factors.

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FACTORS AFFECTING SOLVENT EXTRACTION EFFICIENCY IN FISH LIPID ANALYSIS

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Fish oil is considered as an important source of nutrient because of its health benefiting effect. A correct measurement of fish oil content is important not only from the nutrition stand point of view, but also for the seafood industry wheremuch of trade is often based on the fat content of the fish. However, there is no officially recognized method which gives aconsistent, yet reliable result. There are many differenttechniques available (2, 4, 5, 7), mostly based on solvent extraction, some of which have been modified for improvement (6). This is the reason why there are so many conflicting data published on the fat content. In order to find out what causes such discrepancies in the fat content data, a study should be conducted to investigate the effect of the type of solvent used, the solvent to sample weight ratio, and the solvent ratiowhen a mixed solvent system is used. Recently, in our lab we have demonstrated that the solvent ratio and the sample tosolvent ratio significantly affected the total fat extracted and also that in a given solvent system, the extent of extraction varied with the type of fish (3). The objective ofthe present study was to closely examine various underlying factors involved in fish lipid extraction using Atlantic mackerel (Scomber scombrus) as a representative fatty fish species. Through understanding of the interrelationship among these factors, a simple, rapid, and reliable solvent extraction method can be developed for the determination of the fish lipid content in a single extraction.

MATERIALS AND METHOD

Preparation of fish paste

Fresh mackerel (Scomber scombrus) fillets were run through a Baader Deboner machine (Model 694, North America, New Bedford, MA) to obtain bone and skin free mince. The mince was mixed uniformly with n-propyl gallate and EDTA (Sigma Chemical Co., St. Louis, MO), vacuum packed in cryobags and stored frozen at -20 C to prevent oxidation.

Lipid extraction

Fifty milliliters (50 ml) of a solvent mixtures:chloroform, methanol and water (CHL, MEOH, H2O) were added to 5 g of fish mince. The sample and solvent were blended for 1.5 min using a 100 ml Waring blender equipped with a rubber cap having a vent to release vapor. Caution was taken not to allow any leak during blending. The homogenate was vacuum filtered using a vacuum filter funnel. The filtrate was collected in a graduated separatory funnel and varying amounts of water in the form of 0.5% NaCl solution were added to separate the CHL layer. NaCl was added to prevent emulsion formation by neutralizing electrostatic repulsion necessary for emulsion stability. The total volume of the CHL layer was measured and 10 ml of the CHL layer was collected in a 20 ml beaker and evaporated on a hot plate at a low setting. The remaining was weighed as lipids. The lipids content,
was expressed as either lipid extracted or lipid content using the following formula:

$$\text{Lipid Extraction (LE)} = \frac{(\text{fat in 10 ml CHL})\ (\text{Vol of CHL Layer})}{(\text{Dry Sample weight})\quad (10\text{ ml})}$$

where lipid content was calculated on a wet sample weight basis.

Variation in the solvent composition

The solvent ratio was varied from (45:50:5) to (95:0:5) at the constant volume (5ml) of water phase, where 45:50:5 is equilvalent to 50 ml solvent mixture composed of 22.5ml MEOH, 25ml CHL and 2.5ml H2O. This is based on the condition that 5g sample contributes at lest 2.5ml H2O to the solvent. The water phase was varied from (10:50:40) to (45:50:5) at the constant volume (50ml) of methanol phase by adding an appropriate amount of water to the sample [e.g. 1.7ml to 5 g sample (66% moisture)] to give 10% water phase, where [91.7ml + 3.3ml (sample moisture)] in 50ml = 10%. The solvent to sample ratio and the 0.5% NaCl solution were kept constant at 10:1 and 20 ml, respectively.

Variation in solvent to sample ratio

The solvent to sample ratio was varied from 1:1 to 14:1. Solvent composition and the amount of added NaCl solution were kept constant at 75:20:5 (CHL:MEOH:WATER) and 20 ml, respectively.

Variation in the amount of added water during phase separation

The amount of added water (0.5% NaCl solution) was varied from 5 to 30 ml which allowed a biphase giving solvent ratios from 72:19:9 to 49:13:38. The solvent composition and the solvent to sample ratio were kept constant at 75:20:5 (CHL:MEOH:WATER) and 10:1 respectively.

RESULTS AND DISCUSSION

Variation in the solvent composition

The variation in the solvent composition is illustrated in Fig. 1 using a CHL-MEOH-WATER ternary diagram. The horizontal marked line shows the variation in CHL and water having MEOH constant at 50%. This variation was made in an attempt to determine how lipid extraction is affected by moisture adjustment as one of the critical requirements for complete lipid extraction in the Bligh and Dyer method (Bligh, personal communication). The effect of this variation shown in Fig. 2: there was some increase in LE with an increase in the water level of the sample from 74.6% to 85.5% (1.7 ml to 6.7 ml water added to 5 g sample), however, beyond the 85.5% moisture level (point "B" in Fig.1) LE decreased markedly with a further increase in the moisture level. From this observation, the moisture adjustment does not appear to be necessary when the wet sample (moisture content ranging from 58 to 75%) is used. In the case of a dry sample, it would be logical to hydrate it to the level as suggested before solvent extraction. The reason for the decrease in LE could be explained by the increase in the polarity of the solvent mixture by the increased addition of water Ackman and Eaton (1) found that a great percentage of the lipids in mackerel is composed of non-polar lipids, basically triglycerides. It is understandable that the higher the polarity of the solvent mixture, the less extracting of the non-polar lipids. The diagonal marked line in Fig. 1 indicates the second part of the variation of the solvent composition, where CHL and MEOH were varied with the water level constant at 5%. The effect of this variation is shown in Fig. 3: LE increased with an increase in the CHL and a decrease in the MEOH fraction until it reached a maximum at the solvent mixture of 75:20:5. Based on the same principle as explained above, the increased LE with an increase in the CHL level can be explained by the decreased polarity. The non-polar lipids, namely, triglycerides, should be thus extracted more efficiently as the polarity decreases. As the point reached the solvent mixture of 75:20:5 (point "A" in Fig. 1), the solvent can no longer take additional CHL without having a biphasic state. Bligh and Dyer (2) reported that the monophasic state is necessary in order to achieve an efficient lipid extraction. The monophasic state provides more contact areas for extraction which helps the solvent to act on the lipids, thus explaining the decrease in LE with an increase in CHL fraction beyond the point of 75:20:5.

Variation in the solvent to sample ratio

Fig. 4 shows the effect of increasing the solvent to sample ratio on the lipid extraction. As the solvent to sample ratio increased, LE increased until it reached the 6:1 ratio where the maximum LE was achieved. At the ratios higher than 6:1, there were no further increases in LE. It was interesting to see that the ratios below 6:1 were not able to extract all the lipids in one single extraction and needed a second or third extraction in order to achieve complete extraction.

Variation in the amount of added water during phase separation

In Fig. 5, a ternary diagram shows the variation in the amount of water added (0.5% NaCl solution) for phase separation. The more water added to separate the CHL layer, the closer the system gets to the 100% water corner as the arrow indicates. Fig. 6 shows the effect of water addition on the lipid extraction and the separation time. There was no significant effect of the volume of water on the LE. This means the CHL-lipid phase is separated from the water-MEOH phase so long as the system is in the biphasic state no matter at what point it lies. As to separation time, with an increase in the amount of water added, the separation time increased: the higher the volume of the water added, the higher incidences of emulsion formation, resulting in a longer separation time. Woyewoda, et al. (8) mentioned the problem associated with the emulsion formation during the solvent extraction of lipids from the fatty fish species.

CONCLUSION

Maximum lipid extraction from the Atlantic mackerel (5-25%lipid; 58-75% moisture) was achieved in a single extraction withsolvent mixtures ranging from 65:30 to 75:20 (CHL:MEOH) at a1:10 sample to solvent ratio (5 g sample to 50ml solvent). Under this extraction condition, adjustment of the moisturecontent on wet samples was unnecessary. The yield of lipidextraction increased with an increase non-polar to polar solventratio until it reached a biphase. Maximum lipid extraction canbe achieved with a high ratio of non-polar to polar solvent. Ahigh solvent to sample ratio was required for a completerecovery of lipids in a single extraction. The volume of wateradded for the separation of the chloroform layer should beminimum for a short separation time.

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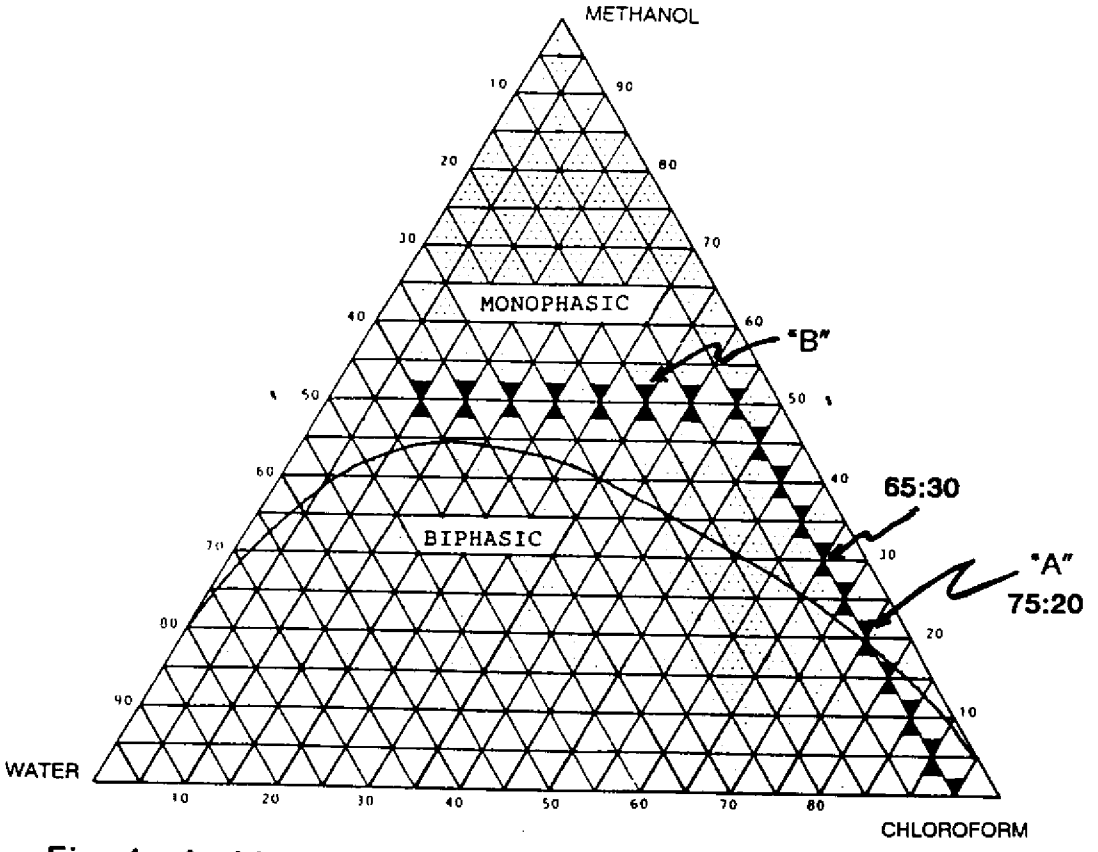
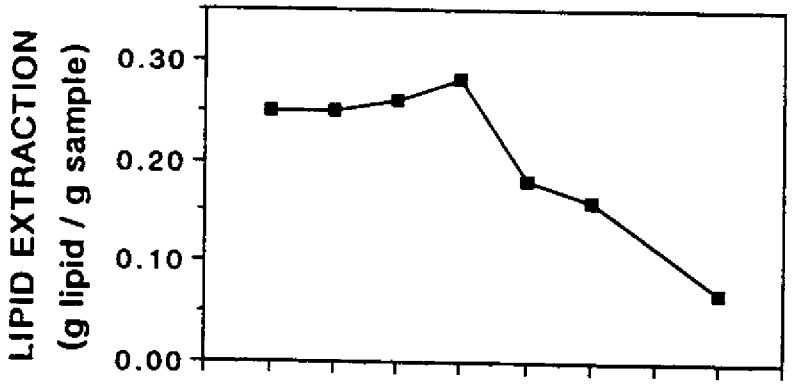


Fig. 1. A chloroform-methanol-water tirnary diagram with % variation in solvent composition

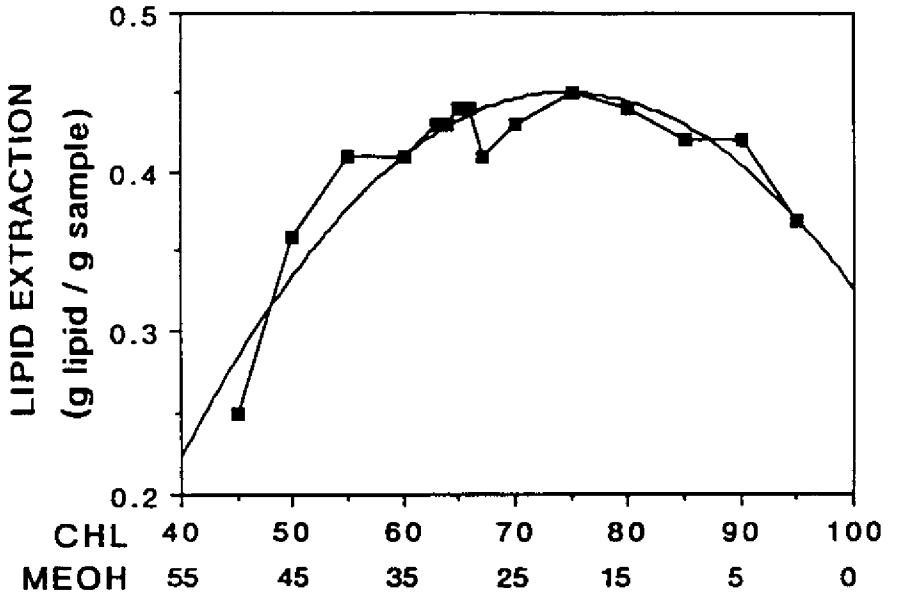
FIG. 2. SOLVENT COMPOSITION ON LIPID EXTRACTION



WATER PHASE(%)	5	10	15	20	25	30	35	40
WATER ADDED(ml) (to 5g SAMPLE)	0	1.7	4.2	6.7	9.2	11.7	14.2	16.7
SAMPLE MOISTURE(%)	66	74.6	81.5	85.5	88.0	89.8	91.0	92.2
CHL PHASE(%)	45	40	35	30	25	20	15	10

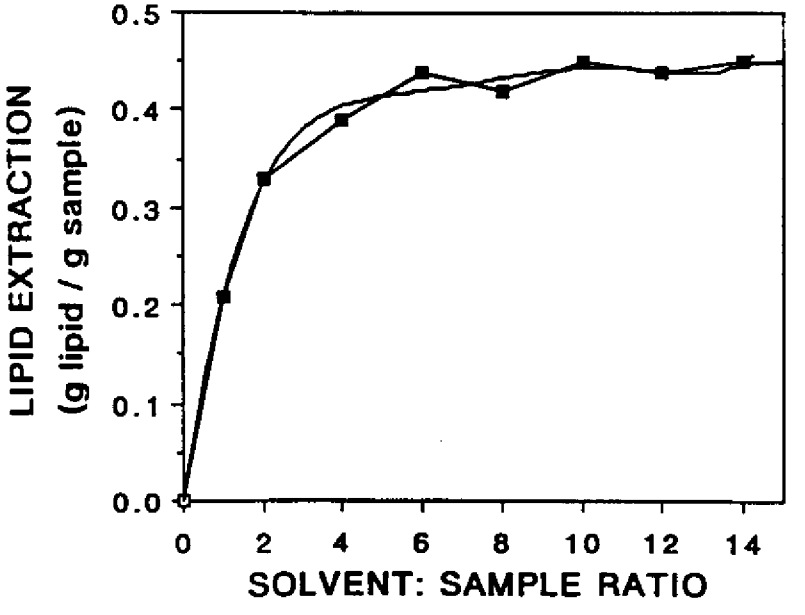
* CONSTANT MEOH AT 50%
CONSTANT SOLVENT TO SAMPLE RATIO AT 10 : 1
CONSTANT 0.5% NaCl SOLUTION AT 20 ml

FIG. 3. SOLVENT COMPOSITION ON LIPID EXTRACTION



CONSTANT WATER AT 5%
CONSTANT SOLVENT:SAMPLE RATIO AT 10:1
CONSTANT 0.5% NaCl SOLUTION AT 20 ml

FIG. 4. SOLVENT:SAMPLE RATIO ON LIPID EXTRACTION



CONSTANT SOLVENT COMPOSITION (75:20)
CONSTANT 0.5% NaCl SOLUTION AT 20ml

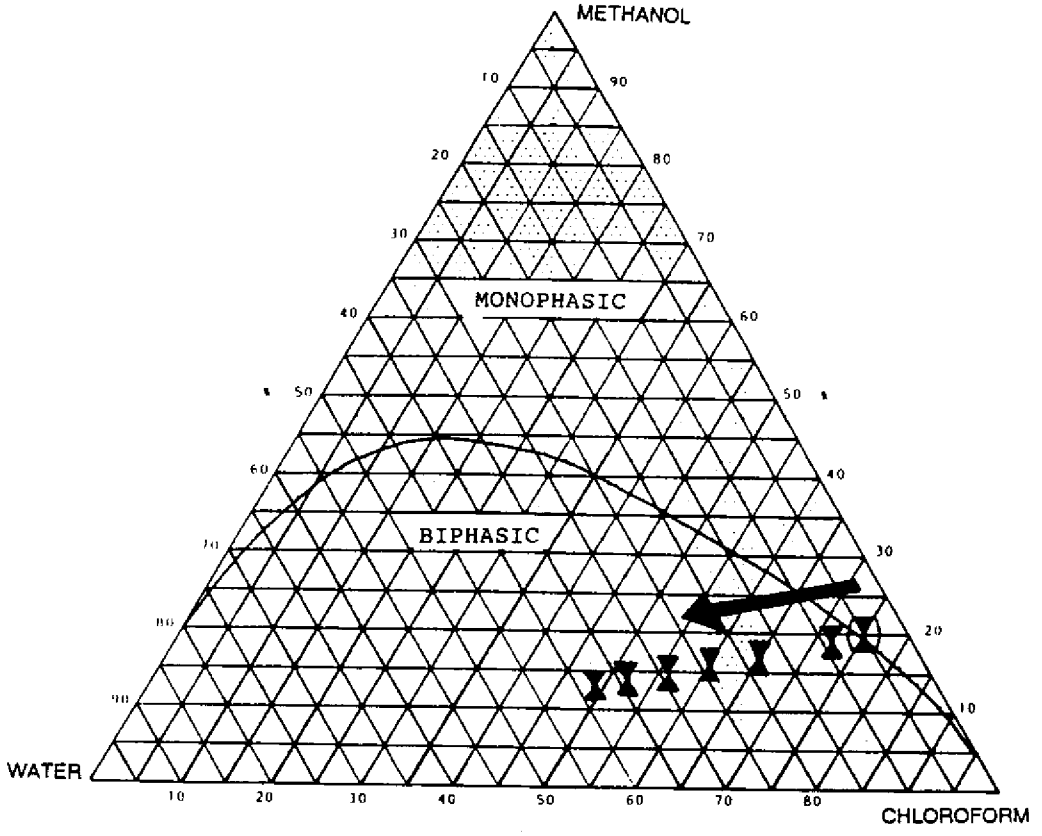
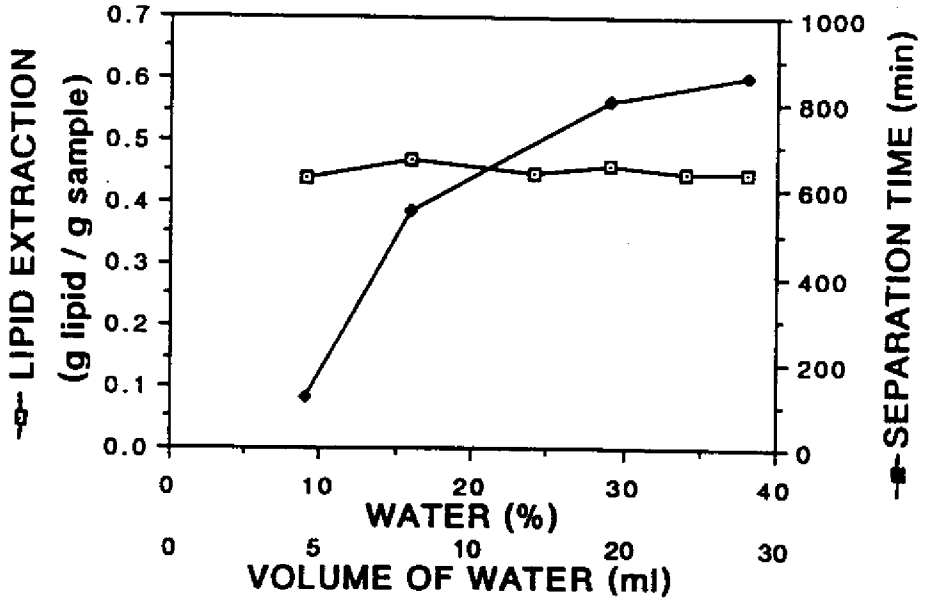


Fig. 5. A chloroform-methanol-water tirnary diagram with % variation in added water (0.5% NaCl solution)

FIG. 6. WATER FRACTION ON THE CHL SEPARATION TIME AND LIPID RECOVERY



CONSTANT SOLVENT COMPOSITION (75:20:5)
CONSTANT SOLVENT:SAMPLE RATIO AT 10:1

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EFFECT OF KOJIC ACID ON PINK SHRIMP PHENOLOXIDASE

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Bisulfites are routinely used in the seafood industry to prevent melanosis, black spot, in shrimp and lobster. Due to health concerns related to residual sulfites, much research has focused on obtaining a bisulfite alternative. A 1% solution of kojic acid, 2-hydroxy-5-hydroxymethly-gamma-pyrone, was found to prevent melanosis in pink shrimp comparable to the customary bisulfite treatment. The effects of kojic acid on pink shrimp phenoloxidase were investigated to evaluate kojic acid as a possible bisulfite alternative.

MATERIALS AND METHODS

Key West pink shrimp, Penaeus duorarum, were dipped in various concentrations of kojic acid and the progression of melanosis followed using the rating scale and method of Otwell and Marshall (1). Phenoloxidase enzyme (PO) was then extracted from pink shrimp by a method similar to that of Rolle et al. (2). Shrimp heads were frozen in liquid nitrogen and ground to a fine powder in a Waring blender. The shrimp powder was added to 0.1 M sodium phosphate buffer, pH 7.2, containing 1 M NaCl (extraction buffer) in a ratio of 1:3 (w/v). An aliquot of 35% Brij 35 was added to give a final Brij concentration of 0.02% (v/v). The suspension was stirred at 0°C for 4 hours and then centrifuged at 23,500g for 30 minutes. The supernatant was fractionated with 40% ammonium sulfate and the fraction precipitating between 0 and 40% saturation was collected by centrifugation as above. The precipitate was then resuspended in extraction buffer and 40% ammonium sulfate. This suspension was homogenized in a glass tissue grinder and centrifuged at 23,500g for 30 minutes. The precipitate was resuspended in extraction buffer and applied to a phenyl sepharose column. The enzyme was eluted in water after a gradient elution profile of 100% extraction buffer, 50% ethylene glycol in water and 100% water.

Basic enzyme assay

A continuous assay was used for measuring enzyme activity. A 70 ul aliquot of the purified enzyme was added to 930 ul of the substrate, 10 mM DOPA, dihydroxyphenyl-alanine, in 0.1 M sodium phosphate buffer (pH 6.5). The formation of dopachrome, which corresponds to the formation of melanin, was monitored at 475 nm with a Beckman Du40 Spectrophotometer. The rate in this study was defined as the change in absorbance (475 nm) per minute. All assays were recorded over 5 minutes at 25°C and run in triplicate.

Kojic Acid Inhibition

The extracted and purified enzyme (200 ul of 13.12 ug/ml enzyme) was added to 10mM D,L-DOPA in 0.1 M sodium phosphate buffer, pH 6.5. The formation of dopachrome was monitored by the increase in the rate of change in absorbance at 475 nm. Increasing concentrations of kojic acid (10, 20, 30, 40, 50, 60 and 80 ug/ml) were then added and the decrease in rate noted.

Reaction of kojic acid with PO over time

In order to determine if kojic acid affected pink shrimp PO over time, kojic acid (25 and 50 ug/ml) was incubated with PO (0.44 ug/ml) for 10, 30, 60 minutes and 24 hours. This mixture was then added to 10 mM DOPA in phosphate buffer (pH 6.5) and the rate of the formation of dopachrome monitored at 475 nm.

Reaction of kojic acid with substrate (DL-DOPA)

To determine if kojic acid reacts with the substrate DOPA, and therefore prevents melanosis, a spectrophotometric scan from 250 to 500nm was made with 4.6 mM DL-DOPA in 0.1 M sodium phosphate buffer, pH 6.5 using a Beckman Du40 spectrophotometer. Shrimp PO (4.4 ug/ml) was added and the scan repeated after a 60 minute incubation period. Kojic acid (19.8 ug/ml) was added and another scan run after a 60 minute incubation period. Also, kojic acid (25 and 50 ug/ml) was incubated with 10 mM DL-DOPA for 10, 30 and 60 minutes and 24 hours. This mixture was then added to 0.44 ug/ml shrimp PO in 0.1 M phosphate buffer (pH 6.5) and the rate of the formation of dopachrome was monitored at 475 nm.

Bleaching of melanin

It was noted that upon treatment with kojic acid, the product whitened. To determine the effect of kojic acid on the preformed melanin, blackened shrimp were soaked in a 10% kojic acid solution for 2 hours. To measure this spectrophotometrically, 140 ul of 10 mM DOPA, 14 ul of 13.12 ug/ml enzyme and 146 ul buffer were mixed and the formation of dopachrome monitored at 475 nm. The substrate:enzyme reaction was allowed to proceed for 15 minutes and kojic acid (1, 10, 25 and 50 ug/ml) was then added.

RESULTS AND DISCUSSION

Extraction and purification

Extraction and purification of the enzyme is summarized in Table 1. Crude extracts were of low specific activity and the majority of the PO was recovered in the 0-40% ammonium sulfate precipitate. Hydrophobic interaction on the phenyl sepharose column resulted in extracts of higher specific activity and an overall 72 fold purification of enzyme activity with a 22% yield of activity.

Kojic Acid Inhibiton

The percent inhibition of phenoloxidase was plotted versus concentration of kojic acid, Figure 1. Phenoloxidase contained 13.12 ug enzyme/ml and the rate of dopachrome formation was completely inhibited with approximately 60 ug/ml kojic acid. This showed that the extracted and purified enzyme was influenced by kojic acid and also gave and idea for concentrations of kojic acid for use in future analyses.

Table 1. Purification scheme for the elution of phenoloxidase from pink shrimp.

STEP	TOTAL VOLUME (ml)	TOTAL PROTEIN (mg)	TOTAL ACTIVITY (units)	SPECIFIC ACTIVITY (units/mg)	YIELD (%)	PURIFICATION (fold)
CRUDE EXTRACT	200	5210	5328	1.02	100	1.0
AMMONIUM SULFATE FRACTION 1	100	946	3120	3.3	59	3.2
AMMONIUM SULFATE FRACTION 2	100	664	2922	4.4	55	4.3
PHENYL SEPHAROSE ELUENT	20	16	1154	72.6	22	72.1

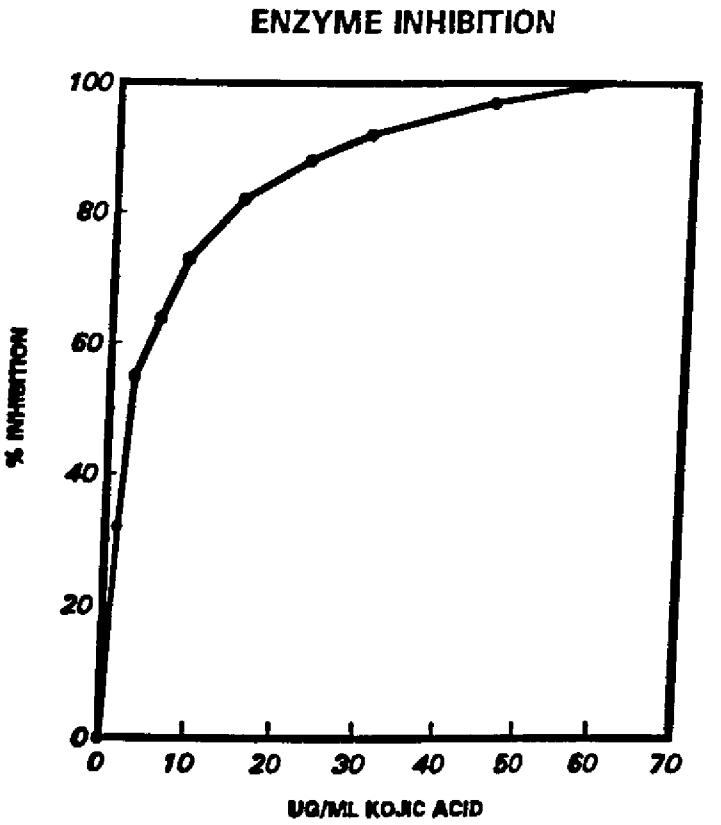

Figure 1. Determination of the amount of kojic acid needed for 100 % inhibition of 0.875 ug/ml pink shrimp phenoloxidase.

Effect of kojic acid on PO over time

When kojic acid was incubated with PO for various times, the kinetic rates obtained were not significantly different, Table 2. This data indicates that kojic acid does not affect the inhibition of the reaction differently if it is added to the PO/DOPA mixture immediately of incubated with the enzyme over a period of time.

Effect of kojic acid on substrate (DL-DOPA)

The absorption spectrum of DL-DOPA remained unchanged following the addition of kojic acid. There was significant reduction in the DOPA peak when PO was added which was expected due to the formation of dopachrome, Figure 2. When kojic acid was incubated with DOPA for various times, a slight increase in the percent inhibition of the PO catalyzed formation of dopachrome was noted, Table 3. At the higher kojic acid concentration, no significant difference in % inhibition was seen. These results suggest that there is no interaction between DL-DOPA and kojic acid.

Table 2. Effect of kojic acid on the phenoloxidase reaction after the incubation of kojic acid with 0.44 ug/ml phenoloxidase and 10 mM DOPA for various times.

Incubation Times	% Inhibition	
	25 ug/ml kojic acid	50 ug/ml kojic acid
1 min	65	82
10 min	69	89
30 min	42	92
60 min	75	93
24 hr	69	89

a Data used in table are average values of triplicate results.
The pooled estimate of standard deviation was = 4.2 %.

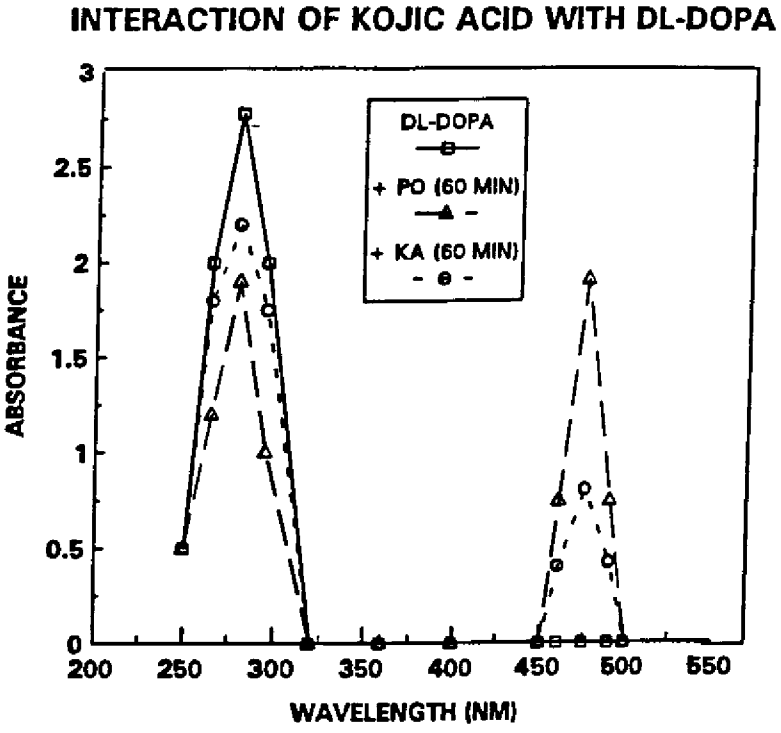

Figure 2. A scan of the wavelength from 200 to 500 nm to determine the interaction of kojic acid with D,L-DOPA.

Table 3. Effect of kojic acid on the phenoloxidase reaction after the incubation of kojic acid with 10 mM DOPA for various times. The concentration of phenoloxidase used was 0.44 ug/ml.

Incubation Times	% Inhibition	
	25 ug/ml kojic acid	50 ug/ml kojic acid
1 min	65	82
10 min	68	82
30 min	69	83
60 min	71	78
24 hr	83	85

* Data used for the table are average values for triplicate analyses. The pooled estimate of the standard deviation was = 3.5 %.

Bleaching experiments

When pink shrimp were allowed to blacken and then soaked in a 10% kojic acid solution, there was a definite reduction of black spots. Upon the addition of increasing concentrations of kojic acid to the PO-DOPA reaction, significant decreases in the absorbance were seen, Figure 3. This suggests that kojic acid acts as a bleach and reduces melanin pigments after they are formed. Kojic acid may also bleach blackspots as they are formed.

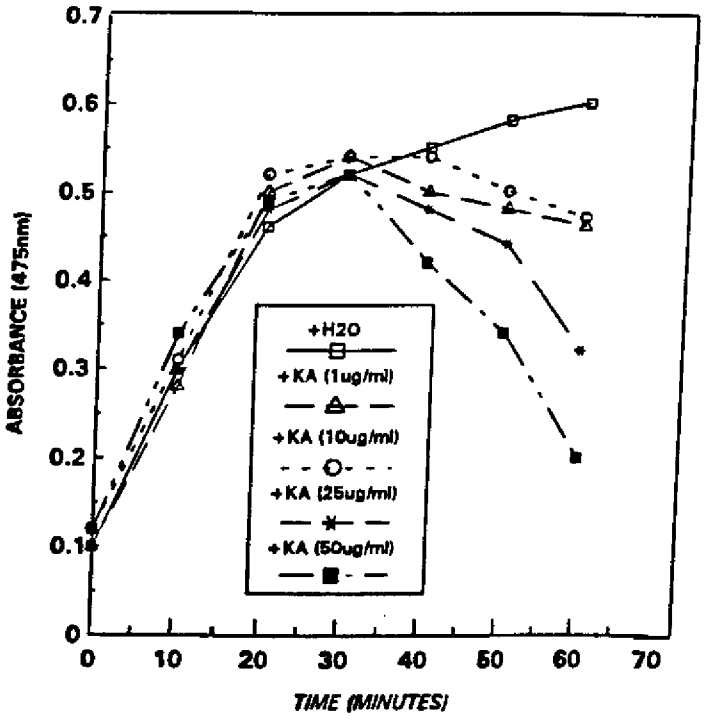

Figure 3. The decrease in absorbance of dopachrome with the addition of various concentrations of kojic acid.

CONCLUSIONS

Kojic acid does inhibit black spot in pink shrimp. A 1% kojic acid solution is comparable to the customary sulfite treatment of shrimp. Approximately 60 ug/ml kojic acid completely inhibits the enzyme activity in 13.12 ug/ml extracted and purified enzyme. Kojic acid does not appear to interact with the enzyme or the substrate over time. Also, melanosis is not affected by the interaction of kojic acid with D,L-DOPA. Kojic acid bleaches the melanin pigments once they are formed and may also inhibit melanosis in this manner.

From this work it was determined that kojic acid does effect pink shrimp PO and may be a possible bisulfite alternative. The toxicity of kojic acid and its production are currently being studied at the University of Florida.

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A NEW PROCESSING AID FOR THE INHIBITION OF SHRIMP MELANOSIS

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Shrimp blackspot, an objectionable surface discoloration, occurs on most commercial shrimp species and decreases the commercial value and consumer acceptance of the shrimp product (1). Blackspot or melanosis is caused by enzymatic formation of the precursors of insoluble polymeric pigments (2). The endogenous shrimp enzyme that catalyzes this reaction, polyphenol oxidase (PPO), remains active during refrigeration, ice storage, and post-freeze thawing. Sulfiting agents have been used since the 1950s to retard blackspot formation (3) and are employed presently on both ice and freezer boats. Current regulations prescribe a dip treatment of 1 minute exposure in 1.25% sodium bisulfite solution with an allowable sulfite residual of 100 ppm (4,5). Adverse health effects associated with consumption of sulfited foods (6), as well as other risks associated with sulfite usage, are well documented. Regulatory pressure to reduce the use of sulfiting agents in foods coupled with consumer awareness of the risks associated with sulfites have led to the need for a safe, functional sulfite alternative for use in the shrimp industry. 4-Hexylresorcinol[1] has been found to be a potent inhibitor of shrimp melanosis and an effective sulfite alternative in both laboratory and field trials.

EXPERIMENTAL PROCEDURES

Procurement of Shrimp

Pink shrimp (Penaeus duorarum) were caught and frozen in Key West, FL and shipped to the University of Florida, Gainesville, FL or to Enzytech Food Ingredients, Cambridge, MA for laboratory trials. Field trials with pink shrimp were performed aboard shrimp vessels based in St. Petersburg or Key West, FL. Fresh Atlantic white shrimp (Penaeus setiferus) were obtained from the Waddell Mariculture Center, Bluffton, SC.

Evaluation of Melanosis Development on Dipped Shrimp

Approximately one pound of shrimp was placed in a nylon mesh bag. The shrimp were dipped into one liter of various test solutions at ambient temperature for one minute. All solutions were prepared in sea water (pH 7.5 - 8) to mimic field conditions and all tests were performed in triplicate. The shrimp were then stored on crushed ice for up to 14 days, a time period typical for shrimp storage. Approximately every other day, the development of shrimp melanosis was evaluated visually by a trained panel and scored according to a previously described system (7) shown in Table I. This rating system can be related to the recommendations of the National Marine Fisheries Service for rating of raw shrimp (8). A score of 4 or greater represents a measurable defect in product quality and a score of 8 or above represents unacceptable product.

[1]Due to the proprietary nature of the 4-hexylresorcinol as a processing aid, the compound was described at the 15th Tropical and Subtropical Fisheries Technological Conference of the Americas under the tradename, EverFresh™.

Table I. Scale used to describe and rate the occurence of melanosis (blackspot) on shrimp.[a]

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

[a]Adapted from (7).

The effective concentration of the inhibitor was determined by dipping shrimp for one minute into solutions containing from 0 to 50 ppm 4-hexylresorcinol. The total amount of shrimp that could be processed using a single solution of 50 ppm 4-hexylresorcinol (the effective concentration of inhibitor, see below) was estmated by repeated dipping of different one pound lots of shrimp into the same one liter solution of inhibitor. In both cases the shrimp were stored and rated as described above.

4-Hexylresorcinol Residual Determination

Residuals of the inhibitor on the edible portion of the shrimp were determined by dipping shrimp into solutions of radioactively labelled 4-hexyl-[14C]-resorcinol, digesting the meat of the treated shrimp, and quantifying the amount of labelled compound present. The 4-hexyl-[UL-14C]-resorcinol was synthesized according to a previously published procedure (9) utilizing [UL-14C]-resorcinol. The residual analyses examined the effect of several variables including dipping of previously frozen vs. fresh shrimp, concentration of inhibitor (50 vs. 100 ppm), heads-on vs. headless shrimp, post-treatment rinsing and/or cooking, extended dip times, storage time, and multiple dips. Data on frozen shrimp (P. duorarum) were acquired for at least 12 samples and from 16 to 24 replicate samples for fresh shrimp (P. setiferus). Following exposure to the radiolabelled inhibitor under the various test conditions, the shrimp were peeled and the shrimp meat was solubilized in Solvable- tissue solubilizer (DuPont NEN, Boston, MA). The solubilized shrimp meat was then added to scintillation cocktail. The samples were placed in a scintillation counter for 30 minutes before they were counted for one minute each. Control experiments employing ten control shrimp and ten shrimp "spiked" with known amounts of 4-hexyl-[UL-14C]-resorcinol showed recovery of added counts from shrimp meat to be 100 2%.

4-Hexylresorcinol Stability in Sea Water

The stability of 4-hexylresorcinol in sea water was determined by high performance liquid chromatography (HPLC). A 50 ppm solution of 4-hexylresorcinol in sea water was prepared and allowed to stand at room temperature. Aliquots were removed at various time points for up to 24 hours and analyzed by reverse-phase HPLC (10).

RESULTS AND DISCUSSION

4-Hexylresorcinol Inhibitory Potency

The results of the inhibition of pink shrimp melanosis as a function of 4-hexylresorcinol concentration (11) is shown in Figure 1. Melanosis develops rapidly in the control shrimp exceeding the target limit score of 4 between 3 or 4 days storage time. The traditional 1.25% (12,500 ppm) bisulfite treatment shows some inhibition of blackspot development; however, the bisulfite-dipped samples exceed the target limit after 7 days. The degree of inhibition seen with 5 ppm 4-hexylresorcinol is comparable to the 12,500 ppm bisulfite dip. Thus, a 2500-fold lower concentration of 4-hexylresorcinol (by a simple weight-to-weight comparison) is as effective as 12,500 ppm bisulfite. Concentrations of either 25 ppm or 50 ppm 4-hexylresorcinol are effective in preventing melanosis development beyond the target limit. A concentration of 50 ppm 4-hexylresorcinol was chosen for subsequent experiments.

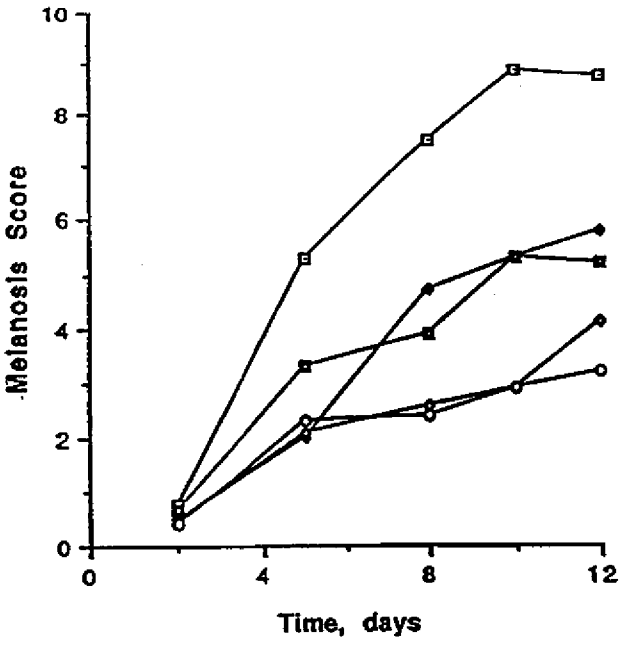

Figure 1. The inhibition of pink shrimp (P. duorarum) melanosis as a function of 4-hexylresorcinol concentration. Pink shrimp were dipped into sea water (—□—), 12,500 ppm sodium bisulfite (—●—), and 5 (—■—), 25 (—◆—) and 50 ppm (—○—) 4-hexylresorcinol as described in Materials and Methods.

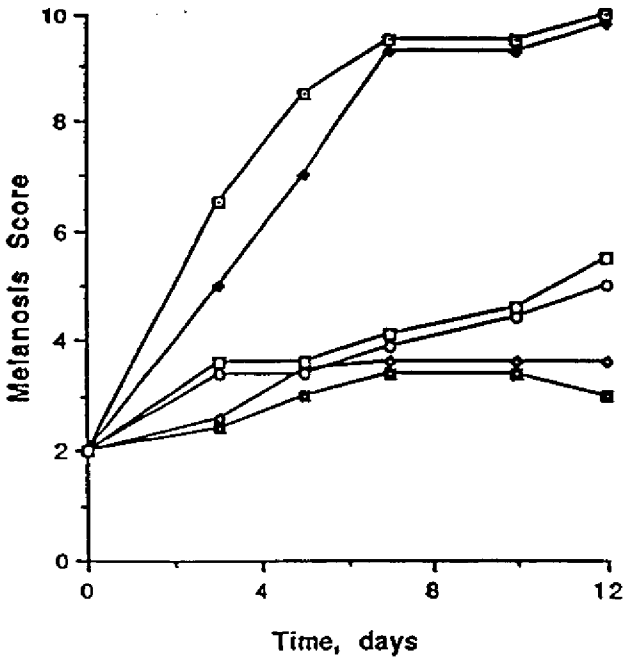

Figure 2. Inhibition of pink shrimp (P. duorarum) melanosis after one (—▫—), four (—◇—), eight (—○—), and twelve (—□—) dips of different one pound lots into the same 50 ppm 4-hexylresorcinol solution as described in Materials and Methods. Sea water (—▫—) and 12,500 ppm sodium bisulfite (—●—) dips are included for comparison.

The total amount of shrimp that could be processed using a single solution of 50 ppm 4-hexylresorcinol was estimated by dipping multiple one pound lots of shrimp into the same test solution. The results are presented in Figure 2. It should be noted that this test was performed with previously frozen shrimp that develop a low level of melanosis upon thawing and give rise to a score of 2 at the initiation of the test. Inhibition of blackspot formation is equivalent from the first dip to the fourth dip; however, blackspot formation does approach the target limit at the end of the 12 day storage period for the eighth and twelfth dip samples. The control samples display a rapid onset of melanosis exceeding a score of 4 by the third day of storage. Assuming a maximum of 10 dips per batch of 50 ppm 4-hexylresorcinol and a linear relationship between the small scale test solutions and actual dip tank usage (50-60 pound/25-30 gallons dip solution), this corresponds to roughly 600 pounds of shrimp processed with a single preparation of inhibitor solution. Hence, the entire daily shrimp catch of a typical fisherman could be processed with one batch of 50 ppm 4-hexylresorcinol.

Parameters such as fresh versus sea water solutions of 4-hexylresorcinol, post-treatment rinsing of dipped shrimp, extended dip times, and the pH of the dip solution were also examined. No differences were observed whether the test solution was prepared with fresh or sea water or whether shrimp received a post-treatment rinse. Dipping the shrimp for 15 minutes did not yield improved results when compared to a one minute dip. Pink shrimp dipped in 50 ppm 4-hexyl-resorcinol solutions with pH values of 2, 5, and 8 showed no difference in functionality across this pH range. In addition to pink shrimp, 4-hexylresorcinol is functional on all shrimp species tested to date including brown (Penaeus aztecus), Kuruma (Penaeus japonicus), nylon (Heterocarpus laevigatus) and rock shrimp (Sicyonia brevirostris) (12).

4-Hexylresorcinol Residuals on Dipped Shrimp

The use of 4-hexylresorcinol for the inhibition of shrimp melanosis results in extremely low residuals on the shrimp meat (13). No significant changes in residual levels as a function of storage time were observed, therefore, the values presented here are the means for specific sample sets over the entire storage period. Under proposed use conditions (one minute dip in 50 ppm 4-hexylresorcinol) the values for residuals on previously frozen pink shrimp were 0.6 0.17 and 1 0.3 ppm for heads-on and headless, respectively. Fresh Atlantic white shrimp exhibited residuals of 0.0.4 0.09 ppm for heads-on samples and 0.9 0.27 ppm for headless. Post-treatment rinsing of dipped samples did tend to reduce the residual values in certain treatment scenarios whereas cooking had no effect. A second one minute dip into 50 ppm 4-hexylresorcinol did not increase the residuals; however, increased residuals were found on shrimp that were dipped for 15 minutes into 50 ppm 4-hexylresorcinol or for one minute into 100 ppm 4-hexylresorcinol. The highest residuals, 2 0.8 were obtained for previously frozen, headless pink shrimp dipped for 15 minutes in 50 ppm 4-hexylresorcinol without a post-treatment rinse.

Due to the low residuals of 4-hexylresorcinol, the taste, texture, visual appearance, and normal development of pink color upon cooking are unaffected by dipping in 4-hexyl-resorcinol. A method for the determination of non-radio-labelled 4-hexylresorcinol residuals on shrimp meat by organic extraction and high performance liquid chromatography has been developed (10).

Stability of 4-Hexylresorcinol in Sea Water

4-Hexylresorcinol is very stable under proposed use conditions. HPLC analysis of 50 ppm 4-hexylresorcinol in sea water (11) shows that greater than 95% of the inhibitor is recoverable 24 hours after preparation of the test solution. Organic matter introduced into the solution by dipping of shrimp had no effect on the stability of 4-hexylresorcinol during this time period (data not shown).

When compared to sulfiting agents, the stability of 4-hexylresorcinol is significant. It is interesting to note that the bisulfite-dipped samples shown in Figure 2 developed blackspot to the same degree and at a similar rate as the control samples. The traditional bisulfite treatment appeared to yield very inconsistent results from test to test even though all solutions were prepared from the same lot of sodium bisulfite. The reason for this inconsistency in performance has not been determined but may be related to the fact that all test solutions were prepared in sea water. Due to its high chemical reactivity, the bisulfite may have reacted with the differing levels of organic matter in the various sea water stocks, affecting its usefulness in the prevention of blackspot.

Safety Evaluation of 4-Hexylresorcinol Usage

4-Hexylresorcinol has been studied for a broad range of toxicological concerns including acute and subacute toxicity, subchronic toxicity, carcinogenicity, mutagenicity (14), and allergenicity (15). The extensive toxicological data on 4-hexylresorcinol have been the subject of a recent review (16). In light of the extremely low residuals on shrimp meat, these studies indicate that the use of 4-hexylresorcinol as a processing aid for the inhibition of shrimp melanosis is safe.

In addition to its functionality at very low concentrations (50 ppm 4-hexylresorcinol vs 12,500 ppm sulfite) and low residuals on shrimp meat (~1ppm), 4-hexylresorcinol has several other advantages over sulfites. Sulfites can "bleach" blackspot encouraging the use of replicate treatments, dips into high concentrations, or even the broadcasting of solid sodium bisulfite onto the shrimp product. 4-Hexylresorcinol does not bleach blackspot and thus, use of excessive

amounts of the inhibitor does not improve the appearance of the shrimp. Since the recommended one minute dip time is adequate for maximal inhibitory effect, dipping of shrimp for longer time periods is not advantageous. Also, sulfites are unstable and decompose to sulfur dioxide gas upon contact with moisture. The exposure of fishermen to high concentrations of sulfur dioxide vapors in the boat hold have resulted in several deaths during actual fishing operations.

CONCLUSIONS

4-Hexylresorcinol is a safe, effective processing aid for the inhibition of shrimp melanosis in all commercial species tested and is applicable to pond-raised as well as trawled shrimp. A one minute dip into a 50 ppm 4-hexylresorcinol solution in sea water is sufficient to inhibit blackspot and maintain a high quality shrimp product for at least twelve to fourteen days. A single dip tank (25-30 gallons) of 50 ppm 4-hexylresorcinol will process 500 to 600 pounds of shrimp, a typical night's catch. 4-Hexylresorcinol is water-soluble, chemically stable, functional at low concentration and residuals on shrimp meat are extremely low. 4-Hexylresorcinol can be used under the same treatment scenarios presently employed for the inhibition of of shrimp blackspot by simple substitution for bisulfite in the dip tank. The use of 4-hexylresorcinol does not necessitate any changes in the post-treatment storage, shipping, or processing of the shrimp product. Due to the numerous safety and functional advantages, 4-hexylresorcinol should prove to be an effective alternative to the use of sulfites in the shrimp industry.

ACKNOWLEDGMENTS

The authors wish to thank Barry Gosselin and Craig Bohmont for leading the sensory panels, Joan M. King for assistance in HPLC analyses, and Paul Sandifer, Steven Hopkins, May Baird and the staff of the Waddell Mariculture Center for providing access to fresh maricultured shrimp. We would also like to thank Bobby Harriman of Bama Sea Products, Inc. and Bobby Canady of Singleton Seafoods, Inc. and the boat captains, Jimmy Hickman of Key West, and John Williams of Holiday, FL, for their assistance in the performance of field trials. This research was funded in part by Sahlman Seafoods, Inc. and the continued support and assistance of William Sandman and Jack Sahlman of Sahlman Seafoods, Inc. is greatly appreciated.

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PHOSPHATE USE IN PROCESSING SEA SCALLOPS, Placopecten magellanicus IN THE MID-ATLANTIC REGION

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INTRODUCTION

The use of sodium tripolyphosphate (STP) in the sea scallop industry has become widespread. Benefits of using phosphates on sea scallop meats include the reduction of drip loss in both fresh held and frozen/thawed meats and maintenance of meat texture and color. Present phosphate application is via a soaking medium in which scallop meats are typically soaked 18-21 hours.

The sea scallop, because of its given textural characteristics and capacity to absorb water, is very susceptible to abuse under current industry practices. Industry usage levels of STP range from 2-8% by weight. The absence of a standard application of STP by industry causes considerable variation in product quality. Further complicating the problem is that phosphate effectiveness on scallop meat fluctuates with seasonal variations corresponding to spawning activities. Mid-Atlantic sea scallops spawn twice annually, in the spring and again in the late fall. Current research suggests that swell potential and the effectiveness of phosphate usage on scallop meats is dependent upon scallop meat condition which varies between pre- and post-spawning.

Results of phosphate utilization depend on phosphate concentration, the usage of salt in medium, dwell time in soaking medium, and the time of year processing occurs. This paper provides preliminary results of an on-going study on optimum phosphate use in processing sea scallops. Specifically, processing enhancements and abuses to scallop quality during pre- and post-spawning periods are illustrated and discussed.

MATERIALS AND METHODS

In conforming to current industry practices, light density, granular, food grade sodium tripolyphosphate (STP) was utilized and application was via a 21 hour soak period. Results of initial investigations demonstrated STP concentrations above 5% by weight were excessive given industry economic concerns and resulting deterioration of perceived product quality. Therefore, STP concentrations of 1-5% by weight with 1% food grade NaCl were investigated. In addition, treatments of freshwater (FW), STP without NaCl, and STP plus 1% NaCl were utilized to illustrate scallop meat swell potential and phosphate/salt synergism.

Freshly shucked scallop meat pH ranged from 6.1-6.3 at 14°C. Treatment solutions ranged from pH 2.26-9.40 at 14C (Table 1).

Soaking mediums were prepared with tap water (14 C), distributed to polyethylene containers to a predetermined amount equaling a 2:1 ratio by weight of solution to scallops, and chilled to 11-12 C. Containers were kept on ice for the duration of soaking.

Table 1. Specific gravity and pH of tested solutions.

	1% NaCl	1% STP	2.5% STP	FW	% STP + 1% NaCl 1	2	2.5	3	4	5
pH (14°C)	7.40	9.40	9.16	7.26	8.92	8.97	8.98	8.98	8.96	8.94
sp. gravity (23°C)	1.007	1.010	1.022	1.001	1.017	1.024	1.029	1.033	1.041	1.049

Scallop shellstock was obtained from commercial vessels in March (pre-spring spawn period) and in May (post-spring spawn period) of 1990. Scallop meats were shucked from shell stock (90-110 mm in shell height), rinsed in freshwater for 10 seconds to remove sand particles, and drained for 5 minutes. Three one pound samples of meats were placed in polyethylene 1/2 inch mesh bags and placed into each container of solution. Control groups (no treatment) were selected and frozen. Scallop meats were soaked in their respective mediums for 21 hours and periodically mixed.

Post-treatment analysis included drained weights, freeze/thaw weights, cooked weights, and organoleptic evaluations of thawed and cooked product. Once removed from soaking mediums, each scallop sample was drained on a half inch plastic grid drain rack for 2 minutes, pat-dried with 100% cotton towels, weighed, placed in a plastic freezer bag, commercially blast frozen to a temperature of -34 C, and placed in commercial cold storage at -23 C. After 30-35 days of frozen storage, samples were thawed by water submersion. Tap water at 20-22 C was introduced to the trough at a rate of 2 gpm. Samples were completely thawed within 2 hours and 15 minutes. Thawed samples were emptied onto and pat dried with cotton towels, reweighed, evaluated organoleptically, and placed into freezer bags for cooking. Cooking was performed by the boil-in-the-bag method. Trial cookings, monitored with thermocouples, indicated an average cooking time of 4.5 minutes was required for the center of scallop meats to reach prescribed temperatures of 73 C (1). Upon cooking, scallop odor was initially evaluated with the opening of each sample bag. Scallops were drained for 2 minutes on 1/2 inch plastic grid, weighed, and further evaluated in regards to appearance, texture, and taste.

Organoleptic evaluations

Organoleptic evaluations were performed on all frozen/thawed and cooked scallop samples. Samples were rated on a five point scale representing scallop characteristics which are most commonly perceived as quality determining characteristics (Tables 2-3). Each scale consists of a five part rating, ratings 2-5 consisting of two elements. The first element pertains to phosphate treated meat characteristics, and the second element pertains to freshwater treated and control meat characteristics. Both thawed and cooked rating scales were based on quality attributes of fresh post-rigor scallop meat, not on a control (i.e. no treatment but processed sample).

Table 2. Organoleptic evaluation criteria; Previously frozen, RAW: scale 1-5.

1.	Appearance, color, odor, and texture same as fresh post-rigor scallop meats.
2.	Color, odor, and texture similar to fresh samples, with slight glassy appearance and slipperiness; or slight discoloration, stale odors, and dryness.
3.	Slight surface translucency, slight to moderate slipperiness and glassy appearance, slight foam; or slight to moderate discoloration, stale odors, and dryness.
4.	Moderate surface translucency and slipperiness, glassy appearance, slight soapy odor, moderate foam; or moderate discoloration, stale odors, and dryness.
5.	Extreme surface translucency and slipperiness, glassy appearance, dominant soapy odor, excessive foam; or extreme discoloration, stale odors, and dryness.

Table 3. Organoleptic evaluation criteria; Previously frozen, COOKED: scale 1-5.

1.	Appearance, color, odor, texture and taste same as cooked fresh post-rigor scallop meats.
2.	Appearance, color, odor, and taste similar to fresh sample, texture slight spongy; or slight dryness and odor.
3.	Appearance, color, odor, and taste similar to fresh sample, texture moderately spongy; or slight stale odors, discoloration, dryness, and slight bland or stale tasting.
4.	Moderate off-odors and/or discoloration, moderate spongy texture, slight soapy taste; or moderately dry, chewy, and bland or stale tasting.
5.	Extreme off-odors and/or discoloration, spongy texture, soapy taste; or extreme discoloration, stale odors, dryness, chewiness, and bland or stale tasting.

RESULTS AND DISCUSSION

Initially, criteria for evaluation of various STP concentrations needed to be established. Recommendations derived from this work were borne with product quality consistency in mind given current industry practices. Therefore, evaluations were based on the performance of the following: (i) treatment-thaw component; (ii) total weight change (initial weight-cooked weight/initial weight); (iii) organoleptic rating at the thawed and cooked state (Tables 4-5). Treatment-thaw relates to initial processors and wholesalers concerns, while total weight change represents the resulting product to the end user. A total weight change at or near zero suggests quality maintenance in the respect of natural juices, vitamins, and soluable proteins being retained, while nothing was added.

Phosphate concentration from 1-5% are represented with the addition of 1% NaCl. One percent NaCl, 1% STP, and 2.5% STP treatments were utilized to illustrate the synergism that exists between STP and salt, and freshwater was used to demonstrate the swell potential of sea scallops and the loss of quality associated with this soaking practice. Organoleptic ratings were assigned to all treatment groups.

Weight changes

Percent weight gain and percent thaw and cooked losses decreased with increasing STP concentrations for the pre- and post-spring spawn periods (Tables 4-5). One percent STP demonstrated the most variation in weight changes throughout processing. Near maximum uptake was experienced coupled with extreme thaw drip loss. Thaw drip loss became less significant at 2% STP and minimal by 5% STP. Treatment-thaw components illustrated performance to be optimal between 2-2.5% STP for pre-spawning periods and between 3-4% STP for post-spawning period. However,

there was a significant transition point occurring in respect to thaw drip loss between 2.0 and 2.5% for pre-spawn period, and between 2.5 and 3% for post spawn period. Total percent weight change nearest to zero was achieved at 2.5% STP during both periods.

Weight changes associated with 4-5% STP and 1-3% STP levels exhibited minimal and maximum variation, respectively. A shift in performance of 1% and 2.5% STP was observed between testing periods (Figures 1-2). Pre-spawning period results indicate phosphate to be more effective in controlling freeze/thaw drip loss than during post-spawning.

Weight changes associated with the control groups revealed greater thaw drip associated with the post-spawn period. This may explain the increase in thaw drip loss consistently observed over all treatments during post-spawn.

Phosphate-salt relationship

Addition of salt into STP solutions increased scallop meat water binding (Figure 3). The use of phosphates or salt alone provided for near maximum weight gains during soaking. However, both of these treatments also produced equally excessive drip losses. Moisture retention is largly associated with the degree of surface protein solubilization. Solubilizing proteins increase meat fluid viscosity which helps to prevent water loss (2,3,4). Proteins effectively become more solubilized when ionic strength and pH of the solution is raised.

The ionic strength of phosphate solution was raised by the addition of NaCl, resulting in increased moisture retention. Alternatively, increased retention experience by the addition of STP to a 1% NaCl solution could be more related to the rise in pH of the resulting solution (Table 1). Either way, the combination of STP and NaCl increased water retention more than each one independently.

Figure 1. Weight Changes Associated With Varying STP Levels

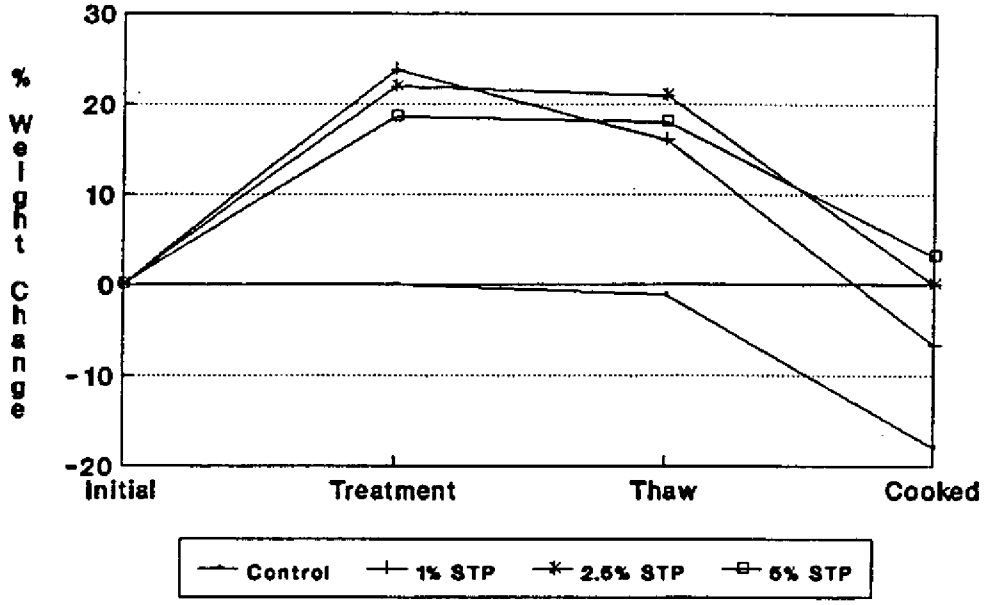


Figure 2. Weight Changes Associated With Varying STP Levels

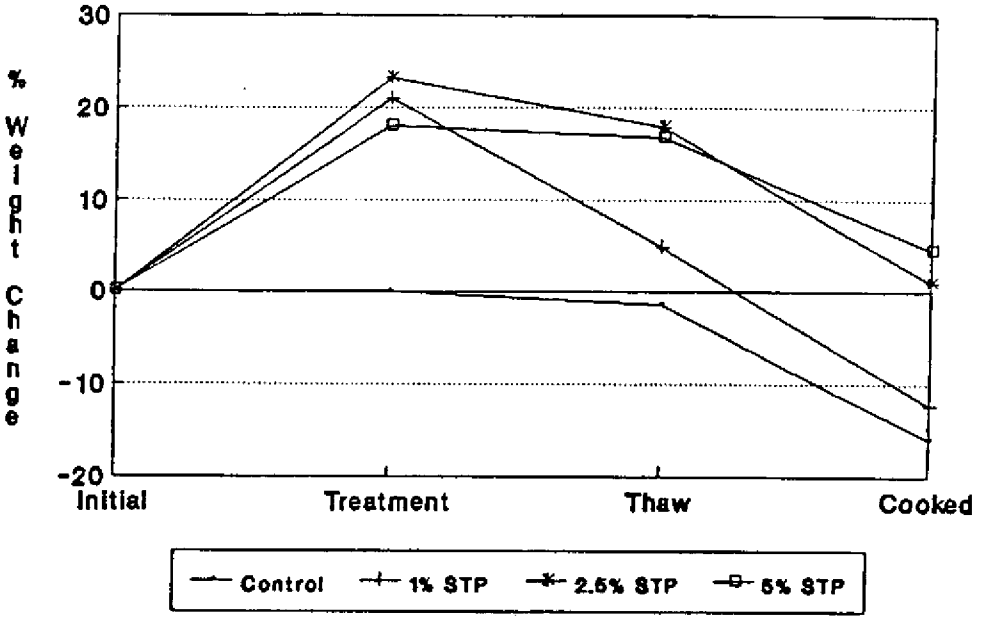
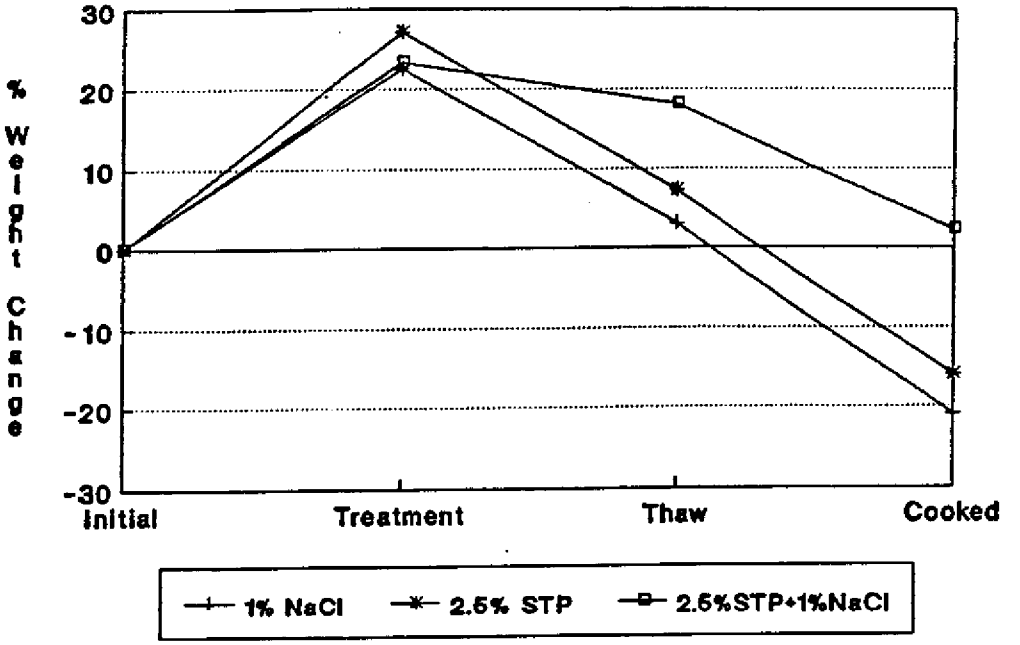


Figure 3. Phosphate-Salt Relationship
(Post-spawn condition)



In this study, increasing pH with phosphates and increasing ionic strength of solution with NaCl maximized water binding and retention. The effect of increasing ionic strength by the addition of phosphate may also explain the differences in drip loss between 1% STP plus 1% NaCl and 2.5% STP plus 1% NaCl solutions when compared against pure STP or NaCl solutions alone (Table 4). Increasing the STP level from 1% to 2.5%, in combination with 1% NaCl, provided superior water retention capabilities.

Organoleptic evaluation

Ratings of perceived quality characteristics were assigned to all treatments (Tables 4-5). Thawed and cooked product quality declined with increasing concentrations of STP for pre- and post-spawn scallop meats. As STP levels increased, translucency, glassy appearance, slipperiness, and foaming increased on thawed meats and spongy texture, soapy taste, and moderate off-odors developed in the cooked meats.

Quality transition points were observed in both thawed and cooked ratings. In the thawed state, noticeable differences were observed in meat translucency, slipperiness, and foaming between 2.0 and 2.5% STP for the pre-spawn scallops, and between 2.5 and 3.0% for the post-spawn scallops. In the cooked state, slight differences were observed in texture between 3 and 4% STP for both spawning conditions.

Control groups displayed equally slight discolorations, dryness, meat shrinkage, and stale odors of thawed and cooked meats. Preliminary tests consistently demonstrated control groups to be adversely affected by processing. The decision to base organoloptic evaluations on fresh post-rigor scallop meat rather than on control processed scallop meats was determined from the preliminary tests.

Freshwater treatment

Freshwater treated meats generated the poorest quality characteristics and ratings (Tables 4, 5). Upon soaking and prior to freezing, these meats displayed various signs of perceived quality abuse including cottony appearance, muscle bundle separation, translucent edges, dry surface, and bland odor. Maximum weight changes were experienced throughout processing, and negatively affected organoleptic ratings. Thawed meats showed slight browning, stale odors, and a dry appearance. Cooked meats were moderately dry, bland or stale tasting, and chewy. Moisture loss at thawing and cooking was extreme during both studies. Trial cookings revealed accelerated cooking; therefore, cooking time was reduced to 4 minutes.

CONCLUSIONS

Results from this study can be perceived differently depending on economic goals or attitudes about phosphate use on seafood in general. The following conclusions are based on current industry concerns and practices, and consumer acceptance.

Examination of the 4-5% STP level demonstrated moderate weight (water) uptake with minimal thaw and cook drip loss, resulting in water weight being passed on to the consumer. More important, perceived quality characteristics of these meats suffered extensively in comparison to lower STP levels. On the other hand, 1% STP provided for near maximum water uptake but also with excessive drip losses. With the loss of added water, natural juices and vitamins are also lost, resulting in a lower quality product.

Sodium tripolyphosphate levels of 2.0-3.0% plus 1% NaCl provided the best results in both study periods. The addition of 1% NaCl to STP solutions should be used in processing to maximize water binding capacity. A 2-3% STP solution was observed to achieve moderate

Table 4. Average percent weight change and organoleptic rating of processed pre-spawn sea scallop meats.

Percent Weight Change	Control	1% NaCl	1% STP	Fresh Water	% STP + 1% NaCl					
					1.0	2.0	2.5	3.0	4.0	5.0
Weight gain (21 hr)	--	24.9	29.9	26.5	23.8	22.6	22.1	20.5	18.7	18.5
Thaw loss	0.8	10.6	12.2	18.4	6.3	1.4	0.8	0.7	0.5	0.5
Thaw rating	3	1	1	3	1	2	3	3	3	4
Treatment thaw	-1.1	14.3	17.7	8.0	17.6	21.2	21.2	19.8	18.2	18.0
Percent cooked loss	17.0	28.6	28.0	40.4	24.2	22.1	21.4	20.0	15.1	12.5
Cooked rating	3	1	2	4	2	2	2	2	3	3
Total percent weight change	-18.1	-14.4	-10.2	-32.4	-6.6	-0.88	0.0	0.1	3.1	5.5

Table 5. Average percent weight change and organoleptic rating of processed post-spring spawn sea scallop meats.

Percent Weight Change	Control	1% NaCl	1% STP	2.5% STP	Fresh Water	% STP + 1% NaCl				
						1.0	2.0	2.5	3.0	4.0	5.0
Weight gain (21 hr)	--	23.5	21.7	27.2	26.5	21.0	--	22.5	23.1	20.5	18.0
Thaw loss	1.3	16.7	16.3	15.7	24.2	13.2	--	4.3	1.8	1.2	1.1
Thaw rating	2	1	1	1	3	1	--	2	3	3	4
Treatment thaw	-1.3	3.0	1.9	7.2	-4.2	4.9	--	18.0	20.8	18.9	16.6
Percent cooked loss	16.0	23.3	27.8	21.6	31.2	16.3	--	14.6	14.9	12.8	10.8
Cooked rating	3	2	2	3	4	2	--	2	2	3	3
Total percent weight change	-17.1	-20.9	-26.5	-15.9	-34.1	-12.2	--	1.1	2.8	3.8	4.5

uptakes together with moderate drip losses relative to other treatments. This resulted in total weight changes at or near zero. During periods prior to spawning, more favorable weight changes and organoleptic ratings were observed utilizing a 2.0-2.5% solution. During periods after spawning STP performance shifted; favorable results were obtained with 2.5-3.0% solutions.

Organoleptic rating provided useful criteria in the evaluation of various phosphate levels. The primary focus in determining scallop quality by primary processors through end users most often lies in how the product is grossly perceived. The proper usage of phosphates can enhance this perceived quality while misuse can detract from it.

The use of freshwater alone should not be used in processing sea scallops. Rapid and excessive hydration is experienced with no moisture binding properties to control drip loss. Quality characteristics suffer tremendous losses throughout all processing stages.

Phosphate utilization in the processing of sea scallop meats produces various results depending on phosphate concentration as well as salt incorporation. The goal of this initial work was to demonstrate phosphates benefits and drawbacks in the hope of developing standard procedures within current industry practices which will provide for a more consistent, quality product. Further work will focus on economic evaluations pertaining to STP usage levels, alternative phosphate incorporation methods, and lower pH phosphate blends.

Support for this work was provided by the Virginia Sea Grant Marine Advisory Program, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, Virginia.

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USE OF BICARBONATE DIP TO IMPROVE WATER BINDING CAPACITY IN FRESH SHRIMP

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Shrimp is one of the most popular and valuable fishery products in the U.S. In 1989, 360 million pounds, valued at $580 million, were landed in the U.S. and per capita consumption was 2.3 lbs (1). To meet this strong demand, an additional 585 million pounds of frozen shrimp were imported.

One of the most important economic problems facing the shrimp industry is drip loss. Because shrimp has a high water content and commands a relatively high price per pound, weight loss due to drip is a major source of profit loss.

There has been considerable research on and patent activity for the reduction of drip loss in shrimp, especially frozen shrimp where the weight loss is even greater than in fresh shrimp (5, 6, 7, 8). Most existing literature deals with the applications of phosphates and polyphosphates, usually as solutions of about 6%. Abuse in the concentration and time of phosphate dips result in absorption of too much water and a spongy, poor texture.

An alternative to phosphates which would avoid their inherent problems and still result in water retention in shrimp is of interest to not only the shrimp industry but consumers as well. Previous work by Curran, Tepper, and Montville (2, 3) in the Department of Food Science at Rutgers University has demonstrated that bicarbonates extend the microbial shelf-life of cod fillets and increase its water-binding capacity, thereby reducing drip loss.

The mechanism by which phosphates increase water binding is well understood. Phosphates alter the isoelectric environment of the fish muscle so that the fibers swell and physically trap more water. Bicarbonates elevate the pH of fish protein to approach its isoelectric point, and thus act via the same mechanism of muscle swelling. The previous work by Curran, et al. (3) with ammonium and sodium bicarbonate showed no anion effect and little concentration dependence above 1%. The increased water binding translated to improved texture in both objective and subjective tests.

In addition to the obvious economic benefit of retaining water, the increased water binding capacity improves the shrimp's texture. Hebbar and Hiremath (4) demonstrated that the improved taste and texture of shrimp dipped in bicarbonates prior to freezing was maintained even after six months of frozen storage. Thus, the application of bicarbonates may be able to minimize drip loss and improve quality characteristics such as texture and microbial quality of fresh shrimp.

This paper reports a preliminary effort to elucidate an optimum practical protocol for the use of bicarbonate dips to improve the water-binding capacity of fresh shrimp and to determine the influence of bicarbonate dips on microbial quality, shelflife, and sensory attributes of fresh shrimp.

MATERIALS AND METHODS

Fresh (never frozen), domestic white shrimp were utilized. Sizes ranged from 21-25/lb. to 31-35/lb. All shrimp were head-off. Both shell-on and shell-off shrimp were investigated.

Four shipments of shrimp were received. The first from a primary wholesaler in Florida via a central New Jersey seafood retailer, the second from a processor in Brunswick, Georgia, the third from the Georgia Sea Grant Marine Advisory Service, and the fourth from a primary wholesaler in Brunswick, Georgia. For all shrimp orders, specifications included "no chemical treatment".

All shrimp were shipped over night on ice and, upon receipt, were refrigerated at 4°C and experiments were begun within 24 hrs. Plate counts were conducted at 0, 24, and 48 hrs., and experiments were conducted to evaluate sodium bicarbonate dips.

Initial plate counts were conducted on the shrimp as received by blending 50 gr. of the shrimp with 450 ml. of cold 0.1% peptone water for 2 minutes. Total bacterial growth was determined by standard plate counts using Plate Count Agar (Difco). Plates were incubated for 2 days at 25°C. Microbial growth on the shrimp was determined at 0, 24, and 48 hrs.

To determine the optimum sodium bicarbonate concentration for minimum drip loss, the drip loss of shrimp dipped in 1, 2, 4, and 8% sodium bicarbonate was evaluated. The various bicarbonate concentrations were compared to untreated shrimp and shrimped dipped in a deionized water, deionized water adjusted to the pH of saturated sodium bicarbonate (pH 8.8), 1.25% bisulfite, and 6% tripolyphosphate. The bisulfite and tripolyphosphate dips are commonly used by the shrimp industry to inhibit melanosis and water loss respectively.

The shrimp were weighed, dipped for one-half hour in solutions as indicated, drained in a stainer, and reweighed. The shrimp were then stored at 4°C and analyzed as described below.

Cooked drip values were obtained by placing 50 gr. raw shrimp in 250 ml. of boiling water, waiting for the water to resume boiling, and then plunging the shrimp into cold water to stop the cooking after 3 minutes. The shrimp were drained in a strainer and weighed again. Cooked weight was determined on both day 1 and after 7 days of refrigerated storage. Percent cooked weight was the weight of drained shrimp divided by the original sample weight, with the resulting weight multiplied by 100.

RESULTS

Trial 1.

The first shipment of shrimp was received with a very marginal initial microbial load of 1.8×10^7 colony forming units (cfu) per gram. The normal weight change pattern observed was a weight gain during dipping and subsequent weight loss during cooking (Table 1). This pattern was followed by all the shrimp in trial 1 except the untreated shell-on shrimp, which actually gained weight during cooking. Desiccation of the original low quality shrimp might explain this unexpected result. There was little difference in the cooked weight of the shell-on shrimp on either day 1 or after one week of refrigerated storage.

In the shell-off shrimp, however, shrimp treated with bicarbonate and tripolyphosphate dips lost less weight on cooking than the control shrimp (Table 1). This trend increased during storage. After a week, the shrimp treated with 8% sodium bicarbonate were 104% of their original weight and the untreated shrimp were 84% of their original weight. Therefore, after a week of refrigerated storage, the shrimp treated with 8% sodium bicarbonate retained 20% more water than the untreated shrimp.

Table 1. Weight retention of raw and cooked shrimp, trial 1. Percent of original weight after dipping (AD) and after cooking (AC) at 1 & 7 days.

TREATMENT	SHELL-ON				SHELL-OFF			
	t=1		t=7		t=1		t=7	
	AD	AC	AD	AC	AD	AC	AD	AC
Untreated	100	101	100	101	100	86	100	84
H_2O	104	102	104	98	107	87	107	87
H_2O, pH 8.8	104	101	105	99	108	86	107	79
1.25% $NaHSO_3$	103	79	104	100	105	80	105	76
6% $Na_5P_3O_{10}$	103	97	103	103	104	100	112	98
1% $NaHCO_3$	103	102	103	102	104	94	104	98
2% $NaHCO_3$	104	100	104	100	103	96	105	81
4% $NaHCO_3$	104	102	103	102	104	93	106	102
8% $NaHCO_3$	103	102	103	104	105	95	105	104

The microbial data for these shrimp were uninformative. Because the initial microbial load was so high, there was no further increase in bacterial numbers regardless of treatment.

Trial 2.

The second shipment of shrimp was received with a very good initial microbial load of 4.0 x 10^4 cfu/gr. The effect of treatments on cooked drip loss was determined as indicated above in triplicate and the water retention results are presented in Table 2. In shell-on shrimp, the differences among treatments were not significant on day one. Although, after one week the average of all bicarbonate treated shrimp had a statistically better water retention (105.8$\pm$.73%) than the controls (except sodium tripoly- phosphate) (103.8$\pm$.62%), these were of no practical significance.

In the shell-off shrimp, the bicarbonate treatments resulted in an average of 10% more weight retained than the control shrimp on day one. There was little difference among the different sodium bicarbonate concentrations and among the various controls, except the tripolyphos-phate. The average weight retention of the controls was 86.5% with a 95% confidence interval, as determined by the Student's t test, of $\pm$1.4, while the average weight retention of the bicarbonate treated shrimp was 96.2$\pm$0.9. This was comparable to the weight retention of tripolyphos-phate treated shrimp.

Table 2. Weight retention of cooked shrimp, trial 2. Percent of original weight after cooking on day 1 and after 7 days of refrigeration.

TREATMENT	SHELL-ON				SHELL-OFF			
	t=1		t=7		t=1		t=7	
	Ave.	Std. Dev.	Ave.	Std. Dev.	Ave.	Std. Dev.	Ave.	Std. Dev.
Untreated	104.3	1.38	104.3	0.78	88.0	1.89	89.7	2.98
H_2O	103.1	0.45	104.1	0.68	86.6	0.85	83.1	1.53
H_2O, pH8.8	102.4	2.22	103.6	0.20	85.8	1.34	83.1	0.55
1.25%$NaHSO_3$	103.1	0.98	103.3	1.67	85.9	0.28	90.7	1.37
6% $Na_5P_3O_{10}$	102.4	2.22	105.6	0.45	96.1	1.24	98.0	3.61
1% $NaHCO_3$	102.7	0.54	104.8	1.02	95.6	0.37	93.0	1.37
2% $NaHCO_3$	103.5	2.27	105.6	0.70	95.7	1.27	96.8	2.00
4% $NaHCO_3$	104.8	0.57	106.7	0.92	96.8	0.61	103.0	1.50
8% $NaHCO_3$	105.1	0.88	106.4	0.45	96.9	1.96	109.3	0.19

After one week of refrigerated storage, the weight retention of the treated shrimp was more pronounced. In this case, there was some concentration dependence in the bicarbonate dips, with the 8% treatment giving significantly better retention than the 1 and 2% dips. If treatments were grouped together as above, the treated shrimp had 100.3$\pm$1.4% water retention while the controls had 86$\pm$5.6% retention. A 20% increase in water retention occurs when the 8% sodium bicarbonate treatment (109.3$\pm$1.4%) is compared directly with the untreated control (89.7$\pm$6.4%).

In the experiments designed to determine the effect of treatment dips on melanosis, there was little difference in the degree of browning among untreated, bisulfite treated, or shrimp treated with 8% sodium bicarbonate (data not shown). Even in the untreated controls, browning was minimal. It may be that brown shrimp would be a better system to examine melanosis since their basal rate of browning is much higher than in white shrimp.

Trial 3.

The third shrimp shipment was received with an excellent initial microbial load of 2.8×10^3 cfu/gr. We followed microbial growth in the untreated samples over 20 days of refrigerated storage and found that they peaked at 1×10^8 between 12 and 15 days.

Triplicate samples were treated with 8% sodium bicarbonate, 1.25% bisulfite, deionized water deionized water at pH 8.8, or were left untreated to examine cooked drip loss and melanosis, and the water retention results are presented in Table 3. The differences in water retention among the shell-on shrimp were not significant, even at the 90% confidence level and after one week. Shell-off shrimp cooked immediately after the dipping had a 12% increase in weight retention compared to the control. The shell-off shrimp treated with 8% sodium bicarbonate had a greater than 20% increase in weight retention compared to the untreated control after one week of refrigerated storage.

Table 3. Weight retention of cooked, trial 3. Percent of original weight after cooking on day 1 and after 7 days of refrigeration.

TREATMENT	SHELL-ON				SHELL-OFF			
	t=1		t=7		t=1		t=7	
	Ave.	Std. Dev.	Ave.	Std. Dev.	Ave.	Std. Dev.	Ave.	Std. Dev.
Untreated	101.3	0.95	99.8	2.61	86.9	1.44	82.2	1.74
H_2O	99.4	1.84	98.7	1.20	82.9	1.81	76.6	0.94
H_2O, pH8.8	100.1	1.54	98.1	1.21	80.4	2.08	77.8	1.99
1.25%$NaHSO_3$	103.6	--	102.9	1.59	82.1	4.05	97.3	0.24
8% $NaHCO_3$	102.8	0.86	105.2	1.73	98.7	1.10	104.9	0.65

The results of the melanosis experiments were similar to those obtained previously; no significant differences among treatments were observed. Shrimp treated with sodium bicarbonate were found to be extremely palatable in very preliminary sensory analysis.

Trial 4.

The fourth shipment of shrimp were received with initial microbial counts of 2.7×10^6 cfu/gr. They were treated with sodium bicarbonate at 4 or 8%, deionized water, deionized water at pH 8.8, or left untreated. Plate counts were determined at 1, 3, 5, and 7 days of incubation at 4°C. All of the samples reached 2×10^8 cfu/gr by three days indicating that sodium bicarbonate dips have no significant antimicrobial effect.

DISCUSSION

These experiments have confirmed that sodium bicarbonate at 8% increases water retention by 20% in cooked, shell-off, fresh domestic white shrimp after treatment and storage for one week at 4°C. No reductions in microbial growth or melanosis were observed. This may be because white shrimp are not especially susceptible to melanosis or because the shrimp, in spite of our specifications, may have been treated with sodium bisulfite. Bisulfite dips are used routinely to inhibit melanosis and are applied at the fishing vessel, rather than processing level. Some experiments with brown and pink shrimp will be conducted to see if an effect on melanosis can be observed in those species. The lack of antimicrobial activity by sodium bicarbonate alone is consistent with previous findings. However, shrimp in trial 3, obtained from a research vessel with absolute assurance of no bisulfite treatment, gave similar results. We will determine the lowest level of ammonium bicarbonate that can be used without adversely affecting the shrimp taste and then determine if that level has antimicrobial properties.

CONCLUSIONS

Sodium bicarbonate dips at 8% consistently decreased water loss during cooking by 20% in shell-off white shrimp using shipments of shrimp from four different sources. The treatments were more effective on shrimp cooked after one week at 4°C than those cooked immediately after the bicarbonate treatment. Shell-on shrimp took up less water during treatments and lost less water during cooking compared to shell-off shrimp. Bicarbonate dips had no significant effect on the water loss of shell-on shrimp. No significant antimicrobial effect was observed for shrimp treated with sodium bicarbonate.

ACKNOWLEDGEMENTS

Our thanks to Kyle Collins of Fiavesa Shrimp, David Lewis of Lewis Crab Factory, and Keith Gates of Georgia Sea Grant Marine Advisory Service in Brunswick, Georgia and Michael Archar of Archar Seafood in Somerset, New Jersey for their cooperation in supplying shrimp. This project was made possible by grants from the Church and Dwight Company of Princeton, New Jersey and the New Jersey Commission on Science and Technology through the Fisheries and Aquaculture Technology Extension Center at the New Jersey Agricultural Experiment Station.

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THE EFFECTS OF ICE AND CHILLED SEAWATER STORAGE ON PINK SALMON QUALITY

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INTRODUCTION

The Alaska seafood industry is increasingly dependent on chilled seawater (CSW) and refrigerated seawater (RSW) systems to hold salmon on tenders and fishing vessels. These systems are excellent for rapid chilling of the catch and maintenance of quality.

Fish received at processing plants are frequently transferred to onshore CSW/RSW systems or iced totes, but salmon transferred from CSW/RSW to ice often lose their quality much faster than those transferred to CSW/RSW. These fish become more fragile, lose scales easier and must be handled carefully and processed quickly.

Are quality changes in the fish associated with transfers between liquid and solid chilling systems? Are there bacteriological changes? Or do other factors, such as handling and sanitation, affect fish quality? This publication reports on an investigation of the quality changes in pink salmon when the fish are transferred between chilling systems (chilled seawater and ice).

MATERIAL AND METHODS

Two hundred and forty freshly caught seine pink salmon were held in ice or CSW totes aboard tenders and delivered within 12 hours. At the dock, they were transferred into fresh ice and CSW and held for up to ten days at 32°F and 31°F, respectively.

On the third day after catch, one hundred fish held in ice were transferred to a fresh CSW system and one hundred CSW-held salmon were transferred to fresh ice. These salmon were then held for seven days.

At periods of 1, 2, 3, 4, 6, 8 and 10 days after catch, salmon were removed from each system for quality evaluation. Quality was measured by visual examination, microbial growth, salt uptake, expressible moisture, weight and texture changes. Fresh fish were examined for visual changes using criteria developed by Crapo et al (3). Microbial growth and identities were measured using methods of Himelbloom (4). Salt uptake was determined using AOAC methods (1). Texture changes were measured with an Instron Universal Testing Machine equipped with a modified Kramer-Lee shear cell and by expressible moisture (Karmas, 5).

After examination, fish were dressed, plate frozen at -40°F and held in 0°F storage for three to six months for taste panel evaluation using a seven point hedonic scale (Moskowitz, 6), texture change and nucleotide determination (Woyewoda, 10).

RESULTS AND DISCUSSION

Visual Changes

On each sampling day, ten salmon were graded as excellent, good, fair or poor condition according to specific criteria (see Appendix). As expected, there were significant changes throughout the ten day storage period (Table 1). Initial quality for both the iced and CSW fish was graded excellent to good with firm flesh, bright skin and fresh odor. Some fish showed slight flesh softness.

Iced salmon retained excellent quality during the first three days. Noticeable changes started to occur at day four with 40% of the salmon judged good to fair. At day six, only 20% of the fish were still in excellent condition. By day 10, salmon held in ice were rated fair to poor, with 50% of the fish showing significant decomposition.

CSW-held salmon deteriorated faster than the iced fish. By day three, fish were graded mostly good to fair. At day four, all salmon were in fair condition showing strong slime odor and moderate softness. By day eight, 80% of the fish were in poor condition and at the tenth day of holding all salmon were judged poor quality with significant spoilage.

Salmon transferred from CSW to ice maintained better condition than the fish held exclusively in CSW. At day four, 40% of the fish were still judged in good condition with neutral odor and slight flesh softening. After day six or three days after the transfer, significant decomposition developed.

Fish transferred from ice to CSW deteriorated faster than the iced salmon and slower than the fish held exclusively in CSW. At day six, most of the salmon (70%) were in good condition. However, rapid quality loss was experienced between days eight and ten. At day eight, only 20% of the fish were judged good. By day ten 60% of the salmon were in poor condition with dark roe and noticeable decomposition.

Microbiological Changes

Bacterial levels in seawater and salmon were monitored for each system (Table 2). During each sampling day, CSW, ice and swab samples from the salmon were collected for aerobic plate counts and identification. Pink salmon held in ice had the highest microbial counts reaching 3,000,000 bacteria per cm^2 after ten days storage. By contrast, the CSW-held fish had only 59,000 bacteria per cm^2. This large difference was due in part to the initial bacterial loads of iced fish being almost seventy times greater than CSW fish. This difference was a result of initial holding conditions on the tenders. CSW-held salmon maintained lower bacterial populations throughout the storage period.

When comparing relative growth rates, it was found that bacteria in CSW systems grew about twice as fast as those held in ice. Bacteria populations of CSW-held salmon grew from 70 to 59,000 per cm^2, an 842-fold increase. By comparison, bacteria in iced fish increased from 6,300 to 3,000,000 per cm^2, a 476-fold growth. The differences in growth rates would indicate that salmon held in CSW would spoil more rapidly than iced fish.

Fish transferred from ice to CSW resulted in an initial reduction of the counts. Some of the adhering bacteria apparently had sloughed off after contact with fresh CSW. After the bacteria had acclimated to this new environment, counts on the salmon reached 210,000 cm^2 at ten days storage. Fish transferred from CSW to fresh ice resulted in a bacterial counts intermediate between those for CSW-held and iced salmon.

Table 1.
Visual Quality of Pink Salmon Held in CSW and Ice

Storage Time (days)	CSW	Ice	CSW to Ice	Ice to CSW
		Fish Condition*		
1	E (80%) G (20%)	E (90%) G (10%)		
2	E (60%) G (40%)	E (90%) G (10%)		
3	G (70%) F (30%)	E (80%) G (20%)		
4	F (100%)	E (60%) G (30%) F (10%)	G (40%) F (60%)	E (20%) G (60%) F (20%)
6	F (70%) P (30%)	E (20%) G (60%) F (20%)	F (100%)	G (70%) F (30%)
8	F (20%) P (80%)	G (40%) F (60%)	F (40%) P (60%)	G (20%) F (50%) P (30%)
10	P (100%)	F (50%) P (50%)	F (20%) P (80%)	F (40%) P (60%)

* See Appendix for descriptions of grading criteria
E = Excellent G = Good F = Fair P = Poor

Table 2.
Aerobic Plate Counts of Pink Salmon Held in Ice and CSW

Storage Time (days)	CSW	Ice	CSW to Ice	Ice to CSW
Salmon:		bacteria per cm^2		
1	480	35,000	--	--
2	70	6,300	--	--
3	450	18,000	150	400
4	1,200	130,000	2,400	3,800
6	800	190,000	2,200	11,000
8	5,900	1,900,000	14,000	20,000
10	59,000	3,000,000	240,000	210,000
Chilling System:		bacteria per ml		
1	1,700	460,000	--	--
2	6,000	1,400,000	--	--
3	13,000	220,000	100	100,000
4	16,000	570,000	9,700	120,000
6	39,000	1,200,000	15,000	510,000
8	130,000	6,800,000	43,000	640,000
10	110,000	39,000,000	180,000	540,000

While the bacterial levels found in the iced fish were much higher than those found in CSW, the iced fish maintained better quality. This was due to the type of bacteria present in each system (Table 3) . Identification of the bacteria revealed some interesting differences between chilling systems. Moraxella species dominated in pink salmon stored in ice or transferred from CSW. These bacteria are not food spoilage organisms.

Table 3.
Types of Bacteria Found on Salmon Held in CSW and Ice

Storage Time and Bacteria Type	CSW	Ice	CSW to Ice	Ice to CSW
		% of bacterial flora		
Day 1				
Moraxella	43%	85%		
Pseudomonas	4	6		
Flavobacterium	26	7		
Acinetobacter	0	2		
Day 4				
Moraxella	15%	76%	8%	59%
Pseudomonas	20	0	26	11
Flavobacterium	40	22	23	5
Acinetobacter	25	2	36	9
Day 8				
Moraxella	19%	66%	63%	29%
Pseudomonas	73	0	29	69
Flavobacterium	10	32	8	8
Acinetobacter	0	0	0	0

The Pseudomonas species that predominated in the CSW systems are potent spoilers of food and produce objectionable odors and flavors that tainted the salmon during storage.

Salt Uptake

One of the concerns with CSW during extended storage is the potential for increased salt content in flesh. Levels greater than one percent are undesirable since salt accelerates the development of rancidity and may affect the flavor of frozen salmon. None of the treatments in this experiment resulted in salt content more than one percent (Table 4). As expected the salmon either stored or transferred to CSW had higher salt contents than iced fish. Salt levels of fish held in CSW doubled from 0.23% to 0.52% during the storage period. Salt content of fish transferred from CSW to ice was slightly reduced, presumably due to the leaching by melting ice. Salt levels of salmon held in ice did not change, remaining between 0.11% and 0.13%

Table 4.
Salt Content (%) of Pink Salmon Held in Ice and CSW

Storage Time (days)	CSW	Ice	CSW to Ice	Ice to CSW
1	0.23	0.11	--	--
2	0.37	0.11	--	--
3	0.39	0.11	--	--
4	0.47	0.11	0.39	0.26
6	0.52	0.12	0.34	0.29
8	0.51	0.13	0.36	0.30
10	0.48	0.12	0.33	0.35

These salt levels compare favorably with values from other investigations. Tertnes et al (7) recorded values as high as 0.7% salt after six days. Tomlinson et al (9) reported salt contents of 0.56% in sockeye salmon held in RSW and 0,14% for salmon held in ice for eight days.

Weight Changes

Another drawback of chill storage is the weight change sometimes experienced during long holding periods. In this experiment, ten salmon in each system were tagged and weighed at each sampling period to determine the extent of the changes (Figure 1). Weight gains occurred in all systems. Weight gain of salmon held in ice was about one percent while CSW-held fish increased almost five percent during the storage period. These weight gains are water absorption in the flesh and result in salmon that are more fragile and harder to handle.

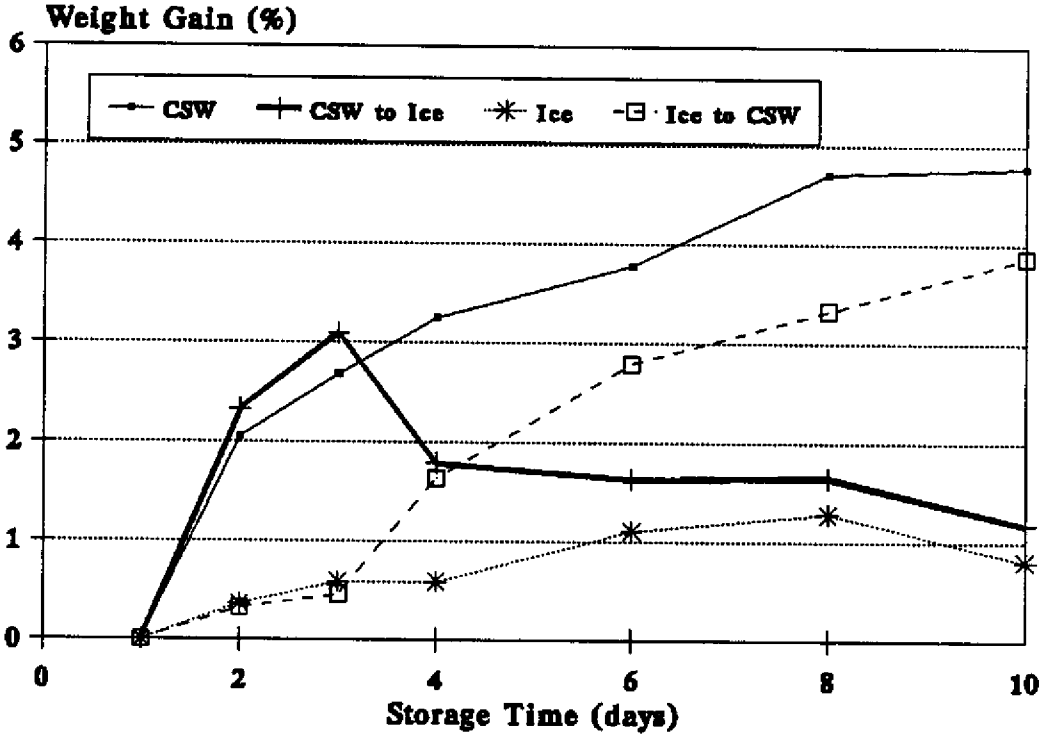

Figure 1. Weight gain of pink salmon held in ice and CSW.

Fish transferred from ice to CSW experienced weight increase similar to CSW-held fish, gaining almost four percent. Salmon transferred from CSW to ice gained weight in the CSW and then lost weight when placed in ice.

Tomlinson et al (8) reported similar weight gains for sockeye and pink salmon held in ice and RSW. After six days storage, pink salmon weights incresed by 3.2% when held in RSW and 0.8% in ice.

Texture Changes

The texture of fresh and thawed frozen salmon was determined using an Instron Universal Testing Machine equipped with a four blade Kramer-Lee shear cell. This machine, specifically designed to measure food texture, recorded the force needed for a four blade shear to cut through a sample. The greater the force needed to cut the fish, the tougher the sample. Results from fresh salmon (Figure 2) showed very little texture difference between treatments. CSW-held fish were slightly softer than those stored in ice, but not significantly. Transferring salmon from CSW to ice and from ice to CSW did not affect overall flesh texture.

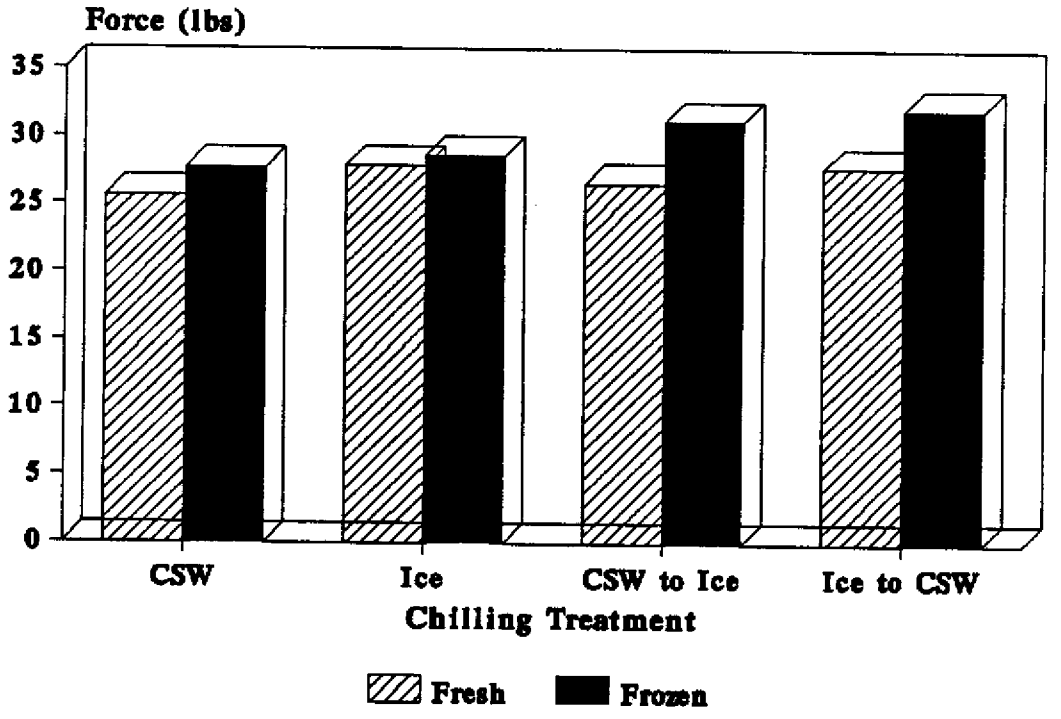

Figure 2. Texture changes of pink salmon held in ice and CSW. Values are average firmness over the ten-day storage period.

Texture of the salmon held in frozen storage for six months at 0°F (Figure 2) showed significant differences among the treatments. After six months frozen storage, iced and CSW-held fish had very similar texture, slightly tougher than the fresh samples. Those fish that were transferred from one chilling system to another showed a large increase in toughness. The fish transferred from ice to CSW had toughened considerably. Handling during transfer may have had an adverse effect on flesh texture in frozen storage. This was not noticeable until the fish had been frozen and stored.

Another measurement of texture changes that occurred was the amount of expressible moisture in the fresh salmon. Expressible moisture is the water that can be forced from a sample by applying pressure. It was expected that as the flesh softened during storage, more moisture could be squeezed from the flesh. The results (Figure 3) showed that expressible moisture increased noticeably in the CSW-held salmon and those transferred from CSW to ice. There was no difference in fish held in ice. The fish transferred from CSW to ice showed a slight decrease in expressible moisture. Fish held in CSW showed increased expressible moisture indicating adverse texture changes.

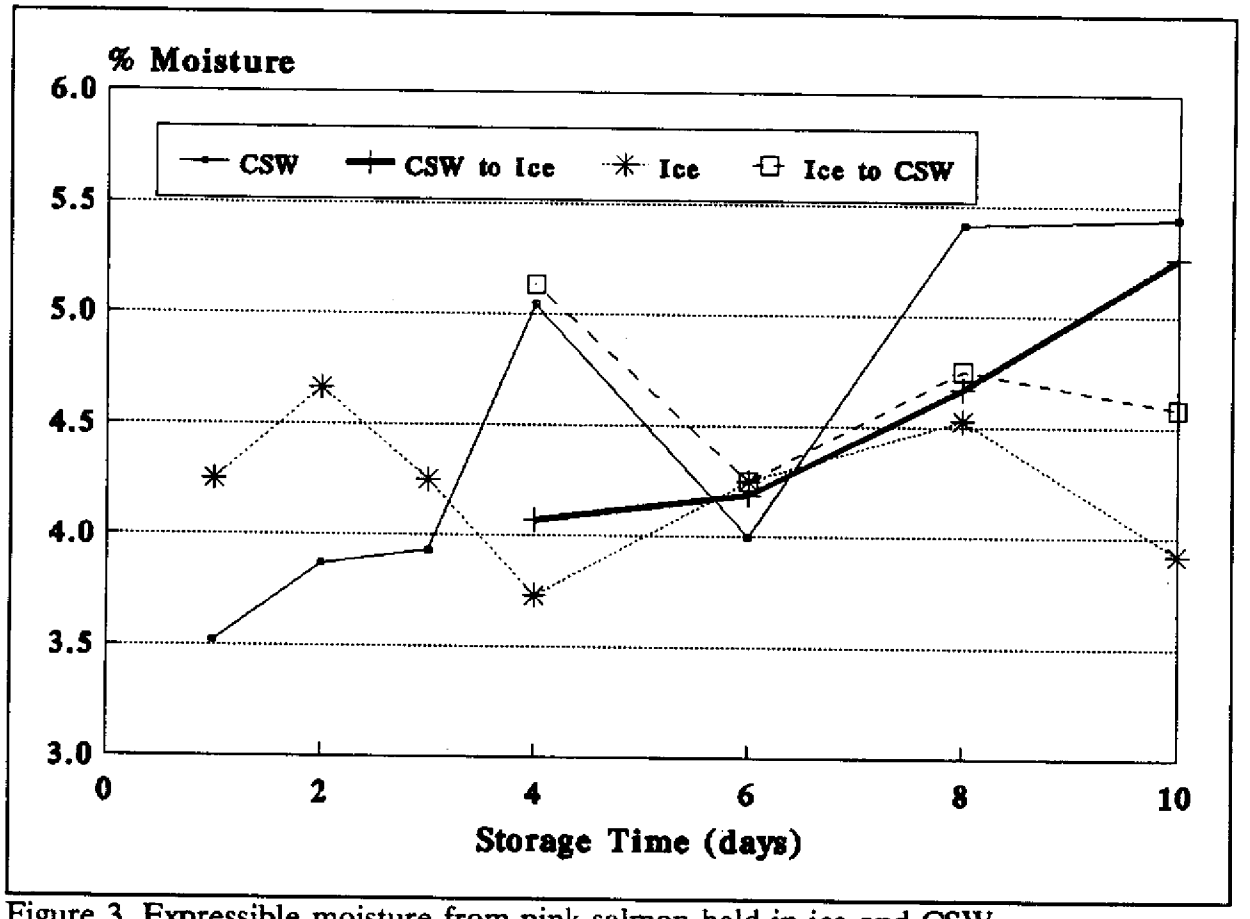

Figure 3. Expressible moisture from pink salmon held in ice and CSW.

Biochemical Indices of Quality

A standard measure of fish quality is the nucleotide levels found in the flesh. Nucleotides are biochemicals essential to the living fish. Once the fish dies, these chemicals degrade to form undesirable compounds. Measuring one of these compounds, hypoxanthine, provides a relative gauge of fish quality.

Hypoxanthine levels were monitored and, as expected, increased in all chilling systems (Figure 4). Salmon held in CSW developed higher levels of hypoxanthine than those held in the other chilling systems. The amounts of hypoxanthine increased four-fold in fish stored in CSW and two-fold in fish held in ice. Fish that were transferred between chilling systems experienced three-fold increases.

Based on hypoxanthine levels, holding fish in CSW resulted in the fastest quality deterioration while iced fish experienced the slowest rate. Salmon transferred into ice or CSW had rate of quality loss intermediate between CSW-held and iced salmon.

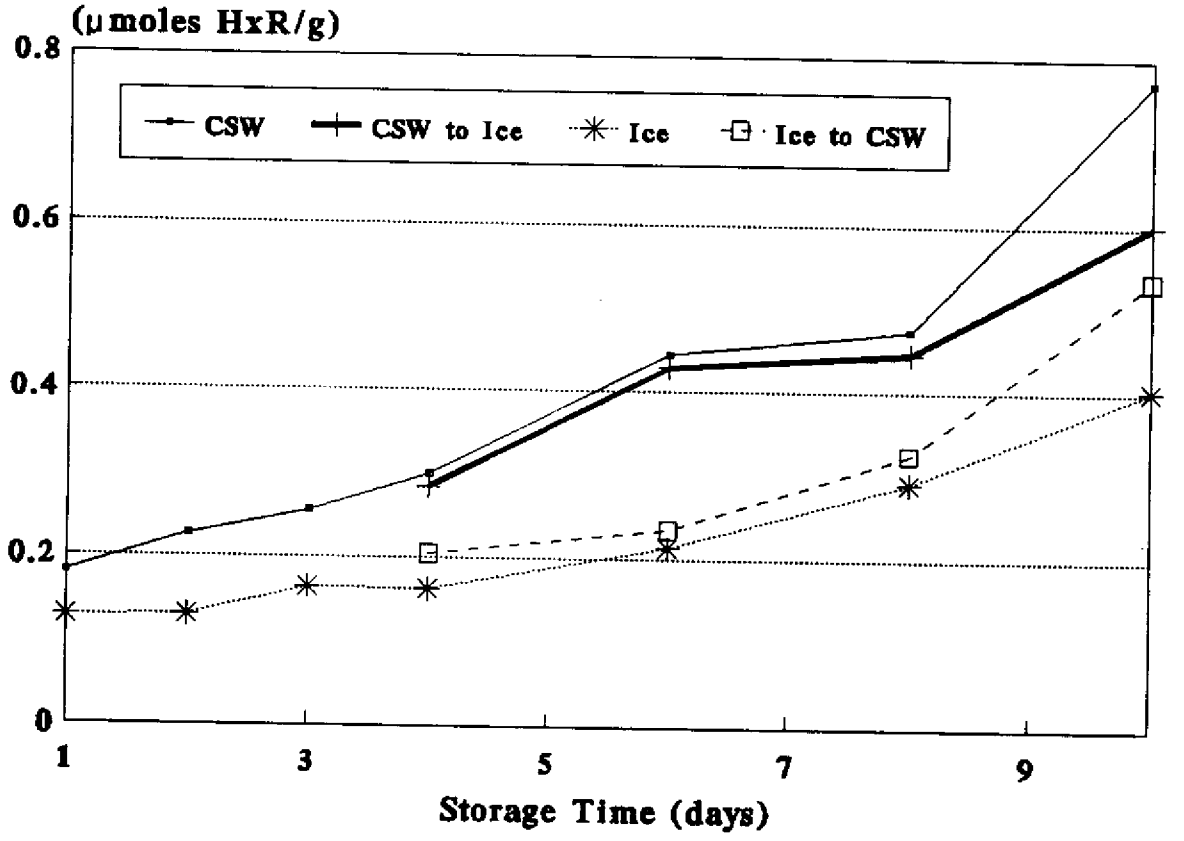

Figure 4. Hypoxanthine levels in pink salmon held in ice and CSW.

Taste Panel Results of Frozen Fish

Salmon were held in frozen storage at 0°F for six months before taste panel evaluation for moistness, flavor, and overall desirability. Results showed that flavor and desirability scores changed with storage time and chilling system.

The best flavor was found in salmon held less than four days. Salmon held more than six ten days suffered a noticeable decrease in flavor. Significant flavor loss (Table 5) occurred in the CSW-held fish and those transferred from CSW to ice and ice to CSW. Fish held in ice maintained acceptable flavor throughout the storage period, no significant changes occurred.

Among the chilling treatments, CSW-held salmon deteriorated fastest, followed by fish transferred from ice to CSW. Fish transferred from CSW to ice held up slightly better and those stored in ice maintained the best flavor of all samples.

Table 5.
Flavor Scores of Pink Salmon Held in Ice and CSW

Storage Time (days)	CSW	Ice	CSW to Ice	Ice to CSW
2	5.45	5.36	--	--
3	5.82	5.63	--	--
4	5.00	4.91	5.73	4.55
6	3.33	4.75	4.50	4.75
8	1.45	4.27	4.09	4.64
10	*	4.82	4.09	3.27

* 10 Day CSW was spoiled.
Higher scores indicate more desirable samples.

CONCLUSIONS

This experiment revealed several potential causes for quality loss when salmon are held in CSW or transferred from CSW systems to ice. When compared to iced fish, salmon held in CSW experienced faster bacterial growth, greater weight gain, and higher expressible moisture and nucleotide levels. Greater weight gain meant more water was absorbed into the flesh making the fish more fragile. Higher levels of hypoxanthine indicated faster deterioration of nucleotides and a more rapid quality loss. Although the bacterial counts were lowest for salmon held in CSW systems, the predominant species were the potent food spoilers belonging to the genus Pseudomonas. All these conditions resulted in salmon that were more susceptible to spoilage and handling damage. These changes occurred within four days after catch which can be a typical tender trip.

Salmon transferred from CSW/RSW vessels to ice are much more fragile when compared to iced fish. Typical handling procedures could easily reduce quality much faster. From the experimental results it is probable that handling is a major influence in the rapid deterioration of these fish.

The recommendation from this experiment is to hold salmon less than four days in CSW/RSW systems. This is in agreement with other RSW/ice studies conducted with salmon. Tomlinson et al (9) and Bronstein et al (2) recommended salmon should be stored no longer than four days in RSW. The fish must

also be handled carefully when transferred from the fishing vessels and tenders to iced totes. Iced storage remains the best method for maintaining fish quality.

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ACKNOWLEDGEMENTS

We wish to thank Mr. Ken Allread and Western Alaska Fisheries in Kodiak for their cooperation and assistance in obtaining the salmon necessary for this project.

APPENDIX: The Effects of Ice and Chilled Seawater Storage on Pink Salmon Quality
Crapo, et. al., 1991

	Excellent	Good	Fair	Poor
Odor	Fresh, seaweedy	No odor, neutral	Strong slime odor	Decomposing, acrid odor
Eyes	Bright, clear opaque	Flat, slightly reddening	Opaque, slight sunken	Opaque, red
Skin	Bright, no bleaching	Slightly dull, wavy	Dull, some bleaching	Dull, bleached
Gills	Bright red, no odor	Pink, slight slight odor	Green-brown, strong slime odor	Brown-White, decomposed
Flesh	Firm	Slightly soft	Moderately soft	Severely soft
Gut	Firm, bright, no decom-position ation	Softening, no decomposition, slight discolor-ening	Slightly liquid, slight decom-position, dark-dark	Very liquid, noticeable decomposition,

OZONE APPLICATIONS FOR COMMERCIAL CATFISH PROCESSING

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Introduction

Current methods of operation in Fish/Seafood/Poultry processing plants include the application of large quantities of chlorine in an aqueous solution, contacting the product directly by spray or submersion, in an effort to control bacterial growth in and on the meat during processing, and subsequently in the fresh meat marketplace; such bacterial content limiting the shelf life of the product (thus limiting shipping radius, storage in transit, export, etc.). The use of chlorine over the long history of this industry, has proven to be ineffective in controlling bacterial growth, resulting in poor shelf life, and inadvertently imposing a potentially harmful chemical on the consuming public.

As an example of the dangers of the use of chlorine, the United States Environmental Protection Agency (USEPA) has in the last two years formally taken a position against the use of chlorine in the public potable water supply due to fact that Trihalomethanes (THM's) are formed when the organics in these water supplies are joined with the chlorine injection commonly utilized in the United States for bacterial control. THM complexes have been proven to be of significant carcinogenic nature to humans. These compounds are very difficult to destroy or modify once formed in their natural state.

The potential for the formation of these compounds in this industry is significant (1), and may be controlled by the alternate use of another oxidizer other than chlorine (i.e. ozone).

Use of chlorine is not an efficient method of bacterial control, or shelf life enhancement, thus additionally encouraging the use and application of another form of control (i.e. ozone).

The presumption in this case is that the application of ozone to the process meat industry would solve all of these current concerns, and provide a much greater degree of operational control and product quality.

Four operating entities were involved in this study: Mississippi State University, Matrix Design, Eco Resources,Inc., and Delta Pride Catfish, Indianola, Mississippi.

The goals, although specific, were aimed at a gathering of information and data, as well as the enhancement of the well being of a rapidly growing industry nationally.

Material and Methods

Delta Pride allowed two operating windows during the testing procedure. The first was in June of 1990, the second in July of 1990. Both were conducted within the plant at Indianola, in a real time environment.

The testing design was done cooperatively among all four parties. Delta Pride directed the locations of selection of the in situ meat for the tests. Eco Resources provided the bioprofile expertise, Mississippi State provided the knowledge of the industry and the goals that had to be accomplished during the testing period, and Matrix Design provided the process treatment design, kinetic projections, and equipment supply/operation during both testing periods.

Multiple levels of tests were scheduled, each encompassing multiple fish, and multiple stages of the processing plant. In Appendix A, the tests noted for the month of June show selected tests and their tracing of the processing procedure through the plant in an effort to find the most evident locations for application. The tests shown for July are reduplications of the June tests (to prove verification of the previous results), and new applications that were discussed among the parties after the initial June tests.

Fish were tested before processing in all cases; and after the selected process treatment. Swab tests were performed on each piece of meat (whole or fillet), to determine cell count (4 sq.in. sample area per sample of meat), with the respective swab recorded, video taped, iced down, and dry iced for shipment to a remote third party lab for results.

The equipment selection was difficult. The need was to provide equipment of a size and capacity necessary to oxidize the bacterial content, but not provide excess production capacity during the testing procedure (altering the results). Monitoring of the in situ process was of major importance.

Delta Pride desired to test the procedure first on the red water chiller, which was now being treated with chlorine. Because of the high organic content, an equipment selection was made that allowed for the inclusion of a unique microprocessor board within the ozone production dielectric system. This board allowed the tests to be performed within a very narrow range of injection rate, constantly controlled by the microprocessor unit.

This unit would sense the condition of the feed air stream; sense the production system (quad dielectric); sense the discharge ozone level and condition; and finally sense the uptake of the ozone in the reaction vessel. All of these values were digitally preset for each bank of tests as shown in the data of Appendix A attached. At present there is only one manufacture of this equipment in the United States with its integral microprocessor unit.

In addition to the ozone generator, compressed air, air dryer, and pumping units were provided. A 30 gallon test vessel (chiller vessel, reactor vessel) was provided to contain the water into which the ozone and processed fish were placed. The ozone was injected through a Kynar educator valve provided as a mixing device. All piping was CPVC, Kynar, or Stainless Steel.

Fish (25-30 pounds of whole fish per tank) were introduced into the test tank (held at a constant temperature range, 32F - 38F), with the introduction of raw (untreated) tap water ice to maintain temperature (as in the present full scale processing plant), for a predetermined amount of time (several time frames, 10-12 min., and several concentrations of ozone, 5ppm-12ppm were allowed). A predetermined amount of unfiltered recycle of the 30 gallon tank was allowed at each treatment level, as the level of ozone injection was altered. A three part variable (digitally created, and controlled) was created for each test: ozone quantity, time of retention, and amount of recycle. At no time was the retention more than that currently exercised by Delta Pride (20-30 minutes).

The goals were the reduction of bacterial count/content on the flesh of the fish being tested, and to increase the shelf life of the product. The scale or extent of the kill, and shelf life was unknown at the onset of the testing, but several assumptions were projected due to the nature of the ozone injection process and its natural superior capabilities in the oxidation of

organics (in direct contrast to that of products like chlorine). Previous testing has shown that the effects of ozone on poultry were significant (2). Real time applications would be used in this case, not laboratory models, or controlled sequences. Attempts were made to duplicate the normal operating conditions of the plant, not laboratory controlled conditions; random fish samples, tap water (untreated), atmospheric air feed, and nominal ozone injection/contact time.

Peripheral areas of application would also be included in the testing procedures. Fillet treatment would be explored, attempting to lower the secondary processing recontamination of the meat for the prepared meat market potential. Equipment bacterial recontamination is a major problem in process plants, and the inclusion of ozone treatment of both the equipment and the fillets would be preformed. Ice production would be treated to try and profile the improvements that would come from treatment of tap water with ozone before ice making.

The first assumption was that the kill rate would be significant, in the whole fish category, and fillet category. This assumption would be defined by specific levels of cell count in each sample. Current (industry records indicate) levels suggest that the cell count of whole fish before exposure to chlorine is well above 100,000 (some have suggested that the count is at times above 150,000). The magnitude of the parallel problem in the poultry industry is detailed in multiple papers (2,3), recording the extent of the bacteria population typical in that industry. After exposure to chlorine the count in both Fish and Poultry can drop below the 35,000-50,000 level. Our goal was to better that lower bracketed number.

Our second goal was to increase the shelf life of the product significantly. Industry numbers indicate a current fresh fish shelf life of 4-5 days. Lastly, we would attempt to show the improvement in the ice cell count, as an application of fresh iced fish transport and marketing.

Ozone is unique. Production is accomplished with only small quantities of electricity, an electrical field, and a prepared air feed (either atmospheric or pure oxygen). No chemicals are required for production. Once produced, ozone provides up to 15 times the killing power of chlorine solutions. In standard potable water applications, the use of ozone can provide a four log kill in a fraction of the time as that required by chlorine under the same conditions. Some researchers have shown the comparisons between various disinfectants to be impressive when considering total kill rates of ozone versus standard operational chemicals (4).

Ozone is a very unstable gas, artificially created (man made in this case) through the exposure of a dried, pressurized atmospheric air stream, to an electrical field. The "lasting character" (half life) of ozone (at temperatures above 32F) is very short. Half life is documented as less than 30 seconds. Oxidation takes place, kinetically, immediate on contact with the target subjects.

No after affects, or detrimental chemical/organic reactions are induced by the exposure of ozone to water streams, or in human consumables. In fact, ozone has been used in potable water treatment (for human consumption) since 1856. Today ozone is widely utilized in the bottled water industry (Ozarka, etc.), soft drink industry (Coke, etc.), and beer and wine industry (Coors, etc.); FDA has authorized the use of ozone in this industry as "Generally Recognized as Safe [Technology], (GRAS).

Ozone has been added to the USEPA Best Available Technology (BAT) list for potable and waste water applications for the elimination of chlorine in the water systems of this country. Recently the largest ozone system in the world was installed for the City of Los Angles potable water supply (>three billion gallons per day production); a second system was recently designed and is currently under construction for the City of Ft. Worth, Texas for its potable water supply.

Chlorine has been identified as older and less efficient technology. The USEPA has confirmed that chlorine (utilized as a bacterial control agent) in public and private water supplies causes the formation of THM complexes that are extremely carcinogenic to humans once ingested. The formation comes from the exposure of organics in the surface water sources to the raw chlorine feed that is intended to destroy the bacterial content of the water source, and to provide a residual of killing capacity control during the distribution of the water to the end user. Typically 5 ppm of chlorine is added, then the water is aerated to remove the overage of chlorine content, retaining at least 1 ppm for distribution. Even at these low levels of injection, the creation of these THM complexes is typical.

The USEPA is now directing public water districts across the United States to stop using chlorine in waste water discharges (thus eliminating the build up of the possible reaction/creation of THM's in the surface water that is eventually used as potable sources), substituting ozone (or other BAT's), or the use of chlorine and a chlorine removal system prior to final discharge into the receiving surface water stream.

Chlorine has always been the oxidant of preference. Its reaction time was slow, as indicated by the typical retention time at a potable/waste treatment plant of 20-30 minutes, before release. Kill rates were eradicate. The chemical itself was a danger to the workers and excesses of injection were of major consideration to the users (due to the human danger involved). Preference for chlorine use is now changing.

Ozone has none of the potential dangers displayed by chlorine. Benefits are many, including the increased efficiency (kill rate, four log kills are typical), and lack of danger to operators, and users alike. The costs are low, due to the lack of depletables (other than the electricity required for operation).

Results

As shown in Appendix A, the results of June and July confirm that the use of ozone (as configured in these tests) is very effective in bacterial control at the Delta Pride facility. Levels of kill fell generally below the 1000 cell count level for June, and well below the 200 level in July; considerably below historical operating standards in the industry.

June test results (see plate #1) were indicative of our desire to test a wide variety of sources, with some narrowing to be expected for the next set of tests in July. Effectiveness of the June tests were generally impressive, especially in the whole dressed category (<5000 w/o ozone, <932 w/ozone), and the fillet category (<5000 w/o ozone, <120 w/ozone). Results of the pre ozone tests show the values of cell count to be greater than 5000. This oversight was corrected in the July tests to show the true values (although the total number of tests were not as comprehensive).

Fillet contamination was shown to be coming from the fillet operation, and its inherent recontamination potential. Fillets that came off this line evidenced very high levels of count, even with the multiple chlorine spray nozzles that are an integral part of the machines utilized for this operation. Application of ozone at this stage of the process proved very effective in the June tests (>75% reduction over conventional treatment).

July proved that the results obtained in June were capable of replication, and could in fact be improved upon once the base data of June was reviewed (see plate #2). July concentrated on the pure ozone chiller results on whole fish (<80 count), fillet treatment with ozone (<190 count), ice made with and without ozone, and shelf life improvements.

Whole fish results ranged from 10 to 80 cell count over multiple whole fish swab tests. All fish tested were deheaded, gutted, and skinned. The fish (six each test, in the 5-6 lbs. size range) were placed in the ozone chiller tank for the allotted time frame, and ozone concentration. Fillets were then made (automatically) of these ozonated whole fish.

Fillets were cut from the whole ozonated fish, but without ozonated spray on the fillet machines, without ozonated pretreatment, and without ozonated ice for packing the whole fish. Fillets created from this flow schematic resulted in low cell counts of 120-190. Fillets without ozone treatment (but with conventional treatment) ranged from 7,500- 85,000 cell count (see plate #3).

Fillets were selected from the ozone treated groups, set aside for a shelf life test, and subsequent taste testing by inplant quality control personnel. The fillets were iced (with tap water ice) and kept at 34 degF and tested for smell, appearance, and firmness. Taste tests were performed at day one for any traces of ozone or off flavor due to the p62 change in treatment. The taste testers were not aware of the change in treatment, and no indication was given as to any off flavor, or degradation in quality over conventionally treated fillets.

Shelf life was shown to be 14 days with ozone treatment (see plate #4). This compares with 4-6 days for conventional treatment of iced fillets. On the fourteenth day, an appearance of odor was present. No reozonation of the fillets was done after the initial treatment on day one; this in contrast to previous studies that showed nominal changes in shelf life (5), were obtained with large quantities of ozone exposure. Shelf life does vary according to fish type, shown previously in the study of ozone on whole fish, and fillets (6).

Ice production was of high quality when raw water was pretreated with ozone (5ppm). The contrast can be seen in plate # 5. Ice made without ozone had cell counts in excess of 250. Ozonated ice showed cell counts less than 5. Ice produced in these tests were for the counts supplied, and not for the further treatment or maintenance of the whole fish, or fillets during the tests in July. Use of ozonated ice is not new. ozone treated raw water sources were first officially noted over 60 years ago in the commercial fish industry in France (7). In that case the shelf life was extended by over 33% with just the use of ozonated ice over fresh fish in the holes of fishing vessels.

Conclusion

Research of the past twenty years has shown that use of ozone in all facets of meat processing (fish and poultry) could be of great benefit to processor and consumer alike. Technology has progressed over those twenty years, and now ozone generation is much more proficient, and accordingly more economical to the end user.

Results as summarized in this paper have shown significant potential gains in shelf life, ice quality production, and evidence of more efficient operation of the red water chiller demands in fish processing plants (with implications for the poultry, and seafood industry). These tests have shown that with current technology, ozone can be applied to those areas reviewed, with impressive results.

Empirical evidence of the data herein offer a multitude of benefits to the industry as a whole. Elimination of chlorine as the prime bactericide is now possible; providing the consumer with a product that has added value, as well as essential health rationale.

JUNE ON-SITE TESTS

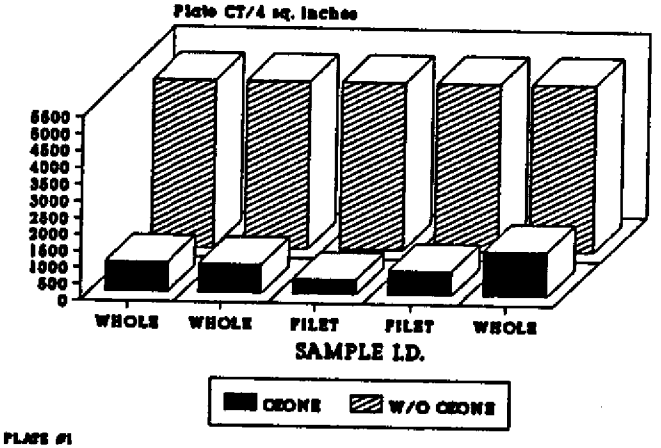

PLATE #1

JULY ON-SITE TESTS
CHILLER TESTS

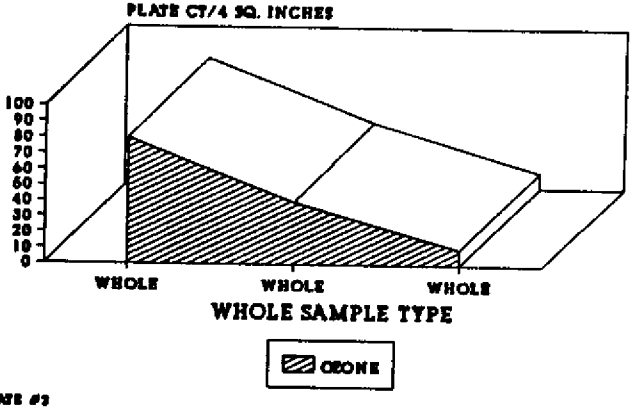

PLATE #3

JULY ON-SITE TESTS
CHILLER TESTS

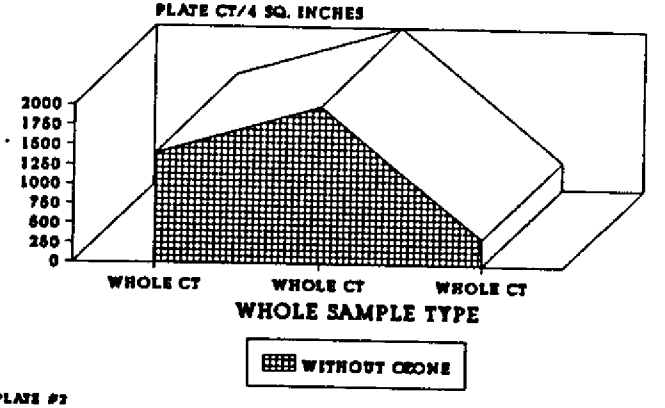

PLATE #2

FILET NON TREATED

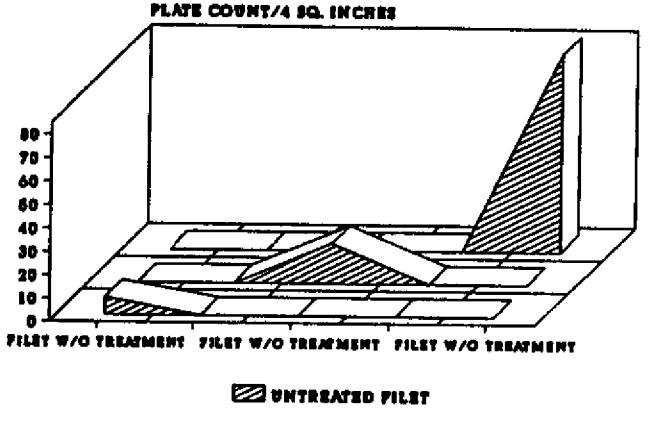

PLATE #5

SHELF LIFE

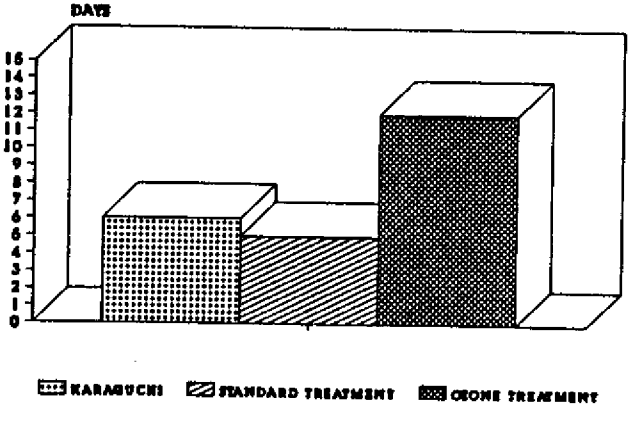

PLATE #4

ICE PRODUCTION
NON TREATED VS OZONE

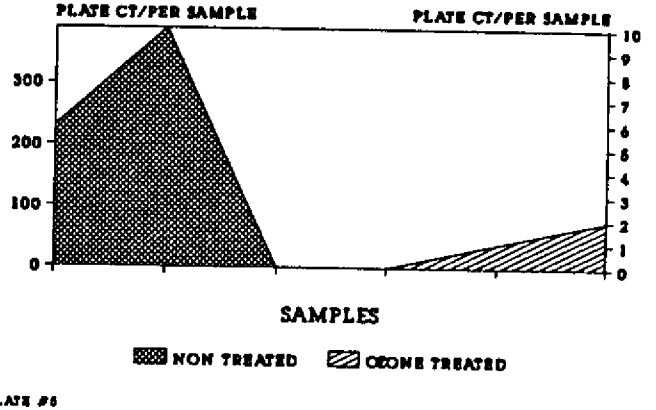

PLATE #6

Appendix A

June Data Set

Location	Cell Cnt.	Coliform	Staph	E. coli
Whole, dressed NT	>5000	+	+	+
Whole, dressed O3	932	+	+	+
Exit Chiller NT	912	+	-	-
Exit Chiller O3	478	+	-	+
Exit Chill Rinse NT	>5000	+	-	+
Exit Chill Rinse O3	912	+	-	+
Fillet NT	>5000	+	-	-
Fillet O3	468	+	-	-
Whole, dressed (belt) NT	>5000	+	-	+
Whole, dressed (belt) O3	750	+	-	+

July Data Sheet

Location	Cell Cnt.	Coliform	Staph	E. coli
Exit Chiller (whole) CT	2000	4	-	<3
Exit Chiller (whole) CT	1400	<3	-	<3
Exit Chiller (whole) O3	80	<3	-	<3
Exit Chiller (whole) O3	40	<3	-	<3
Fillet Belt NT	85,000	640	-	4
Fillet Belt NT	17,000	93	-	<3
Fillet Belt NT	7500	9	-	<3
Fillet O3	120	<3	-	<3
Fillet O3	130	<3	-	<3
Fillet O3	190	<3	-	<3
Ice w/o Treatment	230	<3	-	<3
Ice w/o Treatment	390	<3	-	<3
Ice w/O3	1	<3	-	<3
Ice w/O3	2	<3	-	<3

Notes:

Table definitions are: Cell Cnt., cell count by swab method (4 sq. in. area swabed on each sample); NT, no treatment; CT, chlorine treatment; O3 ozone treatment; multiple tests in each category were made, this is a summary of those tests

Acknowledgements

The authors express their appreciation to Eco Resources, Inc., Dr. K. W. Sharp, Houston, Texas for their financial support, and for their technical capabilities; to Virginia Cooperative Extension Service, Virginia Tech, Dr. Lucina Lampila; and to Delta Pride Catfish, Mr. Larry Joiner and Mr. Bob Winstead, Indianola, Mississippi.

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DETERMINATION OF OZONE PRODUCED OXIDANTS ARTIFICIAL SEAWATER

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INTRODUCTION

Ozonized seawater has been shown to inactivate and/or reduce the levels of bacteria found in seawater and in shellfish (3,6,7). Several studies have been performed to show the effectiveness of ozone to inactivate viruses (5,9), and inactivate toxins associated with red tide blooms (2,4).

The recent position formulated by FDA to classify ozone as a food additive will restrict its use in processing, storage, depuration and mariculture of aquatic food products. If ozone is to be used for shellfish and seafood processing in the future, it is essential to accurately measure the amount of oxidant produced and to determine whether any harmful byproducts are being formed.

An attempt was made to evaluate the relationships between several methods of oxidant determination currently in use. A minimal artificial seawater was used. Concentrations of bromine and ammonia were varied to test the response difference between the assays. The results given are preliminary data from an on going study now being conducted.

MATERIALS AND METHODS

Artificial Seawater

All experiments were conducted in a minimal artificial seawater mix as to reduce the interferences generated by the numerous trace compounds found in natural and commercial mix seawaters. The mix included chloride (30.00 g), bromide (0.097 g), and carbonate buffers (0.192 g as NaHCO3). A standard ammonia solution was prepared and added to the appropriate sample using Eppendorf pipettes.

Glassware Preparation

Oxidant demand free glassware was used in experiments dealing with the quantification of residual oxidant levels. The glassware was placed in a tank containing 20 liters of artificial seawater that was subjected to ozonation for a period of 1 hour. Glassware remained in the ozonated seawater for 24 hours, at which time it was removed and placed in a 108°C drying oven. The glassware was then covered with aluminum foil until needed.

Production and Delivery of Oxidant

Ozone gas was generated by a Welsbach T-408 Ozone Generator supplied with medical grade oxygen (99.5% O_2 with a dew point of -120°C). The gas flow was controlled by a Matheson flowmeter. Ozone was bubbled into artificial seawater via crystalline alumina gas diffusing stones.

Two hundred ml samples were ozonated for a period of 30 seconds under constant agitation. Immediately after ozonation, a 5 ml aliquot was transferred to 5 ml of neutral potassium iodide. Another 10 ml aliquot was transferred to 13 mm cuvette for N,N-Diethyl-p-Phenylenediamine (DPD) analysis. The remaining 185 ml were then amperometrically titrated for residual oxidant using phenylarsine oxide (PAO) as the titrant.

Measurement of Oxidant

Five ml of ozonated seawater sample were added to a neutral potassium iodide (KI) solution (8) at room temperature and allowed to react for 30 minutes in the dark. The absorbance of triiodide produced was measured at a wavelength of 352 nm using a 1 cm path length. A standard curve relating triiodide absorbance at 352 nm to oxidant concentration was generated using the amperometric titration described below.

Amperometric oxidant determination experiments were conducted using the Fisher CAT titration system. An amperometric titration using phenylarsine oxide was used following the procedure outline in APHA: Standard Methods, procedure 408C (1).

The DPD assay followed the procedures outlined in the Orbeco-Hellige test kit. Ten ml samples were drawn and placed in a 13 mm cuvette supplied. DPD tablet number 1 was used to determine the free available chlorine. The Hellige Comparator using disk number 430-D was used to determine oxidant concentration. Tablet number 3 was then added to determine the presence of any combined forms of oxidants.

RESULTS AND DISCUSSION

Amperometric titration, potassium iodide (KI) and N,N- dietyl-p-phenyl-enediamine (DPD) tests were performed to determine their ability to detect ozone produced oxidants. These methods yielded different results when bromine and ammonia concentrations were varied in an artificial seawater environment.

As expected, increasing the bromine concentration in the ASW mix resulted in an increase in the residual oxidant concentration. Figure 1 shows in eight trials consisting of varying the levels of ammonia concentration, all but one showed a marked increase in the final oxidant concentration. In most of the trials, a slight reduction in the rate of increase could be seen when the bromine concentration reached 50 ppm above normal levels. Figure 2 shows the effects increasing ammonium ion concentration after ozonation for the same eight trials. All samples showed an increased oxidant residual with low levels of ammonia added, but decreased levels at higher concentrations. Subsequent work, where ammonium hydroxide was added post ozonation, yielded opposite results.

Figure 3 shows a series of 16 samples where bromine concentration was varied on one axis and ammonia concentration on the other. Each sample was tested by each of the three methods described. Although all tests displayed similar trends, a marked difference in the calculated oxidant levels was noted.

The DPD test yielded the highest estimates. This may be due to the subjective method of determining the residual oxidant. Also, due to the nature of the grading scale, as the oxidant values become larger, the divisions between values becomes larger. Additional color wheels used in the comparator for grading various levels of oxidant would help reduce the variation seen in these experiments.

The KI and amperometric test yielded similar results under the conditions of this experiment, although the titrated values were slightly higher. The values varied approximately 0.5 to 1.0 ppm for all samples.

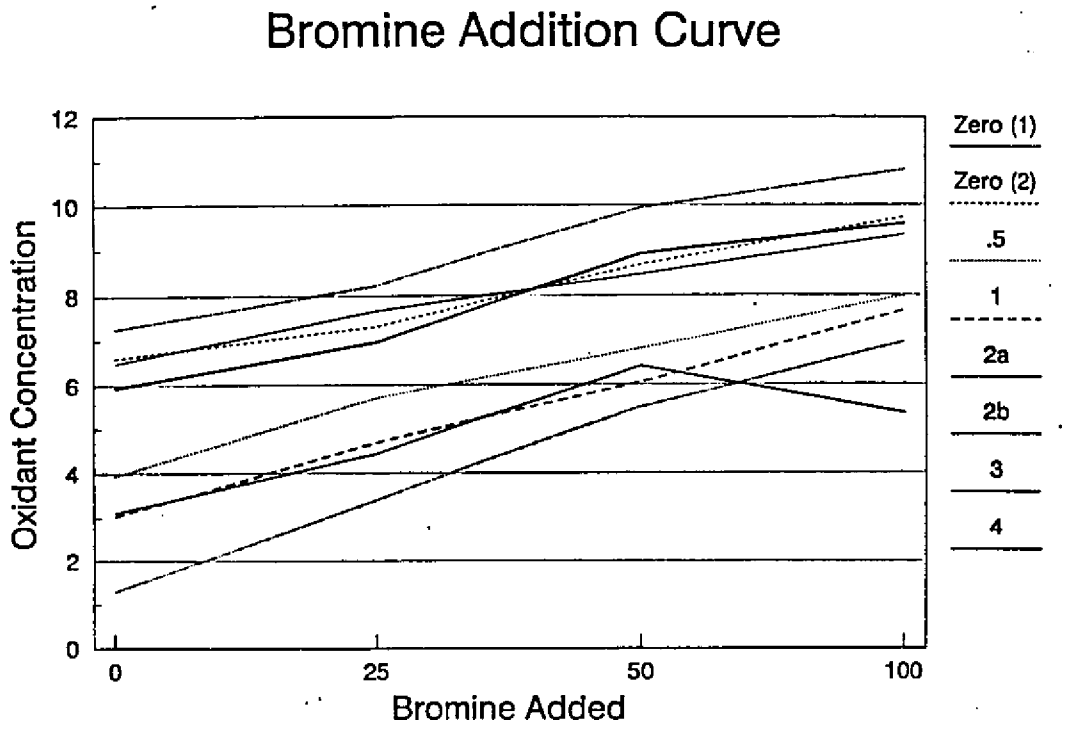


Figure 1

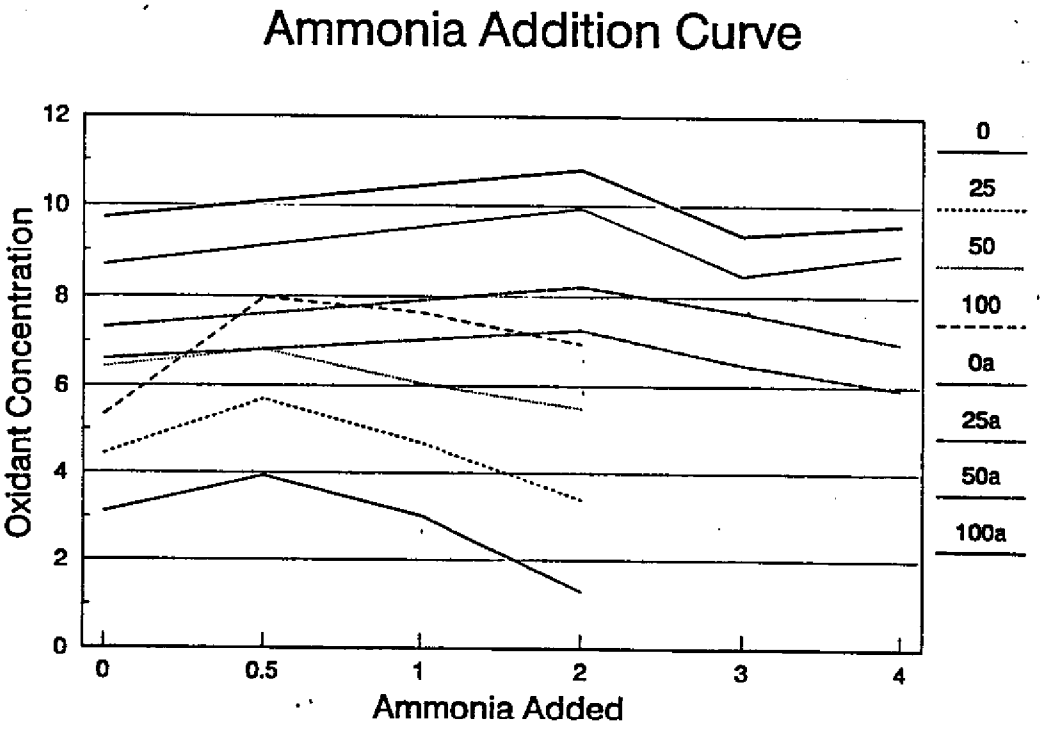
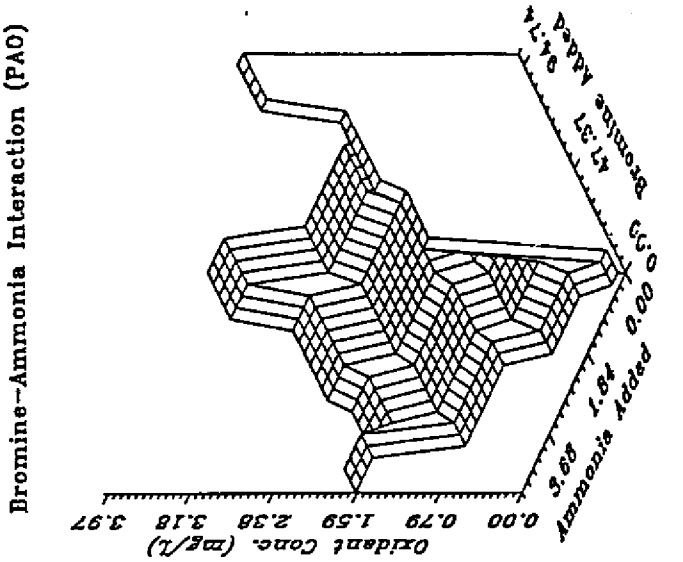


Figure 2


Bromine-Ammonia Interaction (PAO)
Oxidant Conc. (mg/L)
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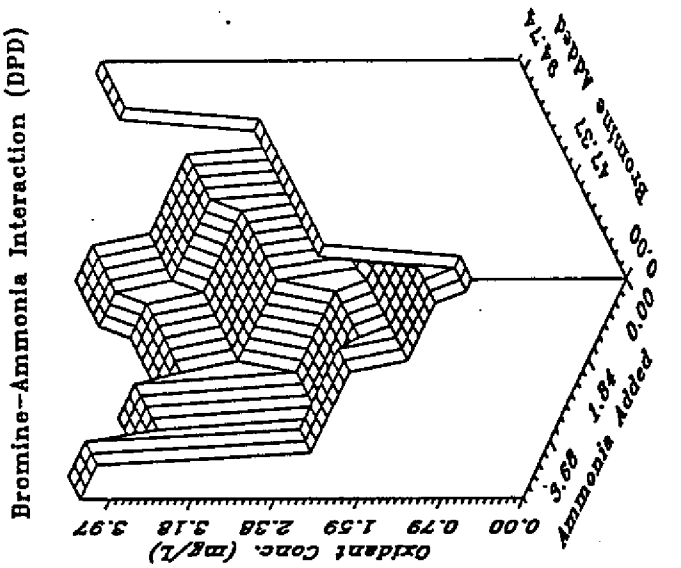
Ammonia Added
0.00 1.84 3.68
Bromine Added
0.00 1.37 2.74


Bromine-Ammonia Interaction (DPD)
Oxidant Conc. (mg/L)
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Ammonia Added
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Bromine Added
0.00 1.37 2.74

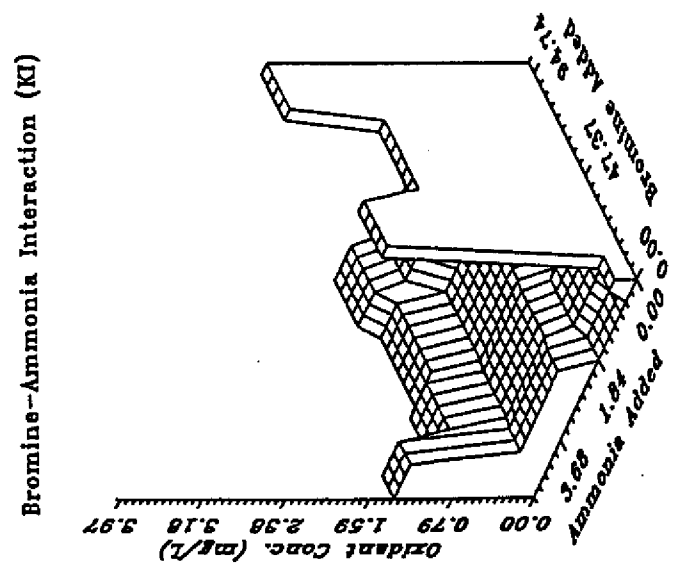
Bromine-Ammonia Interaction (KI)
Oxidant Conc. (mg/L)
0.00 0.79 1.59 2.38 3.18 3.97
Ammonia Added
0.00 1.84 3.68
Bromine Added
0.00 1.37 2.74

Figure 3

CONCLUSIONS

The effective and safe use of ozone in the food industry has tremendous potenial. If the use of ozone can be controlled and measured accurately, the FDA may approve its use in the food industry. Further research in this area is being conducted to examine both pros and cons of the use of ozone as an acceptable processing method.

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METHODS FOR IMPROVING THE QUALITY AND REFRIGERATED SHELFLIFE OF DOGFISH MEAT

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The spiny dogfish, *Squalus acanthias*, is a small shark which had been considered a nuisance to North American fishermen but is now regarded as an underexploited resource. The biomass of dogfish in the northwest Atlantic is estimated at 250,000 to 300,000 metric tonnes (t) with maximum sustainable yields of 20,000 to 40,000 t annually, in the area extending from Cape Hatteras to western Nova Scotia. At present, the Atlantic Canadian fishery is small, being primarily concentrated in the south coast bays of Newfoundland and southwestern Nova Scotia where dogfish are harvested by baited line trawls or 150 mm mesh groundfish gillnets. Products including backs, belly flaps, fins and tails are frozen and sold to foreign markets in the United Kingdom, Germany and the Orient. Dogfish products have been promoted in North America, with the species being renamed as Northern shark, but the consumption continues to be low.

Recently, the Canadian government and fishing industry have promoted the development of underexploited resources by supporting harvesting and processing studies directed at improved quality and increased value of underutilized species. Dogfish research has investigated factors influencing the storage life of fresh, refrigerated and frozen fillets. Woyewoda and Bligh (12) showed that gutting at sea (as compared to storing round without cutting) extended the shelflife of dogfish held at 2°C. The acceptable quality life of the fillets was estimated at less than six days.

Generally, deterioration and subsequent spoilage of fresh dogfish meat has been attributed to the breakdown of urea into ammonia, thus resulting in objectionable sensory characteristics. Dogfish, as well as other elasmobranch species, typically have high concentrations of urea (1-2%) in the blood and flesh. Bacteria play an important role in the spoilage process, being a key source of urease enzymes (2). Other research (3,4,8) supports the conclusion that urea hydrolysis is the primary reaction causing spoilage of dogfish flesh; however, the work of Stansby et al. (10) identified lipid oxidation and rancidity as principal factors. The objective of the present investigation was to test several processing treatments recommended for extending the fresh storage life of dogfish meat with the aim of identifying a simple and practical method that would lead to improved consumer acceptance of the product.

METHODS AND MATERIALS

Processing Treatments and Storage

Dogfish were caught by inshore fishing vessels using baited lines, transferred to large insulated containers, iced and delivered to a fish processing plant in southwestern Nova Scotia within approximately six hours after harvest. The containers were held in a 12°C room overnight

until the following morning. Handling of the dogfish was conducted by plant personnel as follows: fins and tails were removed, the animals were headed and gutted manually, the belly flaps were cut off and then the backs were skinned mechanically. Subsequently, the dogfish backs, or loins, were trimmed and then rinsed in cold, fresh water prior to packaging. All of the fish used in the experiment had been harvested, handled and landed under similar conditions on August 20, 1990, being approximately 18 h postmortem when processed.

Samples of the dogfish backs were taken directly from the end of the production line, treated and then packaged in 13 kg (28 lb) waxed, paperboard cartons which were routinely used for the commercial distribution of frozen dogfish backs. An earlier review of the literature had identified several techniques for reducing the urea content and improving the sensory properties of shark meat. Six different product handling/processing methods, i.e., experimental treatments, tested during the investigation were conducted as follows.

(i) Untreated. Backs taken directly off the processing line were placed in the waxed carton. Temperatures of the meat ranged from 8°C to 14°C at the time of packaging.
(ii) Untreated/Wrapped. Similar to (i) except that each back was placed inside a polyethylene sleeve and than packaged. Internal temperature of the meat ranged from 8°C to 14°C.
(iii) Slush Ice/Wrapped. Backs were soaked for 45 min in a 50%/50% mixture of small ice and fresh water at -1°C and then packaged in an individual sleeve and carton. Meat temperature was reduced to approximately 5°C prior to packaging.
(iv) Brine/Slush Ice/Wrapped. Backs were soaked in a 5% NaCl brine for 10 min at 12°C (pH=6.1), drained and then soaked in slush ice (prepared as described above) for 30 min. Initial temperature of the fish was reduced from 14°C to 6°C prior to packaging in an individual sleeve and carton.
(v) Slush Ice/Acetic Acid/Wrapped. Loins were soaked in slush ice for 30 min at -1°C and then placed in a 5% acetic acid solution at 9°C (pH=2.6) and held for 10 min. Upon removal from the acid, backs were drained, wrapped in individual sleeves and placed in a carton. Temperature of the meat averaged 7.5°C.
(vi) Iced for 1 Day/Wrapped. Backs were placed in single layers of small ice in 100-lb fish boxes. The boxes were covered and then transferred to a refrigerated storage room which was cooled to 12°C. Samples were stored until the following morning, by which time most of the ice had melted and the backs were floating in an ice/water mixture. Fresh ice was obtained and the backs were again packed in single layers of small ice in covered fish boxes.

Two cartons of fish, containing 14 to 15 loins each, were prepared for each of the six treatments. Additional samples of the untreated/wrapped treatment (ii) were packaged and frozen in a blast freezer shortly after preparation. These samples were used as the "controls" or high quality reference samples during the raw grading and sensory evaluation sessions.

Adequate cold storage facilities were not available at the processing plant; consequently, cartons containing the experimental products were enclosed in large plastic bags and stored in ice, in two household chest freezers that had been prechilled to -1°C. A thermocouple was placed inside one sample carton in each freezer so that the product temperatures could be monitored without opening the units. Temperature of the fish was lowered to and then maintained at -1°C during transport to the food science laboratories at the Technical University of Nova Scotia (approximately 26 h). Frozen samples were packaged in an insulated container for shipment. Upon arrival, the containers for experimental lots (i) through (v) were transferred to a 2°C walk-in chill room where they were stored for ll days. Dogfish backs that had been held in ice, treatment (vi) were packaged in individual polyethylene sleeves and placed in waxed paperboard cartons and stored along with the other experimental samples. Frozen reference samples were placed in a computer-controlled cold storage room maintained at -30°C.

Product Sampling and Raw Quality Evaluation

The initial sampling and evaluation of the products was conducted on the day after the dogfish had been delivered to the laboratory, which was approximately 65 h after the fish had been harvested. This first sampling date was designated day 3. Sample quality was assessed every two days thereafter, i.e., on days 5, 7, 9 and 11 of the storage period. On each sampling occasion, three dogfish backs from each treatment were selected randomly from the sample cartons, each loin was placed on an individual tray and assigned a three-digit numerical code. The samples were presented to a group of six judges for determination of product odor, color, texture and acceptability. A labelled reference sample, and a hidden or coded reference sample, were also presented for evaluation. These samples were taken from the product that had been packaged, frozen and stored at -30°C, and were thawed overnight at 2°C prior to each grading session. Panellists were informed that the labelled reference was an example of high quality dogfish and were asked to evaluate the experimental samples in comparison to this reference. An example of the scoresheet used to record each judge's assessments is given in Appendix I.

Chemical Testing

On each testing day the dogfish backs that had been examined for raw quality were used to prepare analytical samples for chemical and cooked sensory evaluations. Each of the three backs from the six treatment lots was cut according to the following procedure.

Three cross section steaks were cut from the front, middle and tail portions of each back, such that each section was approximately the same length as the width of the fish. The cartilage was removed by cutting the meat away from the central "backbone" thus producing two side pieces for each section, i.e., six pieces from each fish. Surface pH was measured on the outer (skin-side) surface of each sample piece with an Orion surface pH electrode. The eighteen pieces of fish from the three backs from each treatment were vacuum packaged in an oxygen impermeable bag and then immediately frozen in a plate freezer. Samples were stored at -30°C for one to two weeks and then defrosted at ambient room temperature prior to chemical analysis.

Each composite sample was homogenized in a domestic food processor. One 50-g aliquot of the homogenate was extracted with 100 ml of 7.5% trichloroacetic acid (TCA) as described by Woyewoda et al. (13). This extract was used to measure the concentrations of total volatile bases (TVB), urea and ammonia (NH_3) present in the flesh. The flesh moisture content was measured in triplicate by the methods of Woyewoda et al. (13) Flesh pH, determined by blending 15-20 g of the homogenized tissues with an equal quantity of distilled water, was assayed in triplicate. The urea and ammonia content of the TCA extracts was determined using a commercial urease/glutamate dehydrogenase urea kit (Boehringer Mannheim Biochemica).

TVB levels were determined by a steam distillation/titration method that was modified by use of a Buchi kjeldahl distillation unit (1). In the distillation flask, 2-5 ml of the TCA extract was added to 50 ml of distilled water and 10 ml of 2M NaOH. The flask was immediately connected to the distillation unit and distilled for 5 min. The liberated bases were collected in 15.00 ml of standard 0.01 M HCl with BDH N-point indicator (BDH Chemicals Canada Limited). After distillation, the excess HCl was titrated with standard 0.01 M NaOH. The TVB-N content of each sample was assayed in triplicate and calculated as mg TVB-N per 100 g of fish.

Sensory Evaluation

The remaining portions of meat from each dogfish back were vacuum packaged and stored at -30°C for one month. Only samples receiving a acceptability rating of 2 or higher during the raw grading tests were submitted for sensory evaluation. Testing of the cooked samples was conducted in triplicate by six experienced staff members. Samples were prepared as follows.

The frozen samples were wrapped in aluminum foil and cooked at 204°C (400°F) for one hour. After cooking, the meat was cut into cubes of approximately 2.5 cm. Three sample pieces of each treatment were placed in an individual coded glass petri dish. During each panel session, four experimental samples were served to the panellists along with a high quality reference sample, labelled "R", and a hidden or coded reference sample. A beverage consisting of a 50%/50% mixture of distilled water and lemon-lime soda was provided for cleansing the palate. Odor, flavor, texture and acceptability of each sample was evaluated using a scoresheet adapted from Kosmark (6) as shown in Appendix II.

Statistical Analysis

Raw grading and sensory evaluation data were analyzed according to Wilkinson (11). For each grading or sensory evaluation characteristic, analysis of variance using Tukey (HSD) was performed to detect differences between treatments for each evaluation day at $P<0.05$. Similarly, analysis of variance using Tukey (HSD) was performed on the data obtained from the chemical determinations, such that differences between treatments for each evaluation day were detected at $P<0.05$.

RESULTS AND DISCUSSION

Raw Quality Evaluation

Quality differences between the dogfish backs were assessed by scoring the various samples for odor, color, texture and acceptability. Generally, characteristics were ranked using a four point scale, wherein a score of four was assigned to samples with superior quality and unacceptable or reject samples were given a score of one. In the color assessment, a score of five was assigned to samples that were white, pink samples were given a four, but neither grade was considered superior to the other. Values lower than four indicated deterioration of sample quality.

Tables 1-4 and Figures 1-4 show the results of the raw grading tests. All of the samples, except those from treatment (ii) were considered highly acceptable on the first day of testing when the dogfish were approximately three days postmortem. Sample (ii) had a mean acceptability rating of 2.7 on day 3, which was significantly different from the scores assigned to the other five treatments. On day 5, both of the untreated lots (i) and (ii) had an ammonical odor making them borderline in acceptability. Treatment (v) was rated at low acceptability on day 5, primarily due to the off odors resulting from the acetic acid treatment. After seven days at 2°C, treatment (vi) was the only sample having a "mid" acceptability rating. Dogfish backs from treatments (v) and (vi) were given a low acceptability score on day 9. All of the samples were unacceptable when examined on day 11.

Although the data indicate that treatment (v) slowed the rate of product deterioration, soaking the backs in 5% acetic acid for 30 min had obvious undesirable effects upon product quality. The samples had an objectionable surface sliminess that was evident upon completion of the soaking step. The product retained this characteristic, as well as a moderately strong vinegar aroma throughout the storage period. With a less severe acetic acid treatment, it may be possible to extend the high quality life of fresh dogfish without contributing objectionable changes to the meat. Overall, results of the raw grading indicate that treatment (vi) was the most successful, in that it added three to four days to the acceptable shelflife of the product in comparison to the untreated samples.

Table 1. Raw Quality Odor of Stored Dogfish Samples

Mean scores for Odor (4.0 = neutral, 1.0 = putrid)

EVALUATION DAY	TREATMENT LOTS 1	2	3	4	5	6
3	3.6^{a}	2.7^{b}	3.4^{a}	3.4^{a}	3.7^{a}	3.3^{ab}
5	1.9^{c}	2.1^{c}	3.1^{ab}	3.2^{ab}	2.8^{b}	3.4^{a}
7	1.6^{c}	1.8^{c}	2.4^{b}	1.8^{c}	2.6^{b}	3.4^{a}
9	1.6^{c}	1.4^{c}	1.8^{bc}	1.7^{bc}	2.7^{a}	2.3^{ab}
11	1.4^{b}	1.3^{b}	1.6^{b}	1.4^{b}	2.8^{a}	1.4^{b}

For each evaluation day, statistically significant differences (P>0.05) between the six treatment lots are denoted by different superscripts. Means bearing the same letter are not significantly different.

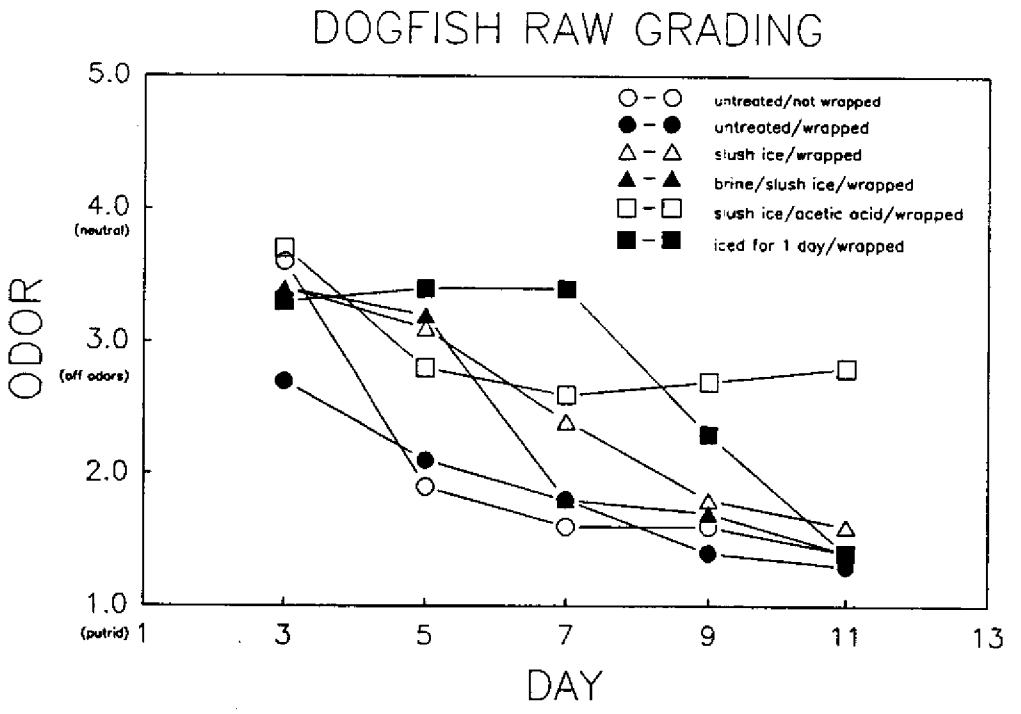

Figure 1. Raw Quality Odor of Stored Dogfish Samples

Table 2. Raw Quality Color of Stored Dogfish Samples

Mean scores (5.0 = white, 4.0 = pink, 1.0 = strong discolored)

EVALUATION DAY	TREATMENT LOTS 1	2	3	4	5	6
3	4.6^{a}	2.8^{c}	4.1^{ab}	3.8^{b}	3.7^{b}	3.8^{b}
5	3.1^{b}	3.7^{ab}	3.7^{ab}	3.6^{ab}	2.8^{b}	4.2^{a}
7	2.2^{c}	2.8^{bc}	3.3^{a}	3.0^{b}	2.3^{bc}	3.8^{a}
9	2.3^{a}	2.8^{a}	2.5^{a}	2.5^{a}	2.4^{a}	2.5^{a}
11	1.8^{a}	1.9^{a}	1.9^{a}	1.8^{a}	1.7^{a}	2.3^{a}

For each evaluation day, statistically significant differences (P>0.05) between the six treatment lots are denoted by different superscripts. Means bearing the same letter are not significantly different.

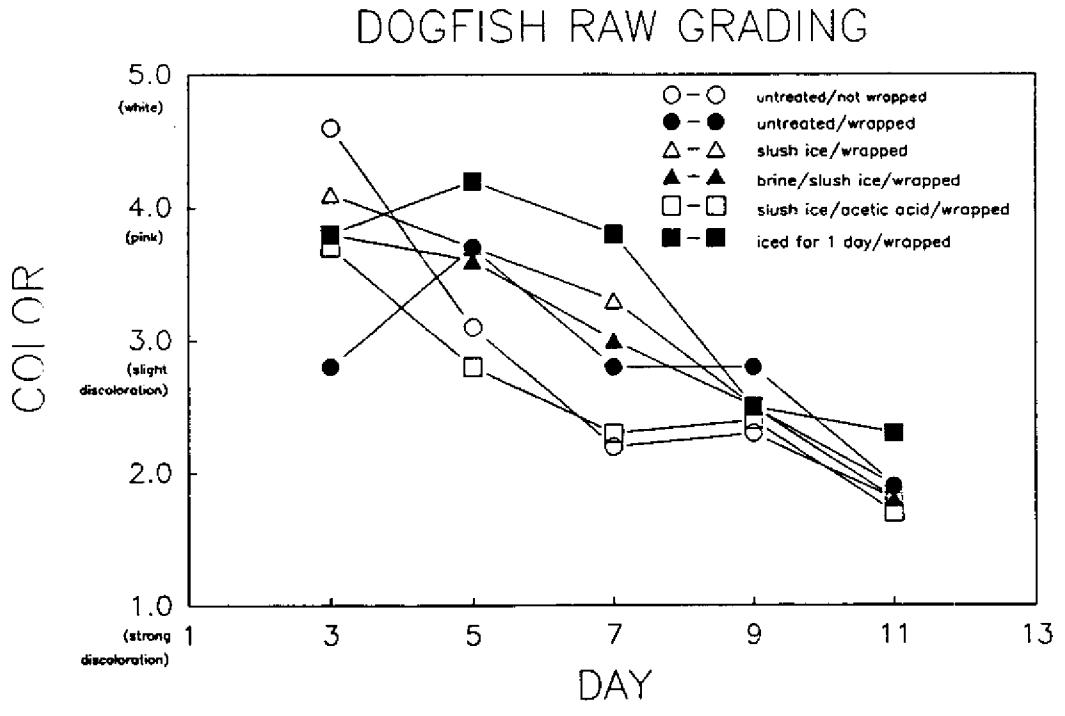

Figure 2. Raw Quality Color of Stored Dogfish Samples

Table 3. Raw Quality Texture of Stored Dogfish Samples

Mean scores for Texture (4.0 = firm, 1.0 = mushy/grainy)

EVALUATION DAY	TREATMENT LOTS 1	2	3	4	5	6
3	3.8^{a}	3.7^{a}	3.8^{a}	4.0^{a}	3.6^{a}	3.9^{a}
5	3.1^{b}	3.7^{ab}	3.7^{ab}	3.5^{ab}	3.3^{ab}	3.8^{a}
7	2.6^{c}	2.8^{bc}	3.1^{abc}	3.4^{ab}	2.6^{c}	3.6^{a}
9	2.2^{b}	2.3^{b}	2.5^{ab}	2.8^{ab}	3.3^{a}	3.4^{a}
11	2.0^{a}	2.0^{a}	1.9^{a}	2.3^{a}	2.2^{a}	2.4^{a}

For each evaluation day, statistically significant differences (P>0.05) between the six treatment lots are denoted by different superscripts. Means bearing the same letter are not significantly different.

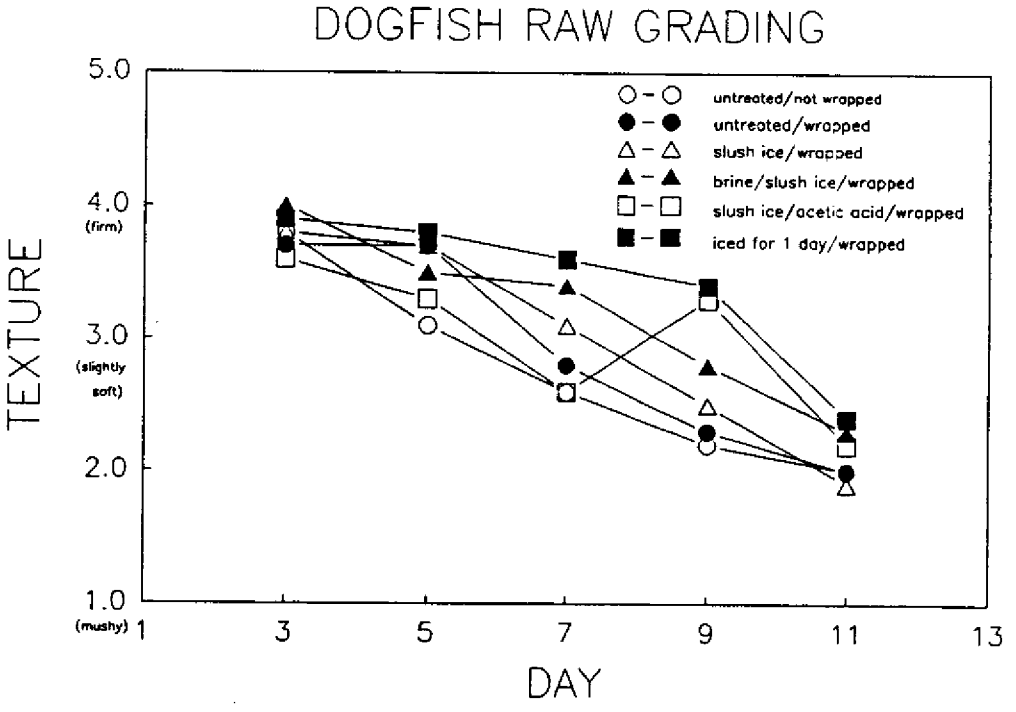

Figure 3. Raw Quality Texture of Stored Dogfish Samples

Table 4. Raw Quality Acceptability of Stored Dogfish Samples

Mean scores for Acceptability (4.0 = high, 1.0 = reject)

EVALUATION DAY	TREATMENT LOTS 1	2	3	4	5	6
3	3.7^{a}	2.7^{b}	3.7^{a}	3.7^{a}	3.7^{a}	3.5^{a}
5	1.9^{b}	2.2^{b}	3.0^{a}	3.2^{a}	2.0^{b}	3.4^{a}
7	1.4^{cd}	1.7^{cd}	2.4^{b}	2.1^{bc}	2.1^{bc}	3.2^{a}
9	1.5^{b}	1.4^{b}	1.7^{b}	1.7^{b}	2.5^{a}	2.3^{a}
11	1.4^{a}	1.3^{a}	1.3^{a}	1.3^{a}	1.4^{a}	1.4^{a}

For each evaluation day, statistically significant differences ($P>0.05$) between the six treatment lots are denoted by different superscripts. Means bearing the same letter are not significantly different.

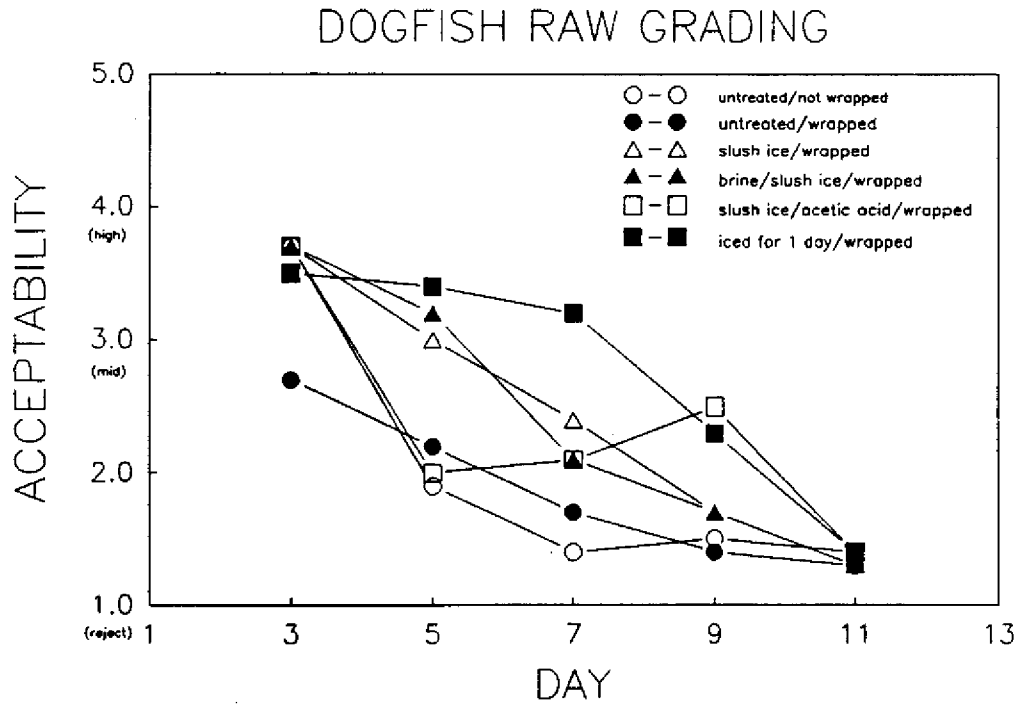

Figure 4. Raw Quality Acceptability of Stored Dogfish Samples

Chemical Indices of Product Quality

Studies investigating the spoilage of shark have identified the production of volatile amines and ammonia, changes in the flesh pH, bacterial growth and oxidative rancidity as key indices of quality loss (3,4,5,7,9,11). Ravesi et al. (8) in a shelflife study with gutted, headed and gutted, and whole dogfish that were boxed, iced and stored at 1.6°C (35°F) reported the following parameters as signalling the end of the useful shelflife for dogfish products: ammonia N content >24-29 mg%N; TVB >26-34 mg%N; pH >6.2-6.3; Torrymeter reading <5-7; TBA number >3-3.8. The TVB value is a well accepted indicator of quality deterioration in seafoods. A TVB concentration of 30 mg/100 g of fish flesh is frequently cited as the threshold level, with samples ranging from 30-40 mg/100 g regarded as being acceptable (5).

The chemical indices monitored in this study included TVB, flesh and surface pH, urea and moisture contents. Ammonia levels were monitored as part of the urea assay, but the combined measurement technique was not sensitive enough to give useful data. (The TVB test measured ammonia along with other end products of spoilage.) Results of the chemical analyses are given in Tables 5-9. Spoilage patterns are shown graphically in Figures 5-9.

Samples of the dogfish taken on the first testing date (day 3) were within the above-mentioned limits of acceptability. All of the treatments had TVB-N levels less than 40 mg/100 g of fish and pH values less than 6.3. Urea content of treatment (vi) dogfish was significantly lower than that of the other lots, which was attributed to leaching of the urea during the initial holding in ice. The urea concentration of the (vi) samples remained at approximately the same level until the final testing date, day 11, when it decreased to 0.813%. The moisture content of treatment (vi) samples averaged 73.20%, which was significantly higher than that of any other treatment. Moisture contents of all six lots varied somewhat over the 11 day storage period.

As is shown in Figure 7, TVB levels in the untreated dogfish, treatments (i) and (ii) increased rapidly after day 3 and continued to rise until termination of the study. Lots (iii) and (iv) exceeded the 40 mg/100 g limit on day 7. Treatment (vi) showed a dramatic increase from 17.77 mg/100 g on day 7 to 105.23 mg/100 g on day 9. TVB levels in the dogfish treated with acetic acid (v) never exceeded the acceptability limit, ranging from 13.69 mg/100 g on day 3, to 17.17 mg/100 g on day 9. It is speculated that treatment (v) successfully suppressed bacterial growth, thereby eliminating a primary source of urease enzymes responsible for the conversion of urea to ammonia.

The pH determinations on the outer surfaces of the backs and flesh homogenates followed spoilage patterns similar to those shown by the TVB measurements (see Figures 8 and 9). Using a pH value of 6.4 as the upper limit for acceptability, the shelflife of the untreated dogfish was three days. Lots (iii) the slush ice treatment, and (iv) the brine/slush ice treatment, had a five day shelflife, and treatment (vi) had a seven day shelflife at 2°C. The pH of dogfish samples soaked in acetic acid was less than 5.7 throughout the study. These results correlate well with those of the TVB tests.

Sensory Evaluation of Cooked Samples

Determination of the edible quality of a food sample is an important step in establishing the efficacy of methods used for processing. In this study, only samples having a raw grading acceptability mean score of 2 or higher were submitted for evaluation of the cooked odor, flavor, texture and overall acceptability. Samples from all six treatments were taken on days 3 and 5. From day 7, treatments (iii), (iv), (v) and (vi) were tested; whereas only lots (v) and (vi) were analyzed from day 9. All of the samples from day 11 were considered unacceptable for sensory evaluation.

Table 5. Urea Content of Stored Dogfish Samples

Mean value (%) with standard deviation given below

EVALUATION DAY	TREATMENT LOTS 1	2	3	4	5	6
3	1.67^{a} 0.04	1.55^{ab} 0.08	1.61^{ab} 0.02	1.45^{b} 0.02	1.51^{ab} 0.10	1.01^{c} 0.03
5	1.59^{a} 0.16	1.55^{a} 0.09	1.51^{a} 0.06	1.50^{a} 0.09	1.54^{a} 0.09	1.06^{b} 0.05
7	1.27^{b} 0.04	1.38^{a} 0.03	1.39^{a} 0.04	1.21^{b} 0.01	1.43^{a} 0.00	1.10^{c} 0.00
9	1.36^{ab} 0.08	1.37^{ab} 0.03	1.42^{ab} 0.14	1.16^{ab} 0.16	1.54^{a} 0.10	1.05^{b} 0.01
11	1.16^{ab} 0.10	1.12^{ab} 0.12	1.10^{b} 0.15	1.14^{ab} 0.04	1.48^{a} 0.04	0.81^{b} 0.01

For each evaluation day, statistically significant differences (P>0.05) between the six treatment lots are denoted by different superscripts. Means bearing the same letter are not significantly different.

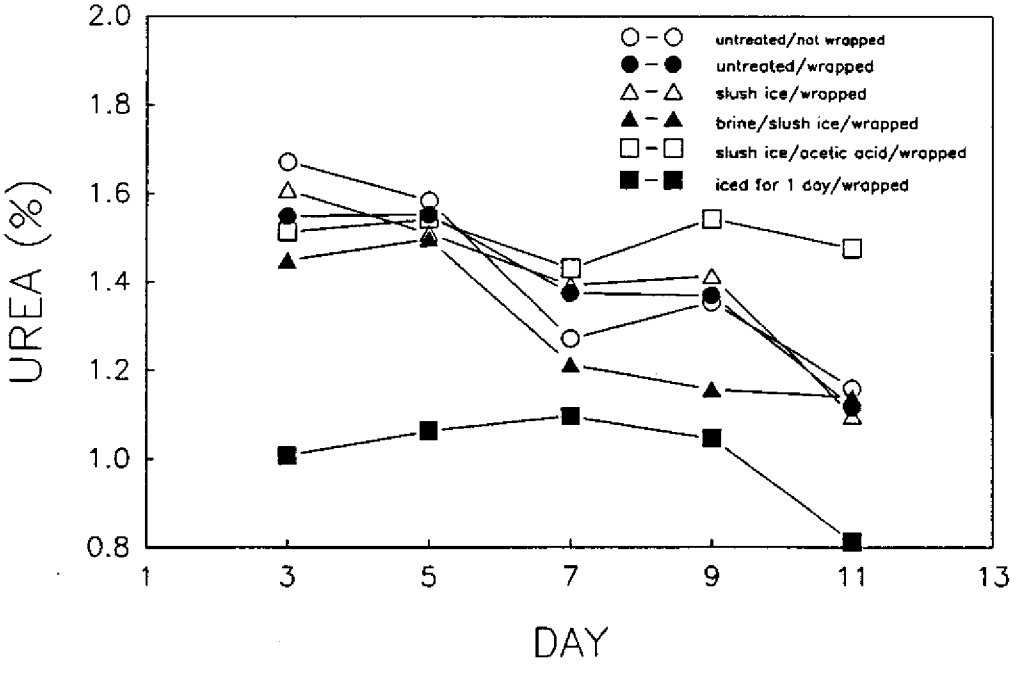

Figure 5. Urea Content of Stored Dogfish Samples

Table 6. Moisture Content of Stored Dogfish Samples

Mean value (%) with standard deviation given below

EVALUATION DAY	TREATMENT LOTS 1	2	3	4	5	6
3	70.18^{cd} 0.13	71.88^{b} 0.10	71.03^{c} 0.09	70.07^{e} 0.10	70.56^{d} 0.15	73.20^{a} 0.12
5	70.61^{cd} 0.10	73.00^{b} 0.03	70.51^{d} 0.12	69.03^{e} 0.05	70.80^{c} 0.05	73.33^{a} 0.03
7	68.65^{f} 0.00	72.24^{a} 0.04	70.87^{c} 0.05	71.77^{b} 0.06	69.98^{e} 0.11	70.65^{d} 0.03
9	71.79^{b} 0.08	74.46^{a} 0.13	71.68^{b} 0.09	74.42^{a} 0.09	70.48^{c} 0.26	69.73^{d} 0.24
11	71.08^{b} 0.08	71.93^{a} 0.25	69.68^{c} 0.07	71.94^{a} 0.10	68.04^{d} 0.15	71.65^{a} 0.07

For each evaluation day, statistically significant differences (P>0.05) between the six treatment lots are denoted by different superscripts. Means bearing the same letter are not significantly different.

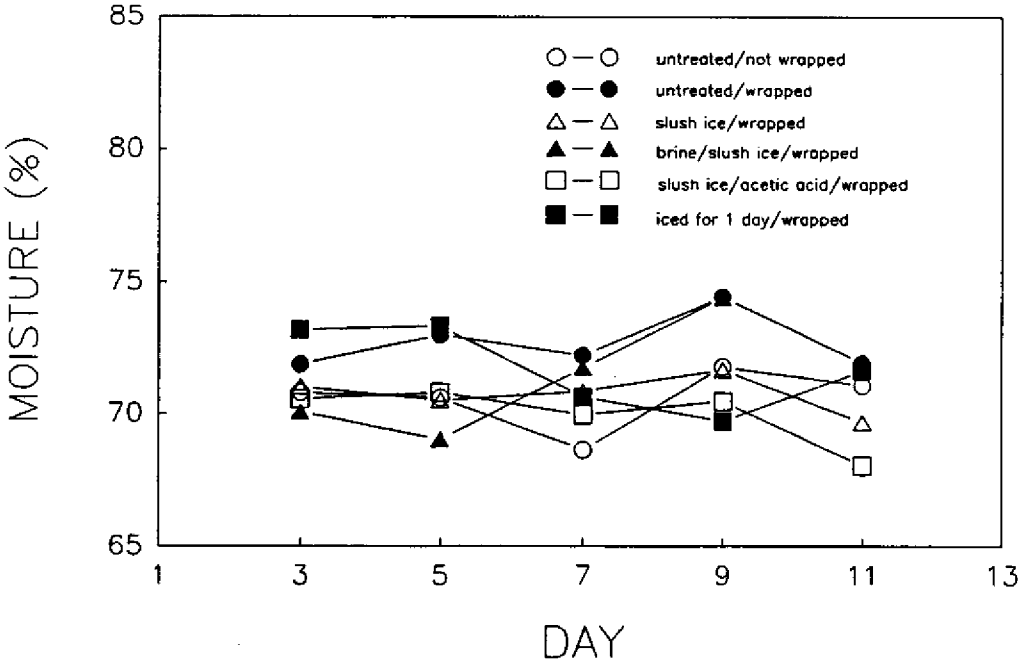

Figure 6. Moisture Content of Stored Dogfish Samples

Table 7. Total Volatile Bases Content of Stored Dogfish Samples

TVB-N mg/100 g fish Mean values with standard deviation below

EVALUATION DAY	TREATMENT LOTS 1	2	3	4	5	6
3	17.11^{a} 0.21	15.28^{b} 0.79	14.22^{bc} 0.44	13.91^{bc} 0.76	13.69^{c} 0.44	10.00^{d} 0.58
5	101.20^{a} 1.44	71.73^{b} 0.55	16.00^{d} 0.47	25.80^{c} 0.94	14.44^{d} 0.98	9.79^{e} 0.73
7	164.99^{a} 1.96	146.61^{b} 2.54	47.91^{d} 2.28	138.54^{c} 1.32	14.99^{e} 1.67	17.77^{e} 0.36
9	223.48^{a} 4.39	226.32^{a} 1.92	151.95^{b} 4.15	222.44^{a} 3.33	17.17^{d} 1.45	105.23^{c} 0.55
11	260.22^{b} 7.18	269.92^{b} 6.87	229.49^{c} 1.44	284.52^{a} 2.91	14.89^{e} 2.17	165.10^{d} 0.73

For each evaluation day, statistically significant differences (P>0.05) between the six treatment lots are denoted by different superscripts. Means bearing the same letter are not significantly different.

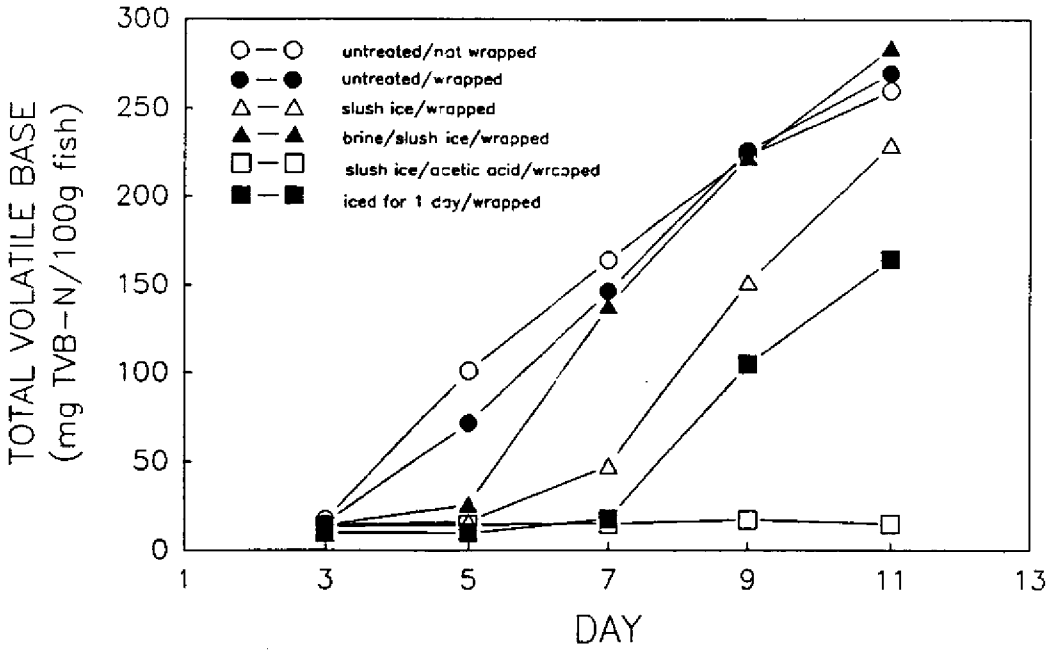

Figure 7. Total Volatile Bases Content of Stored Dogfish Samples

Table 8. Surface pH of Stored Dogfish Samples

Mean value with standard deviation given below

EVALUATION DAY	TREATMENT LOTS 1	2	3	4	5	6
3	6.14^{a} 0.07	6.08^{b} 0.03	6.12^{ab} 0.03	6.02^{c} 0.06	5.53^{d} 0.04	6.03^{c} 0.06
5	7.83^{a} 0.37	7.54^{b} 0.43	6.05^{d} 0.06	6.30^{c} 0.26	5.47^{e} 0.06	6.00^{d} 0.04
7	8.38^{a} 0.16	8.31^{a} 0.09	7.32^{b} 0.48	8.26^{a} 0.21	5.58^{d} 0.05	6.34^{c} 0.36
9	8.56^{a} 0.21	8.47^{a} 0.01	7.88^{b} 0.46	8.44^{a} 0.11	5.69^{c} 0.07	7.88^{b} 0.43
11	8.51^{a} 0.09	8.55^{a} 0.07	8.39^{a} 0.12	8.48^{a} 0.13	5.68^{b} 0.06	8.37^{a} 0.47

For each evaluation day, statistically significant differences (P>0.05) between the six treatment lots are denoted by different superscripts. Means bearing the same letter are not significantly different.

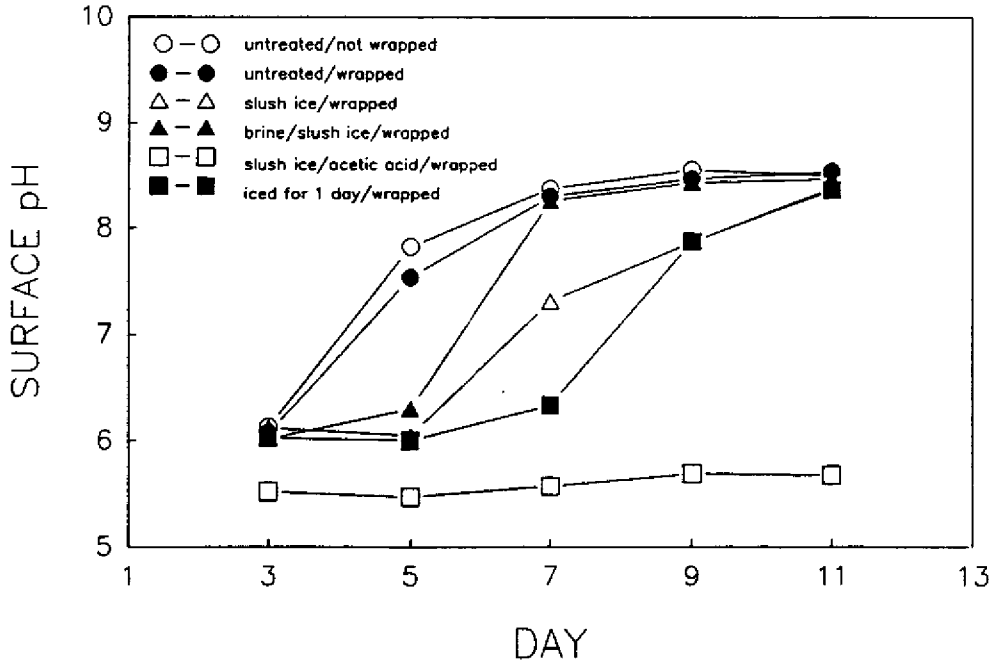

Figure 8. Surface pH of Stored Dogfish Samples

Table 9. Flesh pH of Stored Dogfish Samples

Mean value with standard deviation given below

EVALUATION DAY	TREATMENT LOTS 1	2	3	4	5	6
3	5.80^{a} 0.05	5.70^{b} 0.03	5.71^{b} 0.02	5.58^{c} 0.01	5.07^{d} 0.03	5.65^{bc} 0.01
5	6.67^{a} 0.01	6.40^{b} 0.02	5.68^{d} 0.02	5.76^{c} 0.02	5.01^{f} 0.01	5.61^{e} 0.01
7	7.73^{a} 0.05	7.43^{b} 0.04	6.62^{e} 0.01	7.38^{b} 0.01	5.24^{e} 0.02	5.80^{d} 0.05
9	7.83^{a} 0.00	7.62^{b} 0.08	7.09^{c} 0.03	7.68^{b} 0.03	5.14^{e} 0.02	6.96^{d} 0.03
11	8.16^{a} 0.04	8.13^{a} 0.02	7.79^{b} 0.02	8.11^{a} 0.03	5.28^{c} 0.01	7.73^{b} 0.06

For each evaluation day, statistically significant differences (P>0.05) between the six treatment lots are denoted by different superscripts. Means bearing the same letter are not significantly different.

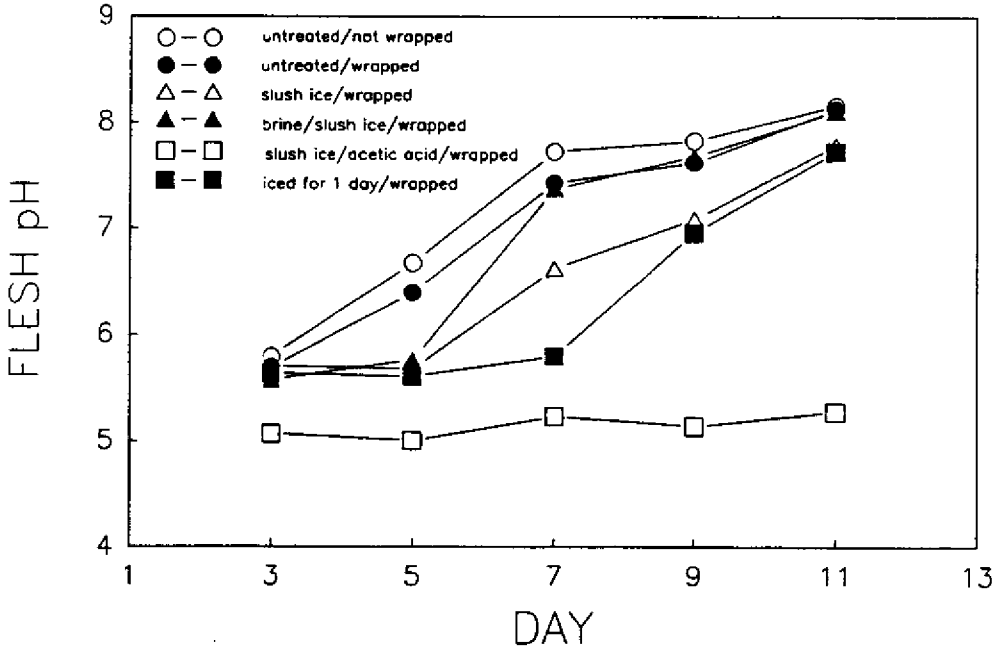

Figure 9. Flesh pH of Stored Dogfish Samples

Samples from all treatment lots except treatment (v), were judged as acceptable in odor, flavor and overall acceptability on day 3 (see Tables 10, 11, 13). Samples from treatment (vi) ranked highest in acceptability, receiving a mean score of 6.1 which was significantly higher than the acceptability of dogfish from treatment (v) which was borderline.

No significant differences in odor, flavor and acceptability of the six treatment lots were detected on day 5. Texture of the unwrapped backs from treatment (i) was slightly tougher than that of the other five lots (see Table 12). This may have resulted from greater air contact with the unwrapped meat surface, thus causing hardening of the tissues.

After seven days at 2•C, ammonia was detected in all of the samples except treatment (vi). The sensory characteristics of treatment (vi) dogfish were ranked as borderline on day 7, whereas samples from the other treatments exhibited obvious signs of spoilage. The dogfish that had been soaked in acetic acid (v) was considered slightly poor in quality after nine days storage, at which time acceptability of product from the one day in ice, treatment (vi) was poor to very poor. Data from the sensory evaluations are given in Tables 10-13.

Earlier studies regarding the spoilage of dogfish have dealt mainly with the shelflives of whole, gutted or bled fish. Handling practices, such as gutting and/or bleeding and then icing of the catch immediately after harvesting were considered critical to achieving the maximum high quality life (8). Ravesi et al. (8) reported a shelflife of 11-12 days for well iced, round dogfish, that could be extended by approximately four days if the fish were gutted immediately. Bilinski et al. (3) found rapid chilling to be essential in preventing the formation of ammonia and trimethylamine in dogfish muscle for up to 12 days. Other researchers observed a six to eight day acceptable storage life for iced, dressed dogfish (7,12).

Results of the present study indicated that treatment (vi), i.e., holding the dogfish backs in ice for one day prior to packaging, contributed approximately three days to the useful shelflife of the product. Soaking the backs in slush ice for 45 min, treatment (iii) also offered benefits, although not as successful as treatment (vi). Commercial application of these treatments may improve the consumer acceptance of dogfish products in North American markets. By implementing these recommendations along with those for harvesting, preprocessing and handling of this species, increased utilization of the resource may be achieved.

SUMMARY AND CONCLUSIONS

In this study, research efforts were aimed at identifying methods to improve the quality and extend the refrigerated shelflife of fresh dogfish meat. Fish were harvested by hand line, iced and delivered to a fish plant for processing. Heading, gutting and skinning were conducted by plant personnel, after which samples of the dogfish backs were treated by holding in slush ice, NaCl brine solution, acetic acid solution and ice for specified periods of time, followed by packaging and storage at 2•C. Samples were assessed periodically for quality by grading, sensory evaluation and measurement of pH, urea, TVB and moisture. Results of the investigation indicated that the quality and shelflife of fresh dogfish was improved by holding the meat in ice for one day prior to packaging. Untreated products deteriorated more quickly than the samples exposed to the experimental processing methods.

ACKNOWLEDGEMENTS

The financial support provided by the Fisheries Development and Fishermen's Services Division, Department of Fisheries and Oceans is gratefully acknowledged. Appreciation is also expressed to D. Singer, J. Kell and K. Wright for their assistance in the execution and completion of this work.

Table 10. Sensory Evaluation Odor of Stored Dogfish Samples

Mean scores for Odor (9.0 = excellent, 1.0 = putrid)

EVALUATION DAY	TREATMENT LOTS 1	2	3	4	5	6
3	5.9[a]	5.9[a]	5.9[a]	5.6[a]	4.8[a]	5.3[a]
5	3.9[a]	4.8[a]	5.1[a]	5.2[a]	4.8[a]	4.9[a]
7			3.6[b]	2.9[b]	4.1[ab]	5.4[a]
9					4.4[a]	3.4[a]

For each evaluation day, statistically significant differences (P>0.05) between the six treatment lots are denoted by different superscripts. Means bearing the same letter are not significantly different.

Table 11. Sensory Evaluation Flavor of Stored Dogfish Samples

Mean scores for Flavor (9.0 = excellent, 1.0 = putrid)

EVALUATION DAY	TREATMENT LOTS 1	2	3	4	5	6
3	5.6[a]	6.0[a]	6.1[a]	5.8[a]	4.5[a]	6.0[a]
5	4.3[a]	4.5[a]	4.7[a]	4.4[a]	4.3[a]	4.6[a]
7			3.7[a]	3.4[a]	3.7[a]	4.5[a]
9					3.6[a]	2.9[a]

For each evaluation day, statistically significant differences (P>0.05) between the six treatment lots are denoted by different superscripts. Means bearing the same letter are not significantly different.

Table 12. Sensory Evaluation Texture of Stored Dogfish Samples

Mean scores (9.0 = optimum, 2.0 = mushy, 1.0 = rubbery)

EVALUATION DAY	TREATMENT LOTS 1	2	3	4	5	6
3	5.6^{a}	6.4^{a}	6.3^{a}	5.8^{a}	6.2^{a}	6.4^{a}
5	4.0^{b}	5.1^{ab}	6.2^{a}	6.5^{a}	6.5^{a}	5.2^{ab}
7			5.2^{a}	4.7^{a}	5.3^{a}	5.4^{a}
9					5.8^{a}	4.0^{b}

For each evaluation day, statistically significant differences (P>0.05) between the six treatment lots are denoted by different superscripts. Means bearing the same letter are not significantly different.

Table 13. Sensory Evaluation Acceptability of Stored Dogfish Samples

Mean scores for Acceptability (9.0 = excellent, 1.0 = inedible)

EVALUATION DAY	TREATMENT LOTS 1	2	3	4	5	6
3	5.2^{ab}	5.5^{ab}	5.4^{ab}	5.5^{ab}	4.3^{b}	6.1^{a}
5	4.3^{a}	4.6^{a}	4.6^{a}	4.4^{a}	3.8^{a}	4.4^{a}
7			3.4^{ab}	2.8^{b}	3.9^{ab}	4.9^{a}
9					4.3^{a}	2.6^{b}

For each evaluation day, statistically significant differences (P>0.05) between the six treatment lots are denoted by different superscripts. Means bearing the same letter are not significantly different.

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APPENDIX I

DOGFISH FILLET QUALITY - SCORE SHEET

Name:_____
Date:_____

CODE	ODOR	COLOR	TEXTURE	ACCEPTABILITY

ODOR
4 neutral
3 sl. off odor
2 ammonia
1 strong ammonia/ putrid

COLOR
5 white
4 pink
3 sl. discolored
2 moderate discolored
1 strong discolored

TEXTURE
4 firm
3 sl. soft
2 soft
1 mushy/grainy

ACCEPTABILITY
4 high acceptability
3 mid acceptability
2 low acceptability
1 reject

APPENDIX II

DOGFISH SENSORY EVALUATION

Name:_____
Date:_____

ODOR	SAMPLE CODE			
Excellent,fresh				
Strong, fresh				
Fresh odor lost				
Almost no odor				
BORDERLINE				
Sl. NH3 /rancid				
Mod. NH3 /rancid				
Strong off odor				
Putrid				

FLAVOR	SAMPLE CODE			
Excellent,fresh				
Fresh flavor				
Freshness lost				
Flat, neutral				
BORDERLINE				
Sl. off flavor				
Mod. off flavor				
Strong off				
Putrid				

TEXTURE	SAMPLE CODE			
Mushy				
Very soft				
Soft-to-tender				
Moderate tender				
OPTIMUM TEXTURE				
Moderate firm				
Firm-to-tough				
Very tough				
Extremely tough				

ACCEPTABILITY	SAMPLE CODE			
Excellent				
Very good				
Good				
Fair				
BORDERLINE				
Slightly poor				
Poor				
Very poor				
Inedible				

REPORT ON EMERGING PROBLEMS IN SEAFOODBORNE PARASITIC ZOONOSES

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INTRODUCTION

The first international conference on foodborne parasitic zoonoses was held November 14th through 17th 1990 at Chiang Mai University in Chiang Mai, Thailand. The meeting was organized by the Southeast Asian Ministers of Education.[1]. The 120 attendees at the meeting were from 26 countries and their areas of research were often cosmopolitan. Major objectives of the meeting are listed in Table I.

Reports at the conference covered nearly all of the inhabited areas of the world, with emphasis on the clinical/epidemiological background as well as veterinary ramifications. The point of view of food science was represented by a minority of papers. The proceedings of the conference will be published as a supplement to the March 1991 issue of the Southeast Asian Journal of Tropical Medicine and Public Health. Copies are available though the Editorial Office, 420/6 Rajvithi Rd., Bangkok, 10400 Thailand. At the conclusion of this meeting, which was judged to be highly successful in documenting foodborne zoonoses, it was proposed that a second meeting be held in conjunction with the XIII[th] International Conference of Tropical Medicine and Malaria, 1993 in Phuket, Thailand. Increased participation by persons with a more direct interest in food science would contribute to a more varied and productive meeting. The nomenclature ending for parasitic diseases -iosis- is that recommended by the International Congress on Zoonosis as a means for standardization. Presented in this paper is a discussion of the seafood aspects of the conference with the addition of some necessary background material.

Results and Discussion

Of interest in relation to seafood parasitic zoonoses were the following reports. The first experimental study to demonstrate spores of the apicomplexa Cryptosporidium parvum in the intestine of an oyster, Crassostrea virginica, was described by Thomas Deardorff. Several speakers commented that there are possibilities of transmitting other protozoa by way of seafood, but there was no data to support the hypothesis.

Table 1. Major Objectives of The First International Conference on Foodborne Parasitic Zoonoses.

1. Assess the current status of foodborne parasitic illness throughout the world.
2. Describe current research activities in foodborne parasitic zoonoses.
3. Assess the impact of zoonotic and other foodborne parasitic illnesses on economics and industry.
4. Establish collaborative research efforts between conference attendees with similar interests.

[1]. The following groups served as sponsors of the meeting: ASEAN-USAID; Faculty of Medicine Mahidol University; Faculty of Medicine, Chiang Mai University; Thailand Ministry of Science, Technology and Energy; Uniformed Services of the Health Sciences, U.S.A. ; Department of Agriculture, U.S.A.; U.N. Food and Agriculture Organization; World Health Organization; International Development Research Center of Canada; Roche Asian Research Foundation; Rockefeller Foundation; EXXON Company International; Amoco Foundation, U.S.A.; and Smith, Kline and French Laboratories, Ltd.

Table 2 lists the helminths transmitted through seafood that were the subjects of reports at the conference. A cartoon depicting the life cycle of each of the nine general groups of seafoodborne helminthic zoonoses together with a reservoir host that allows the parasite to survive without a human host is presented in Fig. 1-8 (since the life cycles of the two species of Angiostrongylus are similar, they are both depicted on one diagram).

Table 2. Helminths Transmitted to Humans in Seafood

Disease	Causative Organism(s)	Seafood
Small Intestinal Fluke Infection		
Echinostomiosis	Echinostoma spp.	Freshwater Fishes
	Artyfechinostomum malayanum	Freshwater Fishes
		Prawns, Crabs
		Freshwater Snails
Heterophyiosis	Heterophyes heterophyes	Brackish Water
	Heterophyes spp.	Fishes, Mullet
	Metagonimus yokogawai*	Trouts
Liver Fluke Infection		
Clonorchiosis	Clonorchis sinensis	Freshwater Fishes
Opisthorchiosis	Opisthorchis felineus	Freshwater Fishes
	Opisthorchis spp.	Fishes
Lung Fluke Infection		
Paragonimiosis	Paragonimus westermani	Fishes, Crabs, Crayfish, Prawns, Mammals
	Paragonimus spp.	Crabs, Crayfish, Prawns
Broad Fish Tapeworm Infection		
Diphyllobothriosis	Diphyllobothrium spp.	Marine and Freshwater Fishes
Larva Migrans		
Gnathostomiosis	Gnathostoma spinigerum	FreshwaterFishes,Frogs,Snakes,Birds
	Gnathostoma sp.	Freshwater Fishes
Eosinophilic Meningoencephalitis		
Angiostrongyliosis	Angiostrongylus cantonensis	Snails, Fish, Crabs, Shrimp, Frogs
Visceral Larval Migrans		
	Angiostrongylus coastaricensis	Mollusks
Gastrointestinal Invasion		
Anisakiosis	Anisakis spp.	Marine Fishes, Octopus, Squid
	Pseudoterranova spp.	
	Contracaecum spp.	
	Phocascaris sp.	
Intestinal Capillariasis		
Capillariosis	Capillaria philippinensis	Marine and Freshwater Fishes

*M. takahashii, H.nocens, H. dispar, Heterophyopsis continua, Pygidiopsis summa, Stellantchasmus falcatus, Centrocestus armatus and Stictodora fuscatum are known from Korea.

General reasons for increased concern with seafoodborne parasitic diseases are listed in Table 3. The increased international trade in seafood is the result of more integrated international markets. Table 4 documents the changes in the U.S. and world markets and in fisheries over the last four decades. This integrated market leads to more international travel and contributes to consumers' increased willingness to experiment with unfamiliar dishes prepared by exotic and often unquestioned methods. Change in the amount of international travel over the last five decades is documented in Table 5. Also, the traveler who is enamored with an exotic dish, often returns home and then exposes acquaintances to it. Fresh seafood has become increasingly available throughout industrialized nations due to improved production and storage conditions aided by the increased availability of air transportation.

The retailer is aware of the of the value added by the market appeal of fresh seafood. Nutritionists have made the public conscious of the value lost by overcooking, and this has contributed to the acceptance of raw or undercooked dishes by the public. For example, *instinctotherapy,* an alternative medical treatment based on the consumption of raw food as a means of curing and preventing disease, was attributed as a major factor to increased risk in a 33 month retrospective epidemiological study in France (Hubert *et al.*, 1). Similar nutritional recommendations in developed countries increase the need for knowledge of the incidence of parasites in our food supply.

Table 3. General Reasons for Increased Concern about Seafoodborne Parasitic Diseases.

1. Increased international trade of seafood.
2. Increased international travel.
3. Nutritional (as opposed to hygienic) concerns may increase consumption of raw or undercooked foods; matters of taste also contribute to the increase.
4. More efficient production, handling and storage conditions of seafood.
5. Market appeal and increased value of fresh seafood.
6. Increased availability of rapid (air)and refrigerated/air transport.

Parasitic diseases have traditionally been diagnosed by the identification of the transmissible stages in bodily excretions or secretions. Current work explores diagnosis based on immunologic and genetic methods. Serum testing capable of distinguishing between closely related digenetic trematodes has been carried out with success in Laos and Korea. However, the development of commercially available diagnostic kits for parasitic diseases of foodborne origin was considered necessary before meaningful advances in reducing foodborne parasitic illness can occur.

Table 4. Summary of U.S. and World Fisheries and Trade 1947-1987.

Year	Unites States Production	World Production	Unites States Imports	Exports
	Thousands of Metric Tons		Millions	U.S. $
1948	2.4	19.6	157	24
1950	2.2	21.1	178	27
1955	2.2	28.9	259	40
1960	2.2	40.2	363	44
1965	2.2	53.2	600	80
1970	2.2	65.6	1,000	304
1975	2.2	66.4	1,381	298
1980	2.9	72.0	2,633	993
1985	2.8	86.5	4,051	1,058
1988	5.0	98.4	8,872	2,275

The parasites listed in Table 2 pose problems for the clinical as well as food diagnostician. Symptoms associated with parasitic illness are generalized and often mimic other more widely recognized diseases. Most symptoms are chronic rather than acute. Medical treatment is frequently not obtained and the illness can persist for extended periods. The only disease listed in Table 2 for which there is not a clinically demonstrated, effective drug is anisakiosis. The previous statement should be qualified with the realization that clinical evaluations are based on small populations in the case of foodborne illnesses. There was a consensus during the conference discussions that delivery of drug treatment to those infected was in drastic need of improvement.

Invasive treatments are effective in treating anisakiosis. Infection in the stomach and upper duodenum as well as certain colonic cases of anisakiosis have been remedied effectively with an endoscope equipped with biopsy forceps. Laparotomy accompanied by surgical resection is the treatment of choice if the nematodes invade an area that is not susceptible to endoscopy or when the disease is misdiagnosed (often as acute appendicitis or gastrointestinal tumor).

When diseases are diagnosed and treated, a change in food preparation habits must occur to avoid reinfection. S. Sornmani reported the successful control of opisthorchiosis in a province in northeastern Thailand through single dose drug treatment and health education aimed at altering eating habits. Comments from the audience indicated that this method had not achieved much success in other areas where it has been attempted. W. P. Carney reported the accidental control of echinostomiosis in the Philippines through the introduction of fish species that prey on the larval stages of the molluskan intermediate hosts. Discussion centered on the possibility of intervening in the life cycles of foodborne parasitic diseases less serendipitously. These two reports represent the only stories of success; in contrast, education efforts in Japan, Korea and China have been unsuccessful in controlling gnathostomiosis, paragonimiosis, and trichinelliosis as well as other foodborne parasitoses. Data is available on the parasites of many of the traditional valued fish species such as cod, herring, pollack, salmon, flounder, sole and haddock. However, data on the parasites of the large species such as tuna and billfishes is sparse. Data on previously underutilized species such as monkfish and orange roughy are lacking. Our knowledge of the parasites of invertebrates and freshwater fishes has many gaps; available information depends on the ease of collection of the species in an area with an active parasitology program at a local institution of higher learning.

Table 5. International Travel.*

Year	United States Travelers Abroad (1,000's)	Travelers to United States (1,000's)
1948	508[1]	not available
1953	827	not available
1958	1,398	472
1963	1,990	847
1968	2,351	1,798
1973	3,885	3,554
1978	6,933	5,764
1983	7,790	7,837
1988	14,529	12,494
1990	15,600[2]	15,360[2]

1. Not summarized from average estimated monthly departures. Census data.
2. Estimated U.S.Travel and Tourism Administration.

The economic impact of foodborne parasitic zoonosis on the production of animals was addressed by K. D. Murrell. F. Van Knapan described the economic impact of a 1987 German television show about anisakid nematodes in Baltic herring; the consumption of fish in Germany decreased 70% in the days following the broadcast. The decrease in consumption did not distinguish between fresh fish, frozen fish, or treated products. This caused the German veterinary services to impose import control measures that virtually eliminated the importation of fish. These regulations were considered discriminatory by other members of the European Economic Community; alternate regulations have been proposed that consider the danger to the public health of fishborne parasites.

Conclusions

The first international conference on foodborne parasitic zoonoses was considered successful in meeting its objectives. The attendees adjourned with a broader understanding of the distribution and ecology of parasitic organisms that are transmitted to humans through foods. The discussion of current research activities led to proposals for collaborative studies among conference attendees. The current economic impact of foodborne illness was assessed, and proposals were made that, if implemented, would reduce the economic impact of foodborne parasitic zoonoses. Table 6 lists some of the long term goals that must be met in order to control foodborne illnesses.The second conference on foodborne parasitic zoonoses should give some indication as to the success of implementing these goals.

Table 6. Major Long Term Goals for Control of Foodborne Parasitic Zoonoses.

1. Develop sources of funding for the needed research on foodborne parasitic diseases.
2. Develop readily available diagnostic methods to accurately estimate the incidence of foodborne parasitic disease in a population.
3. Assess aquaculture to determine what parasite problems, if any, are present in the products.
4. Develop technology to determine the incidence of viable larval parasites in foods.
5. Support collaborative research to improve food safety in both producer and consumer countries.

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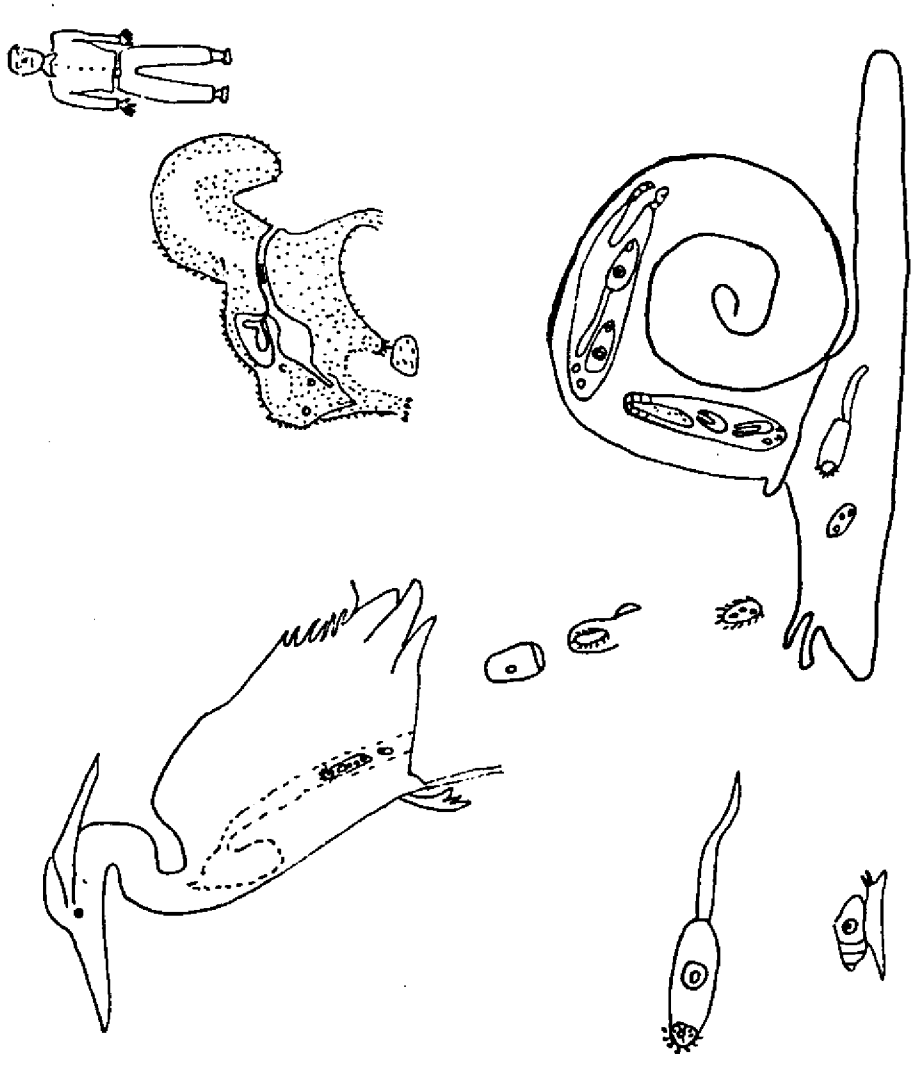

Figure 1. Echinostomatid life cycle. This cartoon depicts the life cycle of the echinostomatid trematodes that reach sexual maturity in the intestines of birds and mammals. If eggs in the feces reach water, the eggs develop and produce a free swimming larva that may penetrate the flesh of a gastropod and develop through two morphologically similar asexual larval stages that again give rise to a motile larvae that encysts and develops into the stage infectious for the homothermic host.

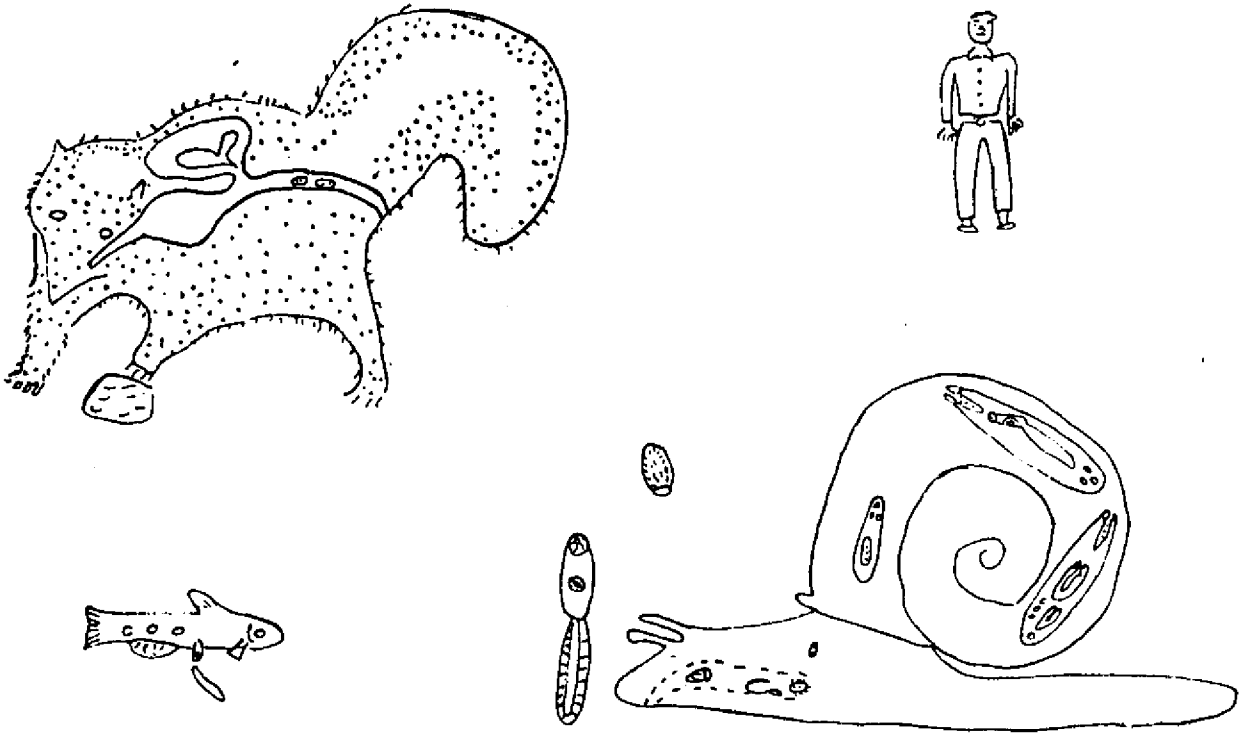

Figure 2. Heterophyid life cycle. This cartoon depicts the life cycle of these small intestinal flukes that mature sexually in the small intestine of humans and other mammals. The flukes mature deep in the intestinal crypts, where some eggs enter the circulatory system and cause cardiac damage. The eggs that exit in the feces may develop into larvae, if they are consumed by a compatible gastropod host, the larvae hatch and penetrate into the snail's tissues where they undergo additional larval development through two morphologically distinct generations. The result of this asexual reproduction is a motile larva that leaves the snail host. If it encounters a fish host, it may penetrate into the tissues and form the mammalian infective stage. The life cycle may be completed if humans or other mammals consume the infected fish hosts in a raw or undercooked state.

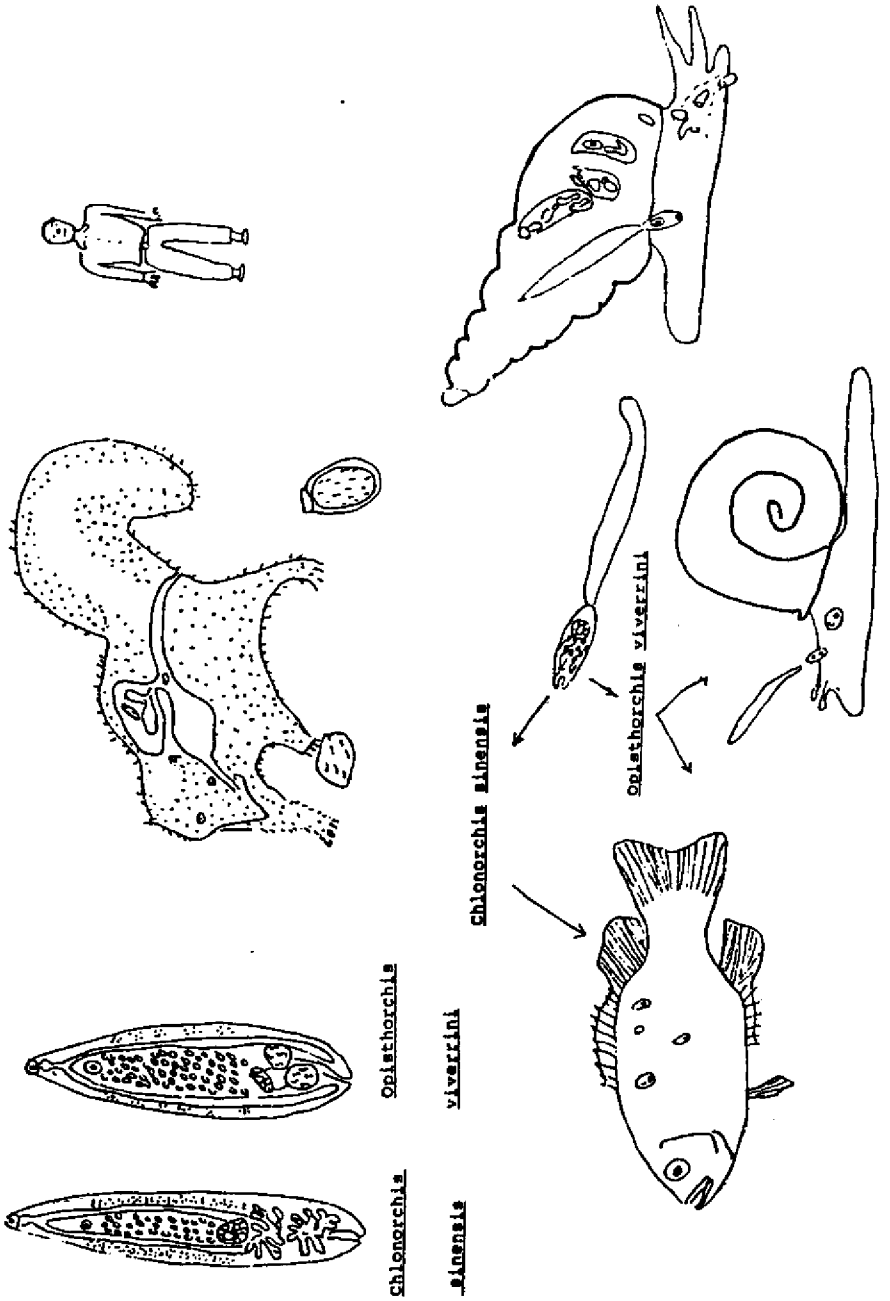

Figure 3. Liver fluke life cycle. This cartoon depicts the life cycle of two trematodes that reach sexual maturity in the liver of humans and other mammals. Eggs enter the intestine in the bile and are incorporated into the feces of the host. If the eggs are ingested by a mollusk, they may hatch and the larvae penetrate the tissues to undergo development through morphologically distinct larval stages that again, by asexual reproduction produce a free swimming larva. These free living larvae of Clonorchis sinensis are capable of infecting only certain fish species whereas, the larvae Opisthorchis sinensis can infect either fish or molluscan hosts. In these hosts the larvae become infective for mammals.

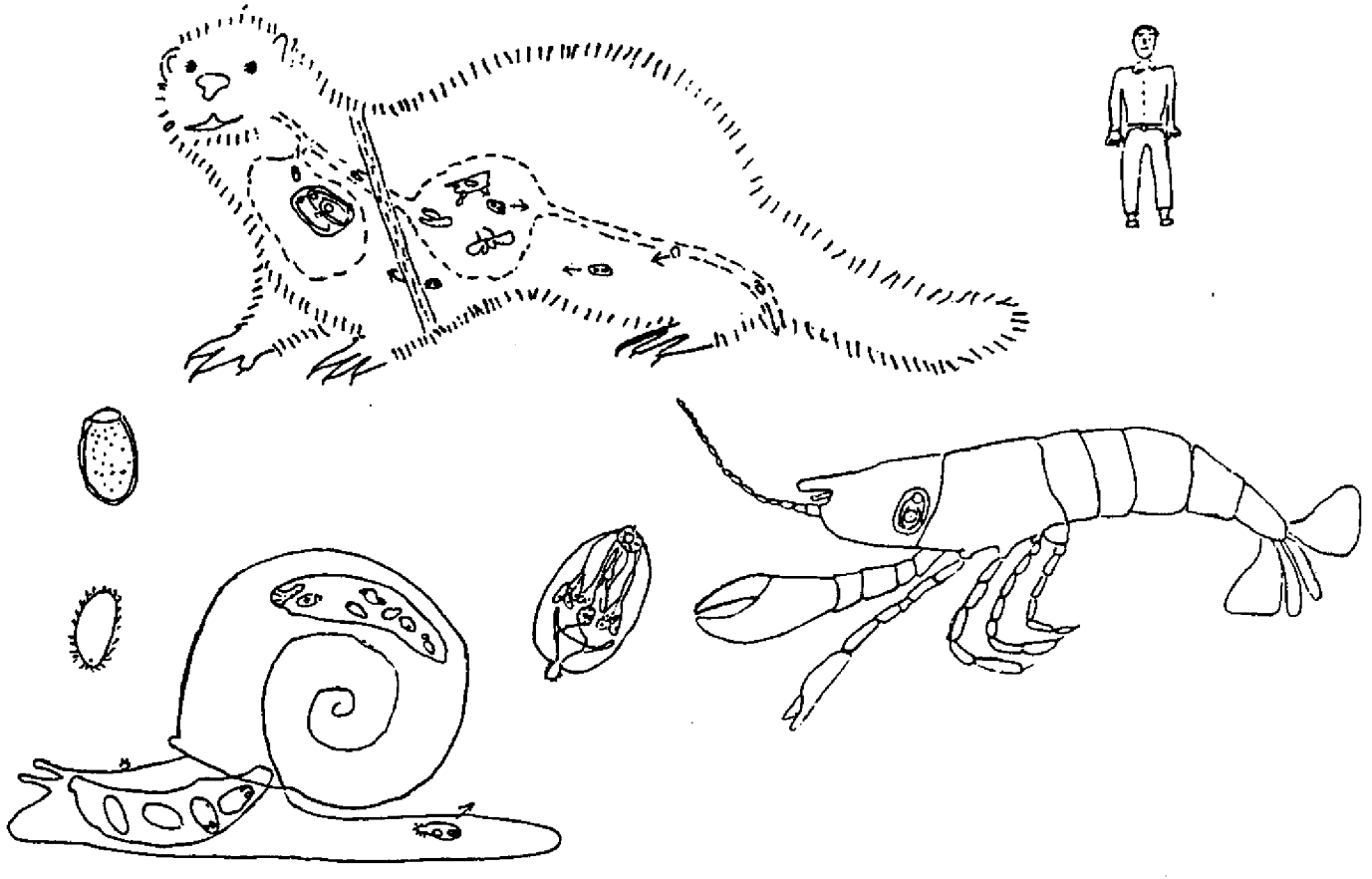

Figure 4. Lung fluke life cycle. This cartoon depicts the life cycle of Paragonimus spp. which reach sexual maturity in the lungs of humans and other mammals. Mature flukes are usually found in pairs in the alveolar sacks of the lungs. Eggs are coughed up and expelled by way of the sputum or feces. A free living larva may hatch from the egg under suitably moist conditions. If this larva encounters a gastropod host, it may penetrate to develop asexually through two distinct morphological forms to produce free living larva. If one of these larvae encounters a crab or crayfish it may penetrate the soft tissues and encapsulate as the mammalian infective stage. When the larva is consumed by a mammal, it may penetrate the intestinal wall and migrate through the tissues. In some hosts, migration continues without further development; however, these larva remain infective to mammals that consume these hosts without cooking. In the proper host the larva migrate to the lungs.

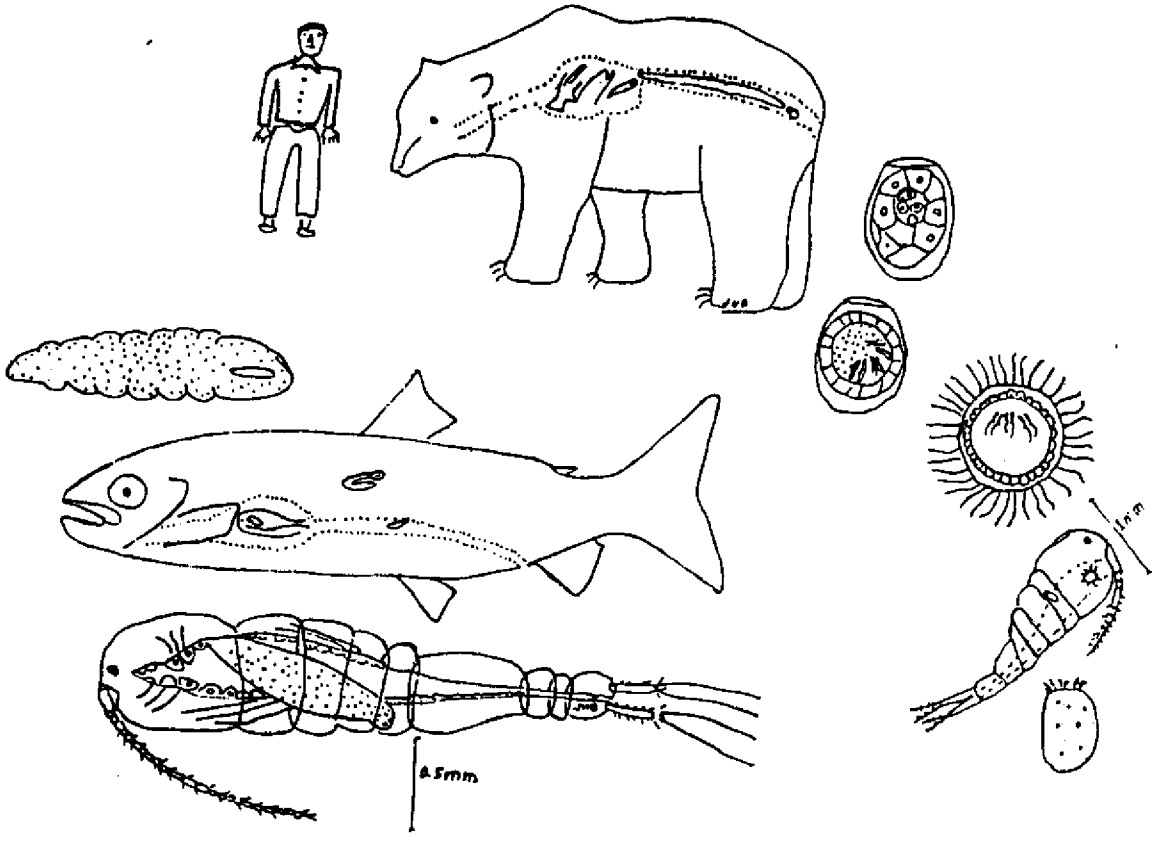

Figure 5. Broad fish tapeworm life cycle. This cartoon depicts the life cycle of the broad fish tapeworms, Diphyllobothrium spp. These large tapeworms reach sexual maturity in the intestinal tract of mammals. Eggs may pass in the feces and develop in water into larvae that hatch and swim freely. If the larva are consumed by a copepod or other suitable crustacean host, they may develop into larva that are infective for those fish that consume the infected crustacean. These larvae develop into larvae that are infective for other fishes or mammals.

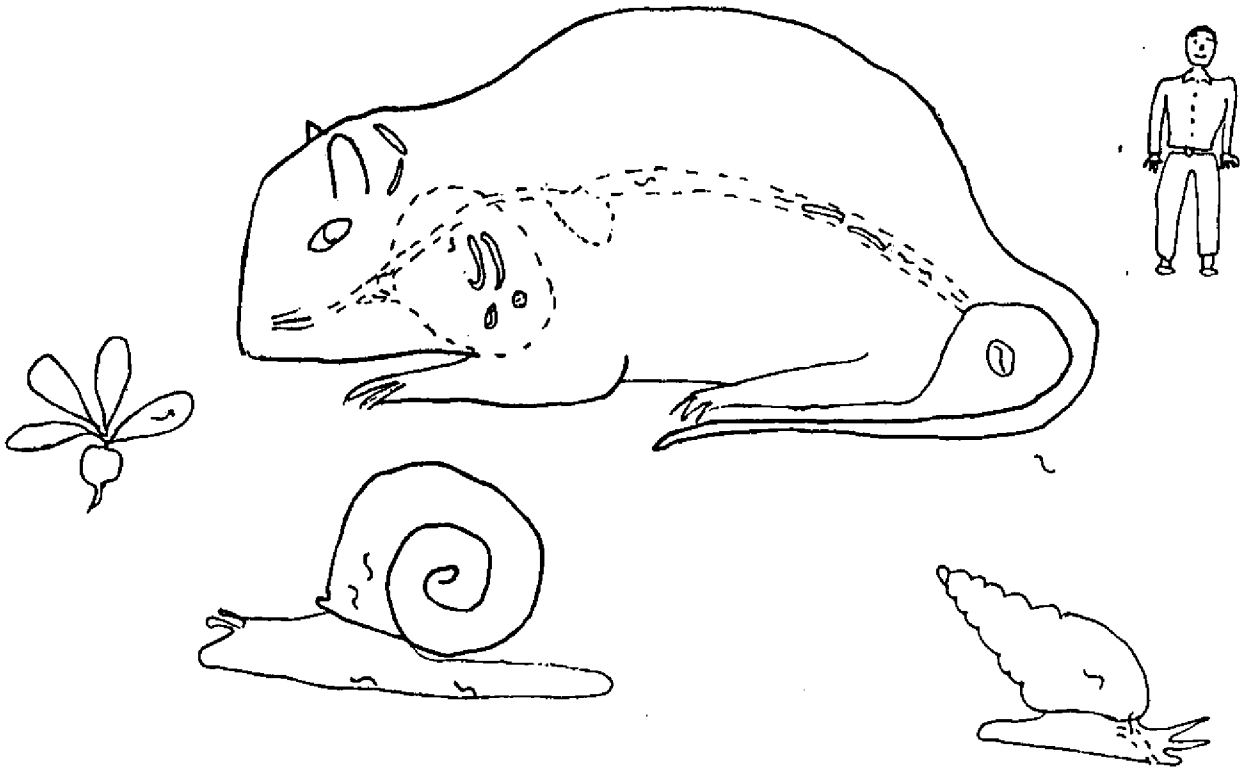

Figure 6. Angiostrongylid life cycle. This cartoon depicts the life cycle of Angiostrongylus spp. Sexually dioecious nematodes mate and produce eggs that pass with the feces or hatch in the intestine. A. cantonensis reaches maturity in the lungs and A. costaricensis reaches maturity in the intestine. The larvae migrate in moist places and may invade invertebrates that they encounter, such as the gastropods depicted. Mammals may encounter infective larvae through the consumption of undercooked infected invertebrates or vegetables. In the mammal, the larva penetrates the intestine and migrates in the viscera. A. cantonensis migrates through the subarachnoid space and develops before migrating to the lungs. In humans, larvae do not migrate beyond the brain. A. cantonensis migrates in the viscera, muscles, and skin, before returning to the intestine of rats. In man it continues to migrate until it dies. The life cycle of the gnathostomatids is similar in that they seem to infect and migrate in almost any intermediate host but only mature in one.

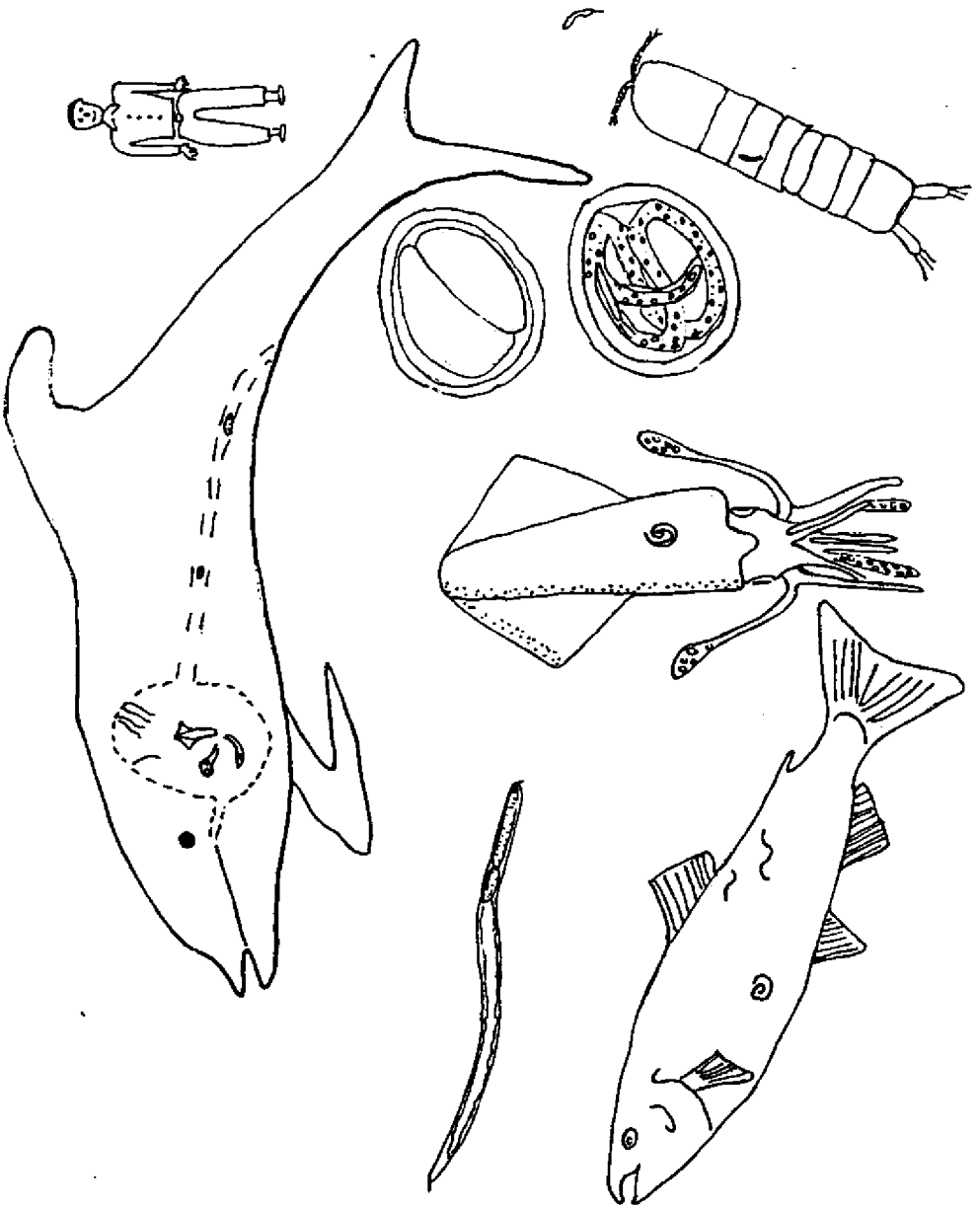

Figure 7. Anisakid life cycle. This cartoon depicts the life cycle of the anisakid nematodes. These dioecious nematodes reach sexual maturity in the intestinal tract of marine mammals. Eggs pass from female worms are expelled into the digestive tract and leave the host in the feces. In a water habitat, the eggs embryonate and undergo at least one molt. The larva that hatches is either free swimming or sessile. These larvae may infect a copepod or other small crustacean that consume them. The larvae penetrate the intestinal tract and grow in the hemocoel. If the infected crustacean is ingested by a larger invertebrate or fish, the larvae may infect that host. Larvae may be transferred from host to host several times. Larvae that have developed sufficiently may infect marine mammals or humans that consume the infected intermediate host. In humans, these nematodes do not normally mature.

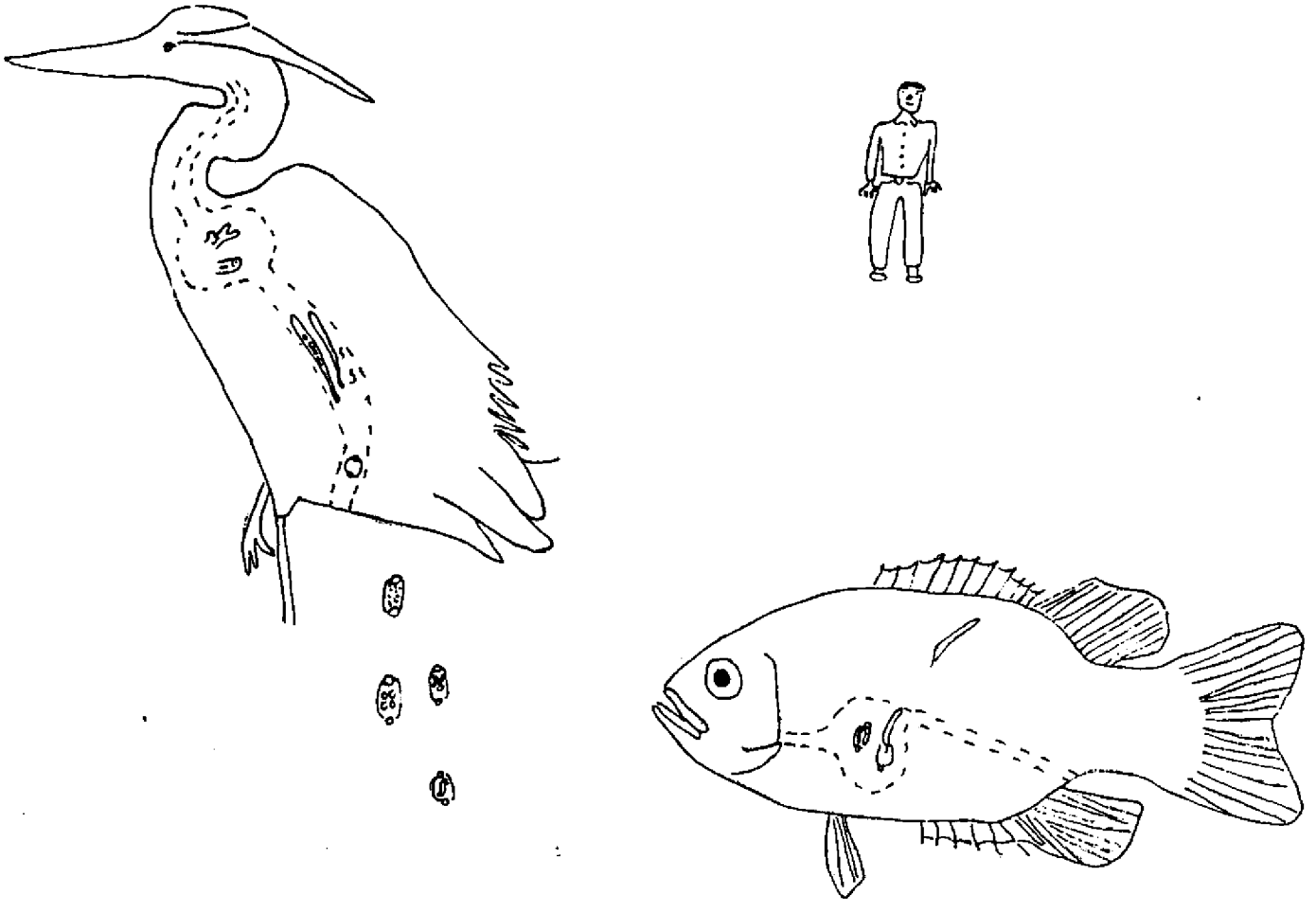

Figure 8. Capillarid life cycle. These nematodes are thought to mature in the digestive tract of wading birds. The females produce two types of offspring. Unembryonated, thick shelled, operculate eggs that are passed in the feces and larvae that complete their larval development and mature in the same host. Autoinfections by these larvae are thought to be responsible for the overwhelming diarrhea that may lead to death in infected humans. If the embryonated eggs are consumed by a fish, they hatch in the digestive tract, penetrate the gut wall, remaing in the viscera and muscles as infective larvae without further development.

DEVELOPMENT OF VALUE-ADDED SALMON PRODUCTS

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INTRODUCTION

Technological advancements, continually changing consumer needs and preferences combined with increased competition from both domestic and foreign players have shortened the life of existing products (4). As a result, firms have become more and more reliant on new products to ensure future sales and continued profit growth. Especially in the food industry in which profit margins tend to be small, successful marketing of new products distinguishes the low profit from the high profit companies (1).

Value-added products when successfully marketed are differentiated goods. They hold a distinctive image in the market and are the key to establishing brand loyalty (1,2). The U.S. trout and salmon processors are now following the lead of the catfish industry by emphasizing development of value-added products to increase their profits. The potential for continued growth in this area is strong in light of continued increases in the U.S. fish consumption and the wide range of value-added fish products demanded by the foodservice industry (3).

This research represents the first stage of a product development plan for new salmon products. The objectives of the study were to (i) develop specific ideas for value-added salmon products, (ii) evaluate consumer response to each product concept, based on the appeal of the idea and the respondent's willingness to buy the product, if available, and (iii) evaluate overall product preferences and characterize one or more prospective target groups of consumers.

MATERIAL AND METHODS

Development of Salmon Prototypes

Six salmon products were developed with special consideration for ease of preparation and processing, product stability, visual and culinary appeal, and dietary concerns. The prototypes evaluated consisted of a six ounce salmon fillet topped with one of the following six sauces: (i) Lobster, (ii) Scallions and Sherry, (iii) Mild Cajun, (iv) Mushroom and Wine, (v) Asparagus and, (vi) Spinach (Florentine). Once topped with the sauce, the raw fillets were individually vacuum packaged and blast frozen at -20 degrees C. Following storage for 1 week the samples were microwave heated in a 700 watt Hotpoint microwave oven for six minutes on high power and prepared for sensory evaluation. Seven semi-trained panelists were asked to evaluate the visual appeal, flavor and texture characteristics of the products. All six scored high in flavor, but the asparagus and spinach sauces lacked the visual appeal of the others. On this basis, the final selection for the consumer survey included the Lobster, Scallions and Sherry, Mild Cajun, and Mushroom and Wine sauces.

The Consumer Survey

With the cooperation of Hannaford Brothers in Portland, Maine, three Maine Shop 'n Save stores were selected for the store intercept interviews. A demonstration booth was positioned near the fresh seafood area and consumers were screened for whether or not they liked salmon. Shoppers who liked salmon were offered a $2.00 gift certificate as an incentive for participating

in the survey. The same two researchers conducted the interviews at each store using a set protocol. Initial questions were designed to gather basic information about shopping frequency, salmon purchasing and frozen fish purchasing habits. After these preliminary questions, interviewers explained they were looking for the respondent's candid reactions to four product ideas consisting of salmon fillets topped with different sauces, which were then vacuum sealed and frozen.

The researcher verbally described each product idea one at a time and presented the respondent with a visual handout displaying two photographs of the prototype as it looked frozen in a vacuum sealed poly bag, and as a prepared entree.

Consumers were instructed to rate the general appeal of the product using a nine-point hedonic scale, with 9 corresponding to "like very much", 5 indicating "neither like or dislike" and 1 being "dislike very much" (5,7). Using a similar scale, (9 = very likely to buy, 5 = not sure, 1 = not likely to buy) they next rated their willingness to actually buy the product, if it was available. Respondents were then presented with a summary handout displaying all four prototypes and asked to rank them in order of their preference, from most preferred to least preferred. Following the interview, consumers completed a brief demographic survey for information on their place of residence, age, number of people in the household, income and education.

Survey responses were coded and statistically analyzed using the SAS statistical package (6).

Product Ratings

A total of 228 consumers were interviewed at the three store locations. The average scores given to each prototype for product appeal and likelihood of purchasing are shown in Table 1. The Salmon in Lobster Sauce and Salmon in Mushroom and Wine Sauce received the highest appeal ratings, with an overall score of approximately 7 out of 9 points. The Scallions and Sherry Sauce product ranked slightly lower, with a rating of approximately 6, following by the Salmon in Mild Cajun Sauce. Since a score of 5 indicated indifference to the product, these ratings were all in the positive field of the scale.

Table 1. Summary of the overall appeal and likelihood of purchasing ratings for the four salmon prototypes.

	Lobster	Sherry	Cajun	Mushroom
Appeal[1,2]	6.87	6.12	5.50	6.66
Likelihood of Purchasing[1,3]	5.42	5.12	4.82	4.83

1 Mean of 228 respondents
2 Nine point hedonic scale: 1 = dislike very much, 5 = neither like nor dislike, 9 = like very much
3 Nine point hedonic scale: 1 = unlikely to buy, 5 = not sure, 9 = very likely to buy

The likelihood of purchasing ratings followed a similar trend with the Lobster Sauce product receiving the highest score. Not surprisingly, the purchasing ratings were slightly more conservative than the appeal ratings.

The second stage of the analysis intended to identify potential target groups; individuals who might be especially receptive to the product and/or those individuals whose demand for the product is latent, but may be motivated through specific marketing strategies. Respondents were classified into groups based on different demographic characteristics, including sex, education, income, age and state residence. Ratings were then compared to determine if differences in the characteristics of the respondents could be linked to variations in how they reacted to the products.

The comparisons showed there were no significant differences in the ratings given by males and females and by respondents in different income categories. In addition, Maine residents and non-Maine residents did not differ significantly in their reactions.

However, further analysis revealed that respondents with different educational levels gave significantly different ratings to the lobster product in terms of both product appeal and likelihood of purchasing the item. Table 2 shows that the Lobster Sauce was more appealing to respondents with a high school education or less, in comparison to those completing college. Similarly, purchasing scores indicate that the college educated group was not as likely to buy the product. As a group, their average purchasing rating was less than 5, in contrast to the more positive purchasing score of over 6 for the respondents with a high school education or less.

Table 2. Appeal and likelihood of purchasing ratings based on level of education for four salmon prototypes

	LOBSTER		SHERRY	
	Appeal[2]	Purchasing[3]	Appeal	Purchasing
EDUCATION[1]				
HS/<HS	7.32 a[4]	6.34 a	5.95	5.34
VO TECH	7.06 ab	5.69 ab	5.57	4.75
BS or higher	6.58 b	4.85 b	6.48	5.20
	0.05	0.05	NS	NS
	CAJUN		MUSHROOM	
	Appeal	Purchasing	Appeal	Purchasing
HS/<HS	5.54	5.11	6.73	6.36
VO TECH	5.20	4.65	6.31	5.57
BS or higher	5.58	4.72	6.75	5.68
	NS	NS	NS	NS

1 Mean of 228 respondents
2 Nine point hedonic scale: 1 = dislike very much, 5 = neither like nor dislike, 9 = like very much
3 Nine point hedonic scale: 1 = unlikely to buy, 5 = not sure, 9 = very likely to buy
4 Means followed by similar letters do not differ significantly at $P \leq 0.05$

Age differences were also associated with rating discrepancies for the Mild Cajun product, as presented in Table 3. The older the respondent, the lower the appeal and purchasing rating. The youngest group, those between the ages of 17-33, gave the most positive reactions to the Mild Cajun Product.

Product Preference Ranking

In addition to analyzing the relationship between selected demographic characteristics and respondent ratings, the product preference rankings give a third perspective on the acceptance of each product. Table 4 reports the distribution of preference rankings for each product, from "most preferred" to "least preferred".

Table 3. Appeal and likelihood of purchasing ratings based on age for four salmon prototypes

	LOBSTER		SHERRY	
	Appeal[2]	Purchasing[3]	Appeal	Purchasing
AGE[1]				
17-33	6.81	5.31	6.17	5.14
34-45	6.93	5.02	5.89	4.79
46-56	6.63	5.42	5.95	4.75
57-84	7.09	6.89	6.42	5.75
	NS	NS	NS	NS
	CAJUN		MUSHROOM	
	Appeal	Purchasing	Appeal	Purchasing
17-33	6.29 a[4]	5.31 a	6.69	5.78
34-45	5.86 ab	5.11 ab	6.53	5.46
46-56	5.11 bc	4.42 ab	6.75	5.86
57-84	4.76 c	4.00 b	6.62	6.20
	NS	NS	NS	NS

1 Mean of 228 respondents
2 Nine point hedonic scale: 1 = dislike very much, 5 = neither like nor dislike, 9 = like very much
3 Nine point hedonic scale: 1 = unlikely to buy, 5 = not sure, 9 = very likely to buy
4 Means followed by similar letters do not differ significantly at $P \leq 0.05$

Table 4. Percentage distribution of respondents ranking the four salmon prototypes in order of preference

		Lobster	Sherry	Cajun	Mushroom
Most Preferred	1	39.5	19.3	16.2	25.0
	2	24.1	29.8	17.5	28.5
	3	22.4	29.8	20.2	27.6
Least Preferred	4	14.0	21.1	46.1	18.9

n = 228

Consistent with the appeal and purchasing ratings, Lobster Sauce was rated "most preferred" by 40% of the respondents giving it the highest incidence of top rankings. Only 14% ranked it "least preferred". Another 25% preferred the Mushroom and Wine sauce above the other three products. Salmon in Scallions and Sherry ranked slightly lower, showing that most respondents ranked it second or third. At the other extreme, the Salmon in Mild Cajun Sauce was ranked "least preferred" by almost half of the respondents.

SUMMARY AND CONCLUSIONS

This project was conducted as an early screening procedure to identify one or more value-added salmon products that demonstrate sufficient consumer appeal to justify product development. This was accomplished by getting consumer feedback on descriptions of four salmon product ideas. Further analysis of the data is required to identify factors influencing respondent ratings. A summary of the results corresponds to each objective.

Objective 1: To develop specific ideas for value-added salmon products

Based on processing potential, product stability, possible consumer dietary concerns, and overall sensory appeal, product ideas were generated with the central theme of a ready to cook salmon fillet in a special sauce. Actual prototypes were developed to test the flavor and eye appeal of six sauces, leading to the selection of four products.

Objective 2: To evaluate consumer response to each product concept, based on overall appeal and the respondent's willingness to actually buy the product.

The survey used both product appeal and consumer willingness to purchase to identify and cross-check consumer reactions. Salmon in Lobster Sauce received the highest appeal and purchasing ratings, followed by the Mushroom & Wine Sauce. Both merit further attention for continued product development. Alternatively, the Scallions and Sherry Sauce received marginal ratings and the Mild Cajun Sauce drew the most negative reactions. Ratings of these two products indicate that neither idea warrants further consideration for developing products intended for Northeast markets.

Objective 3: To evaluate overall product preferences and characterize one or more prospective target groups.

Value-added products of the type represented by the four prototypes are obviously premium items corresponding to a price high enough to provide a reasonable margin. Consequently, it is essential to ensure that consumers exhibiting a preference for the product are willing to purchase and can afford the product. Equally important, analyzing differences in consumer response to certain product ideas allows market planners to sharpen development programs and tailor potential product lines for specific target groups. This entails looking at product reactions from different market segments defined by consumer characteristics such as sex, residence, income, education and age.

The results of the preference ranking were consistent with the product appeal and purchasing ratings. Salmon in Lobster Sauce tended to be ranked most preferred, followed by Salmon in Mushroom and Wine. However, different product ratings were noted for certain consumer groups. One distinction was found between consumers with different educational backgrounds. Respondents in the highest education category gave the lowest ratings to the Salmon in Lobster sauce. Age appeared to be a factor in the ratings of the Cajun Sauce. But even with this consideration, the ratings of the Salmon in Mild Cajun Sauce were comparatively low.

Based on the concepts tested in this project, the two products having the strongest marketing potential are Salmon in Lobster Sauce and Salmon in Mushroom and Wine Sauce. Informal discussion with the respondents and the general survey results illustrate solid market potential for developing value-added salmon products, such as these. Many consumers spontaneously talked about their enthusiasm for a product that would allow them to conveniently prepare salmon at home similar to what they might expect only at a restaurant.

Those interested in developing salmon products should understand that these two products are only representative of the possibilities of this type of product line and pertain to the tastes of consumers tested in Maine. For example, had these ideas been tested in a southern market, Salmon in Mild Cajun Sauce might have received the most favorable ratings. Nevertheless, it is essential to obtain consumer feed back on other product ideas, since the results here indicate that consumers are discriminating in their reactions to Salmon in any particular sauce.

ACKNOWLEDGEMENT

Appreciation is expressed to the Maine Aquaculture Innovation Center for its financial support of this project.

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ALABAMA ROE MULLET INDUSTRY SOCIOECONOMICS

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INTRODUCTION

The Autumn (October through December) roe mullet fishery has, during recent years, provided an important economic boost to numerous small Alabama coastal communities. This relatively new industry now offers fishermen and land-based processing employees the potential for additional work during what historically has been a slow season.

Along the Gulf Coast, roe mullet fishing is conducted predominantly by teams of two or three men fishing gillnets from 16-foot to 20-foot skiffs. This type of fishing often occurs at night and is a nearshore fishery, with gillnets set along beaches, in sounds and bays, and up to the mouths of coastal rivers. Daily roe mullet catches by gillnet fishermen may range from several hundred pounds to more than 4,000 pounds per skiff.

The skiffs which fishermen use to gillnet roe mullet often can be used for crabbing and oyster tonging. During Autumn seasons when blue crabs are still abundant in numbers significant enough to warrant trapping, and when the oyster season is just getting underway, roe mullet gillnetting effectively spreads local fishermen among three nearshore fisheries instead of only two. Conversely, during years when the blue crab season ends during early Autumn, and when oyster harvesting is curtailed or prohibited, roe mullet gillnetting provides local skiff fishermen with an opportunity for continued work.

A limited amount of purse seining is also conducted within numerous inshore sounds and out in the deeper waters of the Gulf of Mexico by a small number of relatively large (100-foot or longer) fishing vessels. While catches of roe mullet may be as large as several hundred thousand pounds per individual purse seine fishing vessel per day, the percentage contribution of purse seine catches to the Gulf Autumn roe mullet industry is relatively small.

These two fishing methods are employed by roe mullet fishermen from Louisiana through Florida. However, all roe mullet catches are not necessarily processed in the fishing ports in which they are landed. Extensive coastwise interstate transportation of roe mullet occurs during the Autumn season. The greatest percentages of total Gulf-wide roe mullet landings tend to accumulate in localities which have the largest processing potential. Coastal Alabama has emerged as one of the North Central Gulf's largest roe mullet processing centers.

Coastal Alabama traditionally has provided a home for many unskilled and semi-skilled seafood processing plant employees. Prior to the development of the Autumn roe mullet industry, many of these laborers went without work, or at best, worked off-season hours which provided only one-third to one-half time employment. However, the establishment of this new "off-season" industry provided additional or even full-time employment for many of Coastal Alabama's seafood processing plant employees.

The domestic portion of mullet roe processing is comprised of eight unit operations. Arriving whole (round) fish are separated according to sex. Female roe mullet are split (cut) along the belly from the pectoral fins to the anal vent. The paired roe sacs and gizzard are removed. The roe sacs are graded into one of five traditional market sizes according to weight. Each roe sac pair is individually wrapped in a small polyethylene bag. The wrapped roe are

packaged in 2-kilogram waxed cardboard boxes, which are then sealed in 20-kilogram polyethylene-lined master cartons. The master cartons are then blast-frozen and maintained at a temperature of 0 degrees F or lower. Frozen master cartons are finally loaded in 40-foot refrigerated containerized freight bodies and shipped to Taiwan.

Once in Taiwan, the female roe are removed from the packaging materials, thawed, and sun dried to a moisture content of approximately 50 percent. Thin slices of sun-dried mullet roe are sold as a traditional holiday gift item in Taiwan and Japan, and may fetch as much as $100 per pound.

Three basic secondary products result from processing roe mullet along the Gulf Coast: whole (round) male mullet; gizzards from female mullet; and, female mullet carcasses. While some very high quality whole male mullet enter local seafood markets or provide the basis for a small seasonal smoked mullet industry, most whole male mullet are packaged in poly-lined 40-kilogram master cartons, blast-frozen, and sold to Egyptian buyers. All of the gizzards, which were removed from the female mullet at the same time as the roe, are packed in 1-gallon plastic buckets, blast-frozen, and shipped to Taiwan along with the female roe. A very small percentage of the female carcasses may be of sufficient quality to enter local seafood markets or smoking operations. Some are rendered into fish meal. Most female mullet carcasses are packaged, frozen, and stored for later use as crab bait.

During the middle 1980's, many Gulf Coast residents, some of whom were affiliated with the seafood industry, began to question the wisdom of an industry which, in their opinion, provided the potential for reducing mullet populations to dangerously low levels. Other coastal residents, who perceived waste as a major problem, complained that many mullet either were being landfilled or simply discarded along river banks. Ultimately, several groups and individuals called for an outright ban on roe mullet fishing and processing in the Gulf region.

In an attempt to achieve a more complete understanding of the situation, the Alabama Department of Conservation and Natural Resources' Marine Resources Division (ADCNR/MRD) conducted a comprehensive three-year study of the Alabama roe mullet harvesting and processing sectors from 1986 through 1988. ADCNR/MRD sought and obtained funding through the National Marine Fisheries Service's (NMFS) Marine Fisheries Initiative (Marfin) Program for project conduct. The Auburn University Marine Extension and Research Center (AUMERC) functioned as a subcontractor, and provided ADCNR/MRD with socioeconomic analyses of the Alabama roe mullet industry during the three years of the project. Additional project funding was provided by ADCNR/MRD and the Alabama Cooperative Extension Service (ACES).

METHODS

The data upon which this report is based were collected from three sources: individual processors, port agents employed by ADCNR/MRD, and NMFS port agents. Regarding data collected at processing plants, some processors provided direct access to their purchase and sales records. Other processors simply provided total product volume and value statistics for the various commodities purchased and sold. The statistics contained in this report reflect the activities of a total of six Alabama roe mullet processors who operated during part or all of the three-year study period. Every effort was made to ensure each processor's anonymity.

RESULTS AND DISCUSSION

Table 1 presents the sources of female roe mullet processed in Alabama in pounds. Roe mullet poundages processed in Alabama varied widely, from 1.6 million pounds in 1986, down to 1.2 million pounds in 1987, and up to 2.1 million pounds in 1988.

Table 1 also illustrates that individual Gulf states' contributions to Alabama roe mullet processors likewise varied greatly during the study period. Louisiana was the most important source during 1986, providing over 44 percent of Alabama's raw materials. However, Louisiana dropped to the third most important source by 1988, supplying little more than 25 percent of Alabama's roe mullet needs. Conversely, Alabama and Florida which, respectively, ranked second and third as suppliers of female roe mullet to Alabama during 1986, attained rankings of first and second, respectively, by 1988.

Table 1. Sources of Female Roe Mullet Processed in Alabama

SOURCE	CONTRIBUTIONS BY YEAR (Pounds) 1986	1987	1988
Florida	371,250	440,000	752,145
Alabama	518,100	325,000	779,134
Mississippi	33,000	75,000	67,500
Louisiana	727,650	375,000	547,200
Totals	1,650,000	1,215,000	2,145,979

Table 2 presents the number of dollars Alabama processors paid to roe mullet gillnetters in the various Gulf States. Louisiana fishermen received 44 percent of Alabama's roe mullet purchase dollars in 1986, but by 1988, Louisiana fishermen received only 25 percent of the dollars. Conversely, Florida grew from third place in 1986 to become the most economically important source of roe mullet for Alabama processors by 1987.

Table 2. Cost of Female Roe Mullet Purchased by Alabama Processors

SOURCE	PURCHASES BY YEAR (Dollars) 1986	1987	1988
Florida	334,125	404,800	827,359
Alabama	466,290	243,750	662,264
Mississippi	29,700	52,500	43,875
Louisiana	654,885	363,750	519,840
Totals	1,485,000	1,064,800	2,053,338

The year by year changes in the relative degree of importance of any state's poundage contributions to the Alabama roe mullet processing industry and the number of dollars paid by Alabama processors to roe mullet fishermen in any Gulf state occurred for two reasons. First, Florida mullet fisheries have the ability to carry Alabama roe mullet processors through a longer processing season. Hence, Alabama processors tended to rely more heavily on Florida fishermen during the latter portion of the study period. Second, Oriental buyers began to favor roe cut from Florida mullet. Thus, Alabama processors adjusted their buying patterns during the latter portion of the study period to accomodate that customer preference.

Table 3 enumerates the number of pounds of female mullet roe which were yielded into each of the five standard (traditional) size classes (grades) during each year. The grades are: under 2 ounces; 2-4 ounces and broken; 4-6 ounces and broken; 6-8 ounces; and, over 8 ounces. Collectively, the three intermediate grades always contained the greatest volume of female mullet roe produced (from 84 percent to 90 percent) regardless of year.

Table 3. Volume of Female Mullet Roe Produced by Alabama Processors

GRADE	ROE PRODUCTION BY YEAR (Pounds) 1986	1987	1988
Under 2 oz	3,360	14,893	7,890
2-4 oz & Bkn	72,520	47,485	77,299
4-6 oz	112,280	74,035	106,955
6-8 oz	68,040	57,600	50,262
Over 8 oz	23,800	19,657	20,516
Totals	280,000	213,670	262,922

Table 4 presents the dollar values of total production for all five standard grades during each year of the study period. Collectively, the three intermediate grades contributed the greatest portion of the value of female mullet roe produced (from 80 percent to 89 percent) regardless of year. However, the larger sizes were worth proportionately more than were the smaller-sized roe.

Table 4. Value of Female Mullet Roe Produced by Alabama Processors

GRADE	ROE VALUE BY YEAR (Dollars) 1986	1987	1988
Under 2 oz	8,064	35,743	18,936
2-4 oz & Bkn	246,568	181,867	445,242
4-6 oz	572,628	387,943	814,997
6-8 oz	408,240	345,600	437,782
Over 8 oz	166,600	197,469	196,953
Totals	1,402,100	1,148,622	1,913,910

Table 5 lists volumes of male and female roe mullet purchased by Alabama processors during the months of October through December from 1986 through 1988. Total roe mullet purchases ranged from 1.3 million pounds to 2.4 million pounds. Annual male roe mullet purchases ranged from 9.6 percent to 19.5 percent of total roe mullet purchases during the study period. As will be discussed later, the volume of male roe mullet purchased became an important determinant of overall Alabama roe mullet industry profitability.

Table 5. Total Volume of Roe Mullet Purchased by Alabama Processors

YEAR	POUNDS Female	Male	Total
1986	1,650,000	402,631	2,052,631
1987	1,215,000	128,500	1,343,500
1988	2,145,979	238,442	2,384,421
Totals	5,010,979	769,573	5,780,552

By combining all three years' female roe mullet purchase volumes and costs in Table 6, several points become apparent. Mean prices paid for female roe mullet varied widely. The Florida mean price was highest due to the previously mentioned quality factor, plus the fact that mullet purchased late in the season from Florida fishermen cost more. Louisiana female roe mullet fetched the next highest price, largely due to the greater mean percentage of roe yielded by Louisiana mullet. Florida and Louisiana fishermen received proportionately more for their catches than did Alabama and Mississippi fishermen whose mullet, which were caught during the earlier parts of each season, generally yielded lower mean sizes and percentages of roe.

Table 6. Total Volumes and Costs of Female Roe Mullet Purchased by Alabama Processors from 1986 through 1988

SOURCE	VOLUME (Pounds)	MEAN PRICE ($/lb)	VALUE (Dollars)
Florida	1,563,395	1.00	1,566,284
Alabama	1,622,234	.85	1,372,304
Mississippi	175,500	.72	126,075
Louisiana	1,649,850	.93	1,538,475
Totals	5,010,979	---	4,603,138

All three years' female roe volumes and values are likewise combined in Table 7. Overall, medium to large-sized female roe were worth proportionately more than were smaller-sized roe. When compared to the percentages of total poundages yielded into the "under 2 ounces" and "2-4 ounces" size classes, those two grades failed to yield proportionate percentages of total sales. Therefore, Alabama processors made every attempt to minimize their purchases of female mullet which might have been expected to contain smaller-sized roe.

Table 8 lists the combined production expenses for all unit operations for the three years of the study period. Year to year differences in most production expenses were minimal, with the notable exception of male mullet purchase prices, which varied from 15 cents to 90 cents per pound. That wide range was created by new, inexperienced buyers who entered the business from time to time during the three-year study period. In general, they tended to purchase too many male mullet at too high a price. Otherwise, Table 8 demonstrates that the costs of female roe mullet and processing labor were the two major contributors to the costs of operation.

Table 7. Total Volumes and Values of Female Mullet Roe Produced by Alabama Processors from 1986 through 1988

GRADE	VOLUME (Pounds)	MEAN PRICE ($/lb)	VALUE (Dollars)
Under 2 oz	26,143	2.40	62,743
2-4 oz & Bkn	197,304	4.43	873,677
4-6 oz	293,270	6.05	1,775,568
6-8 oz	175,902	6.78	1,191,622
Over 8 oz	63,973	8.77	561,022
Totals	756,592	----	4,464,632

Table 8. Total Alabama Roe Mullet Industry Production Expenses Incurred from 1986 through 1988

SOURCE OF EXPENSE	VOLUME (Pounds)	COST/LB ($) Range	Mean	TOTAL EXPENSE (Dollars)
Female Mullet	5,010,979	.50-1.20	.89	4,603,138
Male Mullet	769,573	.15-.90	.50	387,221
Labor	5,780,552	.06-.085	.067	388,582
Packaging	5,716,352	.004-.006	.0052	29,808
Fzn Storage	901,917	.05-.114	.09	80,965
Transport	976,719	.015-.025	.0174	17,021
Rendering	64,200	---	.0086	555
Total	—	—	—	5,507,290

Table 9 enumerates total combined secondary product production volumes and values for 1986 through 1988. (Note that "MM" designates male mullet and "FM" designates female mullet.) The single most economically important secondary product was the frozen female bait carcass. The wide range in sale prices for male mullet in the domestic market resulted both from year to year market price escalation plus variable prices for which male mullet of varying degrees of quality were sold. The wide range in gizzard sale prices was the combined result of inflation and the upward movement of mullet gizzard market prices from one year to the next.Otherwise, sale prices for most secondary products varied only moderately during the three-year study period.

Table 9. Disposition of Secondary Roe Mullet Products by Alabama Processors from 1986 through 1988

PRODUCT TYPE	VOLUME (Pounds)	SALE PRICE ($/lb) Range	Mean	VALUE (Dollars)
Gizzards	66,915	1.60-3.50	2.30	154,036
MM (Fresh Dom)	25,000	.25-1.50	.75	18,750
MM (Fzn Export)	712,094	.21-.25	.22	155,064
FM (Fresh Dom)	253,532	.15-.25	.20	51,912
MM (Fzn Bait)	32,479	.08-.15	.14	4,547
FM (Fzn Bait)	3,869,740	.06-.11	.08	315,668
FM (Rendered)	64,200	-0-	-0-	-0-
Totals	5,023,960	---	---	699,977

Table 10 demonstrates that, by combining all incomes and expenses for the 1986 through 1988 processing years, Alabama roe mullet processors lost a combined total of $342,000. That loss actually resulted from one year's slight income, another year's minor loss, and a third year's major loss. If the total amount of the loss were spread among the three years' production, the loss would amount to only 6 cents per pound. That loss is in the range within which many catfish processors operate.

Table 10. Total Alabama Roe Mullet Industry Losses Experienced from 1986 through 1988

	VALUE	POUNDS	$/LB
Total Production Expense	$ 5,507,290	/ 5,780,552 =	$ .953
Total Gross Income	- 5,164,609	/ 5,780,552 =	- .893
Total Net Loss	$ 342,681	---	$ .06

If the total $342,000 loss were averaged among the six processors over the three years encompassed by the study, each processor's average share of the loss would have amounted to $19,000 per year. Several processors, in fact, stated that their primary objective was not to make vast sums of money during the developmental stages of the Alabama roe mullet industry. Many processors were instead more interested in developing market share during the study period, and would focus on enhanced profitability in succeeding years.

However, many people did financially benefit from the employment offered during the traditionally slow months of October through December from 1986 through 1988. Table 11 demonstrates that an annual average of 208 fishermen in four Gulf states and 144 land-based processing employees in three Alabama coastal communities were employed during each year of the study period in jobs directly related to the Alabama roe mullet processing industry.

Table 11. Employment Related to Alabama Roe Mullet Processing from 1986 through 1988

YEAR	PROCESSING/TRUCKING	FISHERMEN	TOTALS
1986	152	205	357
1987	130	208	338
1988	151	213	364

In summary, the off-season employment provided by this emerging specialty fishery has had a definite, positive economic impact among grassroots seafood harvesting and processing personnel in many small communities in Alabama and all along the Gulf Coast.

REFERENCES

The data for the first two years of the study period (1986 and 1987) were previously reported in the following two articles:

1. HOSKING, W., B.E. PERKINS, R.K. WALLACE, and H.A SWINGLE. 1988. An economic analysis of the 1986 Alabama mullet roe industry. Proceedings: Twelfth Annual Tropical/Subtropical Fisheries Technological Conference. SGR-92. p. 459-465. Florida Sea Grant College Program. Gainesville, FL.

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ACKNOWLEDGEMENTS

The author wishes to express his appreciation to the following individuals and organizations for their support and cooperation throughout the course of this three-year study:

- The National Marine Fisheries Service, Marine Fisheries Initiative, for providing initial project funding.
- The Alabama Department of Conservation and Natural Resources, Marine Resources Division, for providing additional project funds.
- The Auburn University/Alabama Cooperative Extension Service, for providing additional project funds.
- The members of the Alabama roe mullet industry, for providing data and statistics.
- The ADCNR/MRD and MNFS port agents, for providing data and statistics.
- Messrs. Walter M. Tatum and E. Moret Smith, for their personal support, advice, and assistance offered throughout the course of this study.

DETERMINANTS OF IMPORTED SHRIMP AND THEIR ROLE IN THE SOUTHEAST SHRIMP PROCESSING SECTOR

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U.S. imports of shrimp have increased significantly since the mid 1960s with the majority of this increase occurring during the 1980s. Imports averaging 260 million pounds (headless shell-on equivalent weight basis) during 1977-80 for instance, were only about 32% above the 1965-68 average annual imports of 197 million pounds. By 1981-84, imports had increased to 356 million pounds annually and increased again to 531 million pounds annually during 1985-88. Overall, 1985-88 annual imports exceeded 1977-80 imports by about 100% and were 170% above those reported during 1965-68.

The objectives of this paper are twofold. The first objective is to provide a model, developed within an appropriate economic and statistical framework, which explains the growth in the U.S. shrimp import market. The second objective of the paper is to evaluate the use of these increased imports in the Southeast shrimp processing sector.

To accomplish the aforementioned objectives, the paper proceeds as follows. First, the U.S. import market, along with other relevant information, is examined. This examination covers the 1965-88 period. Then, a statistical model used to describe the U.S. import market is presented with findings. Attention then turns to examination of the use of imported shrimp in the Southeast shrimp processing sector. The paper concludes with a summary which highlights significant findings.

REVIEW OF THE U.S. IMPORT MARKET

As noted in the introduction, U.S. imports of shrimp have been expanding since the mid 1960s with a major portion of this increase occurring during the 1980s. Data in support of this claim are formally presented in Table 1. As indicated, 1982 was a pivotal year in this expansion process as was 1986. Overall, imports increased more than 60 million pounds between 1981 and 1982 and increased by 100 million more pounds between 1982 and 1983. After remaining relatively stable during the next few years, imports increased by 40 million pounds between 1985 and 1986 and increased by another 90 million pounds between 1986 and 1987.

The value of U.S. shrimp imports, like poundage, has increased during the 1965-88 period. For instance, shrimp imports entered the U.S. at an average annual value of $142 million during 1965-68 compared to $1.51 billion annually during 1985-88; indicating an approximate ten-fold increase in value. While much of this increased value reflects the increased quantity of imports, the general upward trend in the shrimp import price, at least through the early 1980s, has also contributed to the observed increase in import value. Much of this price increase, however, is inflationary based. Removing this inflationary trend allows for the examination of the historical import value and price on a deflated, or real, basis. Expressed on a deflated basis, the value of

shrimp imports increased from an average of $354 million annually during 1965-68 (1980 represents the base year) to $1.11 billion annually during 1985-88 (Table 1); an approximate 2.1 fold increase. The deflated price per pound, after expanding rapidly during the mid 1960s and into the 1970s, peaked at $3.01 in 1979 and has since fallen sharply (Table 1). This decline, it is generally accepted, is the result of a rapid increase in the world production of shrimp and the subsequent export of much of this increased production to the U.S. and other leading markets.

The sustained growth in world shrimp production, and its more rapid rise during the 1980s, is shown in Table 1. As indicated, annual world production expanded slightly more than one-billion pounds (expressed on a headless basis) between 1965-68 and 1977-80; from 1.0 to 2.3 billion pounds. Since 1981, or in a matter of only seven years, world production has increased by another 1.1 billion pounds and equalled 3.3 billion pounds in 1988.

Expansion in the world production of shrimp during the 1980s, and the subsequent export of much of this increased production to the U.S. market, can be traced to the development of a cultured shrimp industry. For example, production of cultured shrimp in the Latin American region increased from an estimated 7 million pounds (headless) in 1979 to an estimated 110 million pounds (headless) in 1988. Most of this production is Ecuadorian based, as Aiken (1) shows. The rapid rise in U.S. imports of shrimp in the early 1980s, as discussed earlier, can be traced to this emerging shrimp aquaculture industry in Ecuador. Overall, U.S. shrimp imports originating from Ecuador increased from an annual average of 8.2 million pounds (headless shell-on equivalent basis) during 1973-76 to 40.4 million pounds during 1981-84 and equalled 79.1 million pounds during 1985-88.

Production of cultured shrimp in the Asian region, according to Aquatic Farms Ltd. (2), expanded from an estimated 82 million headless pounds in 1982 to about 570 million pounds in 1988. China has recently emerged as the dominant force in the production of cultured shrimp in both the Asian region and the world and is the main reason for the rapid expansion in U.S. imports of shrimp during the 1986-88 period. Overall, cultured shrimp production in China expanded from an estimated 10 million headless pounds in 1982 to more than 200 million pounds in 1988, while the U.S. imports of shrimp from China during the same period expanded from 3.6 million pounds to 117.8 million headless pounds (headless shell-on weight equivalent basis). The U.S. also imports significant quantities of cultured shrimp from Taiwan and Thailand. Overall, the percentage of world shrimp production represented by a farm-raised product equalled about 20% in 1988 compared to about one percent in the late 1970s.

Much of the world's shrimp production is utilized by a relatively few countries. Among these countries, the United States utilized about one-quarter of total production in 1988 while Japan utilized another 22%. Both of these countries, by and large, show a preference for warm-water varieties of shrimp. Western Europe, which has traditionally shown a preference for cold-water shrimp varieties, utilized another 10-15% of the world's 1988 shrimp output.

MODEL OF U.S. SHRIMP IMPORT MARKET

Several attempts at modeling the U.S. shrimp import market have been made in the past. These attempts have ranged from simple equation demand functions (3,4) to more complex models which use multiple equations (5,6). Prochaska and Keithly (8) have provided the most recent and complete analysis of the U.S. shrimp import market, and the analysis presented here is a reformulation and update of their model. The conceptual model, which consists of an import demand equation, an import supply equation, and an identity equation, is specified in the following three equations.

Table 1. Quantity and Value of Total U.S. Shrimp Imports Relative to World Production, 1965-88.

YEAR	QUANTITY IMPORTED[a]	CURRENT IMP. VALUE	DEFLATED IMPORT Value	DEFLATED IMPORT Price	WORLD PRODUCTION[b]
	1,000 lbs	----- $1,000s ----		$/lb	1,000 lbs
1965-68 avg.	196,522	142,100	353,649	1.80	1,043,308
1969-72 avg.	234,217	205,333	427,690	1.83	1,384,559
1973	230,780	281,587	521,984	2.26	1,773,964
1974	267,462	387,336	647,418	2.42	1,861,616
1975	231,522	346,239	530,393	2.29	1,818,693
1976	271,894	463,344	671,415	2.47	2,083,334
1973-76 avg.	250,415	369,627	592,803	2.37	1,884,402
1977	271,811	491,529	669,132	2.46	2,335,700
1978	240,414	421,724	533,469	2.22	2,339,527
1979	269,263	713,238	809,653	3.01	2,158,348
1980	258,069	719,263	719,263	2.79	2,299,525
1977-80 avg.	259,889	586,439	682,879	2.63	2,283,275
1981	259,112	723,875	656,100	2.53	2,216,198
1982	319,596	980,233	837,282	2.62	2,366,773
1983	421,179	1,223,522	1,012,820	2.40	2,493,588
1984	422,340	1,216,350	965,599	2.29	2,608,292
1981-84 avg.	355,557	1,035,995	867,950	2.44	2,421,213
1985	452,232	1,152,912	893,860	1.98	2,897,032
1986	492,005	1,434,337	1,078,461	2.19	3,029,706
1987	583,030	1,710,224	1,240,402	2.13	3,150,444
1988	598,210	1,754,710	1,223,027	2.04	3,326,336
1985-88 avg.	531,369	1,513,044	1,108,938	2.09	3,100,880

Source: Compiled from unpublished Bureau of Census data maintained by NMFS and published and unpublished FAO data.

[a] All imports of shrimp have been converted to a headless shell-on basis using the following conversion factors: 0.63, breaded; 1.00, shell-on; 1.28, peeled raw; 2.52, canned; and 2.40, others.

[b] World production has been converted to headless basis using a factor of 0.62.

Import demand for shrimp is given as: QD = f(RPI, GSL, CL, BI, RDI) (1)

where QD is the quantity of imported shrimp demanded, given in millions of headless shell-on pounds; RPI is the real, i.e., deflated, price of imported shrimp (expressed on a headless shell-on basis) in U.S. dollars per pound (1980 equals the base year); GSL is the Gulf and South Atlantic, i.e., warm water, landings of shrimp in millions of headless pounds; CL is the U.S. landings of cold water shrimp in millions of headless pounds; BI is the beginning U.S. inventories of shrimp in millions of headless pounds; and RDI is the U.S. real disposable income (1980 equals the base year) in billions of dollars.

Export supply of shrimp to the U.S. is given as: QS = f(PI, REXC, JP, WL, FRI, D87) (2)

where QS is the quantity of foreign shrimp supplied to the U.S. market, expressed in millions of headless shell-on pounds; PI is the U.S. price of imported shrimp in current U.S. dollars per pound; REXC is an index of the real weighted foreign exchange rate among principal U.S. suppliers, i.e., foreign currency per U.S. dollar[1]; JP is Japan's import price of frozen shrimp (predominately headless) in U.S. dollars per pound; WL is the world production of shrimp (less production by the U.S. and Japan) expressed on a headless basis; FRI is an index of real foreign income among the principal U.S. shrimp suppliers[2]; and D87 is a trend variable assigned a value of zero before 1987 and 1 and 2 for 1987 and 1988, respectively.[3]

Finally, a market clearing solution is imposed through the identity equation expressed by equation (3): QD = QS = IMP (3)

It is implicitly recognized with this equation, where IMP equals the equilibrium import quantity in millions of headless pounds, that the system is operating in equilibrium; i.e., the quantity demanded is equal to the quantity supplied.

The representation of the U.S. shrimp import market, as outlined in the previous three equations, was developed based on standard economic import theory that import demand is in response to insufficient domestic supplies. Since import demand and import supply are simultaneously determined through an equilibrium price, the model as outlined above was estimated using a two-stage least squares estimation technique as discussed by Pindyck and Rubinfeld (7). Estimation, using a linear functional form for the demand and supply equations, was based on annual observations for the 1965-88 period.

The estimated import demand and supply equations are provided below.

$$QD = \underset{(51.735)}{39.205} - \underset{(17.13)}{108.41RPI} - \underset{(0.318)}{0.903GSL} + \underset{(0.1906)}{0.0065CL}$$

$$\underset{(0.417)}{-1.444BI} + \underset{(0.021)}{0.420RDI} \qquad R^2 = 0.971 \quad D.W = 2.17$$

$$QS = \underset{(64.607)}{-128.850} + \underset{(15.606)}{80.788PI} + \underset{(42.98)}{288.80REXC} - \underset{(15.175)}{67.906JP}$$

$$\underset{(0.026)}{+0.206WL} - \underset{(105.01)}{277.14FRI} + \underset{(14.962)}{87.728D87} \qquad R^2 = 0.983$$

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The equations appear adequate as judged by the high R^2's and the statistical significance of the estimated coefficients (asymptotic standard errors are given in parentheses).

All coefficients in the import demand equation, with the exception of CL, exhibit the signs suggested by economic theory and are statistically significant. The demand for shrimp imports was found to be negatively related to their own real price (RPI) and the results indicate that a one dollar increase (decrease) in the real import price of shrimp would lead to an estimated 108 million pound decrease (increase) in the demand for imports, holding all other factors constant. Similarly, a one-million pound increase (decrease) in warm-water landings of shrimp (GSL) was found to result in a 0.903 million pound decrease (increase) in the import demand for shrimp while a one-million pound increase (decrease) in the beginning shrimp inventories was estimated to result in a 1.44 million pound decrease (increase) in import demand; again holding all other factors constant. Finally, the demand for imported shrimp was found to respond by 0.420 million pounds for a one billion dollar increase in real disposable income (RDI), ceteris paribus, indicating that increases in the demand for imported shrimp are forthcoming with rising disposable income.

All coefficients in the import supply equation exhibited the signs that would be anticipated based on economic theory and were statistically significant. A one-dollar increase in the current import price of shrimp (PI) was estimated to result in an 80.8 million pound increase in the import supply of shrimp, ceteris paribus, while a one-dollar increase in the Japanese import price of shrimp (JP) was estimated to result in a 67.9 million pound decline in the U.S. import supply of shrimp. The U.S. import supply of shrimp was found to respond by 0.206 million pounds to each one-million pound change in the world production of shrimp (less U.S. and Japan's production), ceteris paribus. The U.S. import supply of shrimp was found to be positively related to the real weighted foreign exchange rate (REXC) with a one unit increase in the latter resulting in a 288.8 million pound increase in import supply, ceteris paribus. A one unit increase in the index of foreign real income among principal U.S. shrimp suppliers (FRI) was estimated to result in a 277.1 million pound decline in the import supply, ceteris paribus; indicating increased utilization of shrimp among U.S. suppliers in relation to increases in their purchasing power. Finally, the trend variable D87 was included in the analysis to "capture" the rapid rise in the exports of Chinese shrimp to the U.S. market. The estimated coefficient was found to be positive and statistically significant, suggesting a structural change in the import supply market.[5]

It is also useful to evaluate the parameters estimates in terms of elasticities. An elasticity is defined as the percentage change in the dependent variable, i.e., shrimp import demand or supply in this example, with respect to a one percent change in any of the independent variables, holding all other factors constant (for purposes of this paper elasticities have been evaluated at the mean values of each variable). The elasticity of the import demand for shrimp with respect to the real import price of shrimp (E_{RPI}) was found to equal -0.779, indicating that a 10% increase (decrease) in the real import price of shrimp would be expected to lead to a 7.79% decrease (increase) in the import demand for shrimp, ceteris paribus. Estimated elasticities with respect to other variables in the import demand equation are as follows: E_{GSL} = -0.455, E_{BI} = -0.327, and E_{RDI} = 2.432. The relatively large growth in U.S. disposable income during much of the 1980s has significantly impacted the U.S. demand for imported shrimp as indicated by the large elasticity of demand for imported shrimp with respect to real disposable income. Estimated elasticities with respect to import supply are as follows: E_{PI} = 0.489, E_{REXC} = 1.044, E_{JP} = -0.559, E_{WL} = 1.162, and E_{FRI} = -0.749.

The condition that all other factors remain constant, i.e., the ceteris paribus condition, which was employed in analyzing the relative changes and elasticities as outlined above results in only partial effects because price is held constant in their calculations. The market clearing solution (equation 3), however, implies that quantity demanded must equal quantity supplied in equilibrium. Equilibrium is restored to the system, given a change in the level of any independent variable, through a change in the import price. For example, increases in real disposable income result in an outward shift in the import demand function which results in an increase in import price and a corresponding adjustment in the quantity of shrimp supplied to the U.S. market. The response in supply drives the import price back towards its original level.

Estimation of long-run, or reduced form, elasticities and changes in the system requires that the system be in equilibrium. This can be achieved by equating the import demand and supply equations and solving for an equilibrium price (since the import price in the demand equation is given in real dollars, it is necessary to convert it to a current basis, by inserting the mean value of CPI, before solving for PI). The resulting equation, where price is specified as a function of all independent variables, can then be inserted into either the estimated import demand function or import supply function, yielding the following equation:

$$\begin{aligned} IMP = & - 108.41 - 0.341GSL + 0.0024CL - 0.546BI + 0.159RDI \\ & + 179.61REXC - 42.233JP + 0.128WL - 172.36FRI \\ & + 54.56D87. \end{aligned}$$

In this equation, the equilibrium level of imports is expressed only in terms of independent variables and can be used for the basis of estimating long-run changes and elasticities.

The long-run impact on imports, resulting from a change in any independent variable, i.e., allowing for equilibrium to be restored to the system, is significantly less than the estimated partial effects. For example, a one-million pound increase (decrease) in warm-water landings (GSL) results in only a 0.341 million pound decrease (increase) in the equilibrium import level compared to a 0.903 million pound decrease (increase) initial impact which is calculated before price is allowed to vary. Similarly, a million pound increase in WL results in only a 0.128 million pound increase in the equilibrium level of imports; significantly less than the 0.206 million pound estimated change occurring before price was allowed to vary accordingly.

The long-run, or reduced form elasticities, using the mean levels for imports and independent variables were found to equal $E^*_{GSL} = -0.172$, $E^*_{BI} = -0.124$, $E^*_{RDI} = 0.921$, $E^*_{REXC} = 0.649$, $E^*_{JP} = -0.348$, $E^*_{WL} = 0.722$, and $E^*_{FRI} = -0.466$ (a * next to the E is used to differentiate the long-run elasticities). These are all significantly smaller in absolute magnitude than the short-run elasticities already discussed; especially among the import demand variables. These lower estimates are the result of a relatively lower price elasticity of import supply, 0.489.

USE OF SHRIMP IMPORTS IN SOUTHEAST PROCESSING ACTIVITIES

As indicated in the previous section of this paper, shrimp imports have been expanding during the past two decades and much of the expansion has occurred since the 1980s. In light of previous efforts by U.S. shrimp harvesters aimed at limiting imports, it is important to evaluate the use of shrimp imports in the Southeast shrimp processing sector and changes that can be attributed to their use. This section of the paper provides such an evaluation.

Some anecdotal evidence of the use of imported shrimp in the Southeast shrimp processing sector can be gleaned through the use of published statistics. During 1970-74, for instance, Southeast landings of shrimp averaged 146.8 million headless pounds annually while Southeast processing activities averaged 207.8 million pounds (headless shell-on equivalent weight basis); suggesting a deficit in domestic supplies of about 61 million pounds annually. By the 1980-84 period, processing activities had increased to 223.9 million pounds while landings remained relatively stable at 156.2 million pounds; indicating a deficit of about 68 million pounds. Processing activities expanded to an average of 268.4 million pounds annually during 1985-88 and the deficit increased to 90.5 million pounds annually. Based upon these observations, imports are obviously playing an increased role in Southeast shrimp processing activities.

While the above observations help depict the potential use of imports in Southeast shrimp processing activities, the depiction is far from complete. To obtain a more complete picture of the use of imports in the region's shrimp processing sector, the authors conducted interviews with managers of shrimp companies that were thought to have used and/or are using imports in processing activities. Interviews with management of 31 Southeast shrimp processing

establishments (plants) were conducted in late 1988 and early 1989 and provides the basis for the ensuing discussion (see 9 for a more complete discussion). Because a small number of Southeastern shrimp processors were either not identified or declined to be interviewed, the estimates that are provided may be low. Also, managers of those companies which ceased processing operations prior to this study could not be contacted, and this suggests that information gathered on the earlier years is especially incomplete.

As Table 2 documents, at least 26 establishments were using imported raw material by 1988. A noteworthy aspect of the historical use is the lengthy, uninterrupted usage pattern among Florida Atlantic coast, Georgia, and Florida Gulf coast processors. The year 1982 was the benchmark for other states' experiences. This is the same year that imports began to expand rapidly (Table 1). Alabama, Mississippi, and Louisiana processing industries experienced a large increase in the number of plants that use imports. Among the three states, companies in Alabama began using imports earlier. By 1988, however, the number in Mississippi exceeded the number in Alabama. The number in Louisiana matched that of Alabama by 1988. Much of the increase in the number of Mississippi and Louisiana processors using imported shrimp coincided with the expansion of Asian, particularly Chinese, exports of shrimp to the United States. However, many of the Louisiana companies indicated a much more seasonal basis of import usage than did the companies in Alabama and Mississippi.

The relative importance of imports was identified by comparing the region's total production of processed shrimp and the estimated production of plants that use imported shrimp as raw material, as shown in Table 3. By 1984, the plants that use imports accounted for 56 percent of the region's total processed shrimp output. In the initial year, 1974, they accounted for 31 percent of the total. The estimated use of imported shrimp increased five fold, from 8-9 million pounds in 1974-75, to 50 million pounds by 1986-87. Imported shrimp accounted for 36 percent of production among companies using imported shrimp in 1987 compared with 14 percent in 1974.

The significance of imports to processing plants in the region could also manifest itself through their product mix. Shrimp products were identified in our survey as raw headless shell-on, peeled raw, breaded, and other. Shrimp that are canned, peeled cooked, or dried comprise most of the "other" category. Plants using imported shrimp more than doubled their production during 1974-87, as measured on a raw headless shrimp equivalent weight basis (Table 4). The shell-on, raw peeled, and breaded categories exhibited large quantity increases among processors of imported shrimp (Table 4). By contrast, the other plants in the region experienced growth in only the raw peeled shrimp category during 1974-87 and experienced no growth in total (Table 5) because of a significant decline in breading and other activities. It is noteworthy that the raw shell-on category was a significant growth category for import-using processors during 1974-87 when non-import users had no growth in that category. Use of imported shrimp in the raw shell-on category does not necessarily indicate lack of value added in processing. Processors using imports responded to the usage question concerning their product mix by including repacked shrimp in the raw shell-on product category. In particular, farm-raised shrimp from Ecuador was repacked as raw shell-on product. Other farm-raised shrimp likely to be repacked as shell-on product included shrimp from Taiwan. White shrimp from mainland China were prominent in 1986 and 1987 U.S. imports. The major uses of this supply included peeled raw and repacking.

Table 2. Processing Plants Using Imported Shrimp by States, 1974-88.

	SOUTH ATLANTIC		GULF				
YEAR	Florida	Georgia	Florida	Alabama	Miss.	Louisiana	Texas
1974		2	5		1		
1975		2	5		1		
1976		2	5		1		
1977		2	5		1		
1978		2	5		1		
1979		2	5		1		
1980		2	5		1		
1981	1	2	5		1		
1982	1	2	5	1	1	1	
1983	1	2	5	2	1	2	
1984	1	2	5	2	2	2	
1985	1	2	5	4	2	2	
1986	1	2	5	4	5	3	1
1987	1	2	5	5	7	4	1
1988	1	2	5	5	7	5	1

Source: Primary data collected by authors.

Table 3. Processed Shrimp Products in the Southeast Region and Estimates of Imported Shrimp's Role, 1974-87.

	PROCESSED QUANTITY (MILS. LBS., HEADLESS, SHELL-ON)				
YEAR	A. Total	B. Import-using Establishments	% (B/A)	C. Imported Shrimp Used	% (C/B)
1974	181	57	31	8	14
1975	157	56	36	9	16
1976	203	74	36	14	19
1977	232	83	36	16	21
1978	243	93	38	22	24
1979	219	82	37	23	28
1980	199	64	30	16	25
1981	230	93	47	29	31
1982	219	99	43	31	31
1983	221	92	42	27	29
1984	252	124	56	38	31
1985	255	131	52	44	33
1986	289	158	55	53	33
1987	260	137	53	49	36

Source: Compiled from primary data collected by authors and unpublished data provided by the National Marine Fisheries Service.

Table 4. Shrimp Products of Southeastern Establishments Using Domestic and Imported Shrimp as Raw Material, 1974-87.

YEAR	RAW SHELL-ON	PEELED RAW	BREADED	OTHER	TOTAL
	---------- mil. lbs. heads-off (shell-on) ----------				
1974	15.7	10.2	23.8	7.7	57.0
1975	17.9	8.7	24.8	4.9	56.3
1976	24.8	11.8	29.9	8.0	74.5
1977	28.9	13.1	33.4	7.8	83.2
1978	30.5	17.8	39.0	5.6	92.9
1979	24.1	20.2	34.4	3.6	82.3
1980	17.9	10.2	29.5	6.8	64.4
1981	30.5	10.3	33.0	19.8	93.6
1982	26.6	26.4	36.5	9.4	98.9
1983	25.4	19.8	35.1	11.7	92.0
1984	45.7	21.4	39.6	124.7	
1985	43.8	30.1	39.1	131.2	
1986	59.1	41.7	44.4	158.0	
1987	43.4	44.8	41.3	136.7	

Source: Compiled from primary data and unpublished data provided by the National Marine Fisheries Service, Fisheries Statistics Division.

Table 5. Shrimp Products of Southeastern Establishments Using Only Domestic Shrimp as Raw Material, 1974-87.

YEAR	RAW SHELL-ON	PEELED RAW	BREADED	OTHER	TOTAL
	---------- mil. lbs. heads-off (shell-on) ----------				
1974	52.1	16.8	26.1	29.0	124.0
1975	39.6	17.0	24.6	20.0	101.2
1976	62.2	22.6	17.7	26.0	128.5
1977	83.8	22.0	14.0	29.2	149.0
1978	80.8	30.0	15.0	24.2	150.0
1979	66.3	38.0	14.2	17.9	136.4
1980	61.1	33.3	13.5	26.3	134.2
1981	68.5	36.0	12.0	19.9	136.4
1982	58.5	38.0	12.0	11.5	120.0
1983	61.0	39.0	13.4	15.4	128.8
1984	61.1	41.0	12.1	13.0	127.2
1985	60.8	42.2	12.3	8.3	123.6
1986	59.9	49.0	12.1	9.7	130.7
1987	49.6	49.5	15.0	9.7	123.8

Source: Compiled from primary data and unpublished data provided by the National Marine Fisheries Service, Fisheries Statistics Division.

SUMMARY

U.S. imports of shrimp have been increasing at record levels during the 1980s. The purposes of this paper were two-fold: (i) to develop and present a statistical model which explains the U.S. import market and reasons for the increased imports and (ii) to examine the use of these increased imports in the Southeast shrimp processing sector.

The import market model consisted of both an import demand and an import supply equation. Import demand was specified to be a function of five variables: (i) the real price of U.S. shrimp imports, (ii) Gulf and South Atlantic landings of shrimp, (iii) other domestic landings of shrimp, (iv) beginning inventories of shrimp, and (v) real disposable income. The supply of shrimp imports was specified to be a function of (i) the U.S. import price of shrimp, (ii) an index of the real weighted exchange rate between the U.S. and its principal shrimp suppliers, (iii) the Japanese shrimp import price, (iv) the world production of shrimp (less U.S. and Japan's production), (v) an index of foreign real income among the principal U.S. shrimp suppliers, and (vi) a trend variable taking on a value of 0 before 1987 and values of 1 and 2 for 1987 and 1988, respectively. The import demand and import supply equations were estimated simultaneously using a two-stage least squares technique.

The models performed quite adequately based on standard statistical tests and economic theory. Increases in real disposable income were found to have a very large and positive impact on the demand for imported shrimp while increases in either Southeast shrimp landings or beginning inventories were found to negatively impact the demand for imported shrimp. The real import price of shrimp was found to have a negative influence on the demand for imported shrimp, as would be expected, with a 10% increase in the former resulting in an estimated 7.79% decrease in the later, ceteris paribus.

All variables in the import supply equation were found to be statistically significant and exhibited the anticipated signs. As expected, the large increase in the world production of shrimp during the past decade has been a major contributing factor to the increase in the supply of shrimp imports. The price elasticity of import supply was found to equal 0.489 indicating a relatively unresponsive change in import supply with respect to price.

To examine the use of imported shrimp in the Southeast shrimp processing sector, the authors interviewed management of 31 Southeast processing plants, thought to have used or are using imported shrimp, in late 1988 and early 1990. Increases in processing activities of Southeast shrimp processors were positively linked to increases in shrimp imports. Florida and Georgia processors were found to exhibit a long history of imported shrimp use. By comparison, Alabama, Mississippi, and Louisiana processors started using processed shrimp, for the most part, more recently.

The study found that plants that used imported shrimp more than doubled their output during 1974-87, as measured on a raw headless shrimp equivalent weight basis. Plants not using imported shrimp, by comparison, showed no growth. Among processors of imported shrimp, the shell-on, raw peeled, and breaded activities all exhibited large quantity increases. Among plants not using imports, growth occurred only in the raw peeled shrimp category.

ENDNOTES

[1] The index of the real weighted foreign exchange rate was computed based on ten largest suppliers of shrimp to the U.S. market during the 1974-79 period (mid point of the data series) using the following formula

$$REXC_t = 100 \prod R_{it}^{Wi}$$

where $REXC_t$ is the real estimated weighted foreign exchange rate in year t, R_{it} is the real foreign currency of country i per U.S. dollar in year t indexed to 1980. Wi is the weight assigned to country i based on the average annual 1974-79 import level of country i. Weights are as follows: Mexico 0.427, India 0.255, Panama 0.055, Ecuador 0.052, Taiwan 0.040, Thailand 0.038, El Salvador 0.035, Nicaragua 0.034, Indonesia 0.033, and Colombia 0.030.

[2] The index of foreign real income among principal U.S. shrimp suppliers was computed in a similar fashion as the real weighted exchange rate (see endnote 3). Gross domestic production data was used in lieu of income data due to unavailability of the latter.

[3] Three reasons are advanced for including this trend variable. First, in the absence of a free market economy, there may be little reason to expect that China will direct exports in a manner that would maximize profits. Second, the shrimp that was introduced by China, the black tiger shrimp, represented a new variety to U.S. consumer and reportedly sold at a discount during its introduction to the U.S. market. Finally, the rapid increase in exports to the United States in 1987 and 1988 may have resulted in short-run disruptions in the U.S. import supply market.

[4] The import supply equation has been corrected for serial correlation. Therefore, a Durbin Watson statistic is not given.

[5] It is noteworthy that estimation of the import supply equation through 1986 without the trend variable (D87) yielded almost identical results as those reported in Table 2 for the 1965-88 period. The import supply equation estimated through 1986 and corrected for serial correlation is as follows:

$$QS = -128.15 + 80.643PI + 290.24REXC - 67.07JP + 0.208WL - 285.42FRI.$$

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MEASURING VALUE ADDED TO GULF OF MEXICO SHRIMP BY PROCESSING COMPANIES

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INTRODUCTION

The landings of shrimp from Gulf of Mexico and state waters are known to be large, often exceeding 240 million pounds (head-on equivalent). A distinguishing element of the industry's production is the wide range of sizes produced. The open ended category of 68+ tails to the pound has, in recent years, accounted for over one-third of brown and white shrimp landings National Marine Fisheries Service (9). The most recent data available (1989) had 68+ brown shrimp landings as 39.9 percent of total Gulf landings. The comparative white shrimp figure was 46.7 percent. During the 1978-1989 period an increasing percentage of white shrimp were in the 68+ category. Historical highs were set in 1988 and 1989. The same two years had 68+ brown shrimp below 40 percent. This occurred following six consecutive years of 68+ brown shrimp being 40 percent or more of landings.

The open-end nature of the 68+ category has an important additional characteristic. There could be a shifting toward smaller shrimp. Harvest of smaller shrimp could be occurring even though the relative role of the category remained unchanged. For example, a smaller average size brown shrimp could comprise the 68+ category even though the percentage share of the category did not increase. Review of data collected on brown shrimp harvests from inshore waters of Louisiana and Texas provided insight on the matter. In 1987, for example, 64 percent of the 68+ count brown shrimp landed in Louisiana's inshore fishery were over 100 count National Marine Fisheries Service (8). The corresponding estimate for Texas inshore brown shrimp landings was 80 percent. This exemplifies the multi-state nature of the "small" shrimp fishery. The receipt of "small" Louisiana heads-on shrimp by Mississippi and Alabama processors, while further exemplifying the multi-state nature of the "small" shrimp fishery, adds another dimension of "small" shrimp utilization Roberts, et al. (11). This dimension is the utilization of the shrimp in processing and marketing products for consumer use.

The emphasis on identifying size of shrimp in the 68+ category is warranted because of the prospect of fishery agencies managing for increased economic returns. A means proposed to achieve such returns is to manage for larger, higher priced shrimp. Two major impediments, among many, to an evaluation of the merit in pursuing such a goal are: 1) a lack of a working definition of the size counts considered to be "small", and 2) lack of a comprehensive measure of economic returns to shrimp use other than that reported at the dockside level. Vagueness associated with loosely worded critiques of shrimp harvesting in the gulf of Mexico likely lessen any prospects for change if it were necessary.

Shrimp value, of course, extends past the dock. Processing adds value to shrimp. Per unit harvest value and the per unit processed value can vary considerably depending on the type and extent of processing. Companies involved in processing Gulf shrimp, in turn, often depend

on the size-count of shrimp harvested. The business of processing shrimp certainly occurs in a dynamic international economic environment. Shell-on headless imports have increased recently to the point where they comprise 65 percent of imports compared to 55 percent ten years ago. Aquaculture supplies, primarily imports, in the mid-counts are responsible for not only the shift in the shell-on/peeled import mix, but also for a lessened role for "small" shrimp. Raw material needs for peeling process lines are generally shrimp in the higher counts. From 1977 to 1986 peeled raw shrimp increased from 13 to 26 percent of Gulf processed shrimp share in terms of poundage National Marine Fishes Service (7). Peeled shrimp production increased 40 million pounds for the period or four times as much as the decrease in shrimp usage in canning. Peeled raw imports were essentially static for the period while peeled cooked, mostly European product, increased 20 million pounds. With increased aquaculture supplies from Asia expected to keep pressure on mid-count shrimp prices, the role of "small" shrimp in determining economic returns must be better understood.

Evaluation of public shrimp policy alternatives necessitates inclusion of considerations beyond the harvest level. Size of shrimp at harvest will increasingly receive attention of industry and management agencies. Ex-vessel price as the sole indicator of value or benefit from management measures is a narrow and simplistic basis of comparison. Simplicity in the case of the complex Gulf shrimp industry also translates to incompleteness. Pertinent elements of more complete evaluations are the ramifications of imported products and domestic processing. A shrimp import model was recently completed Keithly, et al. (6). The processing industry is the focus on the material presented herein. The general objective was to propose, evaluate and report on criteria central to the issue of economic value of "small" shrimp. Since the "small" shrimp industry is primarily northern Gulf based, focusing on the Louisiana processing industry provided a good indicator of the total situation.

APPROACH

National Marine Fisheries Service landings statistics and imported shrimp statistics were used to depict size distribution. Import statistics could be represented by size for only the shell-on and peeled product forms. These forms are the majority of imported shrimp. The landings data have routinely ended with an open-ended size category. This category is the 68+ count tails to the pound. All shrimp 68 count or smaller are reported in the category. Use was made of Louisiana catch data which after 1985 disaggregated the 68+ category into three closed size categories and another open-ended category designated 116+ count. There being no standard for demarcation between preferred and "small" sizes of shrimp a subjective approach was necessary.

To accurately estimate value in use requires that the double counting common to processing sales figures be eliminated. Obviously, a processing company's sales reflect success or failure in markets. Sales revenue in the long-run must be sufficient to cover all expenses. Raw material to make the product, labor costs, salaries, supplies, utilities, overhead, and many other things comprise the expenses. To simply subtract from sales the purchase cost of a product or raw material will not remove the duplication. A company to produce a salable product purchases many things from other companies. Double counting of sales occurs when the supplying companies'items are counted again in the sales of the selling companies. Removal of all material and service purchases from a company's sales leaves a residual amount called value added. It is value added not gross margin (markup) that gives insight to net value created from a particular use of a natural resource, shrimp in this case. Definitions highlight in a concise manner the differences between gross margin (markup) and value added.

gross margin (markup): The purchase price of a product subtracted from the sale price of the product yields the gross margin. Thus, gross margin is a term associated with two

levels in the marketing process. The most used measure is that of difference in purchase price and sale price between producer and the primary wholesaler.

value added: It is the part of gross margin that indicates payments to labor (wages), management (salaries), fringe benefits, capital (depreciation and profits), and taxes. The contribution an industry makes to an economy is identified by value added. Value added eliminates the double counting in a sales figure. A sales figure includes the products and services purchased from other companies. These purchases of raw material, containers, supplies, fuel and other items must be subtracted from sales to derive a company's (industry's) value added.

Since a company's purchase and sale prices for a product are critical to the gross margin estimate, some product comparability must be identified. For seafood, a company's purchase price is for raw, unprocessed product. The raw material becomes changed prior to a product being sold by the processor or wholesaler. A fundamental need is to have equivalent products upon purchase by the processor (wholesaler) and eventual resale. Differences between prices of equivalent weight can then be estimated and attributed to processing activities. Gross margin (markup) is the monetary differences between equivalent products sold by fishermen and then resold by processors (wholesalers). Value added as a component of gross margin is consequently also dependent on the equivalent product estimation procedure.

Standard conversions were available through the National Marine Fisheries Service Thompson, (12). Shrimp products evaluated in the project were headless, peeled raw, breaded raw, and canned. The NMFS keeps conversions for headless products on a species basis. Headless white shrimp poundage should be multiplied by 1.54 to attain the head-on quantity. The headless brown shrimp poundage should be multiplied by 1.61. Thus, brown shrimp yield from head-on to headless is less than that of white shrimp. Since quantity by species is not reported in the statistical system beyond the dock level, average for all species conversions were used. On average, it takes 1.59 pounds of head-on shrimp to produce a pound of processed headless shrimp. Corresponding multipliers for other processed products were similarly used to make value added estimates comparable. Such estimates are the best means of identifying the relative economic importance of processing activities Connor, (2). As will be noted in the analysis section, certain processed products are associated with small shrimp. Value added estimates will thereby yield a broader perspective of economic significance than does ex-vessel price.

LITERATURE REVIEW

The review of publications from previous research yielded information of limited usefulness. Previous research in some cases was not specific enough to the issues of interest. In other cases, the literature revealed that a high level of aggregation existed. That is, the research was not specific enough to reveal information of value to the Gulf value added situation.

Two studies based on 1982 as the analyzed period were found. The more comprehensive analyses actually resulted in reports dealing with headless, peeled, breaded, and canned shrimp products Hu (4). The other report is self-critical. That is, the investigator documents an analysis based on inadequate data Perkins (10). The studies reported by Hu were from a contract aimed at eliminating overregulation of seafood processors through regulatory analyses. Reports for the various processed products of southern shrimp included production, import levels, export levels, number of plants, sales employment, and cost components. The processing cost components were identified by size categories of shrimp plants. Value added is comprised of certain costs that reflect payments to labor, management, and capital plus profits. Hu's studies do not provide a complete depiction of value added. The reports are nonetheless useful to indicate the 1982 levels of some value added components.

In the Perkins 1984 report many errors were made that negate the usefulness of findings. Value added was incorrectly defined as "the difference between the raw material cost and the plant sales value of processed shrimp." As noted previously this is actually the correct definition of gross margin not value added. The Perkins findings can not serve to identify the relative economic contribution of shrimp processing establishments. Perkins' findings are also of limited use because as stated in the report, "the responsiveness of processors regarding cost data was disappointing." This essentially necessitated that the processing costs be displayed in percentage of total processing cost terms. The actual processing cost per item or function was not reported. These problems collectively make the Perkins report of no use to those involved in shrimp industry management.

A contract report to NMFS by Danville Research Associates, Inc. (3) dealt with the shrimp processing industry of the southeast. This comprehensive report remains unpublished. It documents the supply procurement, production, transportation, sales and other factors that explain changes in industry structure. Mean processing cost per pound was identified for 1983. Processing costs were reported by state and for the region. A large number of companies were involved. However, this large number of cooperators were not able to attribute costs to functions and supplies. Operating costs were the only costs reported on a pound basis. Management salaries, depreciation, profit, and other components of value added were not reported by pound. The large number of companies reporting; green headless (54), breaded (4) and peeled (21) likely makes the aggregated operating costs reliable. The operating costs reported for the two canners appear very low and not recommended for use. With the exception of canned costs, operating costs increased as expected in relation to level of processing. Green headless processors reported an average of $.22 per product pound operating cost. Peeled product, not distinguished as to raw or cooked, was reported to cost $.36 per product pound. Breaded product, not distinguished as to raw or cooked, cost $.76 per product pound. All operating cost are obviously exclusive of the cost of shrimp.

As described in Connor (2) value added has been recognized as a significant indicator of economic contribution to gross national product. The U.S. Bureau of the Census (1) annually produces a census of manufacturers inclusive of value added estimates. As would be expected the annual publication of the national economy must deal with numerous industries. Consequently, many industries must be grouped to form a manageable number of classifications to track. Only two of the Bureau's classifications are pertinent to this study: group 2091 for Canned and Cured Fish and Seafoods and group 2092 for Fresh or Frozen Prepared Fish and Seafoods. Canned shrimp are identified by the product code 20910-16. All other frozen shrimp products are included in product code 20925. This product code is further defined for headless (2095-21), peeled raw (20925-22), peeled cooked (20925-23), breaded (20925-24). The value added estimates, however, are only reported at the more highly aggregated 2091 and 2092 groups. Value added expressed as a percentage of 1987 canned and cured (2091) sales was 36.9 percent. The corresponding figure for fresh or frozen (2092) was 26.7 percent. These figures indicate that the more highly processed products generate more value added per dollar of sales. Unfortunately value added estimates for specific shrimp products are not calculated.

A contract issued by NMFS to the research company Kearney/Centaur involved estimation of nationwide value added, margin, and expenditure estimates for marine fishery products Kearney/Centaur (5). Data for the report was from secondary sources. Consequently, value added estimates for shrimp products were not specific to the Gulf or shrimp size. The report did estimate 1985 gross margins for headless, peeled raw, peeled cooked, breaded raw, and breaded cooked. Gross margin was reported as a percent based on an arithmetic average. Portraying gross margin as a percent in relation to ex-vessel price is not useful to specific Gulf concerns over shrimp size. This conclusion is warranted because the national study lumped Pacific, New England, South Atlantic and Gulf of Mexico shrimp together. With gross margin expressed as a percent for ex-vessel price for such widely ranging shrimp fisheries, there can be

only limited application of results. The gross margin was also calculated without regard to weighting on the basis of product quantities. This further limits the usefulness of the Kearney/Centaur (5)report for shrimp fisheries involving three coasts and many processed products. Use of the findings about shrimp must be tempered by an understanding of the study's procedures.

Awareness of weakness regarding the shrimp gross margin information is essential when reviewing the Kearney/Centaur value added estimates. The Census of Manufacturer's estimates of value added for 2092 (fresh and frozen prepared fish and seafoods) was used for shrimp, all other shellfish, and finfish. This aggregation results in estimates that can be used only with caution. Group 2092's value added for 1985 was 57.3 percent of gross margin at the primary processing level. There are no secondary data sources available that can be used to separate Gulf shrimp processors and their different products from these national averages. The need in this project to delineate the first ever value added estimates for Gulf small shrimp then must be achieved by collection of primary data.

SURVEY OF PROCESSORS' VALUE ADDED

Secondary data provided the ex-vessel and wholesale shrimp prices. Gross margin was then calculated for the various shrimp products. The value added component of gross margin could not be estimated for 1987 from secondary sources. As previously noted the literature search revealed that previous studies failed to yield value added estimates due to poor research methodology Perkins (10) or excessive aggregation Kearney/Centaur (5). Collection of primary data on costs involved in producing Gulf processed shrimp products was clearly necessary. Fifteen companies responded to a two page questionnaire specifically designed to identify value added.

RESULTS

This analysis was based on 68+ tails per pound as the demarcation point for "small" shrimp. Essentially any point can be criticized as being somewhat arbitrary. There are shrimp management policies in the Gulf focused on size at harvest. Louisiana opens its brown season on the basis of 50 percent of the shrimp being at least 100 count head-on. A Louisiana landing law prohibits smaller than 100 count head-on white shrimp. The Gulf Council's mid-summer brown shrimp closure off Texas produces an annual evaluation report. NMFS's evaluation cites shrimp tails 68+ as small.

There are also trade/market practices that provide guidance. Mechanically processed head-on shrimp yield a peeled raw (PUD) product. Head-on shrimp larger than 41-50 are used only infrequently for PUD market (A. Cuccia, personal communication). This category is equivalent to 68-80 tails. The survey of Gulf processors also provided insight to industry practices. Processors responded to questions about most frequent sizes used in their product and raw material costs. Answers to each gave an indication from two perspectives of the sizes of shrimp used for peeled raw (PUD) breaded, and canned products. The 68+ shell-on tails category was reported to be representative of the PUD and canned products. A range of shrimp from 31-60 count shell-on tails was cited by the processors of breaded products. The most frequent count was 41-50.

Price quotes in NMFS Market News reports are readily available for the 90-100 count PUD produced from the 68-80 tails. The same reports indicate a lessening of price quote availability on shell-on headless for 70 count and smaller shrimp. Gulf supplies of shell-on headless smaller than 70 count shrimp are perhaps limited. The decision was made to denote 68+ headless shrimp as "small" for purpose of the analysis. This certainly would include the brown and white 100 count criteria established in Louisiana and supported by Gulf council action. It also fits the

cited "small" shrimp terminology in the annual report prepared on the Texas brown shrimp closure. From a processed product viewpoint, the 68+ tails choice serves to delineate the shell-on headless and PUD product. Canned product clearly involves use of shrimp in the proposed 68+ category. Just as clearly the breaded products involve use of shrimp larger than 68+ tails in most cases.

Value-in-use of landed shrimp has been portrayed as best estimated as part of the gross margin of initial processors. The focus is on identifying that portion of the margin that represents new value, i.e., value added. Processors responding to a survey provided a direct estimation of value added through delineation of all unit costs. Components of value added are labor expenses, salaries, employment taxes, other taxes, depreciation, rent, interest, and profit. Direct estimation of value added produced numbers by shrimp product that could then be compared to the gross margins. The ex-vessel price of shrimp used in products when subtracted from the processed price received yields the gross margin. Value added is then divided by the gross margin to identify the percentage attributable to value added. This coefficient can then be applied annually to the gross margins by product. Only ex-vessel and processed product prices are needed for the selected year.

Data on processed product quantities and value collected by NMFS are reported annually in Processed Fishery Products, Annual Summary. The most recent volume at time of report preparation was 1986. However, 1987 data were available via personal communication with the NMFS. This source allowed the use of 1987 as the year of most recent data available. Price received for the various Gulf processed shrimp products was derived from the quantity and value amounts. In this way, the primary processing market level's gross margin could be calculated. Thus, the gross margin estimates for shell-on, peeled raw, breaded, and canned products came from NMFS data. This finding will be of use in subsequent analyses. Use of NMFS data will necessitate special requests for ex-vessel and processed product data. This should be anticipated so that potential time delays can be minimized. The Gulf average processed product prices per pound for 1987 were: shell-on headless $4.29, peeled raw $3.43, breaded $3.31, and canned $5.19 (or $35.03 per case).

The value added estimates from the survey of processors are: PUD $.59 per pound, canned $9.96 per case ($1.48 per lb.), and breaded $1.04 per pound (Table 1). Components of value added were grouped into six categories to show the relative contribution (Table 2). Labor costs were the largest category of value added for PUD and breaded products. Production of canned shrimp is more highly mechanized which leads to a lesser role for labor. Net profit at $2.70 and rents/fees at $2.53 are the major categories of value added. The significant role of rents/fees arises due to marketing costs. Gulf canned shrimp are a retail oriented product involving use of marketing services. The rents/fees of PUD and breaded shrimp generally reflect costs only through the primary processing or wholesale level.

Quantifying value added permits other shrimp use relationship factors to be estimated. Value added in relation to gross margin shows the new contribution that a particular processing activity makes to the economic impact of shrimp use. The shrimp cost to produce the product and processed product prices for 1987 provided the estimated gross margins. The value added component from the survey then allows the percentage of new economic activity to be calculated. Additional effort is necessary in order for comparisons among shrimp products to be accurate. This is because each of the final processed products requires different quantities of shrimp input. The shell-on headless amount of shrimp necessary to produce a pound of product was derived from standard NMFS conversions listed in Fisheries Statistics of the United States Thompson (12. Thus, the gross margin and value added estimates are stated on the common basis of shell-on headless in the last line of Table 1. The first four lines of the table relate to product pounds. Value added on a headless shrimp per pound basis was calculated by dividing value added per product pound by the pounds of headless shrimp necessary to produce the product pound. For

Table 1. Gross Margin and Value Added of Gulf Shrimp Processed Products, 1987.

	PEELED UNDEVEINED	CANNED	BREADED
processed price	$ 3.43	$ 5.19	$ 3.31
shrimp cost	2.28	3.31	1.73
gross margin	1.15	1.88	1.58
valve added	.59	1.48	1.04
value added as % of gross margin	51.3	78.7	65.8
value added per pound of headless shrimp used in the product	.46	.73	1.65

The production of a pound of PUD product requires 1.283 pounds of shell-on headless at a price of $1.78 per pound for a shrimp cost of $2.28.

The production of a pound of canned product requires 2.02 pounds of shell-on headless at a price of $1.64 per pound for a shrimp cost of $3.31.

The production of a pound of breaded product requires .629 pounds of shell-on headless at a price of $2.75 per pound for a shrimp cost of $1.73.

Table 2. Total and Component Value Added Per Product Pound Estimated from Survey of Gulf of Mexico Shrimp Processors, 1987.

	$/LB		
	PUD	CANNED	BREADED
labor	.234	.333	.430
salaries	.050	.085	.033
taxes	.026	.132	.040
overhead	.072	.152	.157
rents/fees	.053	.374	.067
profit	.158	.400	.310
total	.593	1.476	1.037

PUD the $.59 value added was divided by 1.283 to derive $.46 value added per headless pound used to produce PUD shrimp. Following the same procedure for the product value added and the conversions yielded the estimates of $.73 for canned and $1.65 for breaded. These results give specific numbers that relate to each other as anticipated. That is, breaded products involve more processing steps and require less shrimp per product pound than either PUD or canned products. Note that canned value added per product pound is actually the highest of the three products. However, 2.02 pounds of headless shrimp are required to produce the canned pound. In making calculations of value in use beyond ex-vessel three findings must be explicitly recognized: 1. shrimp of different sizes are used in the products, 2. the processed products require different amounts of headless shrimp inputs, 3. the combined effect of different sizes with related prices and varied conversions can make the process of evaluation tedious.

The production of frozen shell-on headless shrimp by processing companies is associated with value added generation. A focus of the research was "small" shrimp previously defined as 68+ count. PUD, canned and some breaded products are associated with shrimp in the category. However, the headless product was previously identified as involving little use of 68+ raw material. Processing of this product is a straight forward matter of grading, boxing, and freezing in five pound units. Gross margin and value added levels are comparatively lower in practice than more highly processed products. PUD, canned, and breaded shrimp products utilize more procedures to yield products that serve both the food service and retail sectors of seafood product supply and use. Frozen headless as a product serves the food service aspect of the market. This market is one focused on minimal changes in raw material and large package size. The gross margin for headless products in 1987 was calculated from secondary data. The average ex-vessel price for Gulf shrimp 67 count and larger was $3.91. A gross margin of $.38 per pound results from use of the average headless wholesale price of $4.29 (S. Koplin, personal communication). This verifies the statement that headless product would have lower gross margin than more highly processed products oriented to more finished product uses. Consequently, value added per product pound will also be low in absolute terms. An estimate of headless product value added was made from secondary data Kearney/Centaur (5). Value added as a percentage of gross margin was identified in the report as 57.3 percent. The arithmetic mean of peeled, canned, and breaded products collected as primary data from the survey was 65.3 percent. Using these measures to depict a range for value added of processed headless shrimp yields $.218 to $.248 per pound. These values can be compared to the value added findings in the last row of Table 1. It is the conversion of product weights to a raw material weight basis that facilitates the comparisons. The comparisons or rankings on a raw material basis are consistent with the proposition that more value derives from multi-step processing. Research has quantified the value added and, importantly, identified the amounts by which shrimp product value added differs.

CONCLUSIONS

The focus of the research project was the identification and delineation of the situation in the small shrimp component of Gulf shrimp fisheries. Emphasis was on generation of new economic information from the perspective of value added in processing. The process adds two elements to the deliberations about shrimp size count management. The first is that managers and industry must develop and use information beyond the harvest level. Ignoring the primary processing level in management attempts to improve economic conditions will lead to incomplete information. Decisions would not be based on the best scientific information available. Secondly, size count management must proceed with value added estimates as opposed to price alone. Value added was noted to be more useful in measuring economic results of change. The basis for the statement is that price conveys gross effects while value added specifies new net economic results.

Research results do not determine management objectives. Participants in the management process contribute to statements of objectives. This project contributes results that should broaden and make more accurate both the development and evaluation of objectives. Viewing the data and analysis of this initial research into value added leads to the following conclusions:

1. The contribution an industry makes to an economy can be identified by the measurement of value added.

2. The use of value added methodology necessitates acquiring data from both primary and secondary sources. Routine data collection by agencies will not provide an adequate basis for analysis. Interview data from processors and harvesters is necessary to complete value added research.

3. Agencies should continue to report prices by as large a number of sizes as possible. Future decisions may be far more specific than one involving 68+ tails versus all larger shrimp.

4. The analysis indicated that processed products likely to involve "small" shrimp use generated more value added per pound than other products. Value added on a headless input basis for the various products was estimated to be: $.46 per pound for PUD, $.73 per pound for canned, $1.65 per pound for breaded, and from $.22 to $.25 pound for frozen headless. The PUD and canned products essentially involve use of "small" shrimp. Breaded products involves some use of such shrimp. The frozen headless product essentially reflects use of larger shrimp.

ACKNOWLEDGEMENT

The authors acknowledge funding support of the MARFIN Program for the project "The Role of Small Shrimp in Determining Economic Returns".

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THE NORTH ATLANTIC SWORDFISH FISHERY: PROBLEMS, TRENDS AND THE ECONOMICS OF MANAGEMENT

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The North Atlantic swordfish (Xiphius gladius) has recently been the subject of an increasing number of proposed domestic and international regulatory measures. These measures are designed to reduce current fishing mortality which is believed to place the swordfish stock in danger of recruitment failure. A proposed amendment to the existing swordfish fishery management plan (FMP), as well as an international agreement to limit swordfish harvest, will contribute to the formulation of management measures that are intended to rebuild the spawning stock to a level that will reduce the likelihood of recruitment failure. These new management initiatives are the result of an evaluation of the swordfish stocks by scientific panels, international negotiations, input from public hearings and economic analysis.

This paper will begin with an historical overview of the swordfish fishery. The problems that have developed within the fishery and the growing importance of imports become evident as we discuss production trends. The evolution of the original swordfish management plan is then traced to today's impending regulations. Since the U.S. is the primary importer of swordfish, the role of imports is described within a simple economic model. The economic model is then used to analyze the potential impacts of these proposed regulations on domestic harvesters and consumers of swordfish. The international trade aspects of the proposed regulations will also be discussed.

INDUSTRY BACKGROUND

The swordfish is a slow growing billfish, first spawning at five or six years and living about nine years. These characteristics are typical of species that are susceptible to biological overfishing (2). However, its global distribution, large spawning areas and prolific nature have contributed to the relative resilience of the species. The primary habitat of the North Atlantic swordfish is the Gulf Stream, which flows northeasterly along the U.S. coast, then turns eastward into the Atlantic across the Grand Banks. Tag-recapture data, geographic distribution of Japanese by-catch and larvae distribution suggest that the stock structure of the North Atlantic swordfish is continuously distributed across the Atlantic. Trans-Atlantic migration has been found to be minimal, however, north-south movement along the eastern seaboard is recognized to be very significant (7).

The domestic fishery for the North Atlantic swordfish has historically been dominated by the New England fleet, which fishes along the Atlantic coast from New England to Florida and into the Gulf of Mexico. The Florida fleet, which evolved from techniques and gear used by Cuban-American longliners, is a more recent development. The New England vessels traditionally have been larger than their Florida counterparts because of the distances traveled and time spent at sea. Typically a New England longline vessel, usually 60 to 80 feet long, fishes off New England in the summer and fall, and works its way south as winter approaches. The vessel may fish off Florida's east coast or in the Gulf of Mexico for as long as two weeks at a time (1).

The Florida fleet was developed by shark longliners who began to land a significant amount of swordfish as by-catch. The Florida fishermen saw the high value of swordfish and the large catches by the northern boats as enticements to enter the fishery. However, they also

found that the longline gear used by the New England boats was not as efficient in the swift currents of the Florida portion of the Gulf Stream. In the mid-70's Cuban-American fishermen developed a type of longline that led to more efficient catches in Florida waters. These migrant fishermen were familiar with a traditional swordfish fishery existing for years along the coast of Cuba. The vessels in the Florida fleet are usually 35 to 50 feet long and are geared to make one or two night trips (1).

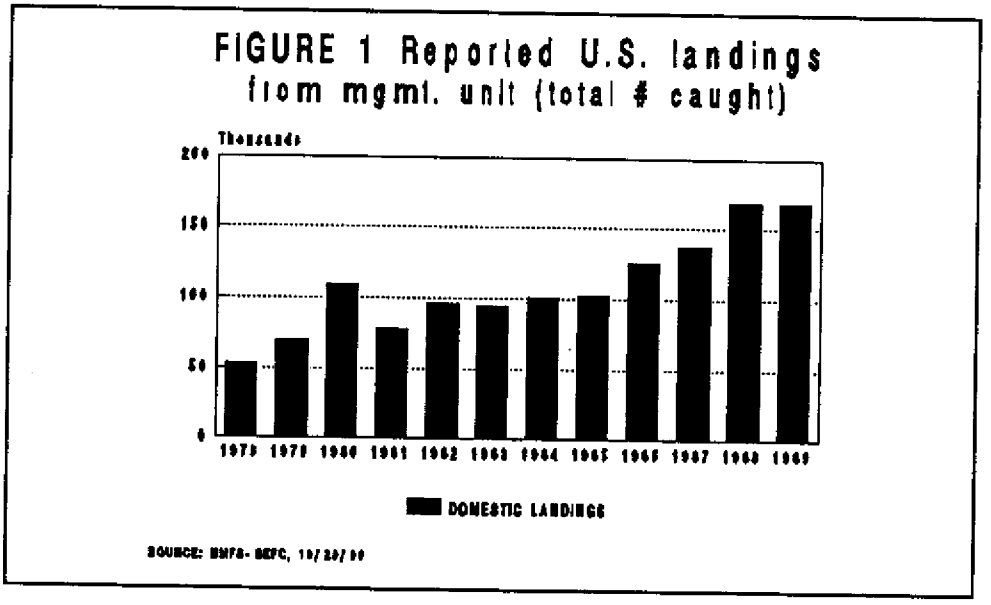
Virtually all commercially landed swordfish are caught by longline gear. This gear consists of baited hooks attached to a main line suspended by a series of floats. The longline can be up to forty miles long. The introduction of artificial lights or lightsticks called "Cyalume" was an important innovation in swordfish longlining. The sticks contain chemicals that emit a glowing green light which, when attached to the hook, attracts swordfish. The lightsticks may also attract bait fish which may enhance the ability to attract the swordfish. The lines generally are released at sunset and hauled in at sunrise to take advantage of the nocturnal feeding habits of the swordfish (1).

The differences in the harvest methods of the two fleets have become less distinct as the fishery has evolved and expanded. As the fishery has expanded into the Caribbean Sea, the Florida vessels have become larger and better equipped for longer trips. The New England boats have utilized shorter longlines with fewer hooks per mile (1). The fishery has also expanded eastward across the Atlantic competing for stocks with Spain, Japan and Canada. The rapid growth of the fishery has led to intense competition between domestic and foreign longliners.

PRODUCTION TRENDS

Historical data indicate four distinct time periods over which significant changes in North Atlantic swordfish harvest occurred. For the period between 1959 and 1970 the average annual catch for the domestic and international fleet targeting the management unit fishery was 8.0 thousand mt. (7). This decreased to 6.3 thousand mt. for the period 1971-1977. Part of this decrease can be explained by the Mercury scare which caused demand to decline and effort to be reduced. The average annual catch increased to 12.8 thousand mt. for the period 1978-1985 as consumers' fear of mercury contamination waned and consumption increased (6). The expansion of the fishery, both in terms of areas fished and fishing effort, led to a stock-wide average annual catch of 19.1 thousand mt. for the period 1986-1988. Most of the tremendous increase in the late 1980's can be attributed to an increase in Spanish effort and to a lesser extent to an increase in U.S. effort in the fishery. Figure 1 depicts the substantial increase in numbers of swordfish caught by the U.S. fleet from 1978 to 1989. Continued high levels of harvest could put the fishery in a situation similar to that of the Mediterranean Sea fishery. High levels of effort, low catch rates and almost complete dependence on one or two year classes of fish characterize the fishery in the Mediterranean Sea (7).

Swordfish imports from countries fishing Atlantic and Pacific stocks have increased from approximately .48 million pounds dr. wt. in 1980 to over 15 million pounds dr. wt. in 1989 (7). Chile has become the largest exporter of swordfish to the U. S. with over 9 million pounds dr. wt. coming from the southeastern Pacific stocks. Imports from Latin America and in particular the tiny Caribbean island nations have increased substantially in the late 1980's (Table 2). This increase may more than offset the decrease of imports from Spain. In addition, Spain as well as Latin American countries such as Costa Rica, Panama, Mexico and Brazil fish from other stocks and this exacerbates the data collection problem since Atlantic, Pacific and Mediterranean swordfish are virtually indistinguishable. Spain has since moved out of the North Atlantic fishery because of decreased yields (4). Figure 2 shows the growing importance of imports from the management unit as compared to domestic landings from the same region. Imports from the management unit increased from 379,000 lbs. in 1980 to 5.5 million lbs. in 1986, the peak year. In 1989 swordfish imports from the management unit were approximately 3.9 million lbs. dr. wt.



Analysis of a recent stock assessment (1987) reflects the effects of the substantial increases in catch. The data indicates the adult spawning stock biomass (SSB) in 1987 was about 40% of the 1978 level, while the mean size of harvested fish has declined continuously from 115 lbs. to 60 lbs. dressed weight (dr. wt.) during the same time period (7) (Figure 3). A 60 lb. fish is approximately 2-3 years of age but not yet sexually mature. If these trends were to continue in the absence of any mitigating factors, yield and revenue projections indicate a dramatic decline in industry returns would result (Table 2). For example, domestic landings are predicted to fall from 10.3 million pounds in 1989 to 4.95 million lbs. dressed weight, which would yield $12.38 million in 1991. If the predicted downward trend in landings occurs due to continued overfishing, only 0.11 million lbs. dressed weight are projected to be harvested during the year 2000. Even taking into account higher prices offered due to decreased supplies, projected revenues would only be $.44 million (4). These reductions in total revenues do not incorporate other negative economic factors resulting from a decrease in catch, such as job losses in the production and processing sectors, and reductions in consumer and producer welfare. The projected landings decreases alarmed resource managers of the danger of recruitment failure and the need for prompt, if not immediate, action.

MANAGEMENT HISTORY

Prior to 1983 the North Atlantic Swordfish fishery was essentially unmanaged and unregulated. However, as landings and fishing effort increased during the late 1970's and early 1980's, the need for management of the fishery became evident. A permit requirement was instituted in 1983, however, no restrictions were imposed on the number of permits or who could obtain one. The number of permits issued have increased continuously with over 700 permits being issued in 1989. Of this number, an estimated 400-500 are active in the directed swordfish fishery, with the remaining permits being allocated among fishermen who target other species, such as tuna, on a full time basis and target swordfish in the off-season.

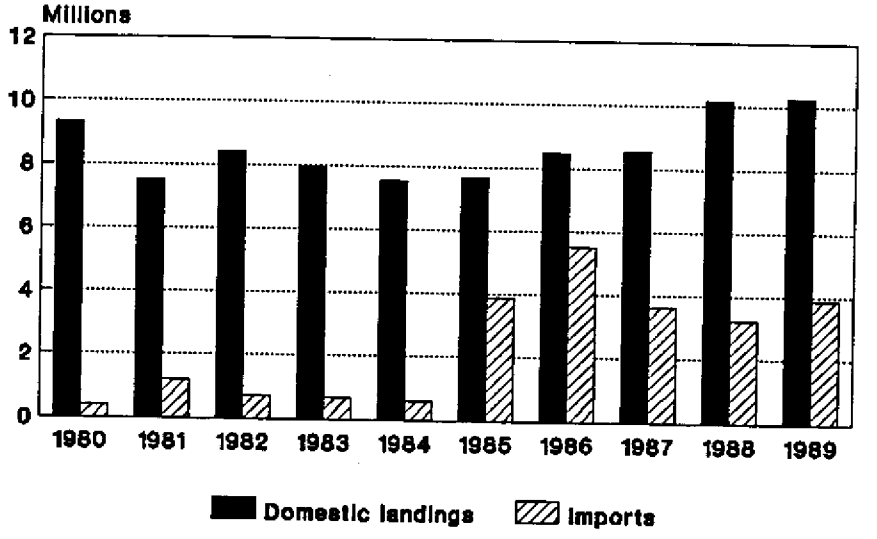
In 1985 the South Atlantic Fishery Management Council (SAFMC) was delegated the responsibility for development of a FMP for the North Atlantic swordfish. SAFMC biologists estimated that fishing mortality for female swordfish had, in 1980, reached the estimated level that maximizes yield per recruit. Based on this estimate, the Council's primary objective was to reduce the harvest of small swordfish [fish < 50 lbs. (dr. wt.)] to the 1980 level. The initial FMP was approved on August 22, 1985. Implementation of Variable Season Closures (VSC's), was a key

TABLE 1. U.S. swordfish imports from the management unit (fresh and frozen in thousands lb dr wt)

	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989
CANADA	266	3		522	506	888	1832	986	828	1186
COSTA RICA*							1		61	54
HONDURAS										6
PANAMA*							1		11	4
TURKS & CAICOS									19	16
BR. VIRGIN ISLANDS								5	66	
BARBADOS									50	156
TRIN. & TOBAGO								176	151	238
NETH. ANTILLES		35	92		1	18	21	13	59	15
VENEZUELA							89	5	6	111
GRENADA										12
ANTIGUA										14
ST. LUCIA										3
SPAIN*				33		2853	3199	1578	271	24
MEXICO*	113	1129	429	20			67	157	358	1091
BRAZIL*		21	212	82	82	121	287	699	1365	933
TOTAL	379	1188	733	657	589	3880	5497	3619	3245	3863

*All or part probably from outside the management unit.
Source: NMFS Fisheries Statistics Division

FIGURE 2 Domestic landings vs. Imports from the Mgmt. unit (lb dr wt)



Source: NMFS Fisheries Stat. Division

FIGURE 3 Reported U.S. landings from mgmt. unit (mean lbs dr wt)

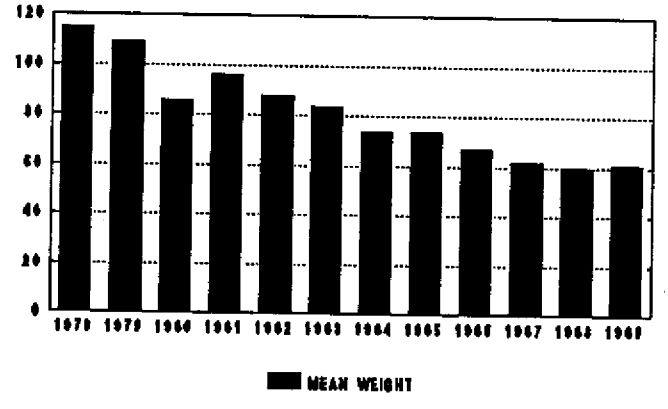


TABLE 2. Projected Yields and Revenues for Domestic Fishery

YEAR	Yield (millions of lbs.)	Price	Revenue (millions of dollars)
1991	4.95	$2.50	$12.38
1992	3.86	$2.50	$9.66
1993	2.98	$2.50	$7.47
1994	2.23	$2.50	$5.59
1995	1.71	$2.50	$4.28
1996	1.30	$2.50	$3.25
1997	.91	$2.50	$2.28
1998	.62	$3.00	$1.86
1999	.34	$3.00	$1.02
2000	.11	$4.00	$0.44

Source: SAFMC Amendment 1 draft

component of that plan but this provision was subsequently sunsetted by the Secretary of Commerce who felt the action was not necessary (7). The VSC would have closed each region a sufficient amount of time so as to reduce the number of small swordfish caught to be within the desired limits imposed by the FMP. As the swordfish FMP is currently written, the only regulations for the fishery are a required permit and a data collection provision. This provision requires mandatory logbooks and dealer reporting of landings data such as total weight, total numbers and average weight.

After reviewing documents concerning Atlantic stocks of swordfish from the National Marine Fisheries Service (NMFS), Southeast Fisheries Center (SEFS) (NMFS-SEFC 1988) and the International Commission for the Conservation of Atlantic Tunas (ICCAT) (ICCAT 1988), the SAFMC developed a revised set of swordfish management objectives. The key objectives were to rebuild the adult biomass to levels that would prevent recruitment failure, and then to increase total yields by shifting fishing mortality to larger fish. The latter objective would be implemented only after significant biomass recovery and stabilization had occurred. Other objectives included the stabilization or reduction of the number of participants in the longline fleet, which may be over capitalized considering present yields. Additionally, the success of any unilateral management plan depends on the emphasis that other countries, who fish the same stock, place on conservation of the resource. Therefore, the SAFMC recommended the establishment of an international management regime to manage Atlantic swordfish.

Initial recommendations by the SAFMC to carry out these objectives would have reduced domestic landings by approximately 78%. This measure would have effectively closed the domestic directed swordfish fishery. Swordfish would then become a bycatch fishery because the total allowable catch (TAC) would be caught inadvertently by tuna fishermen. The announcement of the SAFMC recommendations resulted in hundreds of protest letters and petitions and increased participation in public hearings along the eastern seaboard. Questions arose about the legal and trade implications. The wording of the scientific review panels' reports was also questioned. In response, alternative management proposals by the New England and Mid-Atlantic Councils (NEFMC & MAFMC) contributed to the development of a revised amendment to the swordfish FMP which is now known as Amendment 1. The regulatory measures incorporated in the SAFMC version of Amendment 1 included a three-year phase-in of reduced landings instead of the immediate 78% reduction. A new permit system, gear restrictions and import controls were also proposed. The SAFMC regulations would have become effective

in April 1991, but two very significant events occurred affecting the management process of swordfish regulation.

On November 15, 1990 the 22 nations of ICCAT (International Commission for the Conservation of Atlantic Tunas) agreed to reduce harvest of swordfish in the northwest Atlantic ocean. The principal components of the ICCAT recommendations are as follows: (i) A 15% reduction in fishing mortality on fish greater than 25 kg. (54 lbs. whole weight or 41 lbs. dressed weight) was negotiated, with 1988 as the index year. (ii) A prohibition on the landing of swordfish less than 25 kg. was introduced with a 15% tolerance to account for unavoidable catches of small fish. This minimum size restriction was not part of Amendment 1 because of the fear of undersized discards (4).

Domestically, the Magnuson Fishery Conservation and Management Act was reauthorized, transferring management jurisdiction of the large migratory species (swordfish, tuna, billfish) from the regional fishery management councils to the Secretary of Commerce. The Secretary has three alternatives for management strategies at his disposal: (i) utilize the recommendations prepared and submitted by the SAFMC, (ii) implement ICCAT recommended measures or (iii) incorporate a combination of elements from both institutions into yet another revised amendment. Complicating the decision process is the provision in the amended Magnuson Act that no domestic conservation plan may be more restrictive than its international counterpart.

The three-year phase-in of Amendment 1 will allow fishermen to continue to fish swordfish as a directed fishery, although at a reduced rate. The phased-in reductions protect the resource and allow for the rebuilding of the spawning stock biomass (SSB). The phase-in mechanism will be a harvest quota and an appropriate allowable level of harvest will be set for each year. The U.S. total allowable catch (TAC) can be computed by multiplying the ABC by the U.S. proportion of the stock-wide catch in 1989. Assuming a 60% proportion (see stock boundary discussion below) the U.S. TAC for 1991 is 7.28 million pounds dr. wt. This represents a 31% reduction, as opposed to the initial recommendation of a 78% reduction, from the 1989 catch (7).

In an attempt to better measure effort in the swordfish fishery, Amendment 1 proposes a new permit system. Although this does not limit entry into the fishery, a control date of August 16, 1989 was established for possibly limiting entry in the future. This means that all vessels participating in the directed fishery on or before that date will not be excluded in the event some form of limited entry is adopted. This control date mechanism was designed to prevent speculative entry that would occur if a limited entry scheme was announced. Speculative entry would unfairly penalize present participants.

Amendment 1 also prohibits the use of drift gillnets because of the Marine Mammal Protection Act. Only an estimated 20 vessels, of the more than 700 permitted to land swordfish, use this type of gear (7). Other gear restrictions include prohibition of artificial lights or lightsticks after the season is closed.

One of the more problematic and controversial decisions the SAFMC made was concerning import restrictions. The purpose of instituting import restrictions is to prevent an increase in foreign catch from the same stock to replace the sudden domestic shortfall. The management unit or stock boundary has been the subject of controversy as well. The boundary is now defined as the area north of 5 degrees N latitude, west of 30 degrees W longitude and bounded by the continental land mass on the West (7). Using 1989 landings data the U.S. caught approximately 60% of the total swordfish catch in this region. Amendment 1 suggests reducing U.S. imports caught from the management unit via a quota by the same percentage as domestic landings were reduced by the harvest quotas. The reductions would be computed by applying the quota to a previous three-year average when the imports occurred (i.e. 1987-1989). Imports in the first year of the regulations will be reduced 31% across the board. (Table 3) Total U.S.

imports of swordfish caught within the management unit would fall from 3.9 million lbs. in 1989 to 2.5 million lbs. in 1991. Because there may be inaccuracies in the import figures, any quotas imposed would be subject to revision upon receipt of adequate documentation.

TABLE 3. U.S. swordfish imports from the management unit (fresh and frozen in lbs dr wt)

	AVG. 1987-1989	1991 QUOTA
CANADA	1,000,079	690,055
COSTA RICA*	57,631	39,765
HONDURAS	5,503	3,797
PANAMA*	7,477	5,159
TURKS & CAICOS	17,446	12,038
BR. VIRGIN ISLANDS	35,510	24,502
BARBADOS	102,517	70,737
TRIN. & TOBAGO	188,478	130,050
NETH. ANTILLES	28,830	19,893
VENEZUELA	40,415	27,886
GRENADA	11,761	9,443
ANTIGUA	13,686	9,580
ST. LUCIA	2,626	1,812
SPAIN*	624,337	430,793
MEXICO*	535,250	369,323
BRAZIL*	998,804	689,175
TOTAL	3,670,424	2,532,593

*All or part probably from outside the management unit.
Source: NMFS Fisheries Statistics Division

THE ECONOMIC CONSEQUENCES OF PROPOSED SWORDFISH MANAGEMENT

Given the recent changes in authority regarding jurisdiction over swordfish management, the exact form of the eventual regulatory measures to be imposed on the North Atlantic swordfish fishery is unclear. However, Amendment 1 and the proposed ICAAT management measures have potentially significant impacts on domestic producers and consumers. There may be a substantial decrease in short-run swordfish supply originating from the management unit. The magnitude of this decrease depends, in part, on the ability or willingness of swordfish fisheries outside of management unit (e.g. Pacific, Mediterranean) to increase harvest to meet this shortfall in U.S. supply. For purposes of illustration the subsequent analysis will assume that the domestic and import quotas will be adopted. The effects of such a policy can be shown graphically using a simple conceptual model which highlights the effects of the proposed management harvest restrictions and import quotas.

The economic effect of a change in fishery management policy is measured by changes in domestic consumer's and producer's surplus. Consumer surplus is defined as the consumer's willingness to pay for a product (in this case - swordfish) over and above the existing market price. Thus, the difference between what a consumer is willing to pay and what is actually paid is consumer surplus. Graphically, this is equivalent to the area bounded from below by the market price line and bounded from above by the demand curve shown in Figure 4a. Domestic producer surplus is defined as the amount of income received by producers in excess of all fixed and operating costs incurred to produce a product. Thus, domestic producer's surplus is delineated by the area bounded from above by the market price line and bounded from below by the domestic swordfish supply curve (Sus) shown in Figure 4a. Estimation of the net changes in these surplus values provides a measure of how U.S. consumers and producers fare as policy and market conditions change.

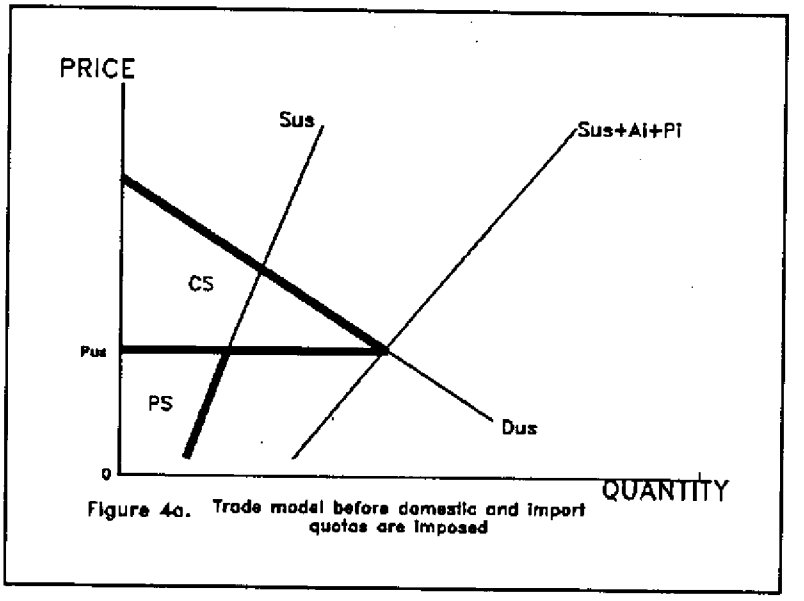


Figure 4a. Trade model before domestic and import quotas are imposed

Figure 4a shows the fishery in its current situation without any harvest or import restrictions. Assumptions in the model include a relatively inelastic domestic supply curve (Sus). This means that domestic quantity supplied will respond with a less than proportional increase to a given price increase. The domestic supply of swordfish, as for other wild-caught species, is determined in the short-run primarily by environmental conditions and fixed harvesting/processing capacities (3,5).

The aggregate supply curve (Sus+Ai+Pi) consists of imports from the Atlantic (Ai) and Pacific (Pi), in addition to the domestic supply, and is obtained by horizontally summing these supply curves (Atlantic and Pacific stock supply curves are not shown). Supplies of swordfish from other sources (i.e. Mediterranean) are not considered. As such, the aggregate supply curve will be more elastic than the domestic supply curve. The aggregate world supply of swordfish may be more sensitive to price changes than domestic supplies due to the wide number of sources, less of a dependence on the environmental conditions for any given region, and a more diverse set of harvesting technologies and capacities. The U.S. price (Pus) of swordfish is derived from the intersection of the domestic demand curve (Dus) and the aggregate supply curve.

Consumer welfare, or surplus, (CS) is shown by the area above the price paid by domestic consumers (Pus) and below the demand curve. Producer surplus (PS) is delineated by the area below Pus and above the domestic supply curve. These values are shown in Figure 4a by the areas highlighted CS and PS. Collectively, these areas will be referred to as domestic surplus.

Initially, assume the Pacific stock supplies will not respond to reductions in Atlantic supplies. In this case, the Pacific stock supply curve is perfectly inelastic and not responsive to price increases resulting from changes in Atlantic stock supplies. Now, consider the quotas imposed by Amendment 1. In Figure 4b, the domestic quota (Q1) is shown as vertically intersecting the domestic supply curve, thus becoming the producers supply above Sus. Next, the domestic quota is combined with the Atlantic stock import quota (Q2) to show the total quota on aggregate supply. If supplies from the Pacific remain constant, then this quota line (Q2) becomes the aggregate supply where it extends above Sus+Ai+Pi. When total supply is restricted, Pus increases to P' (the magnitude of the price increase depends on the price elasticity of domestic demand) with the following domestic welfare implications. The summation of the

areas marked "a","b","c", and "d" represents a loss in consumer surplus (these areas are no longer contained above P'). The consumer surplus loss occurs because of the increased price of swordfish.

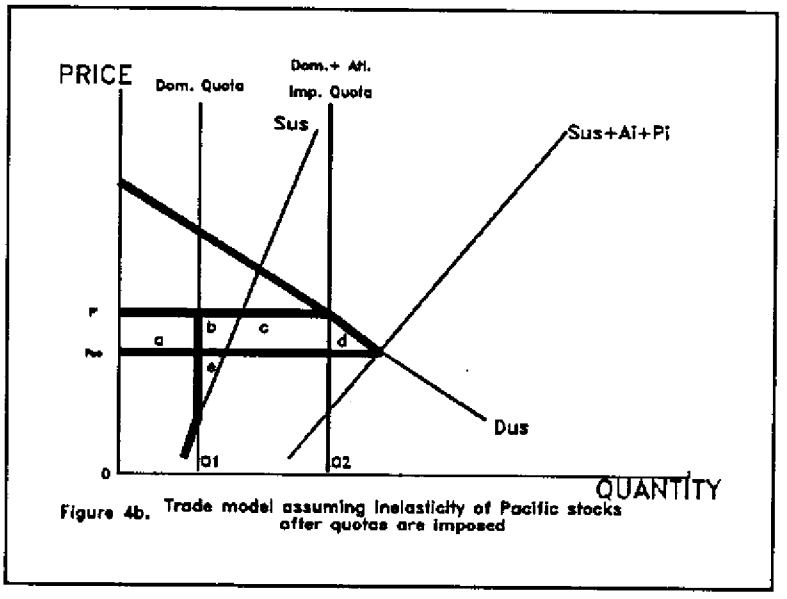


Figure 4b. Trade model assuming inelasticity of Pacific stocks after quotas are imposed

Producers gain area "a" due to higher swordfish prices but lose area "e" because of the harvest quota. The net loss or gain to producers depends upon the relative sizes of areas "a" and "e". If the swordfish demand curve is inelastic, then the proportional change in swordfish prices will more than offset production losses; area "a" exceeds area "e". If swordfish demand is elastic, then swordfish price increases will not offset production losses and the net change in producer's surplus will be negative.

The net change in domestic surplus is measured by the value of the net changes in consumer and producer surplus less any surplus transfers. Thus the net loss in domestic surplus is the summation "b+c+d+e". Area "a" represents a transfer of surplus value from consumers to producers, hence area "a" does not represent any change in net domestic surplus. The actual change in domestic surplus due to potential changes in swordfish management depends, therefore, on the price elasticity of domestic demand and the responsiveness (output elasticity of supply) of the Pacific imports.

In the case where supplies from the Pacific stocks completely replace restricted Atlantic imports (Figure 4c), the effects are potentially less significant. This would occur if supplies from the Pacific stocks exactly offset the reduction of imports from countries fishing the Atlantic stocks. In other words, domestic consumers see no change in swordfish availability in the market. Since aggregate supply is not decreased, swordfish price (Pus) does not increase. Domestic producers are receiving the same price, but because of the domestic quota, are subject to reduced landings. This loss of revenue is represented by the area "e". The magnitude of this loss will again be determined by the domestic elasticity of supply, or the slope of Sus.

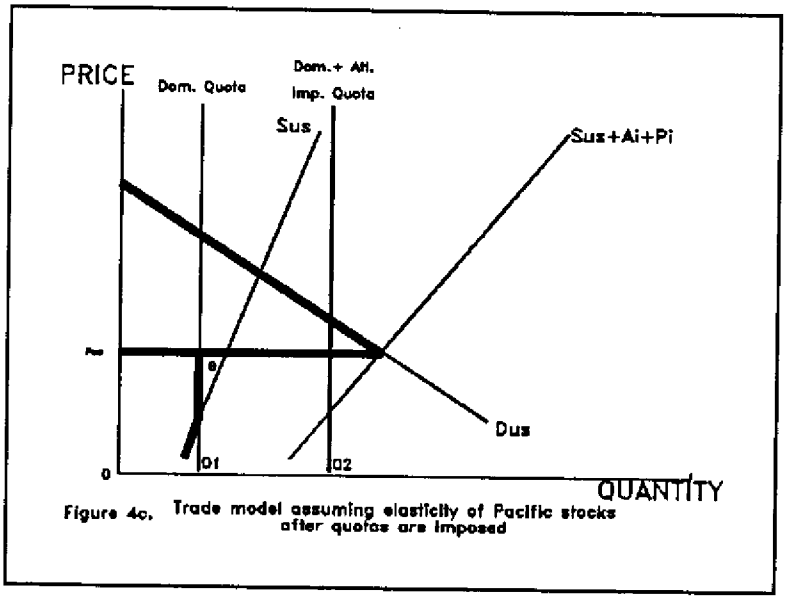


Figure 4c. Trade model assuming elasticity of Pacific stocks after quotas are imposed

Figure 4d represents a more likely scenario where Pacific stock supplies respond partially to the reduced Atlantic supply. S' now represents the aggregate supply curve. The intersection of S' with the demand curve results in a price increase to P'. Loss of consumer surplus is measured by the value "a+b+c+d+f". Again, producer surplus is decreased by area "e" and increased by area "a". A net gain to domestic producers accrues if "a" > "e" and is equal to the value "a-e". The net loss of domestic surplus is the value "b+c+d+e+f". It is clear that consumers and/or producers will suffer net losses in the short-run, but this must be measured against increases in long-run yields, which is the intended objective of proposed regulation.

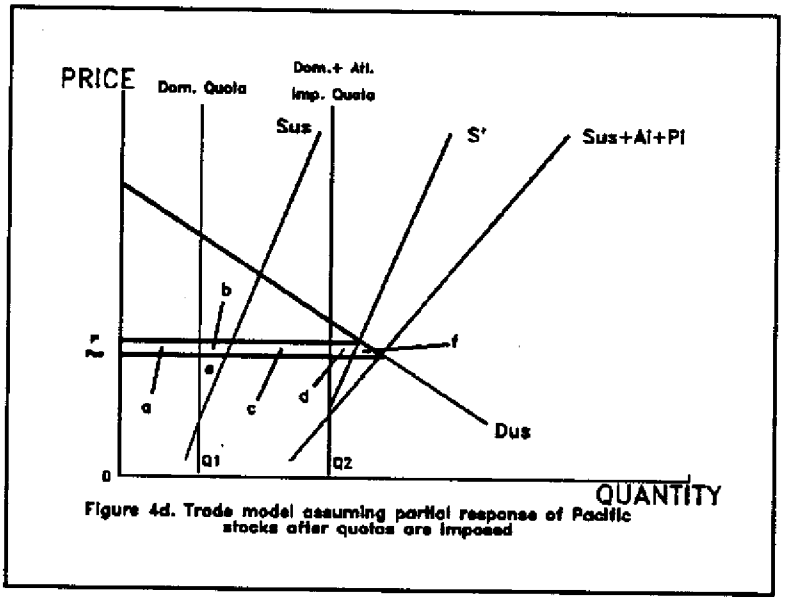


Figure 4d. Trade model assuming partial response of Pacific stocks after quotas are imposed

DOMESTIC AND INTERNATIONAL TRADE IMPLICATIONS

The proposed Amendment 1 restrictions effectively prevent other nations from increasing effort on the same stock since the U.S. is the primary importer and represents the primary stable export market. An opposing argument from the Caribbean nations to Amendment 1 is that these countries are only now developing their offshore fisheries. The restrictions may prevent them from achieving their goal of generating much needed foreign exchange, therefore violating the intent of the Caribbean Basin Initiative (CBI). The small Caribbean countries also complain that Amendment 1 was drafted with U.S./Caribbean trade data that substantially understates the amount of swordfish entering the U.S. from Caribbean sources. The region is consequently penalized by import quotas set at erroneously low levels. The import quotas, however, seem to be acceptable by the General Agreement on Tariffs and Trade (GATT) according to the NMFS's legal/trade representatives because the domestic catch and foreign imports are reduced in similar fashion. Problems with GATT would arise if the Department of Commerce acts in a way that could be construed as protection or promotion of the U.S. market.

An import quota was also chosen because of the potential for U.S. fishermen to re-flag or land their catch in Caribbean ports and export to the U.S., thereby circumventing the domestic quotas. Since early 1988, this has allegedly been occurring in Caribbean island nations such as the Turks & Caicos, the British Virgin Islands, Barbados, Trinidad & Tobago, the Netherlands Antilles, Grenada, Antigua and St. Lucia (4) (Table 2). The reasons for such a trend, in addition to avoiding impending regulations, may be that fishermen are searching for new fishing grounds or more cost effective ports. However, such action may still have a negative effect on the North Atlantic swordfish stocks and reinforce the necessity of import restrictions.

Tag-recapture data suggests that 45 degrees W would be a more appropriate eastern boundary. If this boundary is used in the future as the management unit, unilateral management by the U.S. may be more effective because U.S. fishermen are responsible for 80% of the total landings in this area. However, international cooperation in the management of the fishery remains an objective. The proposed limits agreed upon by ICCAT may have been the result of the stringent regulations proposed by the SAFMC to regulate the fishery. One of the main objectives of Amendment 1 was to encourage international management of the fishery and the success at the ICCAT meetings proves that at least one of those proposed objectives has been met.

Imports from countries fishing both the Atlantic and Pacific stocks account for approximately 45-50% of the total quantity available on the U.S. market, with the remainder coming from domestic landings in the Pacific (10%) and the Atlantic (45-50%). If domestic Atlantic landings and imports from the Atlantic stock are reduced by 31% via Amendment 1, there will be a substantial decrease in short-run supply. In addition, the proposed ICCAT reductions of landings by size class of swordfish will also reduce available supplies from the Atlantic. How will Pacific stocks be affected if effort is increased to replace a decreased supply from the Atlantic stock? If Chile, Ecuador and Mexico increase catch from the Pacific swordfish fishery to meet demand, the Pacific stocks could be depleted to the point where international management of Pacific stocks would be needed. Will overall supply (Atlantic & Pacific stocks) decrease and, given a constant demand, lead to higher wholesale prices and a decrease in consumer surplus? Assuming an inelastic Pacific supply curve and a relatively inelastic aggregate supply curve, the potential for loss of producer and consumer surplus is great given the proposed domestic harvest and import quotas.

SUMMARY

The North Atlantic swordfish stock has been determined by fishery scientists from the U.S. and other international agencies as being over-fished. A variety of management measures have been proposed by ICCAT, SAFMC and the U.S. Secretary of Commerce to rebuild the spawning stock biomass in the management unit to acceptable levels. These measures include percentage reductions in landings of fish within specific size classes, quotas on U.S. landings and restrictions on the importation of swordfish into the U.S. caught within the management unit. At present, the exact form of the management measures to be imposed upon the swordfish industry is uncertain. Regardless of whether the adopted measures are harvest or import restrictions, the U.S. market will likely experience a short run reduction in swordfish supplies.

This paper has shown, within the context of a conceptual economic model, that such a supply reduction will likely have an impact on the economic welfare of both domestic producers and consumers at least in the short run. The long run magnitude of this loss in consumer and producer surplus will depend on two important factors. First, the responsiveness of swordfish producers in other fisheries (Pacific swordfish) to meet the shortfall in the U.S. market must be considered. Second, the short run losses in domestic surplus must be weighed against the long run gains in fishery productivity. The conceptual model provides a means with which to assess the economic consequences of regulatory action in the North Atlantic swordfish fishery, whether Amendment 1 or the less stringent ICCAT proposal prevails.

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RELATION OF WATER-SOLUBILITY OF FISH MUSCLE PROTEINS TO GEL FORMATION IN THE ABSENCE OF SALT

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Fish gels are generally made by mincing fish muscle tissue, washing 3-5 times to remove water-soluble materials, adding relatively high concentrations of sodium chloride (2-3%) and heating. During the last wash, a low concentration of sodium chloride, e.g. 0.15%, is usually added to aid in de-watering. For a product that is to be frozen, sucrose, sorbitol and sodium tripolyphosphate are added to stabilize. It has been generally accepted that the purpose of adding 2-3% sodium chloride is to solubilize contractile proteins (6,7,13,16); these then form a continuous network after partial denaturation and aggregation caused by heating (4,11,19,21).

We have demonstrated that good protein gels can be prepared from fish muscle tissue in the absence of high levels of sodium chloride (2,3,18). If it is essential for proteins to be at least partially soluble to form gels, then it could be predicted that the proteins of washed fish muscle tissue might be soluble at low ionic strength. The objective of this work was to investigate possible relationships between the solubility of fish muscle proteins and their ability to form gels in the absence of high concentrations of salt.

MATERIALS AND METHODS

Materials

Whole fish were purchased from a fish processor in Gloucester, MA the same day of catch during the period ofMarch- - September, l990. The fish were transported on ice to the University of Massachusetts in Amherst where they were hand- skinned and filleted on the day received. The fillets were then placed immediately in polyethylene bags and held on ice for subsequent processing and testing. Three species were usually processed at a time. The order of testing was randomly varied. The species used were red hake (Urophycis chuss),dab (Hippoglossoides platessoides), Atlantic cod (Gadus morhua), Atlantic pollock (Pollachius virens), haddock (Melanogrammus aeglefinus) and Atlantic mackerel (Scomber scombrus). The species were purchased as high quality product became available.

Preparation of de-watered mince and paste

Grinding, washing and de-watering were carried out as previously described (3). Two hundred-gram batches of de-watered mince were chopped in a cold room (4'C) using a pre-chilled (- 20'C) Robot Coupe set at low speed for 2 min. The temperature of the chopped fish was kept below 8'C. The purpose of chopping was to simulate the process whereby salt is ordinarily added although no salt was added here. The material after chopping will be referred to as the "paste".

Extraction of soluble protein

Extraction of the fish paste at low salt concentrations was carried out by first dispersing the paste as thoroughly as possible in a small portion of the extracting solution. The rest of the extracting solution was then added to give a final concentration of 1 part of paste to 20 parts of extracting solution. The sodium chloride concentration in the extracting solution was either 0, 0.025, 0.05, 0.10, or 0.15%. The slurry was then incubated in a shaking water bath (275 rpm) at 4°C for 20 min. The slurry was centifuged at 4°C at approximately 3000xg for 20 min. Supernatant fractions were taken for protein analysis.

Extraction of the fish muscle paste was also carried out in more concentrated salt solutions. This was done byhomogenizing in a commercial blender with NaCl (0, 0.5, 1.5 or 2.5%) or with 1.0 M LiCl (S.D. Kelleher and H.O. Hultin, J. Food Sci., 1991 (in press)). The homogenate was then centrifuged as above and protein concentration determined in the supernate.

Analytical procedures

Protein content of the paste and supernatant fractions was determined by a modified Lowry procedure (9); pH was measured by a glass electrode. Per cent moisture was determined by drying. Approximately 2 gm of fish sample was distributed evenly on weighed aluminum weighing plates and dried in a Fisher Isotemp vacuum oven at 30" of Hg and 70°C for 20 hr. Fold test scores were determined as described by Kudo et al. (5) with a score of 5 the most elastic and one the least. A torsion technique was used to measure stress and strain at breakage (20).

Each species was evaluated at 3 different times during the course of these experiments. Protein extractions from each batch were performed in quadruplicate. Results are reported as mean $\pm$ standard deviation. Linear correlation coefficients, and the significance of each, were calculated as outlined by Steel and Torrie (15).

RESULTS AND DISCUSSION

Recently, a survey has been completed in our laboratory on the ability of 16 species of Northwest Atlantic fish to form gels in the absence of high concentrations of added salt (H. Bakir, personal communication). Six species from those assayed were chosen for the present study including those that formed good gels in the absence of sodium chloride, those that did not, and those that were intermediate. A summary of some of the properties of the fish gels from the chosen species with or without the addition of 3% added sodium chloride are shown in Table 1. A cryoprotectant mixture was not used since the samples were not stored frozen. We also wanted to minimize the number of variables. All species formed a top grade gel as determined by the fold test in the presence of sodium chloride; in the absence of added salt only red hake, cod and dab had a fold-test score of 5. The fold-test score for pollock and haddock was 3 while the value for mackerel, which formed a very poor gel without salt, was 2. The ratio of strain at breakage in the absence and presence of sodium chloride is also given.

Table 1. Properties of fish gels from various species with and without 3% added NaCl

Species	Fold-Test Score -NaCl	Fold-Test Score +NaCl	Ratio ofTrue Strain -NaCl/+NaCl
red hake	5	5	0.89
cod	5	5	0.92
dab	5	5	0.85
pollack	3	5	0.85
haddock	3	5	0.74
mackerel	2	5	0.59

There was a rough correlation between this ratio and the fold- test score in most cases. However, the strain ratio in the absence and presence of sodium chloride for pollock was the same as that for dab, although pollock had a poorer fold-test score than did dab. It is not uncommon that fold test scores and strain at breakage do not correlate (H. Bakir, T. Lanier, personal communication).

The protein extractable from the 6 species of fish at 1.0 M LiCl appeared to roughly correlate to their ability to form gels in the absence of salt (Table 2).

Table 2. Protein extracted from washed, de-watered minced fish by buffered 1M LiCl

Species	g protein per 10g dry wt
red hake	8.01 ± 0.18
cod	6.43 ± 0.49
dab	6.20 ± 1.31
pollack	5.94 ± 1.10
haddock	5.28 ± 0.63
mackerel	4.81 ± 0.22

Solubility at high salt concentrations, however, may have little relation to the gelling behavior of fish muscle proteins extracted in the presence of low concentrations of salt. Thus, experiments were run varying the concentration of sodium chloride in the extracting medium. Lithium chloride is used in our laboratory as a more efficient extractant of fish muscle proteins than is sodium chloride (S.D. Kelleher and H.O. Hultin, J. Food Sci.,l991 (in press)). However, for these experiments it was considered desirable to use NaCl since this is the salt used to make fish gels. Results for red hake showed a high extractibility with no NaCl added to the extracting medium (Fig. 1). This decreased to a low value at 0.5% NaCl and then increased as the NaCl concentration was increased up to 2.5% which was the maximum used. This decrease and increase in protein extractibility was observed in all cases. In some species of fish, the amount of protein extracted at 0% added NaCl was less than it was at the 2.5% level, but a nadir was always present between the values obtained at low and high concentrations of salt.

Since there appeared to be significant solubility at very low ionic strengths and since we were interested in the phenomenon of gel formation at these low ionic strengths, we next examined the solubility of fish muscle proteins in the presence of very low concentrations of sodium chloride. Results of one trial with cod, haddock and mackerel are shown in Fig. 2. The results with cod and haddock were typical of all the white- fleshed fish. There was a large decrease in protein solubility as the extracting solution changed from 0% added sodium chloride to 0.025%; after this, the decrease was more gradual. The initial value for mackerel was very low, and it did not change much with increasing concentrations of sodium chloride.

A summary is given of the averages of all the trials for all of the species giving the amount of protein extracted in water and in 0.15% sodium chloride (Table 3). Normally 0.15% NaCl is present in our washed, de-watered minced fish samples. When the fish paste was extracted with water, a larger amount of protein became soluble from red hake than all of the other species while the water-soluble protein of mackerel was less than that from all the other species. There appeared to be some greater extraction of protein by water from cod and dab which form good gels in the absence of salt compared to pollock and haddock whose gel-forming ability in the absence of salt is less than that of the former two species. One would expect that protein solubility in the presence of 0.15% sodium chloride should be a better reflection of the gel-forming ability of the washed minces than protein solubility in water since the former reflects the amount

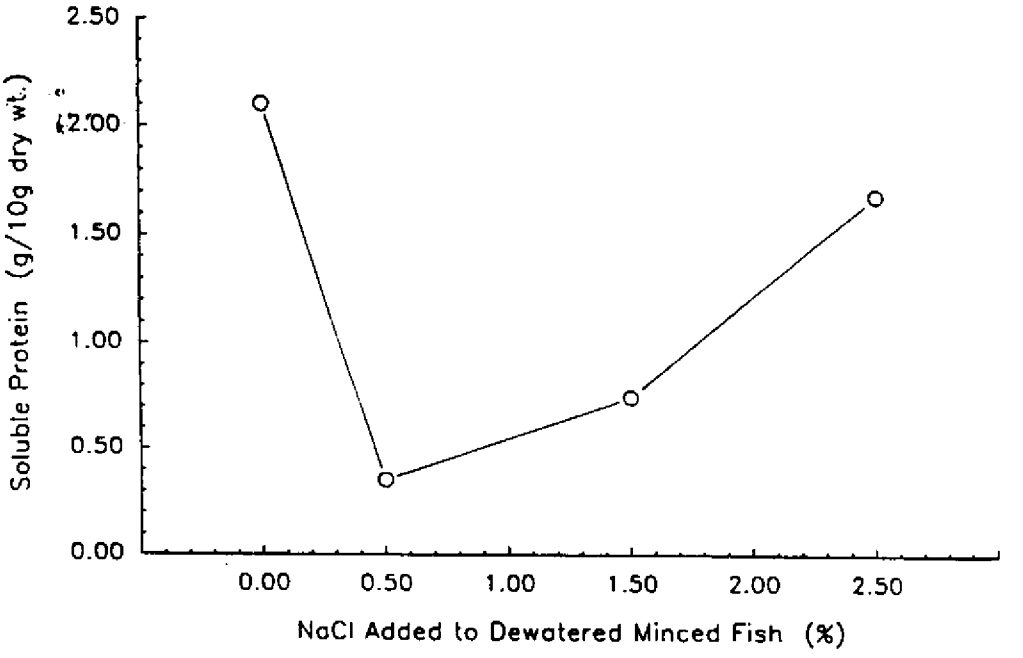

Fig. 1. Solubility of proteins of washed minced red hake muscle over a range of NaCl concentrations from 0 to 2.5%. Results are averages of quadruplicate determinations.

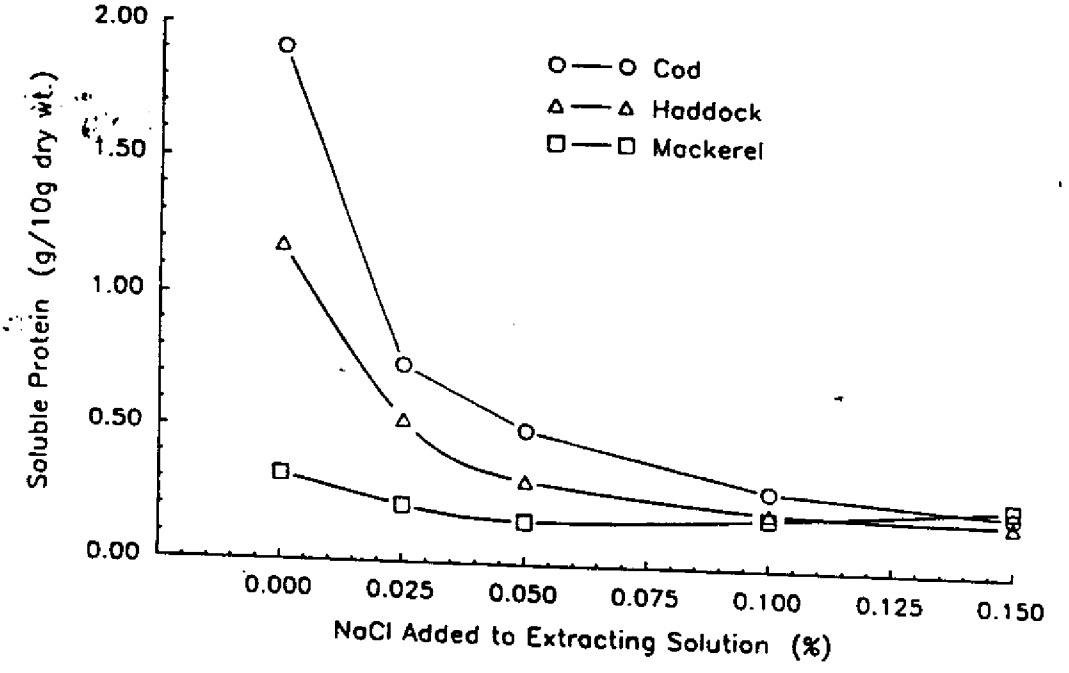

Fig. 2. Solubility of proteins of washed minced fish muscle as a function of NaCl concentration in the extracting solution. Results are averages of quadruplicate determinations.

of salt that was normally present when we made our gels. Protein extractibility for all species was quite low at 0.15% NaCl.

Table 3. Protein extractable from fish pastes in water or 0.15% NaCl

species	g extractable protein/10g dry wt	
	water	0.15% NaCl
red hake	2.9 ± 1.2	0.51 ± 0.31
cod	1.6 ± 0.29	0.30 ± 0.09
dab	1.7 ± 0.07	0.33 ± 0.06
pollack	1.2 ± 0.42	0.26 ± 0.20
haddock	1.1 ± 0.20	0.17 ± 0.04
mackerel	0.38 ± 0.07	0.26 ± 0.01

The ratio of protein soluble in water compared to that in 1M lithium chloride (Tables 2 and 3) ranged from 0.36 in red hake, about 0.25 in cod, dab and pollock, about 0.2 in haddock, to less than 0.1 in mackerel. The solubility of myofibrillar proteins in water has been observed previously. Matsumoto (10) first observed this phenomenon in squid mantle muscle. Stanley (14) found that the extractibility of actomysin from horseshoe crab muscle at very low ionic strengths depended on degree of purity, concentration of protein, the ionic strength of the extracting solution, and substances such as Ca^{+2}, Mg^{+2}, ATP and possibly other compounds. More recently, Trevino et al. (17) reported data indicating that the solubility of protein from sardine surimi was high in water, rapidly decreased at low salt concentrations and increased again as the salt concentration was further increased. They attributed the solubilization in water to the fact that the extractions were done at pH 9.0. In all cases where protein extractibility by water has been observed, including this study, the tissue has been extensively washed. We observed in this study that very low concentrations of salt can prevent the solubilization of fish myofibrillar proteins. Extensive washing of the muscle may be necessary to decrease the normal concentration of salt in the tissue to levels low enough to allow myofibrillar proteins to be extracted by water.

In our studies, the level of salt with "0 added" sodium chloride was calculated to be approximately 1 to 1.5 mM. This was estimated based on the fact that the tissue was de-watered in the presence of 0.15% sodium chloride, and the sample was extracted after a twenty-fold dilution. A concentration of sodium chloride in the range of only 5-6 mM (0.025% added salt) reduced protein solubility by one half or more.

When the data of Fig. 2. were plotted as soluble protein vs. the log of the concentration of added sodium chloride, a straight line relationship was observed (Fig. 3). We define the negative value of the slope as the first order insolubilization constant, k_i. The average values of these constants and their standard deviations are presented for all species in Table 4.

Table 4. Average values of first order insolubilization constants(k_i)

species	k_i (%NaCl)$^{-1}$
red hake	1.6 ± 0.76
cod	0.82 ± 0.22
dab	0.86 ± 0.01
pollack	0.57 ± 0.14
haddock	0.61 ± 0.06
mackerel	0.09 ± 0.03

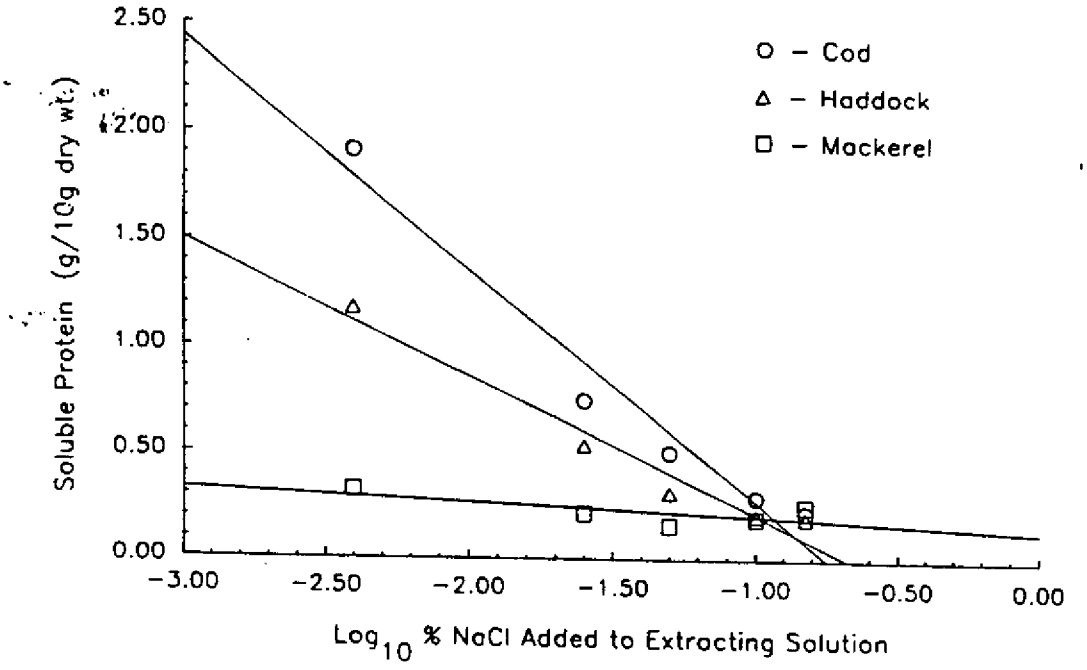

Fig. 3. Soluble protein of 3 fish species plotted against the logarithm of NaCl concentration in the extracting solution (data of Fig. 2).

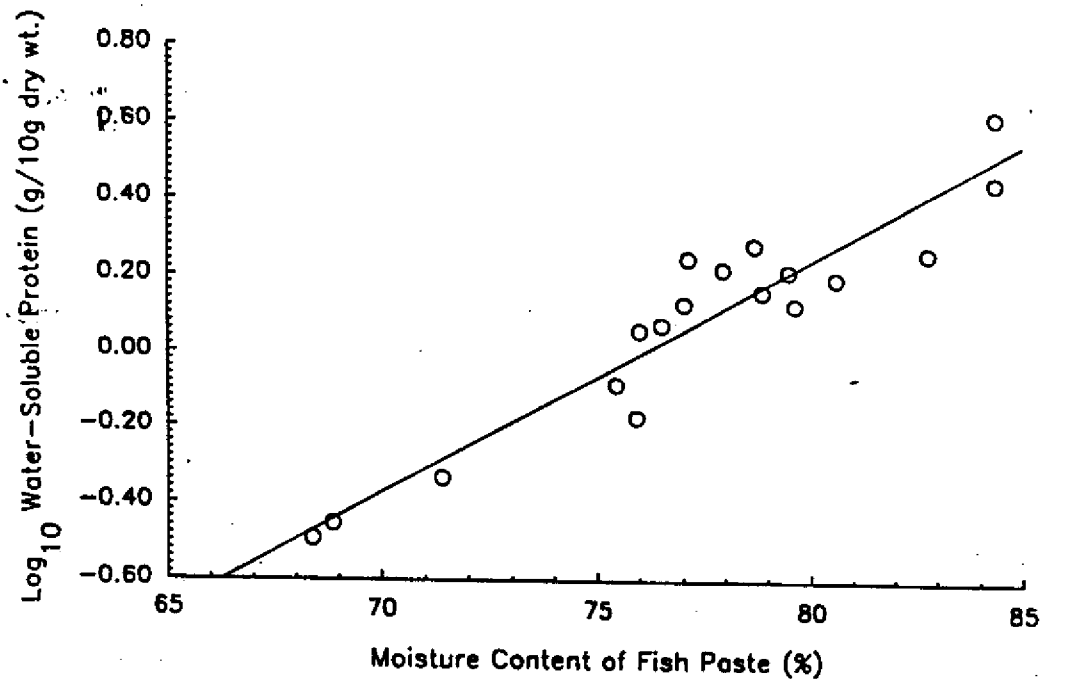

Fig. 4. Logarithm of water-soluble protein plotted against the moisture content of the fish pastes. Data are for all replicates (3) and species (3).

The value of k_i is dependent in part on the solubility of the fish paste proteins at 0% added NaCl. Red hake had the highest k_i value, cod and dab were next, pollock and haddock were slightly lower, and mackerel had a very low value. This last was not statistically significant from zero; there was, however, a clear decrease in solubility of mackerel muscle proteins between extraction with water and the lowest salt concentration (0.025%). In some of the species, the standard deviations were very low. In some they were high; this was particularly true of red hake. Where the standard deviations were large, it was due primarily to large differences amongst the three different batches of each species of fish; quadruplicate samples from the same batch of fish were reasonably constant. The linear correlation coefficients were generally high for the semi-logarithmic plots for all species of white fish; only one sample of cod had a coefficient less than 0.90. Correlation coefficients for mackerel were considerably lower than the others and not statistically significant from zero. This was due in part to the low solubility of the mackerel muscle proteins when the paste was extracted with water.

When the log of water-soluble protein was plotted against the moisture content of the paste, a linear relationship was observed with a correlation coefficient of 0.945 (Fig. 4). It is possible that the factor(s) responsible for good water-binding ability is also the reason why the protein can be extracted from the tissue. Such a factor could be minimum protein denaturation.

A comparison of the average moisture contents of the fish pastes prepared from the 6 different species with the initial pH values of the muscle samples is given in Table 5. These parameters follow closely our observations on the ability of these species to form good gels in the absence of high concentrations of NaCl. What relation a high initial muscle pH might have to protein solubility in water or water retention in the pastes is not known. It is, however, known that pH is an important variable in the stability of the fish muscle proteins (8).

Table 5. Moisture content of fish pastes and pH of minced muscle

species	% water	pH
red hake	83.8 $\pm$ 0.9	7.08 $\pm$ 0.03
cod	78.2 $\pm$ 1.2	7.03 $\pm$ 0.17
dab	79.6 $\pm$ 0.9	7.09 $\pm$ 0.10
pollack	77.3 $\pm$ 1.5	6.66 $\pm$ 0.03
haddock	76.0 $\pm$ 0.5	6.83 $\pm$ 0.14
mackerel	69.5 $\pm$ 1.6	6.34 $\pm$ 0.34

The data indicated that more protein was extractable, i.e., soluble, from the various fish pastes when the extracting medium was water than when a low salt solution was used. If protein solubility is a requirement for the formation of good gels, one would expect that reducing the amount of sodium chloride in the sample would lead to improved gel formation. We tested this hypothesis by preparing washed minced cod muscle which was then "de-watered" by pressing in the presence of a variety of agents (Table 6.)

The use of the term "de-water" is somewhat of a misnomer since it was not possible to effectively de-water washed minced cod with our equipment without the use of salt. Since it was desired to keep the moisture content relatively constant in the samples, we adjusted all samples to be equal to that with the highest mositure content. To de-water we used 0.15% sodium chloride; this is the amount generally used in our laboratory. This was compared to 0.11% LiCl which is equivalent in molarity to 0.15% sodium chloride. Lithium chloride was added because we had previously obtained data indicating that lithium chloride was less likely to denature the proteins of red hake muscle than was sodium chloride (S.D. Kelleher and H.O. Hultin, J.Food Sci.,

1991 (in press)). Two different sugar solutions, 2.5% sucrose and 4% sucrose plus 4% sorbitol, were also used. In additional samples, nothing was added at the final washing step. Samples that were "de-watered" in the absence of salt

Table 6. Effect of dewatering agent on gel-forming ability of washed, minced cod in the absence of 3% added salt

dewatering agent	1% H_2O	Fold Test	True Strain	Stress (pka)
0.15% NaCl	86.5	3.5	1.5	21.0
0.11% LiCl	86.5	3.0	1.4	20.3
2.5% Sucrose	86.9	5.0	1.8	14.8
4% Sucrose + 4% Sorbitol	86.3	5.0	1.9	10.6
None	86.7	5.0	1.7	19.6

produced superior fold test scores and higher values of true strain at breakage. Since both of these parameters are measures of the quality of the protein in the fish gel (1), there appeared to be an improvement in the quality of the gel when salts were omitted from the de-watering step. Park and Lanier (12) recently reported that 3% salt destabilizes and 8% sucrose stabilizes the proteins of washed fish muscle during heating. There was no difference among the gels with respect to fold-test scores or values of strain at breakage when the various sugar solutions or only water was used. Both of the samples treated with the sugar solutions had lower stress at breakage than did the samples washed with either salt or with water. All of the stress values were low due to the high moisture content of the samples.

CONCLUSIONS

A significant proportion of the contractile proteins of five species of fish were soluble in solutions of very low ionic strength, i.e., approximately 0.001. This solubility decreased with increasing NaCl concentrations to at least 0.15% (ionic strength equal to about 0.027).

When sodium chloride or lithium chloride was omitted from the final washing step, the quality of the gels produced from the fish tissue improved.

The extent of "water-solubility" may be related to several factors including solubility in 1 M LiCl, the initial pH of the fish muscle tissue, the percentage moisture in the de-watered washed mince, and the ability of the fish proteins to gel in the absence of 2-3% added salt.

Anomalous behavior of the proteins of mackerel muscle were observed throughout this study. The low solubility of the mackerel muscle proteins may be related to the low pH of the mackerel muscle tissue.

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ACKNOWLEDGMENTS

This work was supported in part by the Massachusetts Agricultural Experiment Station and by contract No. 2-5616-16(A) from the National Coastal Resources Research and Development Institute. Helpful support and suggestions were provided by Dr. Ernest M. Buck and Mr. Stephen D. Kelleher.

PROTECTION OF MACKEREL MINCE FROM RANCIDITY DURING FROZEN STORAGE (-7C) USING ANTIOXIDANTS, BARRIER BAGS AND VACUUM

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INTRODUCTION

A major health benefit of fish leading to their increased consumption has been the presence of omega-3 fatty acids. In addition, fish have a relatively low total fat content and serve as a source of high quality protein (1). Research during the last decade has associated the omega-3 fatty acids with beneficial effects related to the etiology of atherosclerosis, coronary heart disease and other pathophysiological diseases (13). Addis and Romans (4) reviewed the role of omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) (C20:5) and docosahexaenoic acid (DHA) (C22:6), in relation to coronary heart disease. Ackman (2) suggested that high fat fish are those which can contribute at least 2000 mg of C20 plus C22 omega-3 fatty acids per 100 grams of fish consumed.

Atlantic mackerel (Scomber scombrus, L), an underutilized species in the Northwest Atlantic Ocean is a fatty fish. The edible portion constitutes more then 50% of its body weight (15). Several factors: season, geographic location, sex, maturity, and feeding affect the composition of the fish. Mackerel exhibit seasonal variation in its proximate composition, showing minimum lipid content in the months of March and April and maximum levels in the months of September thru November. The fat content of Atlantic mackerel was found to range from 5.1 to 22.6% for the dressed fish (15). Ackman (2) cited the distribution of Atlantic mackerel lipids in the edible parts as 11.3% polar lipids and 74.2% neutral lipids. Atlantic mackerel lipids contain about 70% unsaturated fatty acids of which 30% are polyunsaturated (15). The C20:5 and C22:6 were over 50% of the polyunsaturated lipids (3).

Lipid oxidation is a major quality deterioration problem in mackerel stored frozen, especially when stored as mince. The factors which influence lipid oxidation include the fatty acid composition of the lipids, their disposition, the presence or absence of activators and inhibitors, and external factors such as storage temperature, time, light, oxygen pressure, and packaging conditions (12). Mackerel easily develop rancid flavors and odors from the oxidation and hydrolysis of its highly unsaturated lipids and that leads to a decrease in the storage life and limits its use.

The species is considered underutilized mainly because the cost of a directed fishery for this fish may be almost as high as for currently caught species which have a better market price (16). Extended use of mackerel mince to create high nutritional value food without having an obvious fish character, as suggested by Regenstein (16), combined with means to protect the lipid portion of the fish from rancidity, might increase the consumption of this underutilized species. The minced fish can easily be combined with seasoning to create products, as well as with chemicals to help to maintain the quality and to protect the mince from rancidity. Another advantage of minced fish is the elimination of the bony character of the fish.

Since oxygen is an essential reactant in lipid oxidation, the control of oxygen availability is a critical variable (9). Elimination of oxygen from the packaged fish with vacuum and/or antioxidants of the oxygen scavenger type might be used. Santos and Regenstein (17) found that erythorbic acid, worked well as measured by the TBA test. They found that the exclusion of oxygen from the package by applying a vacuum was more beneficial than the use of the antioxidant in retarding lipid oxidation. Hwang and Regenstein (10) using several antioxidants including ascorbic acid and erythorbic acid

and/or vacuum for menhaden (another fatty species) minces had similar results. Sweet (19) reported on the application of some antioxidants in stabilizing fresh ground fish flesh. TBHQ was beneficial with salmon and TBHQ, BHA and PG with trout. The addition of a metal chelator was helpful with salmon due to the high metal ion content in marine fish. Ascorbic acid (100 ppm) did not show any protection effect. Deng et al. (8) found that ascorbic acid, ascorbic acid + TBHQ, and ascorbic acid + Na_2EDTA protected mullet fillets from rancidity. They found that combinations of ascorbic acid and/or TBHQ with vacuum packaging gave the best protection to skin-on mullet fillets.

Ascorbic acid and erythorbic acid are both water soluble oxygen scavengers. Would an oil-soluble antioxidant similar to ascorbic acid prevent or delay rancidity in fatty fish better by being in better contact with the fish lipids? L-Ascorbyl-mono-stearate, commercially sold as Extract C-100, is an oil soluble antioxidant, which might substitute for ascorbic acid in the oil phase. This antioxidant is approved for use in foods in Japan.

The objective of this work was to compare the use of L-ascorbyl-mono-stearate against ascorbic acid in preventing rancidity of mackerel minces at -7C as well as to evaluate the contribution of the packaging material (barrier vs non-barrier), the atmosphere (vacuum vs air) and the combination of vacuum packaging and antioxidants.

MATERIALS AND METHODS

Raw materials and processing: Two sets of experiments were conducted, one using mackerel caught in November, treated with ascorbic acid (AA) and ascorbyl mono-stearate (AMS) and the other using mackerel caught in February, using AA and vacuum packaging. Fresh mackerel, headed and gutted, were filleted. The skin and most of the visible fat were carefully removed, then the fillets were minced in an Hobart (model N-50, Troy, OH) meat grinder, plate size 0.2".

The November mackerel mince: The minced fish was divided into 5 portions; each received a different antioxidant treatment. Antioxidants: The antioxidants were AA (Pfizer Inc., New York, NY) and AMS (Ogawa Co. Ltd., Osaka, Japan). The concentrations of the antioxidants were calculated on the basis of the fat content and on the basis of sample weight (i.e., two different sets of experiments for each antioxidant). Each portion was treated separately with a different antioxidant or without antioxidant (control). Antioxidant concentrations chosen were: 1) 0.5% of the fat content assuming 12% fat. This level was found suitable for AA in the previous work with fatty fish (10). In order to compare the two antioxidants, AMS was also used at this concentration. 2) 0.1% of the sample weight. This concentration was recommended by the AMS distributor (Arvold C.H., Avron Resources, Oakland, CA, personal communication). AA was also used at this concentration. Antioxidants were used on an equal molar basis, since the active part of the molecule is the same for both. Packaging: After mixing the antioxidants, the minced fish samples were packed in 1 oz Nalgene (Sybran Nalge Co., Rochester, NY) plastic containers. The minced fish was carefully put into the containers to minimize air spaces. Some head space was left. Codes: Samples were coded using AAH for ascorbic acid at a concentration of 0.5% of fat content, AMSH for ascorbyl-mono-stearate at a concentration which matched AA at 0.5% of the fat content, AMSL for ascorbyl-mono-stearate at a concentration of 0.1% of the sample weight, AAL for ascorbic acid at a concentration which matched AMS at the level of 0.1% of the sample weight, and B for the control.

The February mackerel mince: was divided into 2 lots. Antioxidant: AA was used at a concentration of 0.58% of the fat content assuming 12% fat. AA at the above concentration was added to one lot while the other lot served as a control for the antioxidant treatment. Shaping: The treated minced fish was shaped into patties weighing 26-31 g each. Patties were frozen in a walk-in freezer (-20C) for 2 hr. Packaging: The patties were packed in plastic bags. One portion was packed in B-bags (barrier bags, Cryovac, Duncan, SC) and the other portion in L-bags (non-barrier bags, Cryovac). Those which were packed in B-bags were vacuum sealed on a KOCH Multivac AGW machine (Kansas city, MO). Vacuum was set on 5 (almost -1 bar) and sealing time was set on 6. Some of the L-bags

were also vacuum sealed, while the rest were air sealed, using the sealing time set on 5.5. Codes: Samples were coded using B for barrier bags, L for non-barrier bags, V for vacuum sealing, A for air sealing, and AA for the ascorbic acid treatments.

Storage: Samples were stored at -7C (i.e., a mild frozen storage abuse temperature) and were analyzed periodically.

ANALYTICAL METHODS

Fat Content: The A.O.C.S. fat extraction method (6) was used with some modifications. The extractor used was a Soxhlet type, modified for extracting large volumes of materials. The samples were dried in an oven (100-105C, 18 hr). A mixture of chloroform-methanol (2:1) was used to extract the fat. After 6 cycles of extracting, thimbles with extracted samples were removed from the extractor, dried in an oven (100-105C, 2 hr), cooled in a desiccator, and weighed. Fat content was calculated as follows:

% fat = (weight of dry sample - weight of extracted sample)*100/weight of wet sample.

TBA: The TBA test was adopted from Lemon (14) using a ratio of 1:12.5 fish to extracting solution. The absorbance of the samples and the standards were measured at 530 nm in a Hitachi model 100-60 spectrophotometer (Tokyo, Japan). TBA values were calculated as follows:
μmoles MA/g sample=0.065*absorbance at 530 nm*25/g sample.

FFA: The titrimetric method of Ke and Woyewoda (11) was used to determine the free fatty acid content of the fish fat. Fat extraction used the procedure of Bligh and Dyer (7) modified for mackerel. A 20 g fish sample was weighed into a pint Mason glass jar and was blended with 50 ml of chloroform, 50 ml of methanol and 40 ml of water using the Sorvall Omnimixer model 17182 (DuPont Co., New Town, CT). The homogenized mixture was then centrifuged (12 min, 3000 rpm at 4-5C in a Sorvall superspeed RC2-B centrifuge) and the liquid phase was decanted into a separatory funnel. The chloroform extract (bottom layer) was separated and collected in an Erlenmeyer flask after filtering through Whatman #1 filter paper (Whatman International Ltd., Maidstone, England) on which was placed 10 g of sodium sulfate to absorb any water residue. The FFA content was calculated as follows:
FFA (μmoles/g sample)=(S-B)*1000*N*2/g sample
Where S = Volume of titrant for sample (ml)
B = Volume of titrant for blank (ml)
N = Normality of NaOH solution.

PV: The fat extracts from the fish samples were prepared by the same procedure as for the FFA determination. The PV for the extracted fat was determined by A.O.A.C. and A.O.C.S. official method (5,6). The PV of the fish samples was calculated as follows:
PV (milliequivalents of peroxide /1000g)=(S-B)*N*1000*5/g
Where S = Volume of titrant for sample extract (ml)
B = Volume of titrant for blank (ml)
N = Normality of $Na_2S_2O_3$ solution.

Statistical Analysis: Results of the PV and TBA analyses were subjected to two-way analysis of variance using Minitab (Release 7.1, Minitab Inc., State College, PA). Comparisons among means of the treatments combinations were done to find significant differences between the various treatments (18).

RESULTS AND DISCUSSION

Water soluble vs fat soluble antioxidants: Two different antioxidants, ascorbic acid (AA) and ascorbyl-mono-stearate (AMS), at three different concentrations for the AA and two concentrations for

the AMS were tested. The first goal was to compare the protection achieved with AMS to that with AA. The latter is already known to be a good antioxidant in many foods including fish. Since fish contain large amount of moisture (60-80%) (15), AA (which is water soluble) was dissolved immediately and easily in the minced fish. AMS (which is oil soluble) tended to dissolve at a slower rate, although mixing seemed to help.

The fat content of the fish samples was found to average 12.5% for the November mackerel and 5.9% for the February fish. For the earlier experiment, the actual fat content was close to the prior fat level assumed in calculating additive weights on a fat basis. In fact, we actually added AA at a level of 0.56% of fat content instead of 0.5%. In the later experiment using February fish, fat content was about half that assumed. The actual added level of AA was then 1.2% of the fat content, i.e., more then twice as much as planned.

Results for the oxidative parameters (i.e., for PV and TBA values) showed that storage time affected rancidity for all treatments with the highest values being the controls (Fig. 1 and Fig. 2). AA at the concentration of 0.56% of the fat content (AAH), contributed the most to minimizing oxidative changes. PV (Fig. 1) was the lowest for AAH during most of the storage time. AA at the lower concentration (AAL), which matched 0.1% of the sample weight for AMS, showed less protection against oxidation. AMS at a level above the recommended value, i.e., at a concentration to match the 0.5% AA (AMSH), protected the minced fish about equal to AAL. AMS at the recommended concentration, 0.1% of sample weight (AMSL), generally showed the poorest protection. TBA values (Fig. 2) basically showed the same trends with some advantage being shown by AAL as compared to both AMS at the higher and lower concentrations.

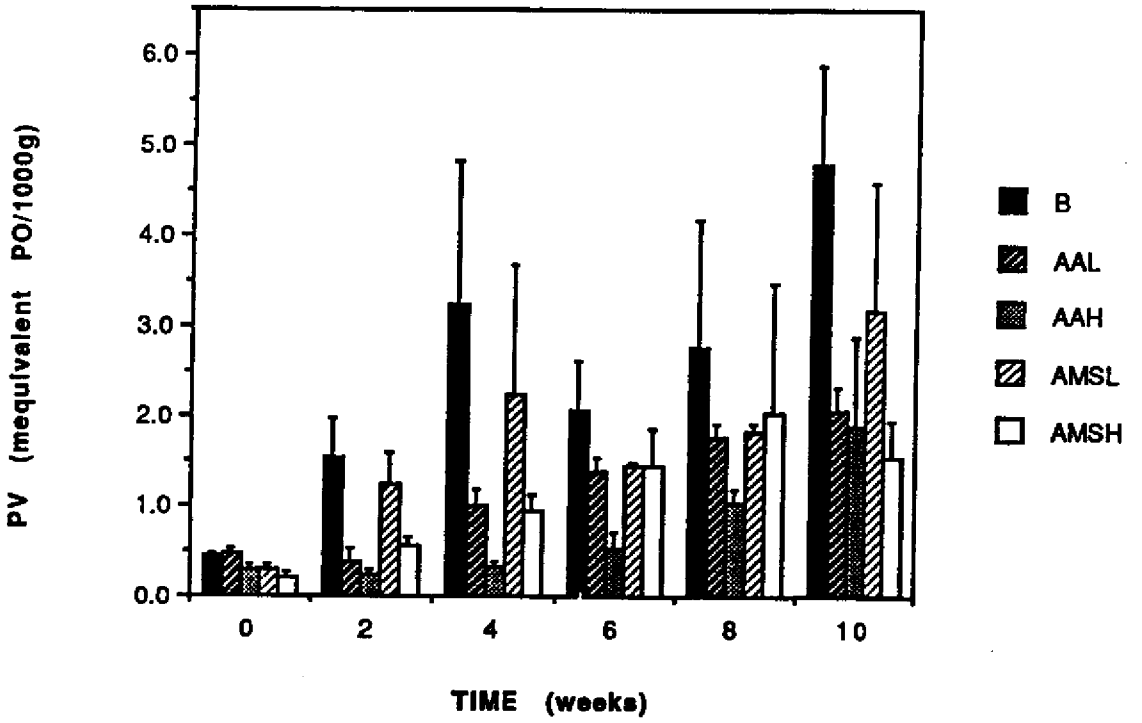

Fig. 1: Changes in peroxide values of the November mackerel during storage at -7C.

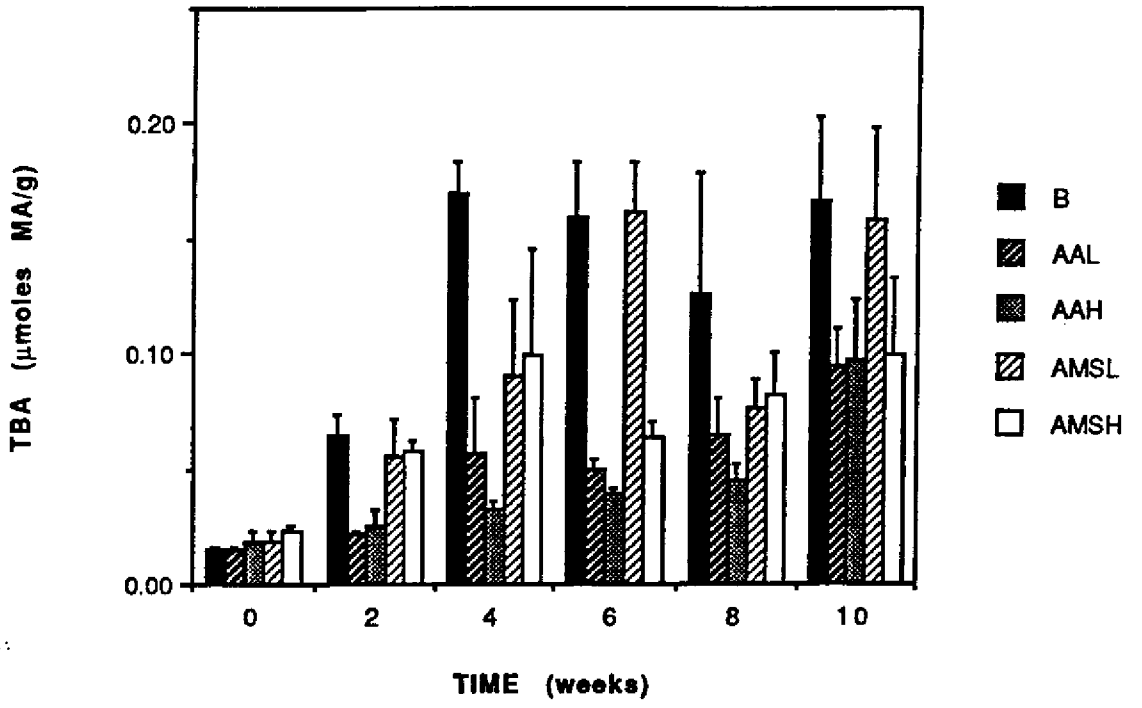

Fig. 2: Changes in TBA values of the November mackerel during storage at -7C.

Results for hydrolytic rancidity (i.e., FFA) showed that storage time led to an increase in FFA values (Fig. 3). None of the treatments was significant in preventing or delaying hydrolytic rancidity. These results agree with those of Hwang and Regenstein (10) for menhaden minces, where they found the temperature to be the only factor affecting hydrolytic rancidity.

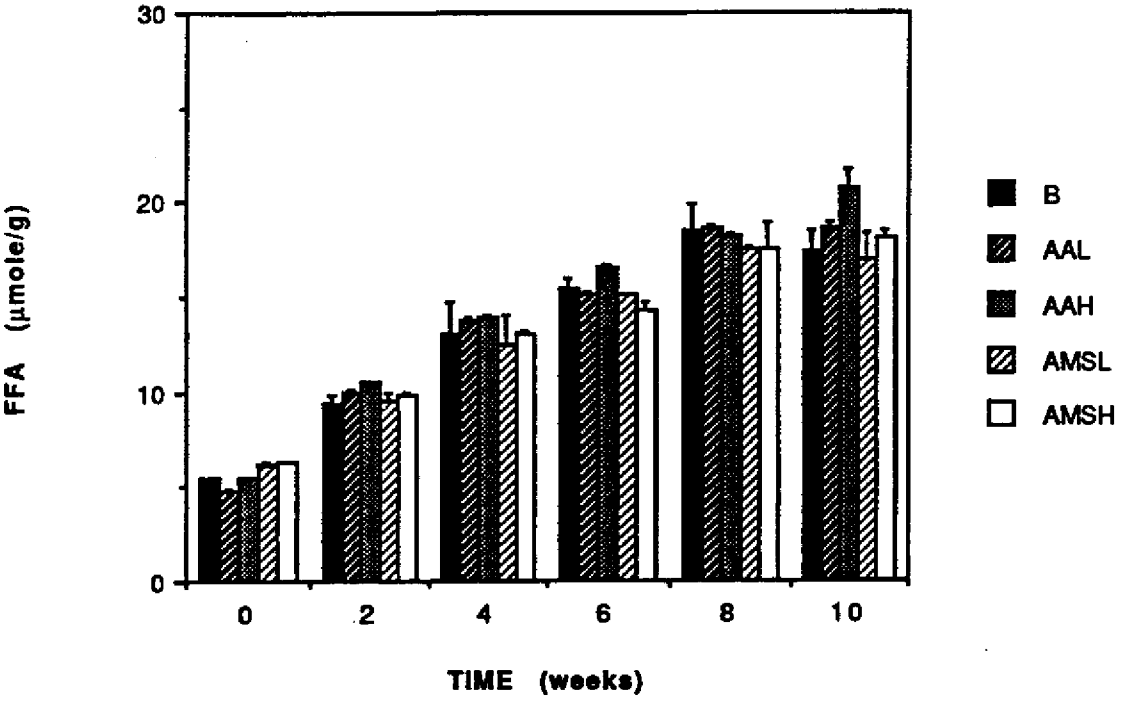

Fig. 3: Changes in FFA values of the November mackerel during storage at -7C.

Results from this experiment suggested that AA at a concentration of 0.56% of the fat content was the best protection against oxidative rancidity. AMS was inferior as compare to AA, maybe because of difficulties in getting the reagent into the oily phase or because the antioxidative activity was needed in the aqueous phase.

These antioxidants, which are oxygen scavenger, failed to protect against hydrolytic rancidity, probably because oxygen does not play a major role in this deterioration process.

Packaging material and the inside atmosphere: Two different plastic bags, barrier and non-barrier, vacuum and air sealed, with and without antioxidant were tested in order to identify the treatments that gave the best results in terms of preventing or delaying oxidative rancidity in mackerel mince.

Effect of antioxidant: Results for PV and TBA (Fig. 4 and 5) were lower for samples treated with AA (BVAA, LVAA and LAAA) when compared to those without AA (BV, LV and LA). During storage, very small changes occurred in the AA treated samples, probably because of the relatively high concentration of the antioxidant (1.2% based on a fat content basis) which could then better protect the fish for a longer time against oxidative deterioration compared to the November experiment.

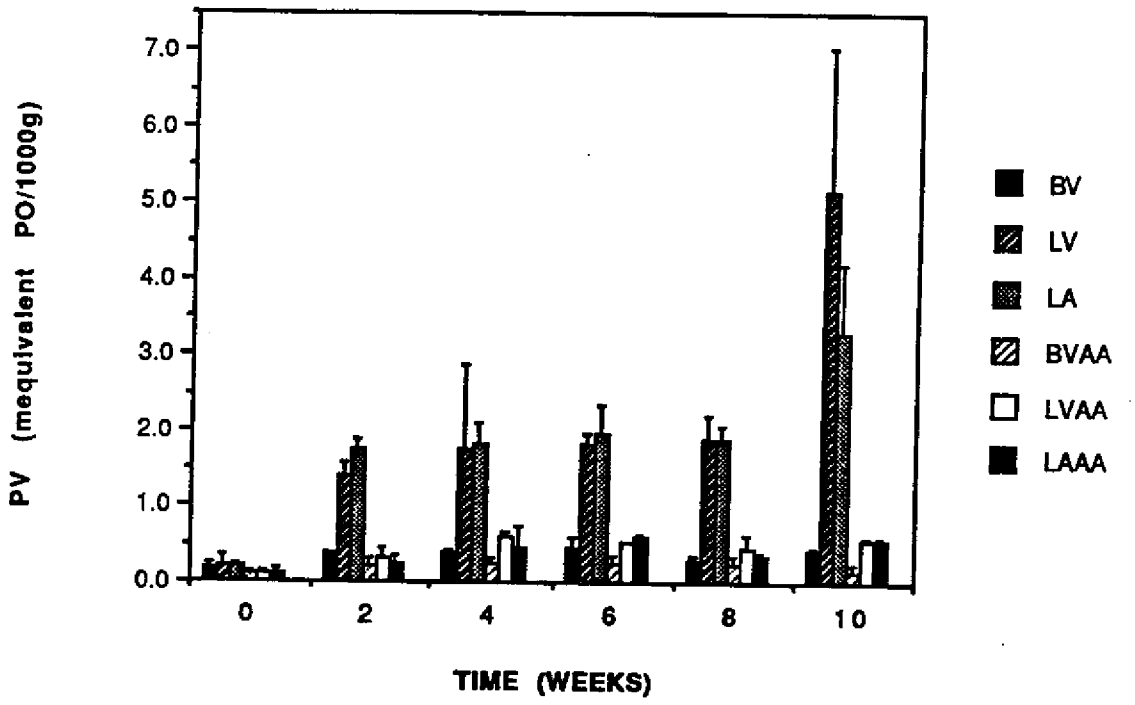

Fig. 4: Changes in peroxide values of the February mackerel during storage at -7C.

Effect of packaging method and materials: Results showed an advantage for the B-bags, which was very clear when samples without AA are compared (Fig. 4 and 5). PV and TBA values were lower for the B-bag samples as compared to both air and vacuum sealed L-bags (BV vs LV and LA). No advantage was noticed for the vacuum sealed L-bag samples as compared to the air sealed L-bag samples (LV vs LA). The same trends were detected for those samples treated with AA (BVAA, LVAA and LAAA), but values for the oxidative parameters were much lower. It seemed to be that the presence of AA minimized to some extend the effect of the packaging material.

The combined effect of packages and antioxidant: Fish patties without any protection (LA and even LV) were more sensitive to oxidative rancidity (Fig. 4 and 5). It seemed that the use of the antioxidant was important to protect the fish. The packaging materials were important, especially when no antioxidant was used, but were less important when AA was used. It can be seen that either B-bags without AA (BV) or L-bags with AA (LVAA and LAAA) protected the patties fairly well, but results suggested that the combination of B-bags and AA (BVAA) was the best protection against oxidative rancidity. TBA values were found to be significantly lower for BVAA as compared to the results for all the other combinations of treatments and as compared to BV, LVAA and LAAA in particular (two different comparison tests). BVAA also seemed to be the best according to the PV results, but no significant differences were found.

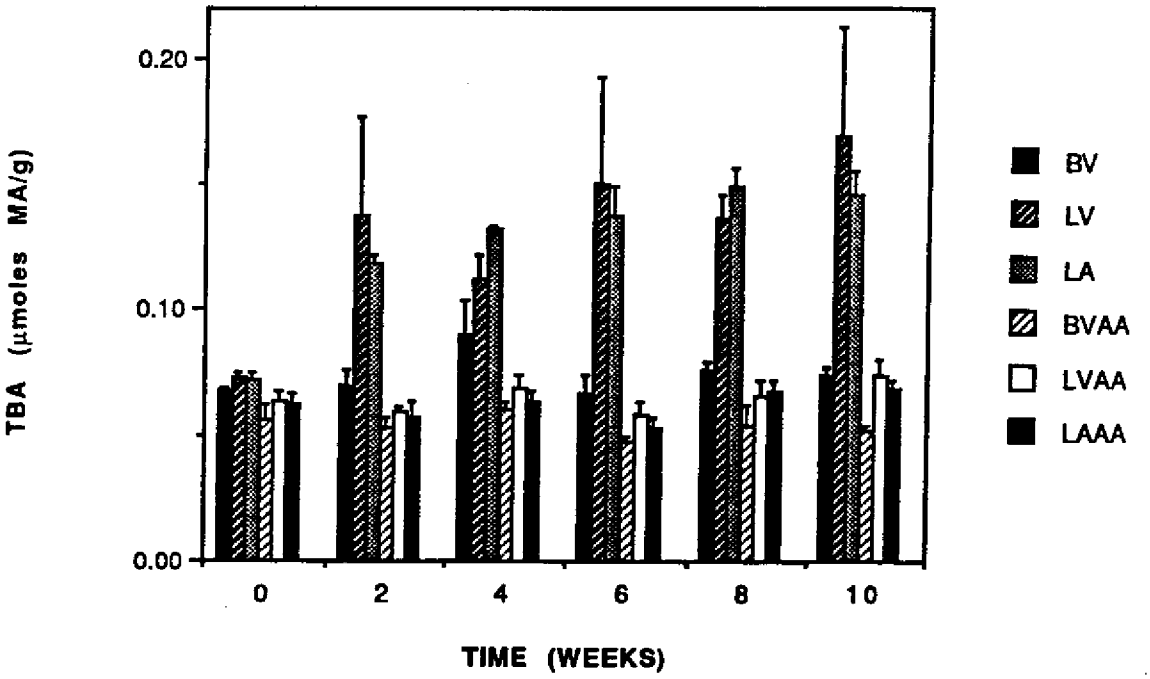

Fig. 5: Changes in TBA values of the February mackerel during storage at -7C.

CONCLUSIONS

Results suggest that the use of antioxidants and/or vacuum is important in controlling the lipid oxidation. AA is a better antioxidant for mackerel minces than AMS. The higher the concentration, the better the protection within the range tested. Vacuum packaging in barrier bags is another good way to control lipid oxidation. It seems that the combination of vacuum and AA gives the best protection. For commercial applications, it is possible that either AA or vacuum might provide protection, depending on the shelf life expected and the packaging/distribution system used.

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THERMAL AGGREGATION OF MYOFIBRILLAR PROTEINS FROM COD, HERRING AND SILVER HAKE

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The commercial importance of gelled fish muscle proteins (kamaboko) has dramatically increased during the past decade. In times of diminishing fish stocks, wider use of under-utilized species in making kamaboko would provide another means to utilise such species for human consumption, and the fish processing industry would undoubtedly benefit from such an opportunity.

The most important functional property of the raw material for kamaboko, surimi, is its gel-forming ability. There are a number of factors that determine the gel-forming ability, such as freshness, fishing method, season, size and species (1). Recent studies indicated that fish myosin determined the gel-forming ability of the fish muscle proteins (2-5). In addition, it was suspected that the differences in the gel-forming abilities among the muscles of various fish species might be attributed to the differences in the cross-linking of myosin heavy chain (6-8).

EDC (1-ethyl-3-[3-(dimethylamino)-propyl] carbodiimide) is a chemical cross-linker and has been used in the study of the structure of the actin-myosin interface (9-11). Recently, there were a number of attempts to use EDC in the study of the changes in the activity of carp myofibrillar ATPase (Konno, 1987, quoted from (12)); on the thermal gelation of oval filefish muscle proteins (12) and the thermal aggregation of cod muscle proteins (4). Gill and Conway (4) reported that about 50% of the cod myosin heavy chain was cross-linked by EDC to form a polymerized complex before the involvement of actin, regulatory proteins or the myosin light chains when the cod myofibril solution was heated at 50°C. In this study, the EDC cross-linking approach (4) was also employed to verify that myosin heavy chain was the main myofibrillar protein involved in the heat-induced transitional changes during setting at 40°C of 3 fish species, including cod (*Godus morhua*), herring (*Clupea harengus*) and silver hake (*Merluccius bilinearis*); and the gel-forming ability of the fish muscle proteins was related to the mechanism of the myosin heavy chain cross-linking.

MATERIALS AND METHODS

Materials

Cod were normally purchased on the same day of catch from a local market in Halifax. Herring were purchased from local fishermen on the same day of catch in the late spring. Silver hake were obtained in May 1989 from a Japanese factory freezer ship.

Preparation of fish myofibrils and myosin

The preparation of myofibrils was carried out according to the method of Olson et al. (13), while myosin was prepared by the methods of Mackie and Connell (14) as well as Watabe and Hashimoto (15) but with the inclusion of 1mM ATP in all buffers used to wash the tissue. Extracted myofibrils and myosin were stored in 50% (v/v) glycerol at -30°C.

Turbidity measurement

Fish myofibrils (5 mg/mL) or myosin (2.5 mg/mL) dissolved in the deaerated assay buffer (0.6M NaCl,0.05M imidazole, pH 6.5) were placed in a plastic cuvette (light path length, 1 cm) and covered with paraffin. The turbidity measurement was performed on a PU 8800 spectrophotometer (Pye Unicam Cambridge, England) equipped with a thermocontrolled cell. The protein solution was heated at 40°C for 40 minutes and the changes in absorbance at 350 nm were measured continuously as the heating proceeded.

Heat treatment of fish myofibrillar proteins

The EDC cross-linking approach developed by Gill and Conway (4) was adopted with some modifications to study the time profile of thermal aggregation of the fish myofibrils and myosin during heating at 40°C. The results of the protein thermal aggregation was monitored by electrophoresing the heat-treated fish muscle proteins either on 10% polyacrylamide or 1.8% acrylamide/agarose composite gels.

For the heat-treated fish myofibrillar proteins being electrophoresed on 10% polyacrylamide gels, the fish myofibrils (5 mg/mL) or myosin (2.5 mg/mL) in 0.6 M NaCl,0.05M imidazole buffer at pH 6.5 was heated at 40°C for 0 to 50 minutes. After the predetermined heating time, 50 μL of 0.505M EDC (Sigma E-7750) dissolved in the assay buffer was added to the fish protein solution to obtain a final concentration of 4.5mM; and the mixture was further incubated at 40°C for 5 more minutes. The incubation was stopped by adding 1 mL of a quenching solution containing 5% 2-mercaptoethanol, 2.5% SDS, 8M urea (pH 8.8). An internal electrophoretic standard, catalase (Sigma C-40) which had been dissolved in the quenching solution (2.04 mg/mL, 0.5 mL), was added to all samples and immediately heated at 95°C for 30 minutes. Samples were dialysed and electrophoresed on cylindrical 5 mm x 115 mm 10% polyacrylamide gels as described by Porizo and Pearson (16).

For the heat-treated myosin being electrophoresed on 1.8% gels, the experimental procedures were the same as above except the final concentration of EDC was 10mM instead of 4.5mM and no internal electrophoretic standard was added. The preparation of 1.8% acrylamide/agarose composite gels with 0.5% agarose was as described by Peacock and Dingman (17).

Examination of electrophoretic results

After staining with Commassie Brilliant Blue R250 (Biorad), the protein bands in the 10% gels were measured densitometrically on the PU 8800 spectrophotometer with gel scanning and peak integration equipment. The intensities of all protein bands in a given gel were first normalized to the intensity of the catalase in the same gel. The degree of cross-linking was then calculated by comparing the normalized densitometric areas of the protein bands of the cross-linked samples with those of the appropriate controls. The molecular weights of the protein bands on the 1.8% gel were estimated by using cross-linked phosphorylase b (Sigma P-8906) as a weight marker.

Protein concentration of each sample was determined by the Bradford method (18).

RESULTS

Turbidity measurement

The turbidity of the protein solutions was monitored by measuring the absorbance of the solutions at 350 nm at half minute intervals. When the myofibril and myosin solutions of the three fish species were heated at 40°C for 40 minutes, the solutions changed from clear to turbid, and their turbidity curves were sigmoidal in shape (Fig. 1). Similar turbidity curves (absorbance vs heating time) were also reported in other heat-treated fish myosins or myosin subfragments suspended in solutions of different pH and/or ionic strength and heated at different temperatures as was done previously in the case of mackerel (19) and carp (20-21) etc.

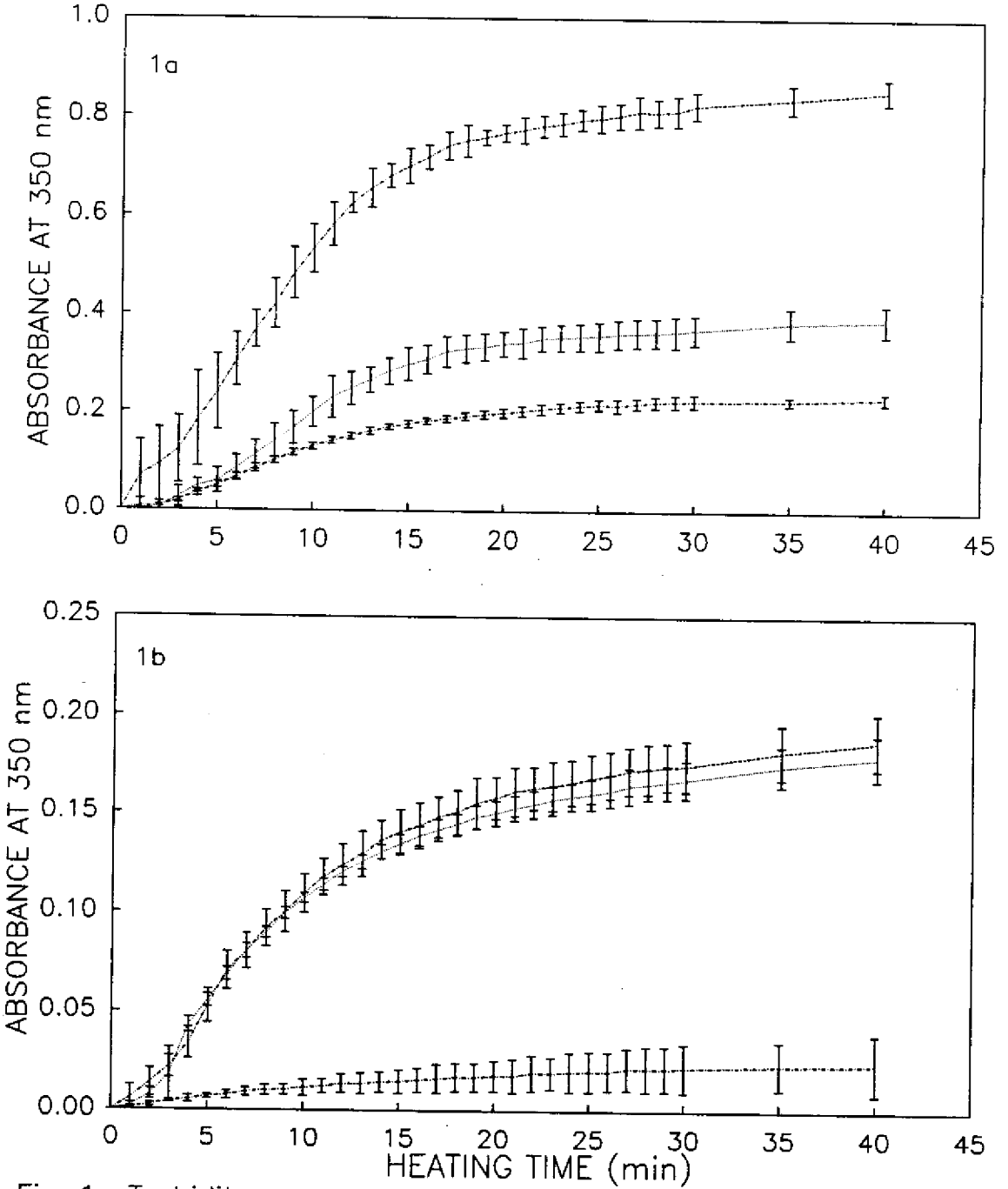

Fig. 1 : Turbidity measurement of the heat–treated solutions of fish myofibril (1a) and myosin (1b) at 40°C. The values were an average of six replicates and one standard deviation error bar is shown.
Cod (——), herring (------) and silver hake (——).

The turbidities of the heat-treated fish myofibril solutions of the three fish species were significantly different from each other. The cod myofibrils had the highest turbidity, followed by silver hake and then herring (Fig. 1a). The turbidities of the heat-treated cod and silver hake myosin solutions were much higher than those of herring (Fig. 1b). The cod and silver hake myosin solutions had similar turbidities for the first 10 minutes of the heat treatment, but after that, the turbidity of the cod myosin solution was slightly higher than that of the silver hake. The extent of thermal aggregation of the myofibrils and myosin solutions was indeed species-dependent.

Fig. 2 illustrates the change in turbidity per unit time ($\Delta A/min$) as a function of the heating time at 40°C. There were more than one transition point in the curves of each individual fish myofibril solution (Fig. 2a). It was most probably due to the presence of a number of myofibrillar proteins in the solution. The curves shown in Fig. 2a consist of 3 different regions : a rapid increase in the first 5 to 10 minutes, followed by a slow decline in the next 10 minutes and then a constant region with little change for the last 20 minutes of the heating. A similar profile was also found in the heat-treated solutions of fish myosins except in the case of herring myosin which showed little change in the rate of turbidity change during the entire heat treatment (Fig. 2b). The increase in the turbidity of the heat-treated solutions of fish proteins was mainly due to the formation of protein aggregates which were large enough to reduce light transmission. Therefore, the rate of turbidity change would reflect the pattern of the fish protein aggregate formation. For example, the thermal aggregation of both the cod and silver hake myosins might involve at least 2 steps : a rapid formation of large sized aggregates which slowly continued to increase in size and/or to link up with each other to form a network. On the other hand, the herring myosin would appear to aggregate in an one-step fashion with a slow build-up of small sized aggregates during the heat treatment. Undoubtedly, the mechanism of thermal aggregation of the herring muscle proteins must be different from the other two fish species.

SDS-PAGE of heat-treated fish muscle proteins on 10% gels

It has been known that EDC is a zero-length cross-linker which can cross-link proteins covalently when the carboxyl and amino groups of the protein are juxtaposed (2-5 Å) and will not cross-link non-interacting proteins. It is generally believed that the mechanism of protein cross-linking with EDC involves formation of an adduct, an O-acylisourea derivatives, between the EDC and the carboxyl group of Glu or Asp followed by a nucleophilic substitution with an amino group (22). Pedemonte and Kaplan (23) reported that the reaction was time and concentration dependent and displayed first order kinetics with respect to time. In our study, it was also found that more protein cross-linking occurred as a result of increasing the EDC concentration in heated solutions of cod myofibrils (Fig. 3). In addition, our previous study showed that degree of cross-linking was related to incubation time (4). In the present study, both concentration of EDC and incubation time (at 40°C) were kept constant in all experiments in order to assure that any protein cross-linking resulted only from the protein thermal aggregation.

The electrophoretic data (Fig. 4) showed that myosin heavy chain (MHC) of the three fish species was found to be the major myofibrillar protein being cross-linked to form a polymerized complex, and the complex could not enter the 10% polyacrylamide gel. Cross-linking of the MHC was observed prior to any other myofibrillar proteins during the heat treatment. There was about 90% to 100% of the MHC that were cross-linked with each other during the entire heat treatment. Cross-linking of the low molecular weight myofibrillar proteins, including troponins, tropomyosins and myosin light chains, was not observed in any of the three fish species. There was around 20 to 25% cross-linking of actin in the cod, 30 to 50% in the silver hake and 20 to 70% in the herring; and the cross-linking of actin was increased with the heating time (Fig. 5).

As reported previously in our laboratory, about 50% of the cod MHC was cross-linked before the involvement of actin, regulatory proteins, or the myosin light chains (4). Any discrepancy between this work and that of Gill and Conway (4) was mainly due to the use of higher concentration of EDC and constant incubation time used in this study. The progressive cross-linking of the MHC during heating at 40°C was also reported in the cod myofibrils (4), Alaska pollock surimi (3,7), Pacific

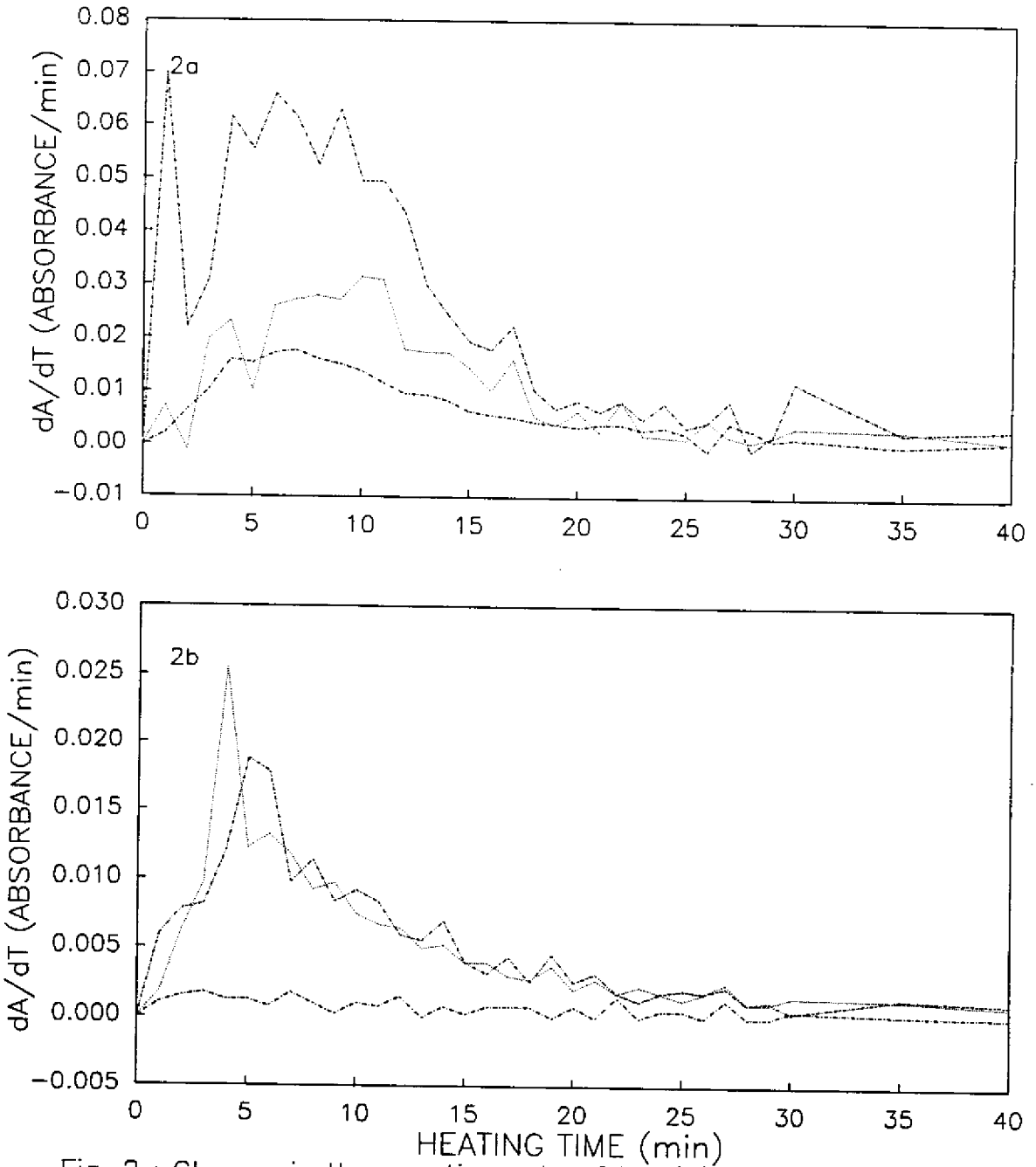

Fig. 2 : Change in the reaction rate of turbidities of the heat−treated solutions of fish myofibril (2a) and myosin (2b). Cod (——), herring (– – –) and silver hake (— —).

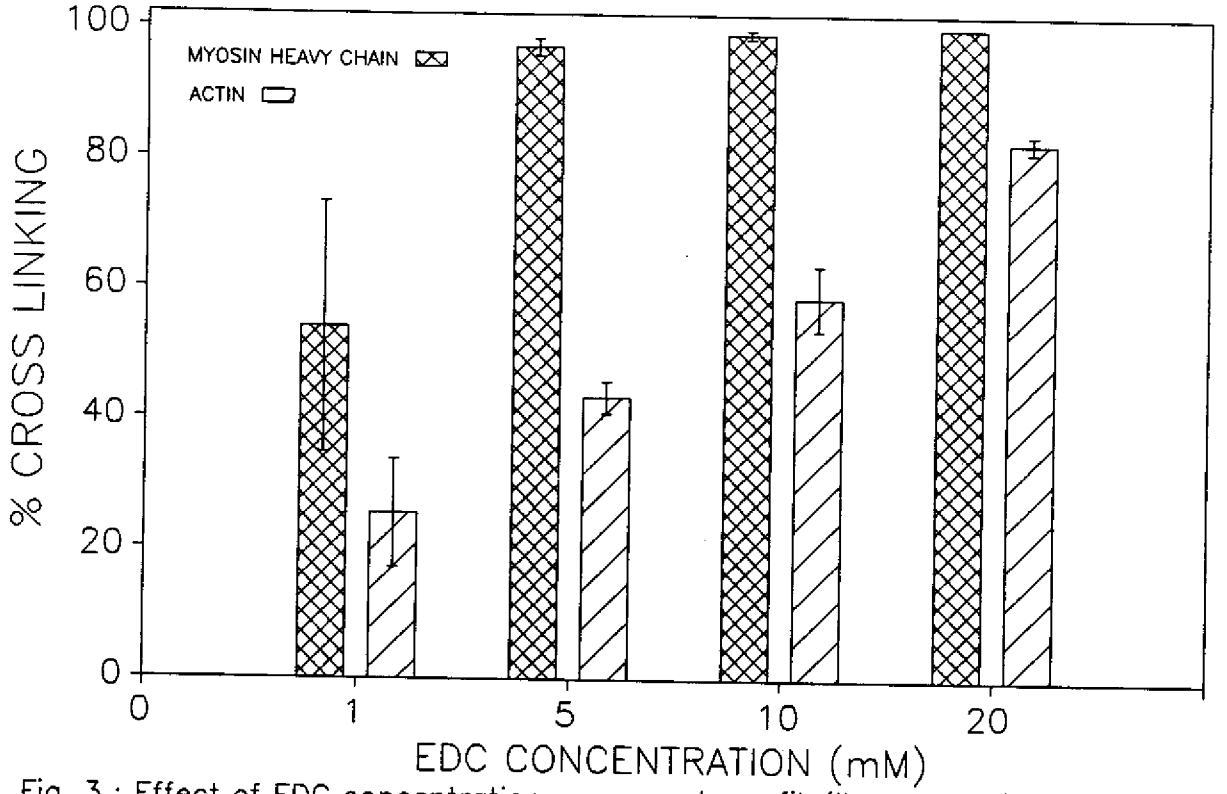

Fig. 3 : Effect of EDC concentrations upon cod myofibrillar cross-linking. One mL of myofibril (5 mg/mL) solution in 0.6M NaCl, 0.05M imidazole buffer (pH 6.5) was heated at 40°C for 30 minutes, then 10 μL of EDC solution was added. The mixture was then incubated at 40°C for a further 10 minutes.

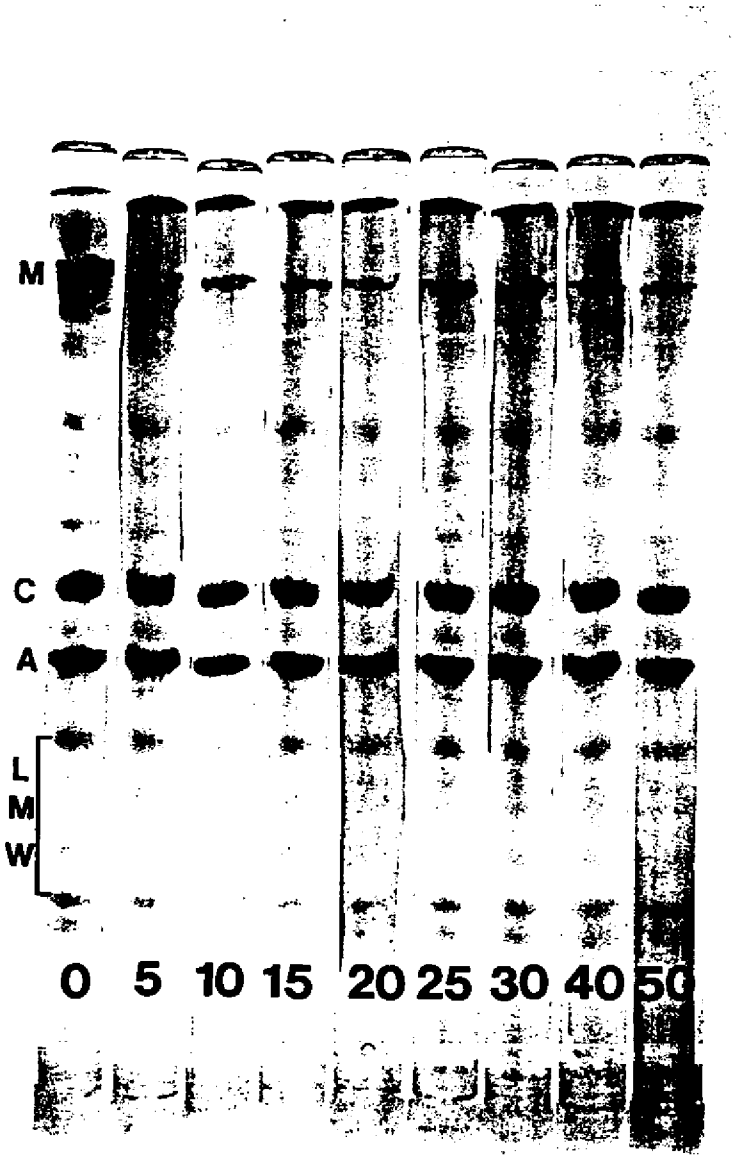

Fig. 4a: A typical SDS-PAGE pattern of fish myofibrils on 10% gels after heating at 40°C for 50 minutes (including incubation with 4.5mM EDC for 5 min). Numbers indicate the heating time. M, myosin heavy chain; C, catalase; A, actin; LMW, low molecular weight components.

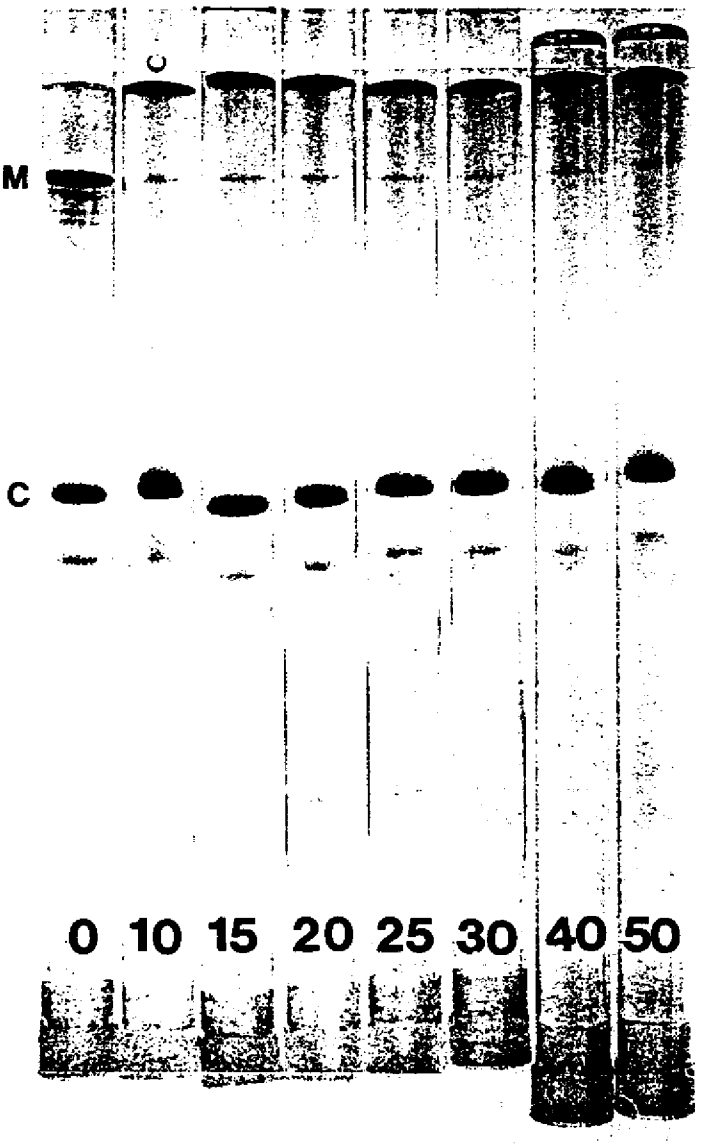

Fig. 4b: A typical SDS-PAGE pattern of fish myosin on 10% gels after heating at 40°C for 50 minutes (including incubation with 4.5mM EDC for 5 min). Numbers indicate the heating time. M, myosin heavy chain; C, catalase.

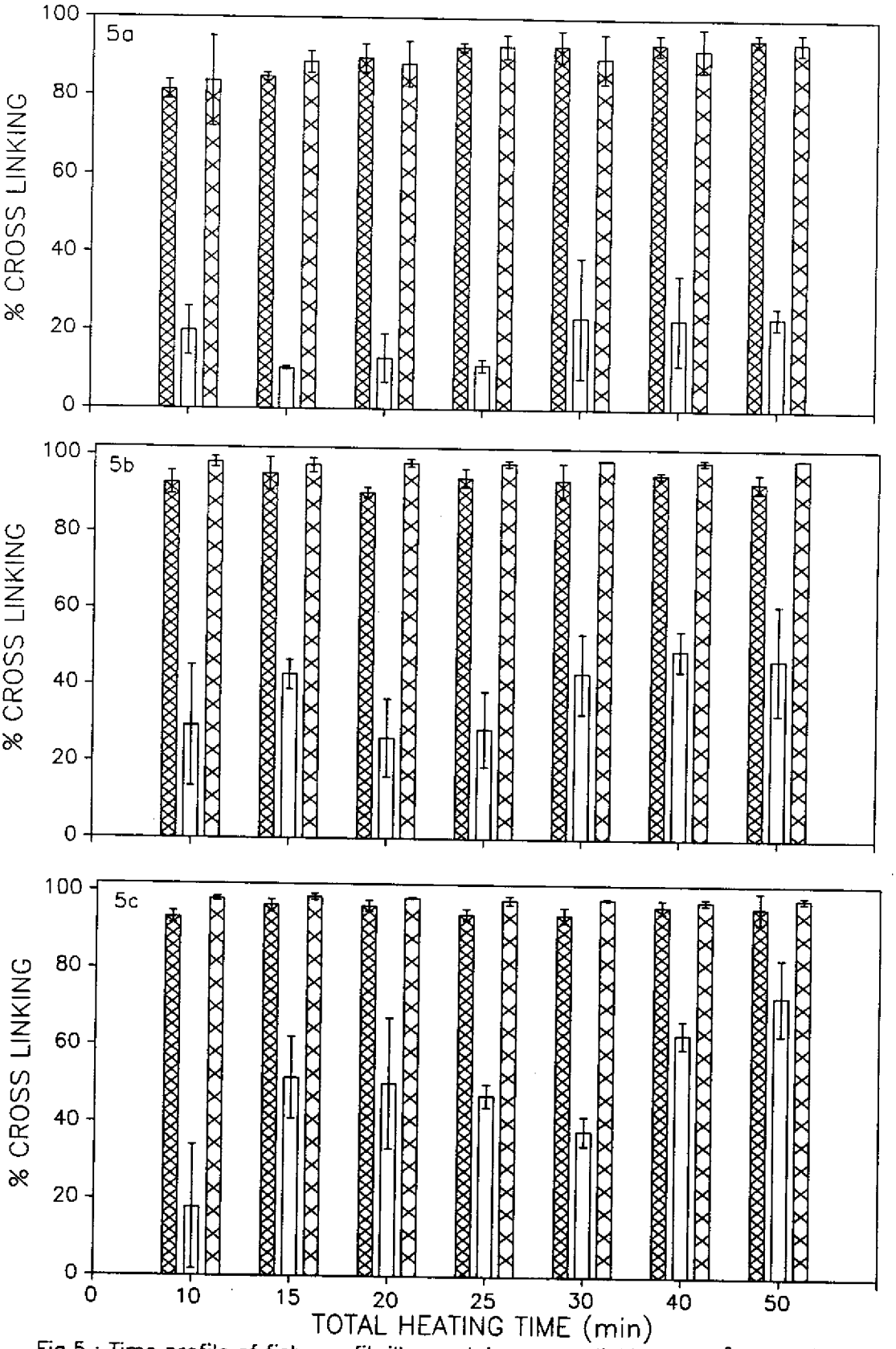

Fig.5 : Time profile of fish myofibrillar proteins cross-linking at 40°C. Cod (5a), silver hake (5b) and herring (5c). Myosin heavy chain of the myofibril (⊠) and myosin (⊠) solutions; myofibrillar actin (□).

mackerel actomyosin (5), and salted meat paste from threadfin bream (8). Differences in gel-forming ability among various fish species have been described in the past (5). In addition, it has been suggested that there is a positive correlation between the cross-linking of the MHC and the gel-forming ability of the surimi from Alaska pollock and threadfin bream (6,7).

The present study indicates that for all three species studied, the MHC becomes cross-linked at a very early stage in heating. Nevertheless, differences exist. It may be that the reduced turbidities observed in the heating of herring myofibrils and myosin resulted from differences in the size of aggregates between herring and the other two species.

SDS-PAGE of heat-treated fish myosin on 1.8% acrylamide/agarose composite gels

In our preliminary work, it was found that a group of MHC polymers ($n>10$) was sometimes observed when the heat-treated silver hake myosin was electrophoresed on 1.8% acrylamide/agarose composite gels (Fig. 6). These MHC polymers ($n>10$) disappeared when the upper reservoir buffer contained 10mM 2-mercaptoethanol. Thus, it was concluded that boiling samples in SDS, urea and 2-mercaptoethanol prior to electrophoresis as described by Porzio and Pearson (16) is not adequate to ensure the complete reduction of myofibrillar proteins. All subsequent electrophoretic experiments incorporated 2-mercaptoethanol in upper reservoir buffers.

Fig. 7 illustrates the electrophoretic patterns of the heat-treated myosin solutions of the three fish species. The conditions of the heat treatment were the same as before except that the EDC concentration was 10mM instead of 4.5 mM. Tailing of the protein bands on the 1.8% gels was so great and problematic that it was difficult to do any densitometric quantitation. MHC polymerization was observed in all three fish species, and polymerization increased with the heating time. The molecular weights of the MHC polymers were estimated by using cross-linked phosphorylase as molecular weight marker. Two to four MHC polymers (bands) were commonly found on 1.8% gels when the heat-treated myosins of the three fish species were electrophoresed. The apparent molecular weights of these MHC polymers were estimated to be 200,000 (MHC), 400,000 $((MHC)_2)$, 600,000 $((MHC)_3)$ and 1,600,000 dalton $((MHC)_8)$. When the cod and silver hake myosin solutions were heated at 40°C, monomers,dimers and trimers of MHC were found in the first 20 minutes and dimers, trimers and octamers of MHC were observed in the last 20 minutes of the heating. For the heat-treated herring myosin solutions, only monomers and dimers of the MHC (with a trace of trimers) were found during the entire heating period.

DISCUSSION

Turbidity measurements have been found to correlate well with some textural measurements of muscle protein gels, e.g. gel strength and hardness (24,25). In addition, the turbidity of heat-treated fish myosin solutions was found to increase with conformational changes of the myosin molecules (27). Therefore, turbidity measurement has been shown to be useful to correlate the extent of thermal aggregation with the gel-forming ability of the fish muscle proteins. When the fish protein solutions were heated at 40°C for 30 minutes, both the extent and reaction rate of turbidities were much higher and faster in the cod and silver hake than in those of herring. It was reasonable to expect that the heated muscle gel of cod and silver hake would have better textural properties than that of the herring. Hastings et al. (27) reported that the functional properties of surimi and kamaboko gels from cod were much better than those of herring. In addition, the recent research in our laboratory showed that the gel properties of cod and silver hake surimi were superior to that of herring (Table 1, personal communication with K.E. Spencer). Consequently, the turbidity measurements of the fish protein solutions could reflect the gel-forming ability of the proteins heated at 40°C. The species specific differences in turbidity for heat treated myofibrillar or myosin solutions may be due to the size and/or composition of the aggregates formed. During the heat treatment, both cod and silver hake myosins were able to form large aggregates which would further cross-link with each other to form a network. However, electrophoretic evidence indicated that herring myosin failed to form such large protein aggregates.

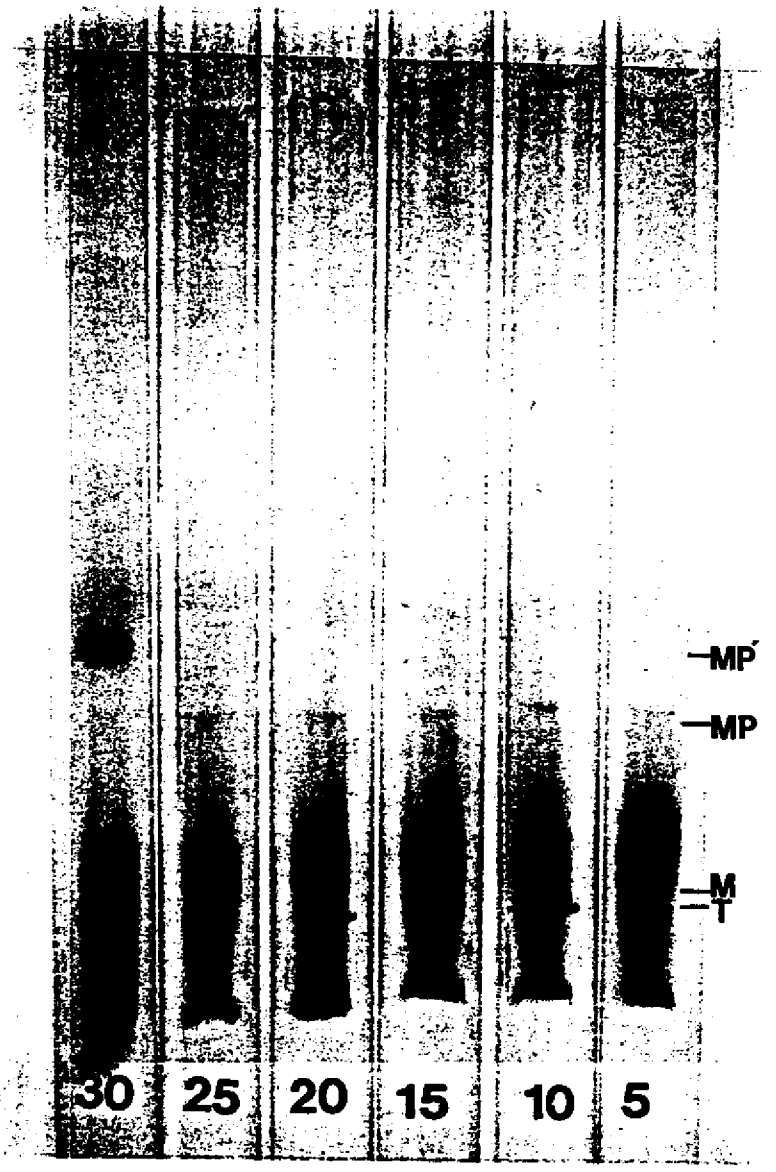

Fig. 6 : SDS-PAGE pattern of silver hake myosin on 1.8% gels after heating at 40°C for 40 minutes including incubation with 10mM EDC for 5 min. No 2-mercaptoethanol was added into the upper reservoir buffer during electrophoresis. Numbers indicate the heating time. M, myosin heavy chain; MP, myosin heavy chain polymers; T, tracking dye.

Fig.7a : SDS-PAGE pattern of cod myosin on 1.8% gels after heating at 40°C for 40 minutes including incubation with 10mM EDC for 5 minutes. The upper reservoir contained 10mM 2-mercaptoethanol. Numbers indicate the heating time. M, myosin heavy chain; B2 to B4, myosin heavy chain polymers; T, tracking dye.

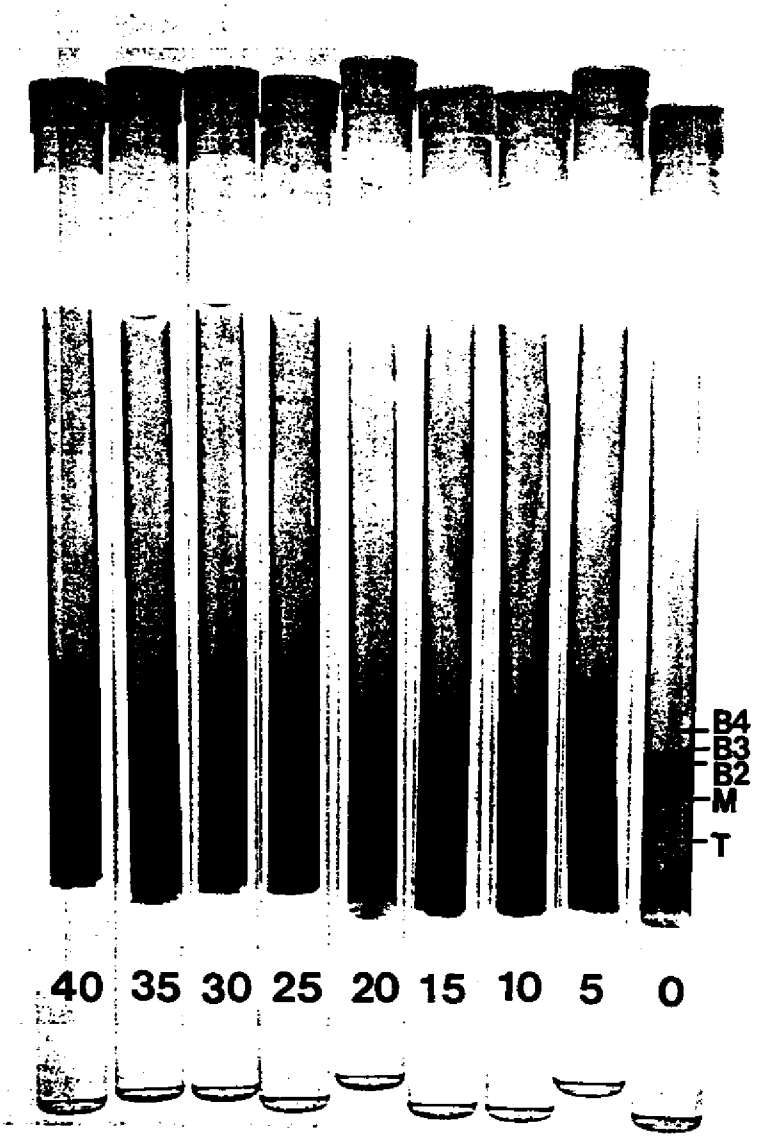

Fig.7b : SDS-PAGE pattern of silver hake myosin on 1.8% gels after heating at 40°C for 40 minutes including incubation with 10mM EDC for 5 minutes. The upper reservoir contained 10mM 2-mercaptoethanol. Numbers indicate the heating time. M, myosin heavy chain; B2 to B4, myosin heavy chain polymers; T, tracking dye.

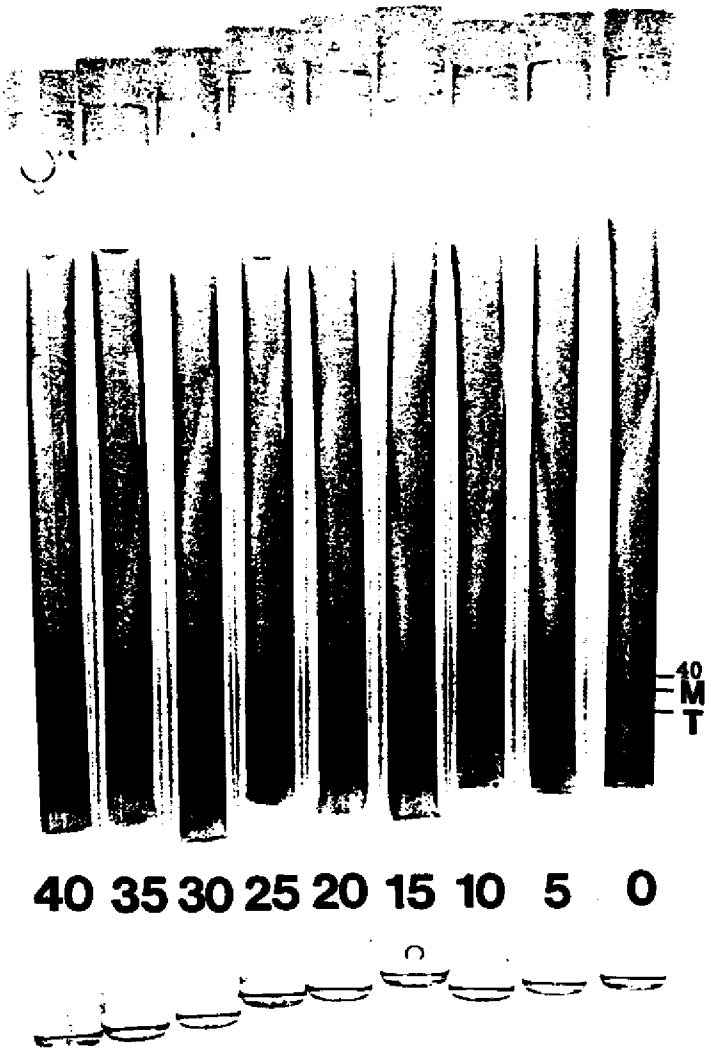

Fig.7c : SDS-PAGE pattern of herring myosin on 1.8% gels after heating at 40°C for 40 minutes including incubation with 10mM EDC for 5 minutes. The upper reservoir contained 10mM 2-mercaptoethanol. Numbers indicate the heating time. M, myosin heavy chain; 40, myosin heavy chain dimer; T, tracking dye.

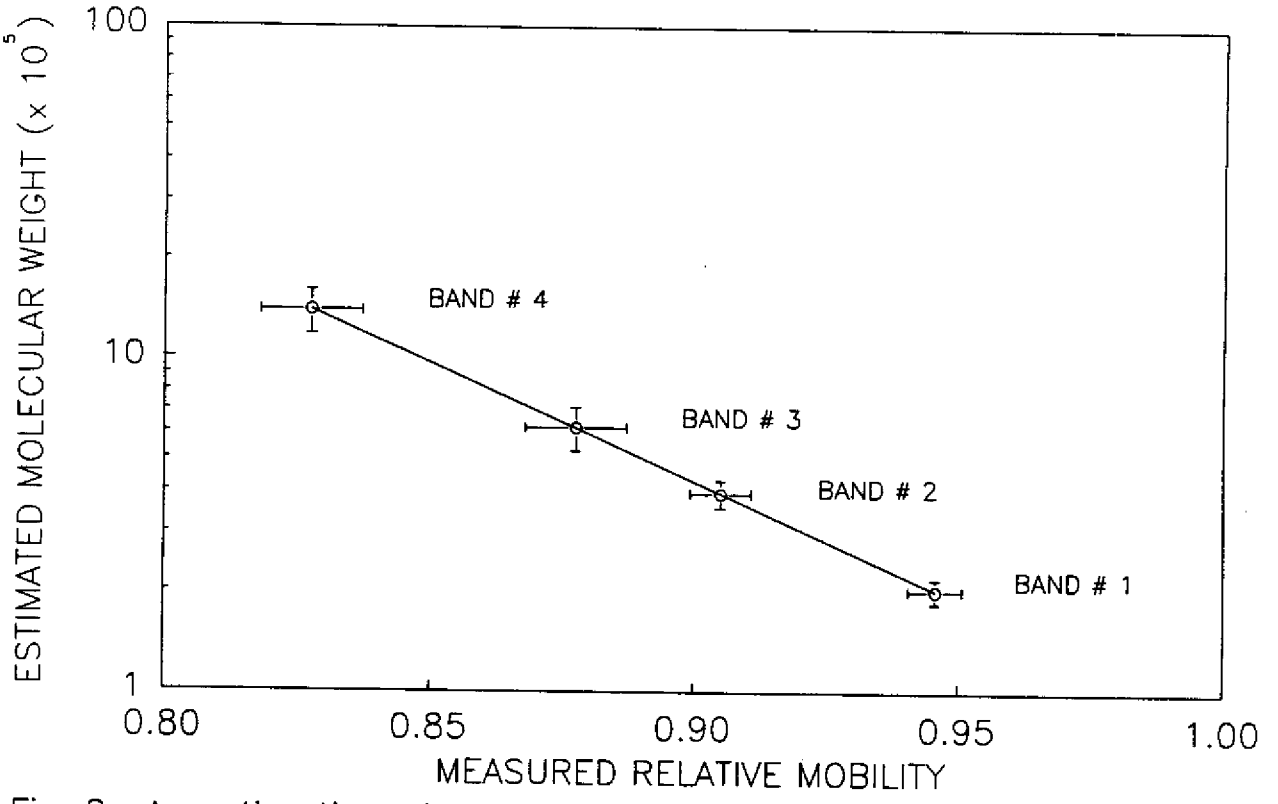

Fig. 8 : An estimation of molecular weights of the protein bands present on the 1.8% gels shown in Fig. 7.

Table 1 : Textural properties of cod, herring and silver hake surimi cooking at 40/90*

FISH SPECIES	PROTEIN CONTENT (%)	TORSION TEST STRESS (kPa)	TORSION TEST STRAIN (kPa)	RIGIDITY (g.cm)	GEL STRENGTH
Cod	15.98	110.60	3.26	33.97	559.80
	0.80	7.82	0.25	1.45	168.80**
Silver hake	14.52	64.46	3.39	18.98	331.73
	0.13	4.66	0.08	0.98	128.40
Herring	13.08	59.72	2.82	21.21	325.20
	0.13	4.09	0.18	0.93	60.97

* 30 minutes at 40°C and another 30 minutes at 90°C.
** standard deviation of six replicates

The myosin heavy chain was clearly the principal myofibrillar protein responsible for the formation of the large protein aggregates during the heat treatment. Numakura et al. (7) reported that there was an exponential loss of MHC and a relative increase in cross-linked MHC during setting of Alaska pollock surimi at 10°C over a 50 hours period. No changes in other myofibrillar proteins were observed. A similar observation was also reported in the heat-treated actomyosin solutions of a number of fish species (5), and was suggested that there might be reduced cross-linking of the MHC's of hard to set species. However, the present study showed that for herring, it was the extent of MHC cross-linking which was important rather than the existence of cross-links per se.

The electrophoretic data (1.8% acrylamide/agarose composite gels) revealed that degree of MHC polymerization at 40°C depended upon the time of heating. Figure 7 illustrates the fact that the thermal aggregation phenomenon at 40°C was due to interaction of the MHC with itself rather than through protein modification mediated by EDC. The polymerization of cod and silver hake MHC was similar and could be divided into 2 stages : (i) the formation of MHC dimers and trimers in the first 20 minutes of the heating at 40°C; (ii) formation of MHC octamers in the last 20 minutes. The polymerization of herring MHC was slower and restricted to the formation of dimers. Turbidity measurements confirmed the two different reaction rates in the absence of EDC.

At least two types protein-protein interaction have been reported during the heating of fish muscle proteins : hydrophobic interaction (2, 4-5, 28) and formation of disulfide bonds (29,30). The relative importance of each is still disputable. This study showed that there were at least two types of protein-protein interactions occurring during the fish muscle thermal aggregation. In the early stages of heat treatment, MHC polymers ($n \leq 3$) were found in all the three fish species, but only the heat-treated cod and silver hake fish protein solutions had high turbidity. In other words, formation of the MHC polymers ($n \leq 3$) was caused by one type of chemical interaction that was present in all the three fish species. However, the observed high turbidities in the heat-treated cod and silver hake protein solutions resulted from another type of interaction. It is speculated that the latter interaction could bring the MHC polymers ($n \leq 3$) together to form large aggregates and build up a 3-dimensional protein network. In addition, this second interaction was much more pronounced in the later stages of the heating. Further studies on the types of protein-protein interactions among the MHC molecules are being carried out in our laboratory.

CONCLUSION

Cross-linking ability of the myosin heavy chain accounted the differences in the gel-forming abilities of the three fish species. Good textural properties found in the heated muscle gels of cod and silver hake were attributed to the fact that fish MHC molecules were able to polymerize in a two-step fashion to form large protein aggregates and build up a protein network during setting at 40°C. The reason for the poor gel-forming ability of the herring muscle proteins is thought to be due to the absence of higher MHC polymers.

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EFFECTS OF DECK TREATMENTS AND THERMAL ABUSE ON WEIGHT CHANGES IN SHUCKED SEA SCALLOPS (PLACOPECTEN MAGELLANICUS) DURING STOWAGE

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INTRODUCTION

The sea scallop, Placopecten magellanicus, supports a valuable commercial fishery throughout much of its range from Newfoundland to north of Cape Hatteras. Over 33 million lbs. of sea scallops with an ex-vessel value exceeding $132 million was harvested in U.S. waters during 1989. In the mid-Atlantic region, commercial harvesting activity increased dramatically since the mid-to-late 1970's with Cape May, New Jersey and Hampton Roads, Virginia, the major ports for sea scallop landings. Vessel activity in Hampton Roads increased rapidly from 5 full-time scallop vessels in January 1977 to over 51 vessels by the end of 1978. The number of scallop vessels operating out of Virginia ports reached an all-time high of 115 vessels during the Fall of 1979. The number of vessels have stabilized at 50-60 vessels since 1979.

Sea scallops are Virginia's most valuable landed seafood exceeding $24 million for the past several years. The mid-Atlantic region has become an important sea scallop resource area contributing to over 50% of the U.S. landings and consequently supports a valuable fishery with an important regional economic impact.

Vessel operations in the mid-Atlantic have some difficult challenges during the summer months. The combination of warm sea water temperatures, hot weather and the tendency for extended trips, often exceeding 15 days, creates the potential for problems relative to product quality. During July and August, sea water surface temperatures between 70-78°F are common and can exceed 80°F for short periods of time. Air temperatures can range from 70-78°F in the protection of shucking houses to over 90°F on surfaces exposed to the sun. Preliminary data indicated that internal bag temperatures at the time of bagging often exceeded 70°F and were recorded as high as 76°F.

At-sea handling practices have profound effect on some of the physical parameters of quality and meat integrity. Little or no information is available with regard to changes in weight during stowage nor is there a good understanding as to how on-deck handling affects these changes. There is a strong suspicion that factors such as thermal abuse, wafering, soaking in fresh water or the use of ice on deck are important, but no quantitative information is available.

In addition to weight changes during stowage, other parameters such as product discoloration and textural changes have to be evaluated relative to on-deck handling procedures and pre-chilling strategies. Scallop quality at off-loading is often determined by coloration, odor, the percentage of meat pieces and texture. Consequently, any modifications of handling procedures should be evaluated relative to the perceptions of product quality at off-loading.

The evaluation of at-sea handling practices as they affect sea scallop quality, volume (weight) and meat integrity cannot stop at the point of offloading. The performance of scallop meats during retail marketing of fresh product or processing to frozen product must also be considered. The questions relating to at-sea handling practices as they affect shelf-life and appearance is poorly understood. A large portion of sea scallop landings are frozen and stored

for future use. Quality control issues such as drip loss and the effectiveness of processing aids such as sodium tripolyphosphate (STP) are poorly understood as they relate to what happens on deck and in the ice holds of fishing vessels. Obviously, even seemingly small effects could be immensely important to plants processing large quantities of sea scallops; however, no quantitative information is available to industry.

METHODS

General Considerations

Three sea scallop fishing trips were conducted during the months of July and August. Fishing vessel operations were conducted in the mid-Atlantic region from Virginia to northern New Jersey in water depths ranging from 22-37 fm. Trips varied in duration from 12-21 days dock-to-dock. Vessels were not allowed to mix scallop meats to obtain a legal average count nor were they allowed to disturb bags of scallops once iced and stowed. Shucking, bag-up times, bagging practices, icing and stowage were strictly controlled to insure conformity to experimental designs and to minimize sources of variability of test results.

Deck Operations

Sea scallops were landed on deck, culled, shucked and processed in accordance with normal industry practices (Figure 1).

Scallops were shucked into buckets with sea water. Hourly, individual buckets were emptied into plastic baskets which facilitated washing with sea water both from the deck hose and in 55 gallon circular trash cans. Scallops were evenly divided and placed into two insulted totes containing ice:seawater (1:2), sea water, ice; freshwater, a solution of 2.5% sodium tripolyphosphate and 1% sodium chloride by weight, or nothing (dry) until bag-up. These are, hence, referred to as "deck treatments." Generally, two deck treatments were used for each deck watch and various combinations of treatments were used throughout the trip (i.e. seawater vs. dry, ice seawater vs. seawater, etc.). Scallop meats were held in insulated totes until bag-up and were not rewashed with seawater prior to bagging. Scallops from different deck treatments were not mixed and were bagged separately.

The use of insulated totes on-deck as holding containers for scallops prior to bagging facilitated the need to control and standardize handling procedures as much as possible. The totes provided cover and protection from the sun and other undesirable elements. They also provided a mechanism by which to keep scallop meats at relatively constant temperatures whether the scallops were kept in sea water or other deck treatments not entailing the use of ice. In the case when ice was used in conjunction with sea water or fresh water, it was desirable to use as little ice as necessary since ice is at a premium during the summer months for extended trips. Generally one basket of ice was used per tote for the duration of a 6 or 8 hour watch and was sufficient for at least 240 lbs. of scallop meats. In order to maintain a consistent 1:2 ratio of ice to seawater, a permanent reference mark for seawater was made on the inside of the tote.

Bagging was done on 6 or 8 hour intervals depending upon watch schedules but remained consistent for the duration of a particular trip. Scallop meats were bailed from the totes and bagged according to normal procedures. Approximately 38 pounds of scallops were placed in linen bags and secured with galvanized metal twist ties. Bags to be weighed were labelled designating deck treatment, date, and time.

Bags were weighed to the nearest 0.1 lb. using a Micro Weigh (R) Series 540 Seagoing Scale with an accuracy of $\pm$ 0.2%. The Micro Weigh Seagoing Scale was used for two of the three trips. Upon offloading, bags were reweighed using the same scale.

Meat counts (meats per pound) were determined at time of bag-up for each deck treatment and immediately after shucking prior to deck treatment. These data were used to assess changes in meat count (weight) attributed to the various deck treatments.

Temperature Determination

Temperatures were determined using an Omega (R) HH-51 Digital Thermometer with Type K Chromel-Alumel Thermocouples (connectors and wire) with a resolution of 0.1 C. Temperature data was obtained during each watch for which tests were being conducted. During the watch and at the time of bag-up, temperatures were determined for (i) shucking house, (ii) surface sea water and (iii) scallops held in totes. An additional thermocouple was located in the ice hold to monitor temperatures during the course of the trip.

At least once during each trip, the internal temperature of 4-6 bags of scallops were monitored from time of bagging, placement in the chill-bin and for the duration of the trip in the ice hold. During the course of the project, internal bag temperatures were determined for 24 bags of scallops originating from the various deck treatments. Thermocouple wires were placed in the center of the filled scallop bags at time of bagging. Connector lead wires were of sufficient length to allow temperature determinations after stowage in the ice hold for the duration of the trip. Generally, bag temperatures were taken just prior to placement in the chill-bin and then on an hourly or frequent basis until temperatures stabilized after 24 hours. Bag temperatures were monitored daily for the duration of the trip.

Sodium Tripolyphosphate Treatment

To test the effectiveness of phosphate on sea scallop meats of various ages and from various deck treatments, replicate half pound samples of scallop meats were recovered at off-loading from 32 bags previously tagged at-sea. Four deck treatments were represented which included: ice-seawater, seawater, dry, and ice-freshwater. Scallop meat age ranged from 2-17 days post-harvest. Scallop meats from shucked live shellstock served as age zero and a control.

A solution of 2.5% food grade STP by weight (specific gravity 1.023 at 15.5%) plus 1% food grade NaCl was prepared with tap water (pH 7.2) and chilled to 11-12 C. The phosphate solution (pH 8.9) was distributed to 9 polyethylene totes representing soak times of 3, 6, 9, 12, 15, 18, 21, 24, 27 hours with a 2:1 ratio by weight of solution to scallops. At each soaking time interval, the respective tote of scallop samples were processed while the other scallops were stirred within their totes and allowed to continue soaking until the next sampling time interval. Mixing of scallop meats occurred every three hours.

Post treatment processing included drained weights and post-freezing, thawed and cooked weights. After soaking, each scallop sample was drained on a half inch plastic grid drain rack for 2 minutes, pat-dried with a 100% cotton towels, weighed, placed in a plastic freezer bag, blast frozen to a temperature of -34 C, then placed in cold storage at -23 C. After 30-35 days of frozen storage, samples were thawed by water submersion. Thawed samples were emptied onto cotton towels, pat dried, reweighed, and placed into freezer bags for cooking. Trial cookings monitored with thermocouples indicated an average cooking time of 4.5 minutes, for the center of various size scallop meats to reach 73 C as prescribed by AOAL methods.

Economic Evaluation: At-Sea Treatments

Examination of the potential economic benefits of various at-sea or deck treatments was restricted to evaluating the marginal product and revenue associated with various deck treatments and stowage time relative to dry or sea water deck treatments.

The benefits were calculated by applying deck treatment gains and daily weight changes during stowage to assumed daily harvests of 800 pounds for 14-21 day trips. Marginal product was estimated in terms of dockside weights of product treated with ice and sea water and ice and freshwater less dockside weights of product that was either processed dry or soaked in sea water. Marginal revenues were calculated by multiplying the marginal product of each treatment by $4.35 per pound, which is the current ex-vessel price for sea scallops in the mid-Atlantic.

RESULTS AND DISCUSSION

Weight Changes of Scallop Meats During Deck Treatment and Stowage

The weight change (gain) of sea scallop meats during stowage is the result of ice melt water being absorbed by scallop meats which are hyperosmotic to the their environment. The degree to which water is absorbed by scallop meats is affected by several factors, some relating to the biological state of the animal and some factors which are man-made. Clearly, the most obvious biological factors are related to the reproductive state of the animal, annual and seasonal environmental changes, fishing area and depth of water. During the course of this project, vessel operations for each trip were conducted over wide geographical areas and water depths which is normal for the mid-Atlantic fishery. As such, no attempt has been made to segregate the data relative to these variables at this time. Previous research has determined that water absorption by scallop meats is highly dependent upon season, water depth, geographic area and reproductive state and subsequently may be directly linked to the physiological state of the scallop. Sea scallops in the mid-Atlantic area during the months of July and August are "recovering" from the spawning episode in May and June.

Man-induced variations on vessels are likely the result of uneven production (i.e. baskets of scallops/hour or lbs. of scallop meats per hour). In addition, variable rates of production were responsible for unshucked scallops remaining on deck from 1-4 hours because harvesting capacity on occasion exceeded the shucking capabilities of the crew. Often the amount of scallops shucked was not evenly distributed throughout the watch. Consequently, the rate at which shucked scallop meats were placed in the totes varied widely, and the residence time of scallops in various deck treatments was not proportionate. This affected the rate at which ice in totes melted, and thus, the relative exposure to 2/3 strength seawater.

Data on the average, minimum and maximum weight change of sea scallop meats incurred during holding in deck treatments prior to bagging demonstrate differences related to the type of deck treatments and its duration (Table 1). Weight gains were larger for similar deck treatments with longer holding times and were the highest for ice:freshwater. The range of minimum and maximum values are indicative of the variation caused by irratic rates of production as mentioned previously. However, it is evident that irrespective of quality issues, the type and duration of deck treatments can greatly alter the weight (volume, and thus, meat count) of scallops from the time of harvesting to bagging and stowage.

Man-induced variations in the on-deck handling procedures would hypothetically affect the rate at which water was absorbed during stowage in the ice-hold. It stands to reason that what happened on deck relative to the degree of exposure to fresh or less saline water and to the degree of thermal abuse or mishandling would ultimately affect what happened to the scallops in the ice hold.

Table 1. Average, Minimum and Maximum Percent Weight Change of Sea Scallop Meats as the Result of Holding in Deck Treatments

Bag-up at 8 hour intervals; August 1990	
Ice:Seawater (1:2)	12.6% (5% min.-18% max.)
Ice:Freshwater	22.0% (19% min.-29% max.)
2.5% STP 1% NaCl	15.5% (11% min.-22% max.)
Bag-up at 6 hour intervals; August 1989	
Ice:Seawater (1:2)	7.0% (5% min.- 9% max.)
Ice:Freshwater	12.2% (9% min.-16% max.)
Bag-up at 6 hour intervals; July-August 1990	
Ice:Seawater (1:2)	9.4% (6% min.-14% max.)

The degree of thermal abuse on deck became immediately evident. Scallop meats held in seawater or dry where temperatures exceeded 72-74 F for more than two hours began to show signs of becoming "wafered." Wafering is the rapid onset of a rigor mortis type reaction during which the scallop meat changes from a cylinder shape to that of a wafer. This phenomenon has been observed frequently, especially after the spring spawning cycle is completed. It has been hypothesized that wafered scallops "behave" differently during stowage with regard to water uptake and subsequent processing on-shore. During the trip conducted in August 1990, air and surface sea water temperatures and temperatures for sea water and dry deck treatments were well above 72 F and the degree of wafering and thermal abuse was described as severe.

Average temperatures at the time of bag-up (Table 2) indicated that the highest temperatures were observed for seawater and dry deck treatments with those during the August 1990 trip reaching above 78 F. Temperatures recorded for the seawater and dry deck treatments closely paralleled air (shade) and surface sea water temperatures. Final temperatures in the ice:seawater and ice:freshwater deck treatments depended upon the quantity of shucked scallops, duration of the deck watch and ultimately the rate at which the ice melted. Ice melt was complete at time of bagging for nearly all 6 hour deck watches and for all 8 hour deck watches. Despite elevated sea water temperatures and sometimes abnormally high deck temperatures above 90 F, common during July and August, the insulated totes with ice treatments were fully capable of maintaining cooler temperatures in the 40-50 F range.

Table 2. Average, Minimum and Maximum Temperatures (F) of Sea Scallops Held in Various Deck Treatments at Time of Bag-up.

	August 1990	July-August 1990	August 1989
Ice:Seawater (1:2)	48.7 (37.6 min.-58.0 max.)	39.5 (32.6 min.-48.2 max.)	44.2 (34.0 min.-53.8 max.)
Ice: Freshwater	50.1 (39.8 min.-57.7 max.)		51.9 (42.4 min.-61.0 max.)
Seawater	78.6 (77.5 min.-79.8 max.)	72.6 (66.4 min.-75.4 max.)	73.1 (69.4 min.-75.6 max.)
Dry	78.3 (75.6 min.-81.3 max.)	72.1 (68.8 min.-74.2 max.)	

Summary Data

Weight changes as a result of various deck treatments and length of stowage for the three trips are summarized in Table 3. Ice:seawater and seawater deck treatments were employed during all three trips where as dry and ice:freshwater deck treatments were employed for only two of the three trips. The use of 2.5% sodium tripolyphosphate as a deck treatment was used on only one trip. The greatest weight changes can be attributed to how the scallops were handled on deck with lesser weight changes attributed to time in stowage. It follows true to the concept that the greater the weight gain achieved on deck the less the gain (even or loss) will be during stowage. However, there are several factors that must be considered when reviewing these results:

(1) Thermal abuse. In the case of extreme thermal abuse, wafering occurred (August 1990) during the dry and seawater deck treatments; average weight change during stowage was minimal (1.8 to 2.1% gain) and statistically different for the 18 day stowage period. During the two trips (August 1989 and July 1990) where thermal abuse was considered as low to moderate with little or no wafering, average weight gains for seawater or dry deck treatments ranged from 3.1 to 6.0% even with shorter stowage periods of 14 and 10 days. Thermal abuse and/or wafering while scallops are shucked and handled on deck can adversely affect product quality and weight gain while in stowage.

(2) Deck-chilling. The use of a ice:seawater (1:2) mixture to hold and chill scallops before bagging conveys two benefits in that (i) of a reasonable weight gain both on deck and in the hold and (ii), minimizing or eliminating the occurrence of thermal abuse and its associated adverse consequences. Weight gains were consistent with bagging intervals and the length of stowage time. For two trips (August 1989 and July 1990) with bagging intervals of 6 hours and stowage times of 14 and 10 days, ice: seawater deck treatment resulted in offloading weight changes of 12.8 to 13.6%. [2] The August 1990 trip with an 8 hour bagging interval and 18 days of stowage resulted in a 16.6% weight change.

The use of ice:freshwater to chill and hold scallops on deck prior to bagging results in large weight gains of 22.0% for an 8 hour bagging interval. However, subsequent weight changes during stowage were minimal for both trips. In fact, the percent weight change during stowage was not statistically different than zero between days 4 and 18 for the August 1990 trip. The use of ice:freshwater as a deck treatment resulted in the greatest weight change compared to other deck treatments. However it also resulted in questionable or less desirable quality and meat integrity characteristics.

[2]Residence time of scallops in various deck treatments depended upon bagging intervals. For 6 hour intervals, residence time range from 30 minutes to at least 5 hours. For 8 hour intervals, residence time ranged from 30 minutes to at least 7 hours.

Table 3. Average weight change (%) of scallop meats as a result of deck treatment and stowage time.

	Ice: Seawater	Seawater	Dry	Ice: Freshwater	STP
	8 hr. bag-up interval; 18 day stowage; Aug. 1990				
Deck Treatment	12.6	0	0	22.0	15.5
Stowage	3.6	2.1[a]	1.8[a]	-0.3[b]	1.9[a]
Total[c]	16.6	2.1	1.8	21.2	16.5
	6 hr. bag-up interval; 14 day stowage, Aug. 1989				
Deck Treatment	7.0	0	-	12.2	-
Stowage	5.4	6.0	-	1.0	-
Total[c]	12.8	6.0	-	13.3	-
	6 hr. bag-up interval; 10 day stowage, July 1990				
Deck Treatment	9.4	0	0	-	-
Stowage	3.9[d]	3.1	4.1[d]	-	-
Total[c]	13.6	3.1	4.0	-	-

[a]Statistical tests indicate weight changes from stowage for seawater, dry and STP are equal.

[b]Percent weight change is not statistically different than zero between day 4 and 18.

[c]Total weight change from shucking to offloading based upon original weight of shucked scallops. Stowage gains are imposed upon deck treatment gains and are not additive.

[d]Statistical tests indicate weight changes from stowage for ice:seawater and dry are equal.

(3) Processing aids. The use of processing aids such as sodium tripolyphosphate (STP) as a deck treatment resulted in weight changes comparable to that of the ice:seawater deck treatment totaling 16.5% and 16.6% respectively. The testing of STP was prompted by questions as to the effectiveness of processing aids at sea and does not constitute an endorsement of use.

Estimated percent weight changes over the time course of each trip are presented in Tables 2-4.[3] The rate at which weight changes occur during stowage was variable depending upon duration of trip, deck treatment and the degree of thermal abuse and scallop meat wafering. However, weight changes during stowage for ice:seawater deck treatment were generally greater than or equal to weight changes for dry or seawater deck treatments and clearly demonstrates the value of pre-chilling and the prevention of thermal abuse during the summer months.

Temperature Records

A series of four temperature records of scallops from shucking to offloading are depicted in Figures 5-8. For each series, one temperature record was for scallops from ice:seawater deck treatments and the other was from a seawater or dry deck treatment at ambient temperatures. In each of the four series, several commonalities are evident.

(1) Deck treatments without ice resulted in bag-up temperatures ranging from 72.1° to 78.6°F whereas deck treatments with ice resulted in bag-up temperatures ranging from 39.5° to 51.9°F.

(2) Despite wide temperature differences between deck treatments with and without ice, internal bag temperatures were nearly the same after 24 hours regardless of the method of deck treatment.

(3) Temperature increases on the order of 3°-10°F were noted for all treatments during the process of bagging and handling scallop bags for placement into the ice hold. These increases were generally larger for pre-chilled scallops but still were in an acceptable temperature range. However, any temperature increase for unchilled scallops could further compromise quality.

(4) The use of chill-bins was very effective in rapidly reducing internal bag temperatures. During a 6 or 8 hour period, internal temperature decreases on the order of 20°-32°F were observed for bags of scallops from un-iced deck treatments.

(5) The internal bag temperatures became stable after 48 hours and temperatures between 33°-35°F were frequently observed for the duration of the trip. On one occasion (August 1990), internal bag temperatures increased 4°-5°F after four days of stowage. This increase resulted when insufficient ice was used to separate one layer of bags from another.

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[3]Percent weight change due to stowage was estimated by several linear and semi-parametric regression models and related statistical tests. Given the voluminous nature of the estimates, they are omitted. The estimates, however, may be obtained from the authors on request.

Economic Evaluation of Vessel Performance

Post-harvest weight changes can occur as the result of how scallops are handled on deck and during stowage over the duration of the trip. Weight changes (gains) on deck may be intentional and motivated only by the opportunity to increase revenues and/or conform to meat count regulations. However, weight changes on deck can also be the result of legitimate efforts to minimize temperature abuse by chilling scallops in an ice:sea water mixture. Consequently, both motives have to be considered in evaluating the effects of various deck treatments on weight changes. The use of ice:fresh water or STP as a deck treatment can be considered as an intentional effort to gain weight and scallop quality may be only a secondary consideration. The use of an ice:seawater deck treatment with the intent to minimize temperature abuse and maintain good scallop quality has an additional benefit, namely the associated weight gains documented in this study. Holding scallops in warm seawater or dry imparts no benefits to the aspects of product quality or an increase in revenues as the result of an increase in weight.

Analyses of weight changes associated with deck treatments suggest that it may be financially advantageous to subject harvested product to one or more of the deck treatment methods. However, financial gains may be short lived or not significant if deck treatments adversely affect quality. Buyers may discount the ex-vessel price or refuse to buy scallops that have been abused.

Evaluation of the economic aspects of the various deck treatments is limited to evaluating changes in landings and revenues associated with deck treatment. The analysis assumes that 800 pounds of meats are harvested per day, and the ex-vessel price equals $4.35 per pound--the current average price in the mid-Atlantic region.

Given daily harvests of 800 pounds and 10, 14, and 18 fishing days, the largest gains in weight are calculated for product treated with ice and freshwater (Table 4). The second highest gains are calculated for product treated with ice and seawater except for the 18 fishing days in which STP was used at-sea. Although ice and freshwater provide the largest weight gains, two aspects need to be considered in the economic evaluation: (i) weight changes for product treated with ice and freshwater are extremely variable--the coefficient of variation was 142.6 and 223.2 for August 1990 and 1989, respectively; (ii) buyers frequently reduce or discount the ex-vessel price for scallops that have been subjected to excessive soaking in ice and freshwater.

Consider the two periods of August 1989 and 1990 and the current market price of $4.35 per pound. Vessels fishing 14 and 18 days and harvesting 800 pounds per day would realize revenues of $55,467 and $75,899 for product treated in ice and freshwater (Table 5). These vessels would harvest 11,200 and 14,400 pounds. In comparison, product treated with ice and seawater would yield approximately $54,201 and $73,054 for 14 and 18 fishing days, respectively. The marginal revenues--incremental revenues from a given deck treatment--of using ice and freshwater would be approximately $5,968 and $13,781 for the August 1989 and 1990 periods, respectively. The marginal revenues of using ice and seawater would be $3,410 and $7,891 for the two periods.

Scallop meats treated with seawater would generate approximately $51,713 and $63,810 in ex-vessel revenues for 14 and 18 fishing days, respectively. On deck weight gains are zero for seawater and dry treatments; all gains are associated with stowage and are approximately equal.

Table 4. Harvests, landings, and changes in product weight associated with fishing days and deck treatment given daily harvest rate of 800 pounds

Trip and at-sea processing stage	Ice and sea water	Ice and fresh water	Sea water	Dry	STP
	----------Pounds of meats----------				
August 1989/14 fishing days					
Harvest	11,200	11,200	11,200		
Deck Treatment	784	1,372	0		
Stowage	476[a]	179[a]	688		
Landings	12,460	12,751	11,888		
June-July 1990/10 fishing days					
Harvest	8,000		8,000	8,000	
Deck Treatment	750b		0	0	
Stowage	285[b]		212	264[b]	
Landings	9,035		8,212	8,264	
August 1990/18 fishing days					
Harvest	14,400	14,400	14,400	14,400	14,400
Deck Treatment	1,814	3,168	0[d]	0[d]	2,232
Stowage	580	-120[c]	269[d]	269	286[d]
Landings	16,794	17,448	14,669	14,669	16,918

[a]Percent weight change from stowage is statistically different for each treatment.

[b]Percent weight change from stowage for ice and sea water is not statistically different than the weight change for dry.

[c]Statistically equals zero for days 4-18.

[d]Percent weight change from stowage is equal for sea water, dry, and phosphate.

Analyses of changes in weight suggest that the influence of deck treatment and stowage on ex-vessel revenue is greatly affected by the physiological condition of the scallop meats. However, use of ice and seawater appears to offer the most consistent or predictable weight changes.

The data generated from the shoreside processing experiments are presented as data based on average weight changes of 100 lbs. of sea scallop meats at harvest as a result of deck treatment and variable processing times with STP (Table 6).

This data does not separate the effects of stowage time but accounts for these changes as an estimated average over 18 days in stowage. Weight changes as a result of shoreside processing are calculated from weight change data at offloading. Consequently, weight changes can be tracked for each stage of processing.

The results indicate certain benefits between processed and unprocessed scallops relative to weight loss during thawing and cooking. For all deck treatments, and for unprocessed scallops there was a significant weight loss from harvest to cooked product. The benefits or changes based upon using a 9 hour, 15 hour or 21 hour process time are not fully realized by extending processing time beyond 9 to 12 hours. The changes observed for weight gain, thaw loss and cooked loss by more than doubling processing time are not large and in many cases not significant. Processors therefore should establish a set of objectives based on desired levels of weight uptake, thaw drip loss and, cooked loss in conjunction with plant processing capacity schedule, and buyers' preferences. For example, processing times to achieve different results vary widely and some set of predetermined objectives should be in place before processing begins (Table 7).

Results indicate that deck treatment had generally minor effects on processing parameters. Weight gain, thaw loss and cooked loss expressed as percent (%) change were remarkably consistent. However, weight changes expressed as net of harvest (which incorporates changes due to deck treatment and stowage) were significantly different relative to the type of deck treatment employed. Essentially, weight changes realized on the vessel carried through to the results of processing. As anticipated, processing weight gains for fresh scallops were significantly greater than for scallops from various deck treatments utilizing ice or freshwater. However, processing gains were also greater than weight gains for scallops from deck treatments not using ice or freshwater (dry and seawater deck treatments). This could very well be due to the fact that these scallops were severely wafered.

Table 5. Ex-vessel revenues given different levels of fishing days, deck treatments, and an ex-vessel price of $4.35 per pound

Trip and at-sea processing stage	Ice and sea water	Ice and fresh water	Sea water	Dry	STP
	----------Ex-vessel revenues----------				
August 1989/14 fishing days					
Harvest	48,720	48,720	48,720		
Deck Treatment	3,410	5,968	0		
Stowage	2,071[a]	779[a]	2,993[a]		
Landings	54,201	55,467	51,713		
June-July 1990/10 fishing days					
Harvest	34,800		34,800	34,800	
Deck Treatment	3,262		0	0	
Stowage	1,240[b]		922	1,148[b]	
Landings	39,302		35,722	35,948	
August 1990/18 fishing days					
Harvest	62,640	62,640	62,640	62,640	62,640
Deck Treatment	7,891	13,781	0	0	9,709
Stowage	2,523	-522[c]	1,170[d]	1,170[d]	1,244[d]
Landings	73,054	75,899	63,810	63,810	73,593

[a]Percent weight change from stowage is statistically different for each treatment.

[b]Percent weight change from stowage for ice and sea water is not statistically different than the weight change for dry.

[c]Statistically equals zero for days 4-18.

[d]Percent weight change from stowage is equal for sea water, dry, and phosphate.

Table 6. Estimated average weight change in lbs. and percent(%) based on 100 lbs. of scallop meats at harvest as a result of at-sea deck treatments and shoreside processing.

	Fresh	Ice and Seawater	Seawater	Dry	Ice and Freshwater	STP
Harvest	100	100	100	100	100	100
Deck Treatment	---	112.6 (12.6)	100.0 (0)	100.0 (0)	122.0 (22.0)	115.5 (15.5)
Stowage	---	116.6 (3.6)	102.1 (2.1)	101.8 (1.8)	121.6 (-.03)	117.6 (1.9)
Off-load Total	---	116.6 (16.6)	102.1 (2.1)	101.8 (1.8)	121.6 (21.2)	117.6 (16.5)
No Process[1]	100.0	116.6	102.1	101.8	121.6	117.6
Thawed	95.7 (-4.3)	107.6 (-7.7)	97.7 (-4.3)	98.6 (-3.1)	108.7 (-10.6)	110.5 (-6.0)
Cooked	78.8 (-17.6)	88.6 (-17.6)	83.0 (-15.0)	85.6 (-13.2)	87.2 (-19.7)	85.3 (-22.8)
Net of Harvest[2]	78.8 (-21.2)	88.6 (-11.4)	83.0 (-17.0)	85.6 (-14.4)	87.2 (-12.8)	85.3 (-14.7)
9 Hour Process	113.6 (13.6)	127.2 (9.1)	111.2 (8.9)	111.6 (9.6)	133.8 (10.1)	128.6 (9.4)
Thawed	108.1 (-4.9)	125.6 (-1.2)	110.0 (-1.1)	110.7 (-0.8)	131.2 (-1.9)	125.2 (-2.6)
Cooked	91.6 (-15.3)	108.0 (-14.1)	97.4 (-11.4)	97.4 (-12.0)	115.2 (-12.2)	99.4 (-20.6)
Net of Harvest	91.6 (-8.4)	108.0 (8.0)	97.4 (-2.6)	97.4 (-2.6)	115.2 (15.2)	99.4 (-0.6)
15 Hour Process	118.2 (18.2)	129.5 (11.1)	115.0 (12.7)	114.4 (12.4)	134.4 (10.5)	131.0 (11.4)
Thawed	111.0 (-6.1)	126.9 (-2.0)	114.0 (-0.9)	113.4 (-0.9)	130.6 (-2.8)	126.4 (-3.5)
Cooked	90.2 (-18.7)	109.6 (-13.6)	99.2 (-13.0)	101.2 (-10.8)	113.6 (-13.0)	108.8 (-13.9)
Net of Harvest	90.2 (-9.8)	109.6 (9.6)	99.2 (0.8)	101.2 (1.2)	113.6 (13.6)	108.8 (8.8)
21 Hour Process	121.6 (21.6)	133.2 (14.2)	118.6 (16.5)	119.0 (16.9)	140.8 (15.8)	136.6 (16.2)
Thawed	111.0 (-8.7)	129.6 (-2.7)	116.3 (-1.9)	116.7 (-1.9)	135.6 (-3.7)	131.8 (-3.5)
Cooked	87.4 (-21.3)	111.6 (-13.9)	98.2 (-15.5)	101.0 (-13.4)	117.8 (-13.1)	111.3 (-15.6)
Net of Harvest	87.4 (-12.3)	111.6 (11.6)	98.2 (-1.8)	101.0 (1.0)	117.8 (17.8)	111.3 (11.3)

[a]Processing times of 0, 9, 15, and 21 hours in 2.5% STP and 1% NaCl solution by weight.

[b]Net of harvest equals difference between cooked and harvested weight.

Table 7. Estimated processing time (hours) to achieve maximum weight gain, minimum thaw drip and cooked loss (%). Estimates are based on landed product for each deck treatment and independ of time stowed. Observations are not withstanding organoleptic evaluations. Scallop meats processed in a solution of 2.5% STP and 1% NaCl by weight.

Deck Treatment	Maximum Weight Gain (Hours)
Ice:Seawater	28.6
Ice:Freshwater	26.4
Dry	27.9
Phosphate	25.3
Seawater	28.1
Fresh	26.2

	Minimum Thaw Drip Loss (Hours)
Ice:Seawater	16.0
Ice:Freshwater	16.9
Dry	15.7
Phosphate	15.2
Seawater	14.8
Fresh	3.0*

	Minimum Cook Loss (Hours)
Ice:Seawater	14.6
Ice:Freshwater	17.2
Dry	10.9
Phosphate	18.8
Seawater	11.0
Fresh	3.0-9.0*

*from observed data.

ACKNOWLEDGEMENTS

This work is a result of research sponsored in part by the Gulf and South Atlantic Fisheries Development Foundation. Additional support was provided by the Virginia Sea Grant Marine Advisory Program and the Virginia Institute of Marine Science, College of William and Mary. This project could not have been successful without the outstanding support and cooperation of the captains and crews of the F/V Ms. Margie, Newport News, Virginia, the F/Vs O'Neal's Pride and Carolina Breeze, Seaford, Virginia and the F/V Neptune, Cape May, New Jersey. In addition, the authors express appreciation to the East Coast Fisheries Association and Seaford Scallop Company for their cooperation and logistical support for vessel operations.

BLUE CRAB PROCESSING WASTE MANAGEMENT: IN-PLANT METHODS

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Landfill space in Florida is rapidly being ex-hausted. Since blue crab processing wastes (by-products) are mostly organic and rapidly putrefy, landfills must handle these wastes with extra soil cover and chemicals to control odor. In the case of Wakulla County, Florida, where thirteen processing plants are located, this represented about 17% of that counties' total waste volume, consuming approximately 25% of their solid waste budget (3). In 1988-89, thirty-one of the forty-five Florida blue crab processors were located in six counties (Franklin, Wakulla, Leon, Taylor, Dixie and Levy) on Florida's big bend Gulf coast (Figure 1). Most of these plants are relatively small waste generators, producing on the average 1.5 - 2.5 cubic meters/day (Wakulla Co./Sea Grant survey, 1988). However, all of them were using county landfills for disposal.

In the near future, landfills will not be available for disposal of seafood processing wastes or by-products. In 1988, the Florida Legislature enacted the Solid Waste Management Act (Chapter 88-130). Section 16 (also Section 403.8075, Florida Statutes) states that commercial food processors "may not dispose of any animal parts, fats, by-products, waste products ... in landfills unless approved by the department" (FL Dept. of Environmental Regulation). This legislation also requires landfills to reduce waste volume by thirty percent by the end of 1994. Therefore, it is likely that crab processing by-products will be prohibited from landfills in the next five years, due to their potential value as fertilizer, animal feeds and nematicides (4, 1, 6). However, these options, among others, have not been fully explored and utilized in Florida.

The goal of this project was to determine potential options for blue crab processing plants to better manage, and utilize, processing by-products at the plant. To accomplish this goal this project had the following objectives.

1. Determine the feasibility of handling crab processing by-products at the plant via (a) grinding and extruding (b) compacting, or (c) anaerobic bioconversion.

2. Evaluate all three methods with respect to volume reduction, storage and handling within current plant operations.

3. Evaluate the fluids from compaction for disposal into septic systems or waste water treatment facilities.

MATERIAL AND METHODS

1. Wet extrusion

Two hundred pounds of blue crab processing by-products were ground in a Hobart (2 hp) sausage grinder and a Fitz-Mill grinder final to a particle size of 1-2 mm. This material was frozen and shipped to the Kansas State University Extrusion Feed Lab in Manhattan, Kansas. The ground crab material was mixed in a ribbon mixer with soybean meal (48% TKN), potato starch and herring oil in two formulas: 1) 45% crab, 41% meal, 10% potato starch, 4% oil, and 2) 35% crab, 60% meal, 5% potato

Figure 1. Big Bend Counties with Blue Crab Processors

starch, and no oil. A Wenger TX-52 wet extruder was used to produce a sinking feed pellet (7 mm dia. x 13 mm long). Feed pellets were run through a Wenger double pass burner dryer.

Approximately 10-15 pounds of the feed pellets, with herring oil, was sent to scientists experimenting with culture of crustacean species. These were spiny lobster, Panulirus argus, (Harbor Branch Oceanographic Institute), shrimp, Penaeus vannamei, (Florida International University), and freshwater crayfish, Procambarus peninsulanus, (University of Florida). These samples were sent to examine the acceptability of the crab containing feed by the above cultured animals.

2. Compacting

A simple box compactor, used by shrimp packers in Biloxi, Mississippi, was custom built for the blue crab demonstration and installed at the Gulfstream Crab Company plant in Chiefland, Florida. The compactor unit consisted of a rectangular box (2'x2'x3') with a movable end pushed by an 18-stroke ram (2500 psi operating pressure). The end opposite the ram was a hinged door to allow the compacted material to be removed. Holes were made in the bottom of the box to allow the compressed fluids to flow downward into a catch basin, and the top had a 1'x2' opening for loading. A heavy metal screen with 1 mm mesh matting was built to insert into the compactor for moisture removal trials.

Compacting trials were run to examine moisture and volume reduction and cohesion of compressed crab scraps in the following comparisons.

a. With screen versus without the screen
b. Ground (using hammermill) versus unground
c. Variable compression times (1 to 8 minutes)

Between .06 and .08 cubic meters (15 to 20 gallons) of crab by-product was weighed and poured into the compactor box. Top compacting pressure of 600 psi was maintained for the trial times of 1:00 to 8:00 minutes. Following the sessation of pressure, the fluid volume was measured and the compacted crab scraps were pushed out of the box via the ram into a trash basket. This material was poured back into a 20 gallon barrel (.08 m3) for weighing and volume estimate.

3. Anaerobic bioconversion

Raw crab scraps and compressed fluids from the compactor trials were collected and evaluated in the following stepwise series of studies:

a. physical and chemical analyses of the wastes;
b. assessment of the ultimate conversion and rate of conversion via the biochemical methane potential (BMP) assay;
c. process development and optimization using bench-scale digesters.

Through this approach, results generated could be used as a basis for systems calculations and decision-making for incorporation into future design, and operaton of demonstration and full-scale bioconversion facilities.

Where appropriate, analysis procedures outlined in standard methods (2) were used to determine total and volatile solids, total nitrogen (TKN), phosphorus (P) and chemical oxygen demand (COD) of the raw waste. Ammonia-kjeldable concentration was determined by steam distillation followed by titration with standard sulfuric acid, using boric acid as an indicator.

The ultimate anaerobic biodegradability of the waste was determined by BMP assay, conducted in accordance with a modified procedure of Owens et al. (7). In this assay, approximately 180-220 mg of the substrate (waste) volatile solids were placed in 250-mL Wheaton serum bottles and incubated at 35 C, following addition of an active seed inoculum and defined nutrient medium. Measurement of gas production and gas composition were made at regular intervals until gas production ceased, using a measured syringe.

The bench-scale reactor used in this study was a non-mixed, 20-liter capacity vertical flow system equipped for leachate recirculation. The body of the reactor comprised an epoxy-coated 8-in dia. carbon steel barrel, 30-in long, fitted with carbon-steel base, top flange and head plate. The barrel is slotted to accommodate a 1-in wide sight glass for the full height of the reactor. Inside the reactor are perforated substrate support and leachate distribution plates at the bottom and top respectively. The substrate is placed on the lower plate, and the upper plate placed on top of the substrate.

The head plate of the reactor is fitted with 3/8-in dia. ports controlled by needle valves, for gas collection, head space gas sampling, and head space purging. Feed and leachate recirculation ports complete the openings in the head plate. The bottom plate is also equipped for leachate recirculation. The reactor is operated at a controlled temperature of 35 $\pm$ 1 C, heating being accomplished by two external heater strips. A storage reservois and leachate recirculation pump, temperature controller, and manometric Berlometer (TM) gas meter complete the bench-scale equipment used.

Gas composition analysis was performed on a Fisher Model 1200 Gas Partitioner chromatograph. For this determination, a 30-mL aliquot of gas was injected manually into a sampling loop from which 0.25 L was passed through two stainless steel columns, one a 6-1/2 ft. x 1/8-in. OD column packed with 80/100 mesh Poropak Q used for the separation and detection of CO2, N2 and CH4. Helium was the carrier gas, maintained at 30 mLs/min. Determination of the separated gases was accomplished by comparison with standard gases using thermal conductivity detectors. This instrument was used with a Perkin-Elmer LC-100 Laboratory Computing Integrator.

Volatile fatty acids were analyzed using a Shimadzu 9-AM gas chromatograph equipped with a flame ionization detector. Samples and standards were acidified with 20% (V/V) H3PO4 to a final concentration of 2% (V/V), centrifuged, and the supernatant transferred to 2-mL vials and preserved for analysis. Chromatographic separation and analysis were accomplished using a glass column packed with 10% SP-1000 on 100/120 Chromosorb. Analyses were conducted under the following conditions: N2 carrier gas 30 mL/min., h2 30 mL/min., air 25 mL/min., injector temperature 160 C, detector temperature 200 C, and oven temperature 140 C. Baseline separation of acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids was accomplished by this procedure with 12 minutes. Acid concentrations were computed by a Perkin-Elmer LC-100 Laboratory Computing Integrator connected to the gas chromatograph.

RESULTS

1. Wet extrusion

Of the two formulations, the one which contained herring oil flowed the best off the extruder. Without herring oil, the pellets did not form properly and not enough pellets were produced for feeding trials. All formulations were extremely high in moisture (mean = 19.2%), even after two passes through a forced air dryer. The pellets began to mold within fourteen days and also began to float, approximately 50 percent. One set of feeding trials was attempted with spiny lobsters at Harbor Branch Oceanographic Institute (Bill Lellis, personal communication). The lobsters readily accepted the pellets, however, the pellets tended to break apart before they could be totally consumed. This led to tank fouling and water quality problems. The remaining pellets had become too moldy for feeding trials with shrimp and crayfish.

2. Compacting

Volume reduction of crab scraps was highest via grinding, achieving 42.5% reduction. Compacting without the screen achieved the best volume reductions for both ground and unground crab scrap (25.0% and 28.6%, respectively). However, volume reductions via compacting were subjective estimates only, whereas the weight reductions, were measured using a digital scale.

Weight reduction, representing the fluid removed, was highest for ground by-product without the screen (20.8%). However, there was only one observation in this category, because it was found that using the screen provided a more even compaction of the material and was determined to be the preferred method for future trials. Of the remaining trials with the screen, the highest weight reduced was at 1:00 with ground material (12.3%). However, there appears to be a trend of increasing percentage of weight lost with increasing time of compression in the remaining trials for the ground crab scraps. Therefore, this high value at only one minute may be due to the high variability in moisture content of the raw scraps. The unground scraps, appeared to have no correlation of weight lost with time of compression, indicating a high degree of variability in moisture content of the raw crab scraps.

3. Anaerobic bioconversion

For this study, the total waste stream (TWS) and the liquid fraction (LF) were analyzed separately. Evaluation of these results are indicated below. Physical analyses of the samples indicated an average total solids (TS) concentration of 33% for the TWS, and a volatile solids (VS) concentration of 50% (day weight) of the TS. This indicates a very high ash content (average 50%) for this waste stream. In the case of the LF the comparable results were 10% and 72% respectively.

Chemical analyses indicated COD value of 256 g/L for the liquid fraction, highlighting the pollutional potential of this waste stream. For the TWS, the TKN value was 2.01%, NH3-N was 1.96%, and total phosphorus 0.09%. In the case of the LF, the TKN was 1.5%, and the NH3-N was 0.3%.

Additional anaerobic bioconversion studies are being continued with Sea Grant funds, and final data are not available at this time. Unadjusted preliminary results from the BMP assay indicate a methane yield of approximately 0.3 m3/kg for the liquid fraction, and 0.1 m3/kg for the TWS. The gas being produced is a high grade gas composition at 65% methane, as compared to solid waste garbage which runs at 50-55% methane.

As for total volume reduction from this method, it is estimated that between 30 and 50 percent reduction can be expected.

DISCUSSION

The best method examined for volume reduction at the plant level is grinding. Of the type grinders used the hammermill would be the easiest to use. These are already being used in those plants that have claw machines. The sausage type grinder, or Fitz-Mill would take more manpower and time to operate, although can produce a finer particle size, hence a slightly greater volume reduction. If these crab by-products were to be used in an extrusion process for making feeds, the finer particle size would also be preferred. Other types of grinders should be examined, such as cork screw types, as alternatives.

Accurate volume reductions were difficult to determine following compacting due to the type of compactor used. However, despite the low pressure applied during compacting (600 psi), it was noted that the ground crab by-products held their compacted form much better than the raw crab scraps. Design changes would be necessary to improve the compacting capability and maintenance of the

compacted block of crab scraps, yet, the potential is there to reduce the volume up to twenty-five percent via compacting leaving the fluids for anaerobic bioconversion (Table 1).

TABLE 1. Compacting Trials: Mean Percent Reduction By Weight in Lbs. (no. of trials)

	UNGROUND		GROUND	
TIME (MINS.)	W/O SCREEN	W/SCREEN	W/O SCREEN	W/SCREEN
1:00	4.9(1)	--	--	12.3(1)
2:00	6.2(3)	6.7(1)	--	8.1(2)
3:00	10.1(1)	--	--	--
4:00	8.3(2)	8.8(1)	--	9.4(3)
5:00	10.0(1)	--	--	--
6:00	7.8(2)	8.7(1)	20.8(1)	10.6(3)
8:00	--	11.3(1)	--	10.5(2)

Of the methods examined, compacting and grinding are the methods that could be implemented immediately using existing manpower at the plant and would not take a large amount of training to put into practice. However, these methods do not eliminate the by-products, only change their form and make them easier to store.

Wet extrusion and anaerobic bioconversion both need additional product development research before they can be implemented at the plant level. These methods do utilize the by-products, however, and can produce economically viable end-products that need to be examined further. Feed pellets made of crab by-products has potential for finfish culture for species such as red drum and tilapia hybrids which need the crustacean proteins in their diet for the red color. Unfortunately, the pellets broke apart too quickly in seawater for crustaceans to eat, also creating water quality problems; therefore, these feeds may not be suited for crustacean culture. Nutritional studies and diet analyses would be necessary to determine the appropriate ingredient ratio for final product production.

The biogas area has the greatest potential since this process could address the energy uses of the crab processing plant, as well as the by-product disposal. It does not require additional ingredients, as does extrusion. It is highly recommended that additional work be funded to examine this method to its fullest potential, such as with anaerobic wastewater treatment systems (Ellers, 1990). Several modifications to improve compaction would also enhance the development of biogas generators, since the fluids were found to be more useful in this process than the solids.

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NUTRIENT AND CHEMICAL COMPOSITIONS OF ATLANTIC SNOW CRAB OFFALS

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An abundance and variety of crab species exists off the eastern coastlines of Canada. Nearly 25% of the total Canadian catch of snow crab (Chionoecetas opilio) in Atlantic Canada comes from Newfoundland coastal areas. Only 20-30% of the weight of crab is processed for human food consumption. The remaining 70-80% is generally discarded by hauling into the ocean or in-land dumping.

Slow biodegradation of shell discards from snow crab fishery as well as their growing quantities has become a major concern for processors and environmentalists. However, by-products of crab processing could be used for the preparation of value-added products such as chitin and carotenoid pigments.

This study was undertaken to investigate the chemical composition and nutrient value of snow crab processing discards in Newfoundland. Mineral, amino acids, fatty acids and carotenoids compositions of crab offal are also reported in this paper.

MATERIALS AND METHODS

Raw materials

Different parts of shell wastes, separated during the commercial processing, of snow crab (Chionoecetas opilio) at Quinlan Brothers Plant, Bay de Verde, Newfoundland. The shell wastes were ground using a Waring blender (Model 33 BL 73, Dynamics Corp., New Hartford, USA), vacuum packed in plastic bags, and kept frozen at -20•C until used.

Methods

Moisture content in shell wastes was determined by oven drying of about 1 g of the sample at 105•C until a constant weight was obtained (1). Total nitrogen and ash contents were determined using the AOAC (1) methods. Protein content in shell wastes was determined after 2 hr extraction of a known amount of sample with 2.5% KOH solution at 90•C. The protein extract was separated from insoluble matters in KOH using a coarse glass-sintered funnel and then was diluted to 250 ml with water. The protein content of the solution was determined according to Kjeldehl (1) method. Residual protein in chitin was extracted by base followed by quantification according to Lowry et al. (5).

The content of chitin in the shells was determined by demineralization of 1 g of shells, deproteinized with 5% KOH solution for 2 hr at 100•C, in 20 ml 5% HCl for 2 hr at room temperature. Chitin was separated on a coarse glass-sintered funnel, washed with water to a neutral pH and then 3 x 25 ml of acetone followed by oven drying at 105•C

The content of amino acids was assayed in proteins extracted from shell waste and freeze dried. The individual amino acids were generally determined after their hydrolysis with 6M HCl for 24 hr at 110•C (2). The amino acids were then separated and identified using a Beckman 121 MB amino acid analyzer. Tryptophan was determined separately according to method of Penke et al. (6). Sulfur-containing amino acids were determined after performic acid hydrolysis.

Total lipids were extracted from shell wastes according to the method of Bligh and Dyer (3) using a chloroform-methanol-water mixture.

Total carotenoids in the offal were determined in the lipid fraction isolated according to Bligh and Dyer (3) by the method of Saito and Regier (7). The concentration of carotenoids was calculated from the equation.

$$CPPM = \frac{A_{468nm} * V_{extract} * Dilution\ factor}{0.2 * W_{sample}}$$

where 0.2 is the A_{468nm} of 1 μg/ml of a standard astaxanthin solution.

Carotenoids fraction were then separated using thin-layer chromatography. Silica gel TLC plates (Analtech, Inc., Newark, Delaware) were spotted with the carotenoid extracted in chloroform and were then developed using a benzene-petroleum ether-acetone (10:3:2, v/v/v) mixture. Each component was then extracted 3 times in 3 ml of chloroform. The absorbance of carotenoids in the centrifuged solution diluted to 10 ml was then read on a Beckman DU-8 spectrophotometer. Concentration of carotenoids was then calculated using linear equations of standard curves prepared for individual carotenoid standards in chloroform.

Mineral composition was determined after ashing and residue was used for the analyses after acid dissolution by the atomic absorption spectroscopy. Minor mineral constituents were determined by an X-ray fluorescence method using the facilities of the Department of Earth Sciences and Centre for Earth Resources Research at Memorial University.

RESULTS AND DISCUSSION

The proximate composition of Snow Crab (Chionoecetas opilio) shells waste is given on Table 1. The protein content in shell discards from whole crabs was about 11.0% and ranged from 8.0% in legs to 12.7% in shoulders. Amino acids composition of discard proteins presented in Table 2 show that crab shell proteins contain all essential amino acids. However, the amount of leucine, isoleucine, lysine, and methionine were lower as compared with the FAO/WHO standards. For this reason, crab shell wastes should be supplemented with other proteins, if they were to be used for preparation of animal feed. Among amino acids, aspartic and glutamic acids were major components of the shell discard proteins.

The lipids of crab shell wastes ranged from 0.1% in legs to 1.4% in backs. The highest content of lipids and high content of proteins in backs as compared with shells from other parts of crab were due to the presence of higher amounts of residual meat. The major fatty acids of lipid components of crab wastes are summarized in Table 3. Monounsaturated fatty acids comprised about 51% of the total lipids. Additionally, crab discard lipids contained 24.7% polyunsaturated and only 13.7% of saturated fatty acids. Almost 74% of polyunsaturated fatty acids fraction were of the ω-3 type. The significance of these fatty acids in the diets of salmonids has been demonstrated by Yu and Sinnhuber (8). These investigators showed that certain physiological changes in rainbow trout, such as increased liver respiration rate, lower hemoglobin content and increased content of water in the muscles were related to the dietary deficiency of these acids. Poor feed conversion was also attributed to the same factor.

Table 1. Proximate Composition of Snow Crab Offal.[a]

COMPONENT %	SHELLS FROM WHOLE CRAB	FROM DIFFERENT PARTS OF CRAB SHELLS	
		Minimum	Maximum
Moisture	42.5	29.7	48.6
Protein	11.0	8.0	12.7
Lipid	0.5	0.1	1.4
Ash	17.6	15.7	20.5
Chitin, on dry basis	26.0	18.7	32.2
Carotenoids, on dry basis (μg/g)	53.1	16.4	139.9

[a]Results are mean values of at least a dozen determinations.

Table 2. Amino Acid Composition of Snow Crab Offal Proteins.[a]

AMINO ACID	g/16g NITROGEN	FAO/WHO STANDARDS
Alanine	6.83	
Arginine	6.66	
Aspartic Acid	11.75	
Cysteine	0.52	
Glutamic Acid	11.08	
Glycine	6.28	
Histidine	3.58	
Isoleucine	2.67	4.0
Leucine	5.14	7.0
Lysine	2.51	5.5
Methionine	1.93	3.5[b]
Phenylalanine	5.38	6.0[c]
Proline	5.64	
Serine	4.99	4.0
Threonine	4.74	1.0
Tryptophan	0.78	
Tyrosine	6.02	
Valine	7.07	5.0

[a]Results are mean values of 3 replicates.
[b]Methionine and Cysteine.
[c]Phenylalanine and tyrosine.

Table 3. Fatty Acid Composition of Snow Crab Offal Lipids.

FATTY ACID	% OF TOTAL LIPIDS
14:0	1.0
14:1	0.4
16:0	9.4
16:1 n-7	4.1
18:0	3.3
18:1 n-9	25.8
18:2 n-6	3.7
20:1 n-9	13.3
20:2	1.3
20:4 n-6	2.0
22:1 n-11	7.4
20:5 n-3	12.7
22:5 n-3	1.4
22:6 n-3	5.6

The major mineral components of crab discards were calcium and phosphorus in amounts of 14.95, and 2.88%, respectively (Table 4). Moreover, crab shells contained Na, K, Mg, and Sr in quantities of less than 1% of the shells. Other minerals given in Table 4 were present only in trace quantities.

Table 4. Mineral Composition of Snow Crab Offals.

MINERAL (CONCENTRATION)	CONTENT
Ca (%)	14.95
Na (%)	0.95
K (%)	0.25
Mg (%)	0.92
P (%)	2.88
Sr (%)	0.23
Mn (ppm)	13
Fe (ppm)	70
Cu (ppm)	6
Zn (ppm)	17
As (ppm)	27
Ba (ppm)	20

The total content of carotenoids in the shell discards was 5.28 mg%. Crab backs contained the highest amount of pigments when compared with other segments of the waste. The distribution of the individual pigments in the waste is presented in Table 5. Astaxanthin diester was the major carotenoid present in the amount of 19.7 μg/g in shells. Astaxanthin and its monoester were present in smaller amounts of 16.2 and 1.8 μg/g, respectively.

Table 5. Carotenoid Composition of Snow Crab Offal.

CAROTENOID	μg/g SAMPLE[a]
Astacene	1.5
Astaxanthin	16.2
Astaxanthin Monoester	1.8
Astaxanthin Diester	19.7
Canthaxanthin	0.1
Lutein	5.6
Zeaxanthin	1.9
Unidentified	0.1

[a]On dry basis.

The proximate composition, amino acid profile, fatty acid composition, and carotenoid content of crab discards indicate that these offals are suitable for inclusion as a dietary component for animal feeds including fish feed formulations. However, the quality of its proteins suggests that it should be used in combination with other proteins so that a balanced dietary formulae could be prepared. Crab discards could also be used as an excellent source for production of chitin with a yield of about 84%. The characteristics of chitin produced under optimal conditions of deproteinization with 2% KOH solution (2 hr, 90°C, with a shell to KOH solution ratio of 1:20, w/v), and demineralization with 2.5% HCl solution for 1 hr at 20°C with a solid to solvent ratio of 1:10 (w/v) are provided in Table 6. We consider this chitin to be of technical/standard grade (4).

Table 6. Characteristics of Snow Crab Chitin.

SPECIFICATION	CONTENT/QUALITY
Moisture (%)	0.60
Nitrogen (%, on dry basis)	6.42
Ash (%, on dry basis)	0.10
Protein Residue (%, on dry basis)	0.49
Lipids (%, on dry basis)	0.00
Specific Gravity (16-35 mesh, g/cm^3)	0.17
Hunter Values: L	92.62
a	3.54
b	4.06

ACKNOWLEDGEMENTS

We are grateful to the Department of Fisheries and Ocean, Newfoundland Inshore Fisheries Development Agreement for financial support.

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THE EFFECTIVENESS OF RECOMMENDED FAT-TRIMMING PROCEDURES ON THE REDUCTION OF PCB AND MIREX LEVELS IN LAKE ONTARIO BROWN TROUT (SALMO TRUTTA)

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INTRODUCTION

Concern over detectable levels of organochlorine contaminants in Lake Ontario fish flesh has existed since at least the mid-1970's (20). In New York's Great Lakes waters, levels of polychlorinated biphenyls (PCB's) and most other pesticides and environmental contaminants have declined in fish during this time (3, 18, 8, 17) In 1989, however, controversy over the risk associated with eating Great Lakes fish was renewed with the issuance of a fish consumption health advisory by a private environmental group, the National Wildlife Federation (6, 12). This non-governmental advisory suggested that the health risk from consuming Great Lakes (Lake Michigan) fish greatly exceeded the risk estimated by state and provincial health agencies in the Great Lakes Basin.

One response to this controversy by the angling and fish-consuming public has been renewed interest in the effectiveness of certain fish trimming and preparation techniques in reducing the contaminant burden in the edible portion of Great Lakes fish, particularly the more popular but more contaminated salmonid species. A series of earlier investigations found that organochlorines were concentrated in the fattier portions of fish, and therefore could be removed by using special fat-trimming and cooking methods (14, 19, 10, 15, 16, 24, 5, 9, 23).

Because of their well-conceived experimental research design, which analyzed and compared contaminant levels in both pre- and post-trimmed fillets from the same fish, and their focus on some pervasive chemical contaminants and commonly caught species, a series of studies by Skea et al. (15, 16) were at least partly responsible for the removal of a ban on possession of Lake Ontario salmonids in 1978, and led to the direct mention of trimming recommendations in New York State's fish consumption health advisory and to a separate New York State Department of Environmental Conservation (NYSDEC) brochure on trimming methods (7, 13). The research led by Skea is still the most frequently cited on the topic in the literature, and for over a decade has remained the most robust scientific analysis of the effectiveness of fillet trimming in reducing organochlorine contaminant burdens in anadromous species in general, and in Great Lakes (Lake Ontario) salmonids and smallmouth bass in particular.

To date, however, no replicates of the Skea studies have been carried out to validate their findings. Furthermore, significant declines in many environmental contaminants such as PCB's and DDT have been observed in Great Lakes fish since the Skea studies were conducted, but no investigations have explored whether trimming techniques remain consistently effective when carried out on fish exhibiting relatively higher or lower initial contaminant levels.

PURPOSE

The primary purpose of this study was to verify the efficacy of the New York State recommended fillet-trimming technique on reducing organochlorine contaminant levels in the fillets of Lake Ontario brown trout (*Salmo trutta*). The hypothesis to be tested was that recommended fillet-trimming methods would significantly reduce PCB and mirex levels in the trout fillets, and that such reductions would approximate those observed in the Skea studies. PCB's and mirex were selected for the study because they are generally considered to be the contaminants of primary health risk concern in Lake Ontario fish, and represent two of the three contaminants analyzed in the Skea studies. Brown trout was selected as the test species because this popular and commonly caught Lake Ontario salmonid was the most thoroughly examined species in the earlier work by Skea et al. and would thus facilitate and strengthen comparison of results.

Beyond the attempt to verify the results of the Skea work, a secondary purpose of the study was to provide some basic answers to questions often posed by fish consumers and educators concerned with the fish contaminant issue. These questions included:

> What are the edible yields that result after recommended fillet-trimming procedures are carried out?
>
> What bearing might such variables as fish fat content, sex, age, physical condition, weight/length, and location of fish capture in Lake Ontario have on accumulated contaminant levels and on the effectiveness of trimming methods?

No attempt was made to determine the effects of cooking procedures on contaminant levels. Previous studies suggest that cooking trimmed fillets can further reduce contaminant residues, although the exact amounts and concentrations may vary widely depending on cooking method, fat loss and moisture loss during cooking (22, 14, 19, 15, 16, 24, 9, 1, 2).

METHODOLOGY

The sample

Thirty-six (36) Lake Ontario brown trout caught close to shore (depth < 20 feet) by rod and reel in late April/early May 1990 were collected by members of the Lake Ontario Charter Boat Association (LOCBA) for use in the study. The fish were taken from four geographically distinct locations dispersed along the Lake's southern shore, including Fair Haven, Sodus Point, Rochester/Irondequoit, and the Niagara River/Wilson areas (see Figure 1).

Each fish was wrapped individually in aluminum foil, frozen within 24 hours of capture, and held in frozen storage at 0°F-10°F. After transport to the preparation laboratory, the fish were thawed, numbered, and then 20 fish (5 from each of the 4 areas of capture noted above) were randomly selected for further preparation and analysis.

Sample Preparation

Total (whole) weight, total length, sex[1], age[2], and condition factor[3] of each of the 20 randomly selected trout were determined and recorded. Each fish was gutted, scaled, and then prepared by alternately selecting and removing its left or right side, resulting in an entire fillet portion (inclusive of skin and half rib-cage) labeled the "standard fillet." This standard fillet was described and used by Skea et al. in their earlier work, and is also currently utilized as the standard test sample by fisheries and health agency contaminant analysis programs in New York and the Great Lakes states. The opposing fillet of each fish, labeled the "trimmed fillet", was trimmed of the half rib-cage and fatty areas (skin, lateral line muscle and dorsal and belly muscle) according to methods developed by Skea et al. and recommended by the NYSDEC in its publication, Reducing Toxics: Fish Filleting Guide (13) (see Figure 2). Weights of the standard and trimmed fillets and all trimmings were taken and recorded. Standard and trimmed fillets for each fish were rewrapped in foil, numbered and labeled, and held in frozen storage (0•F-10•F) for further analysis.

Analytical Procedures

All chemical analyses were conducted at the Toxic Chemicals Laboratory at Cornell University. A total of 40 samples (1 standard and 1 trimmed fillet from each of the 20 fish) was analyzed for total lipid content and PCB and mirex residues. Each of the samples was ground, mixed, and sub-sampled, and the total lipid content was determined using Association of Official Analytical Chemists (AOAC) methods for measuring total lipid in fish and other foods. Each sample was then tested for PCB and mirex levels using standard isolation and analytical gas chromatographic techniques as outlined and described by the U.S. Food and Drug Administration (21).

Specifically, each sample was freeze-dried and subjected to Soxhlet lipid extraction with hexane for 8 hours. Compound isolation was carried out on a Florisil column according to procedures described by Mills et al. (11). Electron capture gas chromatography was employed for dissemination of PCB's (quantitated as Aroclor 1260) eluted from a 2m x 4mm column packed with 1.5%SP-2250 and 1.95%SP-2401 on 100/120 mesh Supelcoport at 200•C[4]. Mirex eluted from a 15m x 0.5mm fused silica megabore capillary column with DB-608 as the liquid phase operated at 200•C. The detection limits of the method were set at less than 0.6 ppm (dry weight) for Aroclor 1260 and less than 0.02 ppm (dry weight) for mirex.

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1 Sex of individual fish was determined from gonad inspection via ventral incision from vent to isthmus.

2 Scale samples were taken posterior to operculum and superior to lateral line. Scales were examined microscopically (40X) for age determination.

3 Condition factor (K) was determined for individual fish using the formula: $K = 100W \div L^3$, where W = total body weight and L = total length (4).

4 It should be noted that in the Skea studies, PCB's were quantitated as Aroclor 1254. Characterization of either Aroclor 1254 or 1260 is such that there is considerable overlap of the mixture of PCB compounds, so that the PCB compounds present in aged residues of Aroclor 1254 and 1260 are not significantly different. Aroclor 1260 most likely represents a better measure of the long-term, environmentally-weathered PCB residues. It was assumed that reference to Aroclor 1260 in this study would not compromise or preclude comparison of trimming results with those recorded by Skea.

RESULTS

Sample Characterization

All data measured, calculated, and recorded for the sample of 20 brown trout, including specimen identification number, total weight, total length, sex, age, physical condition, approximate date(s) of capture, and areas of capture are displayed in Table 1. With a mean weight of 1595.3 ± 189 grams (3.5 ± 0.4 lbs.) and weight range of 542-3637 grams (1.2 to 8.0 lbs.); a mean length of 465.1 ± 14.5mm (18.3 ± 0.57 inches) and length range of 362-610 mm (14.3 to 24.0 inches); 1:1 ratio of males to females; a mean age of 2.95 years (range: 2-4 years); and a condition factor of 1.45 ± 0.05, the sample was judged as very typical of the late April-early May springtime angler catch of brown trout common for the south shore of Lake Ontario (personal communication, L. Wedge, Senior Aquatic Biologist, New York State Department of Environmental Conservation, Region 7, Cortland, NY).

Fillet Yields

Yields, expressed as a percentage of whole fish weight, were calculated for standard fillets and trimmed fillets. Because each fish was to supply a standard and a trimmed fillet for analysis, fillet yields were extrapolated for each fish using the following formulas:

$$\text{Standard Fillet Yield} = \frac{\text{weight of standard fillet} \times 2}{\text{whole fish weight}} \times 100$$

$$\text{Trimmed Fillet Yield} = \frac{\text{weight of trimmed fillet} \times 2}{\text{whole fish weight}} \times 100$$

Yields, expressed as a percentage of standard fillet weight, were calculated for the removed skin, the removed trimmings (including excised rib cage and the dorsal, ventral and lateral line 1/4" trimmings as noted in Figure 2), and the fully trimmed fillet.

All yields are reported in Table 2. In general, standard filleting resulted in retention of an average of 58% of the whole fish, while trimmed filleting resulted in an average retention of 34% of the whole fish. An average of 40.2% of the standard fillet weight was lost when skin and fat were removed to produce a trimmed fillet. The skin portion represented an average of 26% of the standard fillet. Other trimmings averaged 14.2% of the standard fillet. This left 59.8% of the standard fillet remaining as edible flesh after trimming.

Fillet Fat Content and Contaminant Concentrations

Total fat content (g/100g wet weight basis) and PCB and mirex concentrations (ppm-wet weight basis) for the standard and trimmed fillets, as well as the calculated percentage reductions in fat, PCB and mirex levels due to trimming, are presented in Table 3. Standard fillets averaged 12.1% fat, 1.05 ppm PCB's, and 0.05 ppm mirex. Trimmed fillets averaged 4.9% fat, 0.57 ppm PCB's, and 0.03 ppm mirex. All PCB concentrations for both standard and trimmed fillets fell well below the current federal tolerance limit for PCB's (2.0 ppm), and only 2 of the 20 standard fillets were found to exceed the current federal tolerance limit for mirex (0.10 ppm).

Trimming Effectiveness and Fat-Contaminant Correlations

On average, approximately 62%, 46% and 44% of the total fat, PCB and mirex levels respectively were removed from the standard fillet when the recommended trimming procedure was used (Table 3). Comparision of the mean reductions using the paired t-test showed significant differences in total fat, PCB and mirex levels
($P \leq .001$) in trimmed fillets when compared to the corresponding standard fillet.

TABLE 1. CHARACTERIZATION OF SAMPLE

SPECIMEN NUMBER	AREA OF CAPTURE	CAPTURE DATE (S)	AGE (YEARS)	CONDITION (K)	SEX	WEIGHT IN GRAMS (LBS.)	LENGTH IN MM (INCHES)
1	Niagara/Wilson	4/25-4/28	3	1.26	F	1240 (2.7)	462 (18.2)
2	Niagara/Wilson	4/25-4/28	4	1.72	F	2742 (6.0)	542 (21.3)
3	Niagara/Wilson	4/25-4/28	3	1.26	F	1606 (3.5)	459 (18.1)
4	Niagara/Wilson	4/25-4/28	3	1.40	M	1558 (3.4)	479 (18.9)
5	Niagara/Wilson	4/25-4/28	2	1.55	M	962 (2.1)	396 (15.6)
6	Sodus Point	4/26-5/5	3	1.52	M	1751 (3.9)	487 (19.2)
7	Sodus Point	4/26-5/5	3	1.23	M	1156 (2.6)	455 (17.9)
8	Sodus Point	4/26-5/5	3	1.17	M	862 (1.9)	419 (16.5)
9	Sodus Point	4/26-5/5	3	1.51	F	1070 (2.4)	414 (16.3)
10	Sodus Point	4/26-5/5	3	1.73	F	1528 (3.4)	445 (17.5)
11	Fair Haven	5/12	2	1.38	F	840 (1.9)	393 (15.5)
12	Fair Haven	5/12	2	1.14	F	542 (1.2)	362 (14.3)
13	Fair Haven	5/12	3	1.37	F	1568 (3.5)	485 (19.1)
14	Fair Haven	5/12	3	1.78	M	2069 (4.6)	488 (19.2)
15	Fair Haven	5/12	3	1.70	M	1778 (3.9)	471 (18.5)
16	Rochester/Irondequoit	4/27-5/6	2	1.38	F	883 (2.0)	393 (15.5)
17	Rochester/Irondequoit	4/27-5/6	4	1.61	F	3637 (8.0)	609 (24.0)
18	Rochester/Irondequoit	4/27-5/6	4	1.57	M	3553 (7.8)	610 (24.0)
19	Rochester/Irondequoit	4/27-5/6	3	1.07	M	1121 (2.5)	472 (18.6)
20	Rochester/Irondequoit	4/27-5/6	3	1.16	M	1440 (3.2)	462 (18.2)
			$\bar{x}$=2.95 ± 0.14	$\bar{x}$=1.45 ± 0.05	M:F=1:1	$\bar{x}$=1595.3 ± 189	$\bar{x}$=465.1± 14.5

TABLE 2. FILLET YIELDS

SPECIMEN NUMBER	STANDARD FILLET YIELD	TRIMMED FILLET YIELD	SKIN YIELD	TRIMMINGS YIELD	TRIMMED FILLET YIELD
	(AS % OF WHOLE WEIGHT)		(AS % OF STANDARD FILLET WEIGHT)		
1	56.8	32.6	22.7	18.4	58.9
2	47.6	31.7	16.6	14.7	68.7
3	57.8	35.6	20.0	17.1	62.9
4	59.9	33.5	33.8	6.6	59.6
5	58.4	35.8	28.0	9.5	62.5
6	58.8	35.3	24.5	14.1	61.4
7	56.4	33.4	35.6	9.4	55.0
8	58.7	33.9	30.2	11.9	57.9
9	59.1	36.6	29.2	8.6	62.2
10	53.1	37.7	25.3	12.9	61.8
11	59.3	35.0	20.5	19.3	60.2
12	56.1	33.6	26.1	18.8	55.1
13	57.4	33.7	18.4	15.1	66.5
14	58.6	36.9	25.6	14.8	59.6
15	59.4	30.9	27.4	18.0	54.6
16	58.4	31.0	29.2	14.4	56.4
17	62.6	31.8	23.0	16.0	61.0
18	55.3	29.6	27.7	12.0	60.3
19	60.5	34.3	27.9	14.4	57.7
20	65.8	32.2	28.3	17.3	54.4
MEAN	58.0	33.8	26.0	14.2	59.8
RANGE	47.6-65.8	29.6-37.7	16.6-35.6	6.6-19.3	54.4-68.7

TABLE 3. FILLET FAT CONTENT AND CONTAMINANT CONCENTRATIONS (WET WEIGHT) AND PERCENT REDUCTION

SPECIMEN NUMBER	FAT CONTENT grams/100 grams			PCB CONCENTRATION ppm (ug/g)			MIREX CONCENTRATION ppm (ug/g)		
	STD	TRIMMED	% RED.	STD	TRIMMED	% RED.	STD	TRIMMED	% RED.
1	15.2	4.5	70.4	0.77	0.55	28.6	0.033	0.017	48.5
2	11.4	4.5	60.5	1.54	0.88	42.9	0.064	0.031	51.6
3	15.8	7.8	50.6	0.89	0.55	38.2	0.039	0.023	41.0
4	12.3	4.7	61.8	1.56	0.86	44.9	0.078	0.039	50.0
5	14.3	5.9	58.7	1.74	0.87	50.0	0.129	0.076	41.1
6	10.7	4.3	59.8	1.65	0.98	40.6	0.065	0.055	15.4
7	12.8	4.0	68.8	1.14	0.39	58.8	0.037	0.017	54.1
8	7.0	2.4	65.7	0.45	0.27	31.3	0.022	0.010	54.5
9	13.0	5.7	56.2	1.14	0.61	46.5	0.054	0.028	48.1
10	16.1	7.4	54.0	1.00	0.57	43.0	0.044	0.033	25.0
11	8.9	3.6	59.6	0.75	0.45	40.0	0.030	0.025	16.7
12	7.6	1.9	75.0	0.37	0.17	54.1	0.017	0.007	58.8
13	8.5	3.4	60.0	0.69	0.30	56.5	0.034	0.015	55.9
14	17.4	7.2	58.6	0.80	0.41	48.8	0.046	0.020	56.5
15	12.9	4.8	62.8	1.15	0.51	55.7	0.047	0.029	38.3
16	10.3	1.1	89.3	0.73	0.27	63.0	0.025	0.010	60.0
17	17.3	12.4	28.3	1.40	0.93	33.6	0.080	0.058	27.5
18	15.4	6.9	55.2	1.61	0.99	38.5	0.113	0.073	35.4
19	3.4	0.7	79.4	0.68	0.35	48.5	0.039	0.018	53.8
20	12.3	4.9	60.2	0.86	0.45	47.7	0.043	0.021	51.2
MEAN S.E.	12.1 ±0.83	4.9 ±0.60	61.75 ±2.71	1.05 ±0.09	0.57 ±0.06	45.6 ±2.07	0.052 ±0.01	0.030 ±0.01	44.2 ±3.07
RANGE	3.4-17.4	0.7-12.4	28.3-89.3	0.37-1.74	0.17-0.99	28.6-63.0	0.017-0.129	0.007-0.076	15.4-60.0

Correlation analysis revealed strong, positive and statistically significant ($P \leq .05$) correlations between percentage reductions in fat and PCB's and between percentage reductions in fat and mirex; between fat and contaminant levels in the standard fillets; and between fat and contaminant levels in the trimmed fillets (Table 4).

Variance Among Areas of Capture

Analysis of variance among the 4 locational groupings of fish indicated that these groupings were homogeneous subsets, having no significant differences ($P \leq .05$) in standard fillet mean fat content or in PCB and mirex levels. In short, there were no significant differences in fat or contaminant levels across collection sites.

Weight, Length, Age, Sex and Condition Relationships to Contaminant Concentrations and Reductions

Correlation analysis was conducted between several physical attribute variables (weight, length, sex, age and condition), and fat, contaminant, and percentage reduction variables. Results are shown in Table 5.

Based on the general patterns and relationships of stronger or statistically significant correlations, it can be generally stated that as brown trout increase in size (weight, length), "fatness" or healthiness (condition), and age, fat content and fat-soluble contaminant levels in the edible portions of the flesh also increase, and contaminant-reducing trimming techniques become less effective. It also appears that the sex of the brown trout had little bearing on fat content, contaminant levels, or any observed reduction in these variables due to trimming.

COMPARISON WITH THE SKEA STUDIES AND FURTHER DISCUSSION

The reductions in fat content, PCB and mirex levels determined in this study were compared to those reported by Skea et al. (15, 16) (Table 6). Fat and contaminant reductions observed in this study were similar to those found in the Skea studies, which were also found to be statistically significant at the $P \leq .001$ level. Contaminant reduction percentages found in the two studies were equivalent despite the fact that initial standard fillet contaminant levels in the two studies differed markedly[5]. These

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[5] It should be noted that Skea et al. reported mean PCB levels in the standard fillets of their brown trout (collected in 1976) that were substantially higher than those reported here (2.85 ppm vs. 1.05 ppm). In contrast, Skea noted mean mirex levels in brown trout standard fillets that were considerably lower (0.027 ppm vs. 0.052 ppm) than those observed in the present study. The difference in PCB levels between the two studies appears to reflect the documented decline in PCB levels occurring in Lake Ontario and Great Lakes fish in general. The difference in mirex levels is more difficult to explain. Trend data for mirex in Lake Ontario fish shows only a slight decline at best over the last 14 years, but there is clearly no evidence of a general increase in levels. The mirex levels in Skea's standard fillets appear to be considerably lower than average levels in Lake Ontario brown trout standard fillets observed in 1978 (0.09 ppm), while the present study's standard fillet mirex levels are in close keeping with the most recent State findings for brown trout collected in 1987. One is therefore left to speculate that the marked difference in standard fillet mirex levels between the two studies may simply reflect the significant fluctuations seen in Lake Ontario brown trout mirex levels over the last 14 years, which, in turn, could be due to variation in forage, or to environmental perturbations that may release and expose mirex-contaminated sediments to the food chain or water column. In addition, random sampling error cannot be ruled out since standard deviations overlap.

TABLE 4. SIGNIFICANT ($P \leq .05$) PEARSON CORRELATION COEFFICIENTS BETWEEN FAT CONTENT AND CONTAMINANT CONCENTRATIONS

	FAT CONTENT
I. **STANDARD FILLETS**	
PCB CONCENTRATIONS	0.459
MIREX CONCENTRATION	0.444
II. **TRIMMED FILLETS**	
PCB CONCENTRATION	0.588
MIREX CONCENTRATION	0.566

TABLE 5. MATRIX OF PEARSON CORRELATION COEFFICIENTS*

	FAT (STD)	FAT (TRM)	% RED FAT	PCB (STD)	PCB (TRM)	% RED PCB	MIREX (STD)	MIREX (TRM)	% RED MIREX
WEIGHT	.528*	.697*	-.657*	.562*	.674*	-.3394	.5420*	.5778*	-.3032
LENGTH	.409*	.590*	-.576*	.5202*	.630*	-.3603	.4819*	.5042*	-.2336
SEX	.0772	.1253	-.1145	-.2912	-.1570	-.1020	-.3494	-.2818	-.0642
AGE	.3539	.5297*	-.5510*	.4198*	.5353*	-.4377*	.3215	.3197	-.1136
CONDITION	.6985*	.6450*	-.5290*	.5216*	.5219*	-.0653	.4203*	.4591*	-.3917*

* Significant at $P \leq .05$ level

findings tend to support two conclusions: (1) fat-soluble organo-chlorine contaminants such as PCB's and mirex are removed when recommended fat-trimming procedures are carried out on brown trout, and (2) the efficacy of contaminant removal by trimming is consistent despite wide variation in the initial standard fillet contamination level.

Study results also reinforce the trimming and risk management advice dispensed through health agency fish consumption advisories, and other public educational programs undertaken by university-based outreach efforts. These programs have long emphasized that larger and older (as indicated by such variables as weight, length, and age) fish are likely to be more contaminated than smaller and younger specimens, and therefore should be eaten less often, if at all. Findings in this study clearly support this straightforward and simple advice.

Although confined to a limited sample of 20 fish, the study also suggests that springtime contaminant levels in Lake Ontario brown trout do not significantly differ between locations along the south shore of the Lake.

Application of the recommended fillet trimming technique substantially reduces the yield of the edible portion (by about 40%). However, it must pointed out that a significant portion of the trimmings, including skin and rib bones, would not normally be eaten. Therefore, the actual waste stemming from the trimming procedure may not be as great as yield calculations suggest or as the consumer may think.

Despite significant mean reductions in contaminant levels achieved through the trimming procedure, wide variation in reductions occurred between the individual fish--from 29% to 63% in the case of PCB's, and from 15% to 60% in the case of mirex. While this may make it difficult to confidently assure a fish consumer that trimming a specific Lake Ontario brown trout reduces his or her ingestion of PCB's or mirex by "almost half," mean data clearly indicate that, over time and over numerous meals of brown trout, significant reductions in a consumer's intake of PCB and mirex residues would most assuredly occur.

Reasons for significant variability encountered in trimming effectiveness between individual fish, and for the unexplained variation observed in correlation analysis of fat, contaminant and physical attribute variables are unclear. One possible cause could be the "static" nature of step #6 (Figure 2) in the State-recommended trimming method in relation to the size of the fish (i.e. the prescribed advice to trim away exactly 1/2 inch of flesh centered along the lateral line regardless of the size of the fish or fillet). This advice may too often result in the retention of more red muscle (which is known to be fattier) on the fillets of larger fish. A future test of this hypothesis could involve analysis of the fat and contaminant reductions achieved when trimmed fillets are stripped of <u>all</u> observable red muscle along the lateral line.

A second possible cause of the variation observed in the results may be that complex and distinctly different contaminant migration-deposition patterns and processes may be occurring within individual fish. Such patterns and processes may be dependent upon a multitude of physical, dietary and environmental factors at play on or in the organism. Exploration of these aspects would appear to be fruitful areas for further research, and could result in additional and more effective trimming or fish selection guidelines for the consumer.

TABLE 5. LAKE ONTARIO BROWN TROUT FAT/PCB/MIREX CONCENTRATIONS (WET WEIGHT) AND PERCENT REDUCTIONS: SKEA ET AL. (1979, 1981) VS. VOILAND ET AL. (1990)

	SKEA (N=60)	VOILAND (N=20)
FAT CONTENT (G/100G)		
STANDARD FILLET	16.5 (N.A.)	12.1 (3.7)
TRIMMED FILLET	8.8 (N.A.)	4.9 (2.7)
% REDUCTION	53.0 (N.A.)	61.8 (12.1)
PCB CONCENTRATION (PPM)		
STANDARD FILLET	2.85 (1.15)	1.05 (0.42)
TRIMMED FILLET	1.62 (0.73)	0.57 (0.26)
% REDUCTION	43.2 (N.A.)	45.6 (9.25)
MIREX CONCENTRATION (PPM)		
STANDARD FILLET	.027 (0.02)	.052 (0.03)
TRIMMED FILLET	.015 (0.01)	.030 (0.02)
% REDUCTION	44.5 (N.A.)	44.2 (13.7)

Standard deviation in parentheses.

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EVALUATION OF FREEZING TREATMENT ON VIBRIO PRESENT IN SHELLSTOCK OYSTERS AND CLAMS

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INTRODUCTION

Oysters and clams are filter feeders that pump large volumes of seawater and therefore can accumulate bacteria in their tissues. If eaten raw and/or improperly cooked they can serve as a vehicle for transmitting Vibrio spp. from water to man. These Vibrio spp. are primarily aquatic, occurring in estuarine and marine habitats and in association with aquatic animals (1). One example is Vibrio vulnificus which is a lactose fermenting, halophilic bacteria that has been isolated from waters in the Gulf of Mexico, the Atlantic and Pacific Oceans (2). Vibrio vulnificus is a significant health hazard to "at risk" individuals such as liver disease and/or compromised immune systems (3) and once transmitted it has been identified as the causative agent of primary septicemia that is often fatal. Because it represents a much less significant risk to the population in general, it is difficult to establish regulations concerning its presence in shellfish. Significant foodborne cases involving Vibrio spp. have attracted both regulatory and public attention. Since the presence of this pathogen in oysters and clams may potentially cause serious health problems in individuals consuming contaminated oysters and clams, various processing methods have been attempted to lower the level of the organisms. Freezing would be a possible method of inactivating this microorganism in oysters and clams. The purpose of this study was to evaluate the effect of freezing and thawing treatment on the level of microorganisms.

MATERIALS AND METHODS

Oysters and clams were harvested from Apalachicola Bay near Apalachicola, Florida and transported in box to the University of Florida within 18 hours. Shellstock oysters and clams were frozen and stored in freezer at -10 to -16°F. Thermocouples were used to monitor temperature changes in freezing of oysters and clams using different substrates (metal pan, styrofoam and cardboard). Microbial tests were performed at 0, 5, 10 and 15 days after freezing. Frozen oysters and clams were thawed at room temperature, and by placing the oysters and clams in plastic bags under running tap water. During the thawing, the temperature changes were measured using thermocouples. With the frozen shucked oysters the microbial tests were done without thawing.

Sample preparation

The thawed oysters and clams were scrubbed under running tap water, then opened with an alcohol flamed oyster knife. The contents of oyster and clam were transferred to sterile blender jars weighed, mixed with an equal amount of peptone water and homogenized.

Total plate count

Standard plate count agar was used for the determination of total aerobic plate count. The spread plate method was used and plates were incubated at 37°C for 24 hours (4).

MPN of Vibrio-like bacteria and Vibrio vulnificus

A 3-tube MPN procedure using 1% alkaline peptone water (2.5% NaCl) as the enrichment media was used to enumerate Vibrio (4). One loopful of enrichment broth from positive tubes (incubated at 37°C for 16-24 hours) was streaked onto TCBS and CPC agar. All isolates that

grew on TCBS (incubated at 37°C for 16-24 hour) were considered to be "Vibrio-like bacteria" and were used to calculate the MPN of " Vibrio-like bacteria". The yellow colonies found on CPC (incubated at 40°C for 16-24 hours) were considered to be V. vulnificus and were used to calculate the MPN of V. vulnificus.

RESULTS AND DISCUSSION

Temperature change in freezing

Figure 1 shows the rate of temperature change in freezing oysters with different substrates. Specifically a metal pan was the best conductor when compared to cardboard and styrofoam. The temperature of oysters reached -10°F in the freezer after about 105 minutes while styrofoam took 165 minutes to reach -10°F. With clams, it took 120 minutes to reach -10°F using the metal pan, but took more than 210 minutes when using cardboard (Figure 2). The freezing rate varied with the size of oyster or clam.

Temperature change in thawing

Thawing using running tap water was faster when compared to air (Figure 3). Room temperature thawing took approximately 165 minutes in reaching a temperature of 70°F. However, using running tap water, oysters and clams reached 70°F after 75-90 minutes of thawing. Water thawing was more efficient than air thawing at room temperature.

Total plate count

There was no significant difference in total plate counts between air thawing and water thawing. In shellstock oysters total plate count decreases gradually with time (Figure 4). On day 15, the total plate count was reduced 4.5 log units in shellstock oyster. The total count of shucked oysters without thawing was reduced by 1.6 log units on day 14 (Figure 5). This indicates that the reduction rate of total plate count depends on if oyster is shellstock or shucked and if the oyster is thawed or frozen. In shellstock clams, two log unit reductions were obtained on day 10, but on day 15, total plate count increased when compared to day 10 (Figure 6).

MPN of Vibrio-like bacteria

Freezing reduced the total "Vibrio-like bacteria" by 2.4 logs 15 days after freezing in shellstock oysters (Figure 7), while in shucked oysters without thawing there was a 2.0 log unit reduction on day 14 (Figure 8). In shellstock clams (Figure 9) the MPN of total Vibrio was lowered by 2.7 logs on day 15. There was no significant difference in the level of total "Vibrio-like bacteria" between air thawing and water thawing. After 15 days of storage at -10 to -16°F "Vibrio-like bacteria" were found to be culturable.

MPN of Vibrio vulnificus

After 15 days of storage at -10 to -16 °F, most V. vulnificus was not recovered in shellstock oysters (Figure 10). There was a 2.8 log reduction in MPN of V. vulnificus. In shucked oyster without thawing a 1.0 log reduction was obtained on day 14 (Figure 11) and on day 21 V. vulnificus was not detectable. It is likely that the reduction rate depends on shellstock or shocked and thawed or frozen. The MPN of V. vulnificus in control clams was 390, but on day 15 the organism was not detected (Figure 12). There was no significant difference between thawing methods.

CONCLUSION

After 15 days of storage at -10 to -16°F, total aerobic plate count of shellstock oysters was reduced by 4.5 log units and 2.0 log units in shellstock clams. The MPN of "Vibrio-like bacteria" of shellstock oyster showed a 2.4 log unit of reduction while shellstock clams showed a 2.7 log unit of reduction. The MPN of V. vulnificus in shellstock oysters was lowered by 2.8 log units while in shellstock clams the organism was not detectable.

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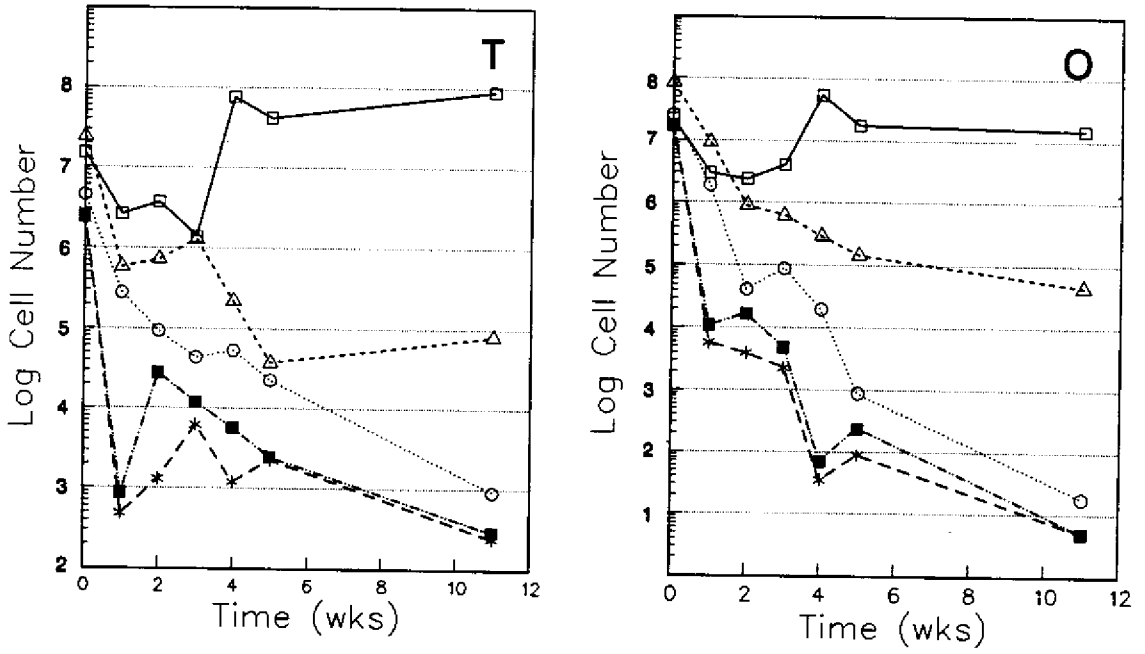
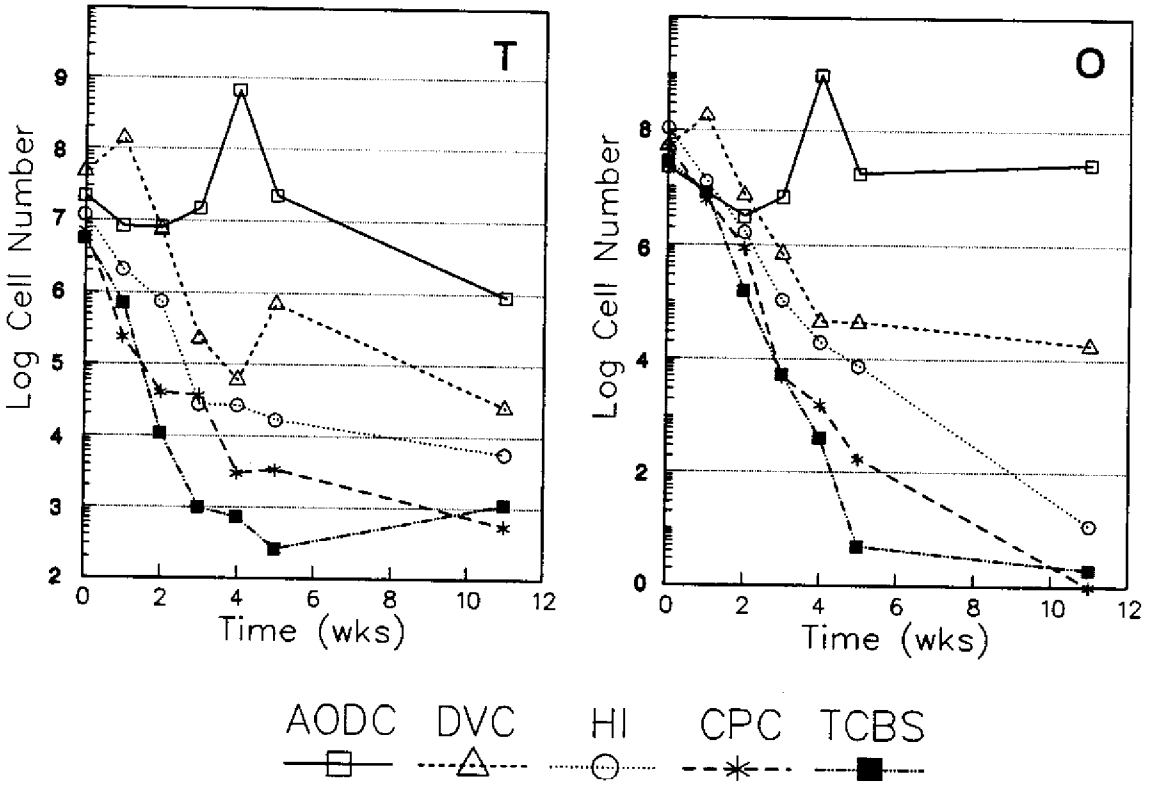
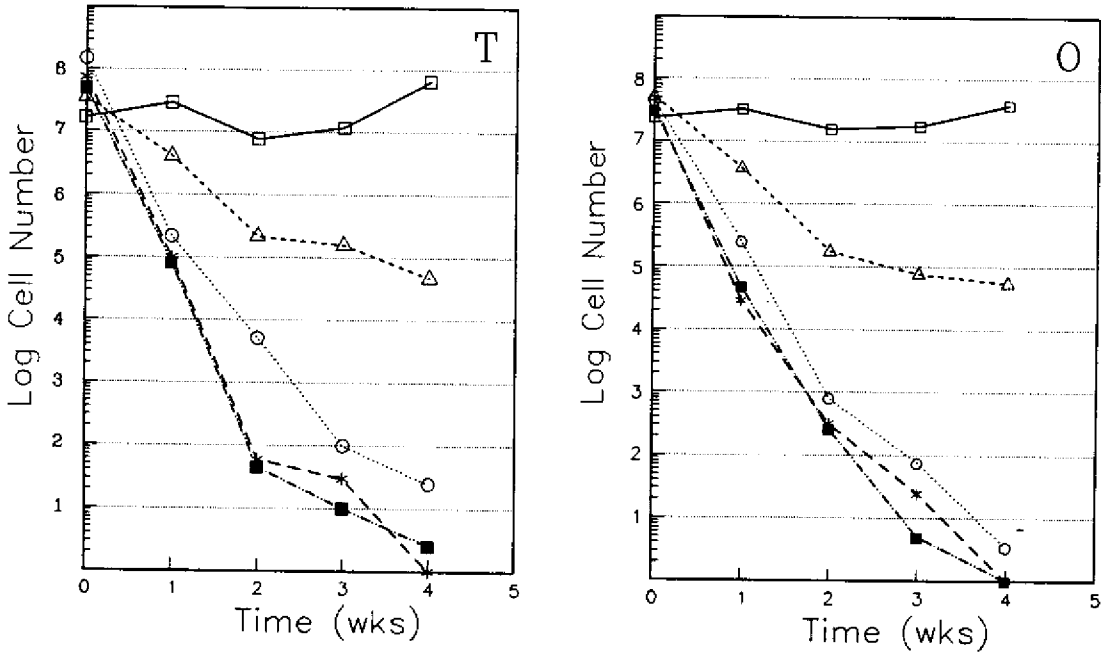
Graph 1. Survival of V. vulnificus in Artificial Seawater Microcosms upon Incubation at 25 C.



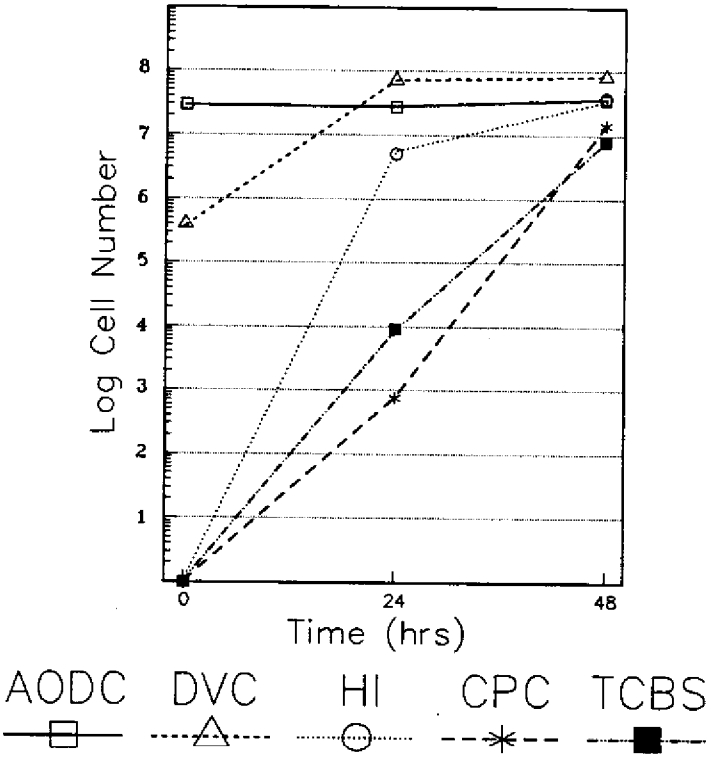
Graph 2. Survival of V. vulnificus in Artificial Seawater Microcosms upon Incubation at 37 C.



Graph 3. Survival of V. vulnificus in Artificial Seawater Microcosms upon Incubation at 4 C.



Graph 4. Resuscitation of V. vulnificus VBNC artificial seawater microcosms after incubation at RT



SEASONAL FLAVOR VARIATIONS IN RANGIA CLAMS

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Rangia clams are abundant (2) in the estuarine marsh areas of Southern Louisiana and have been consumed by local fishermen and native Louisiana Indians for hundreds of years. Microbiological profiles of Rangia from the harvest areas in Southeastern Louisiana have confirmed the ability of this species to meet the standards established by the National Shellfish Sanitation Program (5) either through direct harvesting from unrestricted waters or by relaying the clams for 14-days in clean water, when harvested from marginal or restricted waters (1). During certain times of the year Rangia have been noted to develop a musty/earthy or "muddy" flavor after cooking. This muddy flavor is considered an unique and/or off-flavor. A muddy flavor compound, geosmin, has been identified in Rangia clam meat taken from Louisiana waters (3). Geosmin has been a problem in the catfish growing industry, with processors testing every every catfish pond harvest for muddy flavor before accepting delivery. Catfish are evaluated by subjective sensory panelist or individuals trained to recognize the muddy flavor and determine acceptability of the product. The purpose of this study was to determine what effect the muddy flavor has on flavor acceptability of Rangia clams and using this information determine if seasonal changes occur in Rangia and establish harvesting times when flavor acceptability was maximum.

MATERIALS AND METHODS

Rangia clams were harvested monthly from an open estuary in Southeastern Louisiana from July 1989 through June 1990.

Clams were stored on ice and delivered to the Department of Food Science, Louisiana State University, Baton Rouge, LA.

A trained ten-member sensory panel evaluated fresh steamed clams for muddy flavor intensity and hedonic scale of acceptability each month of the study. Using a randomized complete block design(RCBD)(4), each panelist evaluated all samples in duplicate. Least square means of sample scores were used to determine monthly flavor differences and establish a relationship between muddy flavor intensity and overall acceptability of Rangia clam flavor. Acceptability of Rangia clam flavor was scored on a 15 point scale with acceptable scores being above 5.0. Muddy flavor intensity was scored on a 15 point scale with 0 as no muddy flavor and 15 as extreme muddy flavor.

RESULTS AND DISCUSSION

Monthly flavor scores of fresh steamed Rangia clams during the 12 months of this study indicated that there was a statistically significant decresase in muddy flavor during the cooler winter months from October 1989 through February 1990 (Fig 1). Muddy flavor scores dropped from a moderate intensity range of 5.0 to 7.0 to a low intensity of less than 5.0. Acceptability scores for this same period (October - March) were significantly improved (Fig 1). Acceptability scores increased during the same period from low acceptability less than 5.7 to moderate acceptability range of 5.7 to 7.0. Muddy

flavor scores were found to be negatively correlated with acceptability in fresh clams (Fig 2). When muddy flavor intensity of Rangia scored above 5.5, the clams were rated as unacceptable by the sensory panel. When the muddy flavor was scored below 4.0, the clams were found to be highly acceptable with hedonic scores above 7.0 (Fig 2).

CONCLUSION

Rangia clam muddy flavor scores, for the 12 months studied, indicated a significant decrease in muddy flavor with an increase in Rangia clam flavor acceptability during the late fall and winter season from October thru February. From the experimental results to date: it is suggested that Rangia clam peak flavor occurs during the winter season. Further evaluation of seasonal changes in Rangia flavor would be necessary to confirm these findings. Muddy flavor in steamed clams does affect the acceptability of Rangia clam flavor. Studies are now being done to reduce muddy flavor perception in Rangia using various processing techniques or by using the clams in a highly flavored spiced product. The source of muddy flavor has not been definitively identified. In fresh water species such as catfish, muddy flavor is often associated with pond algal blooms that produce geosmin, a muddy flavor compound. In brackish water, no source for muddy flavor in Rangia clams has been identified. Identification of the source of muddy flavor in these clams could lead to a rapid method for predicting and pinpointing specific harvesting times when flavor is at its peak.

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Fig 1: Monthly Flavor Scores For Rangia cuneata, Louisiana "cajun" clam

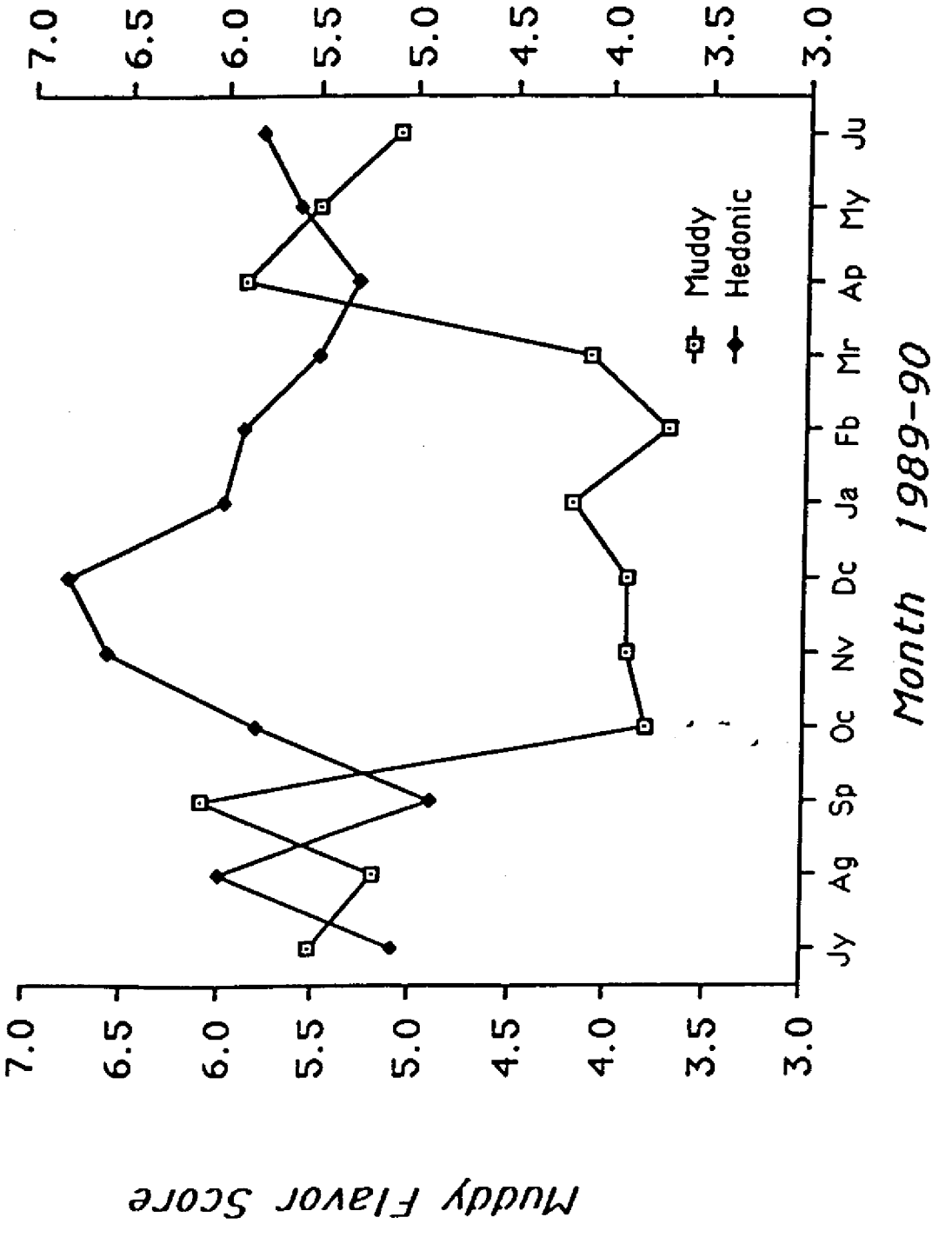
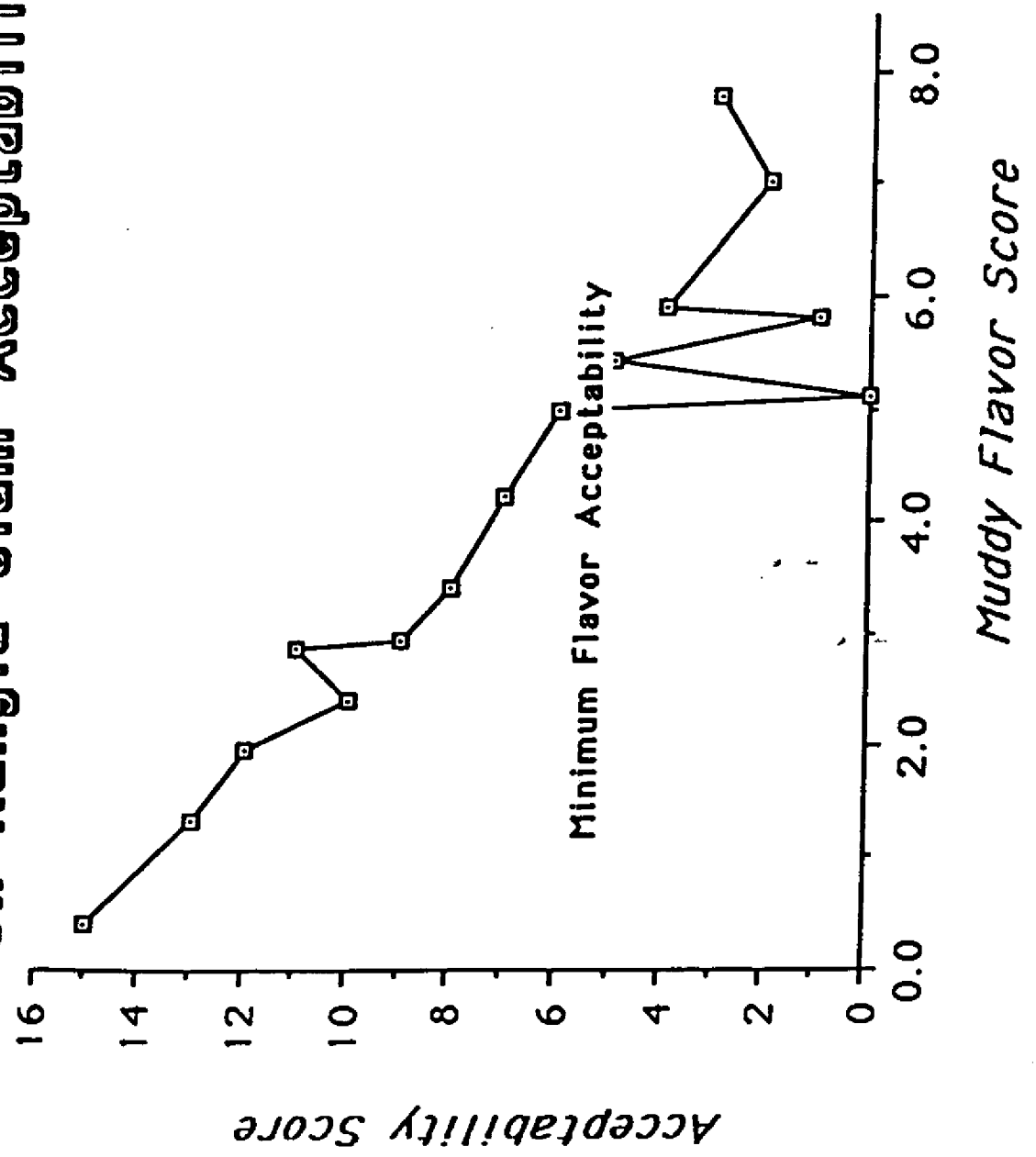


FIG 2: Effect of Muddy Flavor On Rangia Clam Acceptability



EFFECT OF PACKAGING ON STORAGE QUALITY OF ICED CHANNEL CATFISH

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According to USDA statistical reports (USDA, 1983; USDA, 1988), 127 million kg of catfish were processed in 1988 compared to 62 million kg in 1983. The increase in marketing of catfish indicates that production of catfish with good storage characteristics in retail outlet is very important to the industry. However, an increase in fattiness in fish coming from production ponds has been identified as a current trend (CFAMA, 1986). Since fish lipids are highly unsaturated and susceptible to oxidation, they could cause rancid flavor resulting in reduced product quality and market value. Earlier studies proved that vacuum packaging would substantially reduce oxidative deterioration in fish and fish products (Moyer, 1960; Hardy and Hobbs, 1968; Lindsay, 1977; Morris and Dawson, 1979). In order to keep refrigerated catfish products safe and of high quality, packaging with materials exhibiting a low oxygen transmission rate might be an alternative way. However, little information regarding the effect of consumer packaging on storage quality of refrigerated catfish fillet are available.

The objective of this investigation was to determine effects of different packaging methods on chemical and microbiological qualities of farm-raised channel catfish during 21 days of iced storage.

MATERIALS AND METHODS

Materials

Channel catfish (Ictalurus punctatus) fed commercial-type feed containing 24 and 32% protein were used for the study (Huang et al. 1991). Fish, averaging 1.5 kg, were harvested by net from Auburn University aquacultural ponds in September, 1989. Upon harvest, fish was deheaded, eviscerated, skinned, and filleted by using laboratory scale equipments, and then placed on ice for packaging.

Packaging Treatment and Storage

Fillets, averaging 350g, were placed on polystyrene trays and were packaged in one of the following methods: (1) Overwrapping with Saran^{R} film (Polyvinylidene chloride, PVDC); (2) Vacuum packaging with Eva bag^{R} (Cryovac Co., Duncan, SC) using a Multivac AG 900 vacuum packaging machine (Multivac Sepp Haggenmuller KG, West Germany) followed by a heat shrink process which required dipping in hot water (88^{0}C) for 2 sec as reported by Teixeira et al. (1986); and (3) Vacuum skin packaging with Intact Skin Packaging film (ISPF, Trigon Packaging Co., Redmond, WA) using a Trigon Intact RM 331 Mark III Mini Intact machine. Specifications for the packaging material are in Table 1.

All packaged fish samples were held at about 2:1 ice to fish ratio in ice chests provided with holes for drainage. Ice chests were housed in a refrigerated room at 4°C to slow ice melt. Additional ice was added to chests as needed.

Quality Evaluation

Iced samples were removed and evaluated after 1, 7, 14, and 21 days of storage for chemical analysis and microbial count. Chemical tests consisted of determining pH, thiobarbituric acid (TBA) number, ammonia concentration, and free fatty acid content. Microbiological evaluation was determined by psychrotrophic plate count. Triplicate samples were subjected to each analysis.

Chemical Analysis

Minced fish flesh (20g) was blended with 80 ml of distilled water for 1 min in a food processor (Regal La Machine I). The homogenate was filtered through glass wool to collect 50 ml of filtrate. The pH of the filtrate was measured at room temperature with a glass electrode using a Fisher Accumet Model 950 pH Meter.

Millivolt readings from an ammonia ion selective electrode coupled to a Fisher Model 950 pH meter were used to determine sample ammonia concentrations. Readings were taken after 1 ml of 10 N NaOH was added to 50 ml of filtered homogenate as previously described for pH determinations. Ammonia levels, expressed in millimolar concentration, were determined from an ammonium chloride standard curve, and converted to mg NH_3 per gram of fish.

The distillation method of Tarladgis et al. (1960), with the following modifications, was used to determine TBA numbers. Antioxidant solution [200 mg of butylated hydroxytoluene (BHT) in 1.8g of propylene glycol] was added to the sample before blending to prevent further oxidation as suggested by Yu and Sinnhuber (1967). Instead of glacial acetic acid, distilled water was used to make the 0.02M 2-thiobarbituric acid solution. Absorbances (A) of the TBA-malonaldehyde chromagens were read against a blank at 538 nm on a Bausch and Lomb Spectronic 20 Spectrophotometer. Results were expressed as TBA number (mg of malonaldehyde/kg of sample) = A x 7.8.

Fat Content and Free Fatty Acids

Lipids were extracted from fish using a mixture of solvents and determined as described by the AOAC (1984). A separatory funnel instead of graduate cylinder was used to separate the filtrate. The mixed oil was then titrated with 0.25N NaOH and data are reported as percent free fatty acids expressed as oleic acid.

Microbiological Analysis

Triplicate 25g samples were obtained aseptically and homogenized for 30 sec with 125 ml sterile Butterfields phosphate buffer in Fisher brand polyethylene sample bags using a Tekmar Stomacher Lab-Blender 400. Appropriate serial dilutions were plated onto Standard Methods Agar by the spread plate method. Psychrotrophic counts were determined by counting the colony forming units after the plates were incubated at 7°C for 10 days (Speck, 1984).

Analysis of Data

Statistical analyses (SAS, 1987) were performed on data of chemical and microbiological tests by means of PC SAS. The Duncan's multiple range test was used to determine significant differences among samples.

RESULTS AND DISCUSSION

Channel catfish fed 24% protein feed had significantly ($p<0.05$) higher fat content (10.6%) than those (8.6%) fed 32% protein feed (Huang et al., 1991). Although the fillet samples from fish fed 32% protein had a lower pH than these from fish fed 24% protein on day 1, no significant difference ($p<0.05$) was found at the 7th day (Table 2). The pH of fillets from fish fed 24% protein except the overwrapped sample decreased at day 7 and then increased to the same value throughout the entire storage period. However, fillets from 32% protein diet fish had significantly higher pH at day 21 than that at day 1 regardless of packaging methods.

Ammonia content of samples increased sharply from day 1 to day 7 and remained constant to 21 days of storage. There were no significant differences ($p<0.05$) among samples with different packaging methods (Table 3). Results were in agreement with that of Hearnsberger et al. (1987) who reported an initial increase of ammonia production then decreased slightly and again to increase. However, protein levels in the feed had no effect on ammonia production of catfish during 21 days of iced storage.

Although there was a significant difference ($p<0.05$) in fat content of catfish fillets (Huang et al., 1991), no significant difference in TBA numbers was found among samples in different packaging methods for 1, 7, and 21 days of storage (Table 4). TBA numbers remained constant throughout the storage period.

Table 5 shows the effect of packaging materials on free fatty acids in ice stored catfish. Regardless of the fat content, after 14 days of storage, the vacuum skin packaged samples and the E-bag^R samples had the lowest and highest free fatty acid content, respectively in fish flesh.

Samples in vacuum skin packaging and in vacuum bags showed significantly lower ($p<0.05$) psychrotrophic bacterial counts than Saran^R overwrapped ones after 7, 14 and 21 days of storage regardless of fat content in the muscle (Table 6). All tested samples had bacterial counts increased as the days of storage increased. However, the bacterial count remained constant after 14 days of storage.

In summary, both vacuum skin packaging and vacuum bags did retard the psychrotrophic bacterial growth of iced catfish. Although no effect on lipid oxidation was found, vacuum packaging did slow the catfish lipid hydrolysis. Vacuum packaging can not only provide a better external appearance but also prevent leakage of fish juices. Cross-contamination of displayed fishes and the odor problem in retail markets would be reduced. Results revealed that the quality of iced fresh catfish fillets could be improved through vacuum packaging with low oxygen permeable materials.

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TABLE 1.
SPECIFICATIONS OF PACKAGING MATERIAL[a]

Packaging method	Composition	Oxygen transmission rate[b]	Water transmission rate[c]
Overwrap	Polyvinylidene	5 (at 20^{0}C, 43% R.H.)	1.00 (at 20^{0}C, 85% R.H.)
Vacuum bag	Low density ethylene and vinyl acetate copolymer	4,000 (at 22.8^{0}C)	10.08 (at 37.8^{0}C, 100% R.H.)
Vacuum skin	Surlyn (low density polyethylene)	930 (at 23^{0}C, 75% R.H.)	4.00 (at 37^{0}C, 95% R.H.)

a Specifications from technical literature provided by manufacturers.
b Transmission rate = cc/m^2/24 h/atm
c Transmission rate = g/m^2/24 h/atm

TABLE 2.
EFFECT OF PACKAGING METHOD ON pH IN ICE STORED CHANNEL CATFISH

Packaging treatment	Days of storage			
	1	7	14	21
Catfish (fed 24% protein level)				
overwrap	6.72a	6.65a	6.54d	6.75b
vacuum bag	6.72a	6.52a	6.79a	6.86a
vacuum skin pack	6.72a	6.68a	6.72b	6.75b
Catfish (fed 32% protein level)				
overwrap	6.59b	6.60a	6.68bc	6.88a
vacuum bag	6.59b	6.65a	6.68bc	6.74b
vacuum skin pack	6.59b	6.56a	6.64c	6.74b

a,b,c,d Means in a column followed by the same letter are not significantly different at level of 0.05

TABLE 3.
EFFECT OF PACKAGING METHOD ON AMMONIA CONTENT (MG NH_3/G SAMPLE) IN ICE STORED CHANNEL CATFISH

Packaging treatment	Days of storage			
	1	7	14	21
Catfish (fed 24% protein level)				
overwrap	0.04	0.49	0.38	0.46
vacuum bag	0.04	0.54	0.37	0.45
vacuum skin pack	0.04	0.48	0.40	0.52
Catfish (fed 32% protein level)				
overwrap	0.09	0.36	0.40	0.53
vacuum bag	0.09	0.43	0.38	0.47
vacuum skin pack	0.09	0.50	0.39	0.55

TABLE 4.
EFFECT OF PACKAGING METHOD ON TBA NUMBER (MG MALONALDEHYDE/KG SAMPLE) IN ICE STORED CHANNEL CATFISH

Packaging treatment	Days of storage			
	1	7	14	21
Catfish (fed 24% protein level)				
overwrap	1.72a	1.14a	1.05ab	0.52a
vacuum bag	1.72a	1.63a	1.04ab	0.82a
vacuum skin pack	1.72a	1.39a	0.93b	0.98a
Catfish (fed 32% protein level)				
overwrap	1.98a	1.24a	1.26a	0.79a
vacuum bag	1.98a	1.58a	1.93a	1.58a
vacuum skin pack	1.98a	1.65a	1.67ab	1.43a

[a,b] Means in a column followed by the same letter are not significantly different at level of 0.05

TABLE 5.
EFFECT OF PACKAGING METHOD ON FREE FATTY ACID CONTENT (MG/G SAMPLE) IN ICE STORED CHANNEL CATFISH

Packaging treatment	Days of storage			
	1	7	14	21
catfish (fed 24% protein level)				
overwrap	0.04a	0.05a	0.24a	0.59a
vacuum bag	0.04a	0.05a	0.16bc	0.43ab
vacuum skin pack	0.04a	0.04a	0.11d	0.14c
catfish (fed 32% protein level)				
overwrap	0.03a	0.04a	0.27a	0.28c
vacuum bag	0.03a	0.05a	0.17b	0.32bc
vacuum skin pack	0.03a	0.04a	0.13cd	0.17c

a,b,c,d Means in a column followed by the same letter are not significantly different at level of 0.05

TABLE 6.
EFFECT OF PACKAGING METHOD ON PSYCHROTROPHIC BACTERIAL COUNT (LOG CFU/G SAMPLE) IN ICE STORED CHANNEL CATFISH

Packaging treatment	Days of storage			
	1	7	14	21
Catfish (fed 24% protein level)				
overwrap	5.1a	7.7a	9.2b	9.2a
vacuum bag	5.1a	7.2b	8.3d	8.4b
vacuum skin pack	5.1a	7.3b	8.7cd	8.8b
Catfish (fed 32% protein level)				
overwrap	5.4a	7.6a	9.4a	9.1a
vacuum bag	5.4a	7.1b	8.9cd	8.6b
vacuum skin pack	5.4a	7.4b	9.0bc	8.7b

a,b,c,d Means in a column followed by the same letter are not significantly different at level of 0.05

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INHIBITING MELANOSIS IN TRAWLED AND POND-REARED SHRIMP SPECIES

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INTRODUCTION

Melanosis ("blackspot") continues to pose a significant quality problem for numerous shrimp species from traditional harvests and cultured production. The consequences can be reduced product value, estimated to range from 10 to 25 percent less in dockside payments depending on the degree of melanosis, or product rejection for excessive blackspot (1). As an adverse cosmetic consequence of the natural, indigenous polyphenoloxidase enzyme activity, the expression of blackspot varies per species, harvest and handling methods. Thus a necessary preventative control should offer simple and general application for all species under a variety of harvest and handling conditions. The principle approach of this work was the inhibition of melanosis rather than the resolution or bleaching of developed blackspot.

METHODS

A standard test procedure, designed to exemplify common commercial practices, was used to monitor the effectiveness of a polyphenoloxidase inhibiting compound (current trade name, Everfresh) in prevention of shrimp melanosis. Prior work with a variety of similar compounds concluded Everfresh$_{TM}$ was effective for pink shrimp (Penaeus duorarum) treated in dip concentrations as low as 0.0005% (3). A similar dip treatment for one minute in concentrations ranging from 0.0005% to 0.05% was tested for use on harvested shrimp (Penaeus aztecus, P. duorarum, Sicyonia brevirostris and Heterocarpus laevigatus) and cultured shrimp (Penaeus japonicus, P. monodon, P. setiferus and P. vannamei). The product to dip ratio was approximately 300 grams/liter. Trials involved replications per species through small scale, one liter dips, and common commercial scaled tanks of 100 liters (approx. 30 gallons). As noted in prior work (3,4) the salinity of the dip water did not influence the expression of melanosis or effectiveness of the dip. In most instances the dip water was taken from the harvest waters.

Heads-on, whole untreated shrimp were used in all tests to represent the most problematic product form. The treatments were applied immediately post-harvest on vessels or at pond side, or when thawed from frozen storage necessary for transhipped samples. All frozen samples were specifically harvested and rapidly frozen without any prior treatment. As noted in commercial practice, frozen storage tended to accentuate the onset of melanosis when the samples were thawed.

Melanosis on the treated samples was scored in comparison with controls (untreated) and sulfite treated (1.25% sodium bisulfite dip) samples per the respective batches of shrimp species. Scoring was based on a proven (3) 10 point melanosis scale (Table 1) which referenced the grading criteria of the National Marine Fisheries Service (2). Results were based on progressive scoring by a trained panel observing one pound samples held in mesh bags stored in ice for at least 12 days. The mesh bags provided exposure to leaching melt water typical for commercial iced shrimp. Each bag was scored as a test unit. Any deviations from these procedures are so noted in the results.

Table 1. Melanosis rating scale used to describe the occurrence of 'blackspot' on shrimp.

Score	Description	Commercial Consequence	USDC[1] Equivalence
0	absent		
2	slight, noticeable on some shrimp	possible reduced value	A
4	slight, noticeable on most shrimp	reduced value	B
6	moderate, on most shrimp	definite reduced value	B
8	heavy on most shrimp	unacceptable	C
10	heavy, on all shrimp	unacceptable	C

[1] U.S. Department of Commerce equivalence based on respective quality ratings and melanosis descriptions used by National Marine Fisheries Service volunteer inspection program (CFR, Title 21, Part 265).

RESULTS

All initial results indicate Everfresh$_{TM}$ dips effectively retarded melanosis on all shrimp tested (Table 2). The results were not conclusive for some of the less problematic cultured shrimp. Effective dip concentrations contained less than 0.005% Everfresh$_{TM}$. Prevention was equivalent to or better than the results scored for comparative tests utilizing the current recommended dip procedure of 1.25% sodium bisulfite for one minute. The Everfresh$_{TM}$ dips were odorless and colorless, and caused no noticeable organoleptic alterations of the shrimp in raw, fresh, frozen or cooked states.

CONCLUSIONS

Everfresh$_{TM}$ in dip concentrations less than 0.005% for 1 minute offer effective prevention of shrimp melanosis for harvested or cultured shrimp, and three separate shrimp genera. The method of application is simple, controllable and suitable to current commercial practices. Dip preparation and use does not pose a health risk or irritant to users. Dipped whole, heads-on shrimp exhibit no noticeable organoleptic alterations in the raw, fresh, frozen or cooked product forms.

Table 2. Consolidated results for shrimp species treated for prevention of melanosis during 1990.

Species[1]	Source[2]	Total days storage with melanosis ratings less than '4' Controls	Sulfited	Everfresh$_{TM}$
P. duorarum harvested pink shrimp	2v.t.-Key West 2v.t.-St. Pete 3f.t.-FL	2-6	6-12	12
P. aztecus harvested brown shrimp	1v.t.-Freeport 1f.t-TX	4-5	5-7	14
P. setiferus cultured white shrimp	1p.t.-SC	9	10	10
P. vannamei cultured white shrimp	1p.t.-HiltonHead SC	9	10	10
P. japonicus cultured kuruma shrimp	1p.t.-Japan	positive Everfresh$_{TM}$ inhibition confirmed through collaborative work with aquaculture firm in Japan.		
P. monodon cultured tiger shrimp	1f.t.-Tawian	No dip application. Positive Everfresh$_{TM}$ inhibition of polyphenoloxidase extracted from the shrimp samples.		
S. brevirostris harvested rock shrimp	1v.t.-FL	3-4	5-6	9
H. laevigatus harvested nylon shrimp	1f.t-Hawaii	5	14	14

1. Genera designations P.-Penaeus; H.-Heterocarpus; and S.-Sicyonia.
2. Trial designations: v.t.-vessel trials, on-board, utilizing fresh, immediate post-harvested shrimp; f.t.-frozen trials utilizing untreated shrimp, rapidly frozen and delivered to the lab; and p.t.-pond side trials utilizing fresh, immediate post-harvested, cultured shrimp.

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ACKNOWLEDGEMENTS

Product procurement and access was provided through assistance of Bob Canady (Singletons - Key West, FL), Bobby Harriman (Bama Seafood - St. Petersburg, FL), Jan Harper (S&H Shrimp - Freeport, TX), Ron and Mark Herndon (Coastal Seafood - Aransas Pass, TX), Food Industry Research and Development Inst. (Hsinchu, Taiwan), Paul Sandifer and Steve Hopkins (SC Wildlife and Marine Resources, Waddell Mariculture Center) and John Doyle (Alaska Sea Grant Program). Vessel captains included Jimmy Hickman ('Little Hobo' - Key West), Johnny Williams ('Capt. Adam' - St. Petersburg, FL), James Hewett ('Capt. Dexter' - St. Petersburg, FL), and Ken Simpson ('Miss Davi Nicole' - Freeport, TX).

INHIBITORY EFFECT OF KOJIC ACID ON SOME CRUSTACEAN POLYPHENOLOXIDASE

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INTRODUCTION

Unfavorable darkening of many seafood products resulting from enzymatic oxidation of phenols to *o*-quinones by polyphenoloxidase (PPO) has been of great concern to seafood processors and consumers. Compounds capable of inhibiting melanosis in these products through the interference of PPO mediated reactions or through the reduction of *o*-quinones to diphenols have been identified. However, the use of chemicals in inhibiting melanosis is limited due to off-flavors, off-odors, toxicity, and economic feasibility (5).

Kojic acid (5-hydroxy-2-hydroxymethyl-γ-pyrone) is a fungal metabolite produced by many species of *Aspergillus* and *Penicillium* (8, 12). It possesses antibacterial and antifungal activities (11). Saruno et al. (13) demonstrated kojic acid from *Aspergillus albus* inhibited mushroom tyrosinase activity. Since only limited information was available on the inhibitory effect of kojic acid on PPO, this study was undertaken to investigate the inhibitory activity of this compound on crustacean (white shrimp, grass prawn, and Florida spiny lobster) PPO and to elaborate mechanisms involved.

MATERIALS AND METHODS

Extraction of crustacean PPO

Nonsulfited shrimp cephalothorax and lobster tail cuticle were frozen in liquid nitrogen and ground into fine powder. The powder was suspended in 3 volumes (w/v) of 0.05 M sodium phosphate buffer (pH 7.2) containing 1 M NaCl (extraction buffer) and 0.2% (v/v) Brij 35, and stirred at 4°C for 3 hr.

Purification of lobster PPO

The suspension was centrifuged at 8,000*g* (4°C) for 30 min and crude lobster PPO (1.0 mL), after dialysis against 0.05 M sodium phosphate buffer (pH 6.5), was applied to nondenaturing preparative polyacrylamide gel electrophoresis (PAGE) tubes (12 cm length x 14 mm i.d.) containing 5% acrylamide gel, and run at a constant current of 10 mA/tube. PPO was visualized using a specific enzyme-substrate staining method (3) using 10 mM DL-β-3,4-dihydroxyphenylalanine (DL-DOPA) in distilled H_2O as substrate. Gels containing PPO were sectioned and homogenized in 0.05 M sodium phosphate buffer (pH 6.5). Following filtration through a Whatman No. 4 filter paper, the filtrate was concentrated using an Amicon stirred cell fitted with a YM 10 filter. The PPO was further purified by subjecting the concentrated filtrate to electrophoresis using 7.5% acrylamide gel.

Purification of shrimp PPO

Following centrifugation of the suspension at 23,000*g* (4°C) for 30 min, the supernatant was fractionated with $(NH_4)_2SO_4$ between 0 - 40% saturation; the precipitate was collected by centrifugation at 23,500*g* (4°C) for 30 min. For white shrimp, the precipitate was dissolved in 0.05 M sodium phosphate buffer (pH 7.2) and dialyzed overnight against the same phosphate buffer. The dialyzed PPO was loaded onto a DEAE-cellulose (0.95 meq/g) column. Elution of PPO was performed using a 300 mL 0.05 M sodium phosphate buffer (pH 7.2) containing a linear gradient (0 - 1.0 M) of NaCl.

Fractions possessing PPO activity were pooled and concentrated. The PPO was further purified by a Sephadex G-100 gel column.

For grass prawn, the precipitate was resuspended in extraction buffer containing 40% $(NH_4)_2SO_4$. After a repeat homogenization and centrifugation at 23,500*g* (4°C) for 20 min, the precipitate was subjected to a preparative Phenyl Sepharose CL-4B column. PPO was eluted with a step-wise gradient of elution buffer [100% extraction buffer (9 mL), 50% extraction buffer in water (24 mL), and 10% extraction buffer in water (24 mL)], 50% ethylene glycol (12 mL), and then distilled water (150 mL) at a flow rate of 0.2 mL/min. Four-mL fractions were collected and fractions exhibiting PPO activity were pooled and concentrated.

Protein quantitation and enzyme purity determination

Protein content of all PPO preparations was quantitated using the Bio-Rad Protein Assay kit. Enzyme purity was examined using a Bio-Rad mini gel system. PPO (20 μg protein/well) was loaded and electrophoresized at a constant voltage (200 volts) for approximately 35 min. The purity of enzyme preparations were determined by comparing gels stained with 10 mM DL-DOPA in H_2O and then with a Commassie brillant blue R-250 solution.

Enzyme activity assay

PPO activities were measured by adding 0.1 mL enzyme to 1.4 mL 10 mM DL-DOPA in 0.05 M sodium phosphate buffer, pH 6.5. The reaction was monitored at 25 °C and 5 min for grass prawn and lobster PPO, while 40°C and 5 min for white shrimp PPO. Maximal initial velocity was determined as (ΔA_{475nm}/min) and one unit of PPO activity was defined as an increase in absorbance of 0.001/min.

Effect of kojic acid on PPO activity

One hundred-μL PPO was incubated with 1.0 mL of 0.05 M sodium phosphate buffer (pH 6.5), and 0.9 mL kojic acid solutions (20 - 200 μg/mL) at 37°C for 15 min. Following equilibration to ambient temperature, 1.0 mL 10 mM DOPA or catechol in the same phosphate buffer (pH 6.5) was added to the mixture and the reaction was monitored at 475 or 395 nm, respectively, for 5 min. For control sample, an equivalent volume of H_2O was used to replace kojic acid solution. Percentage inhibition (I) was expressed as $[(T - T^*)/T] \times 100$, where T^* and T were enzyme activities in the presence and absence of kojic acid, respectively (13). For white shrimp PPO system, the reaction was performed at 40°C, while 25°C was used for grass prawn and lobster PPO system.

Enzyme kinetics study

Michaelis constant, K_m, for PPO was determined using the Lineweaver-Burk equation (10). L-DOPA or catechol (1.5 - 7.0 mM) in 0.05 M sodium phosphate buffer (pH 6.5) was used as substrate for white shrimp PPO (5,400 units/mg protein); while DL-DOPA or catechol (1.67 - 9.92 mM) in the same phosphate buffer (pH 6.5) was used for grass prawn PPO (900 units/mg protein) and lobster PPO (7,000 units/mg protein). The assay conditions was carried out similarly as previously described.

The inhibitory mechanism of kojic acid on enzyme activities was also investigated. Five hundred-μL kojic acid (0.28, 0.56, and 1.06 mM) replacing phosphate buffer solution were used in the assay systems. Prior to the addition of the substrate, the enzyme-inhibitor mixture was incubated at 37°C for 15 min. The assay conditions were performed as previously described. Kinetic parameters of K_{mapp} and K_i for enzyme activities were also determined according to the equations of Lineweaver-Burk and Dixon (4, 10).

Effect of kojic acid on O_2 uptake by PPO reaction

A 0.1 mL lobster PPO was added with 0.1 mL kojic acid solution (0.28, 0.56, and 1.06 mM) into the sample chamber of an oxygen monitor. Following incubation at 25°C for 30 min, 2.9 mL of 10 mM DL-DOPA or catechol in 0.05 M sodium phosphate buffer (pH 6.5) was added to the sample chamber. The reaction was allowed to proceed at 25°C for 10 min and the consumption rate of O_2 was monitored. For control sample, H_2O was used to substitute kojic acid. Background O_2 consumption for kojic acid or substrate, or their combination was also carried out. The O_2 consumption rate was defined as $[(U - U^*)/U] \times 100$, where U and U^* was the rate of O_2 consumed by the enzymatic reaction in the absence or presence of kojic acid, respectively.

Effect of kojic acid on the reduction of Cu^{2+}

Following incubation (10 min) at 25°C, the solution containing 1.0 mL kojic acid in deionized H_2O and 0.5 mL 0.4 mM $CuSO_4$ in 0.05 M sodium phosphate buffer (pH 7.0) was added to 0.5 mL of 4 mM aqueous bathocuproine disulfonic acid. The final concentration of kojic acid in the mixture was 0.02 to 1.40 mM. After the mixture was incubated at 25°C for another 20 min, the absorbance at 483 nm was determined. For control sample, kojic acid was substituted with 1.0 mL deionized H_2O.

Effect of kojic acid on phenolic substrates and quinone products

Three respective solution: (i) 1.2 mL 10 mM DL-DOPA, (ii) 0.6 mL kojic acid (5.63 mM), and (iii) 0.2 mL lobster PPO, all in 0.05 M sodium phosphate buffer (pH 6.5) at a total volume of 2.0 mL, was spectrophotometrically scanned. In addition, mixture containing (iv) 1.2 mL 10 mM DL-DOPA and 0.6 mL kojic acid (5.63 mM) in phosphate buffer (pH 6.5), and 0.2 mL phosphate buffer, or (v) 0.6 mL kojic acid, 0.2 mL lobster PPO and 1.2 mL phosphate buffer following an incubation at 25°C for 30 min was also scanned.

A reaction mixture containing 1.2 mL 10 mM DL-DOPA and 0.2 mL lobster PPO in 0.05 M sodium phosphate buffer (pH 6.5), and 0.6 mL of the same phosphate buffer was incubated at 25°C for 30 min. Following red color development, the mixture was spectrophotometrically scanned. The effect of kojic acid on dopaquinone was also studied by adding 0.6 mL 5.63 mM kojic acid to the solution containing dopaquinone formed by 0.2 mL lobster PPO and 1.2 mL 10 mM DL-DOPA. Following incubation at 25°C for 30 min, the mixture was scanned as previously described.

RESULTS AND DISCUSSION

Effect of kojic acid on PPO activity

White shrimp PPO activity on L-DOPA and catechol was inhibited by 25.5 and 21.5%, respectively, when kojic acid at 20 μg/mL was added (Fig. 1a). Comparatively low inhibition was also observed for the oxidation of DL-DOPA (23.5%) and catechol (13.3%) by grass prawn PPO when kojic acid at the same concentration was used (Fig. 1b). However, 80 and 70% inhibition of the oxidation of DL-DOPA and catechol, respectively, was observed using lobster PPO and kojic acid at 20 μg/mL (Fig. 1c). When kojic acid at 100 μg/mL was added, the inhibition on lobster PPO exceeded 90% (Fig. 1c). Under the same treatment conditions, the inhibition was only about 50% for white shrimp PPO and 80% for grass prawn PPO (Figs. 1a and 1b). Kojic acid appeared to be more effective in inhibiting lobster PPO activity than inhibiting white shrimp and grass prawn PPO.

Enzyme kinetics

The kinetic parameters (K_m, K_{mapp}, and K_i) for PPO utilizing DOPA or catechol in the absence or presence of kojic acid, and the type of inhibition as verified by the Dixon plot are listed in Table 1. The Michaelis constants for the oxidation of L-DOPA and catechol by white shrimp PPO were

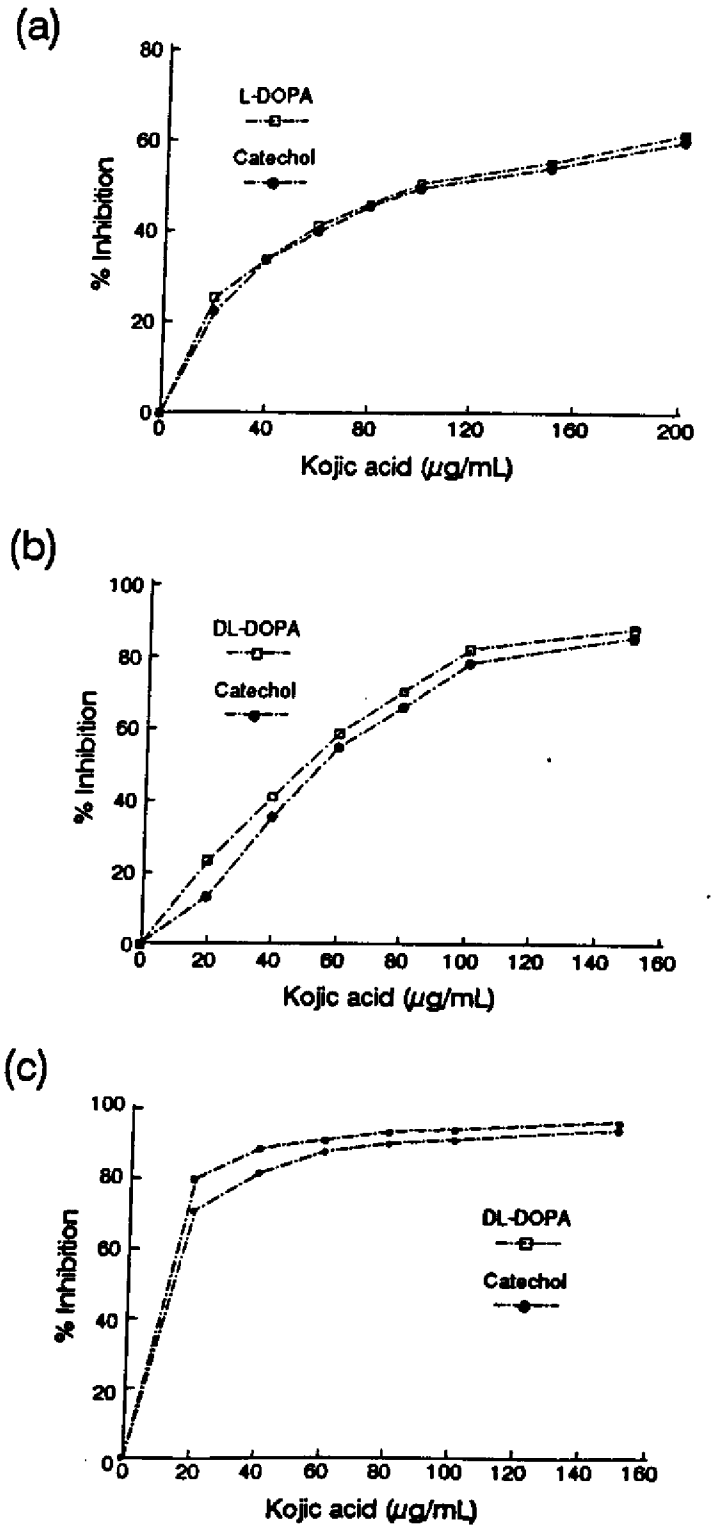

Figure 1. The concentration-related inhibitory effect of kojic acid on (a) white shrimp, (b) grass prawn, and (c) spiny lobster polyphenoloxidase activity.

determined to be 3.48 and 4.27 mM, respectively. Regarding lobster PPO, the K_m values for oxidation of DL-DOPA and catechol were 3.27 and 4.98 mM, respectively. For grass prawn PPO, the K_m values for DL-DOPA and catechol were 3.64 and 5.29 mM, respectively.

Table 1. Inhibitory mechanism of kojic acid on crustacean polyphenoloxidase

Enzyme	Substrate	K_m (mM)[a]	Type of inhibition	Apparent K_m (mM)	K_i (mM)[b]
White shrimp	L-DOPA	3.48	Mixed	4.29	0.30
	Catechol	4.27	Mixed	5.18	0.33
Grass prawn	DL-DOPA	3.64	Mixed	7.77	0.05
	Catechol	5.29	Mixed	7.78	0.07
Spiny lobster	DL-DOPA	3.27	Mixed	4.37	0.07
	Catechol	4.98	Mixed	7.31	0.10

[a] Michaelis constant
[b] Inhibitor constant

When kojic acid was added as an inhibitor, a mixed-type inhibition was observed for the oxidation of both substrates by these three crustacean PPO. The inhibitor constant for the oxidation of DL-DOPA by grass prawn PPO was 0.05 mM and 0.07 mM for catechol. Similarly, the K_i values were determined to be 0.07 mM for DL-DOPA and 0.10 mM for catechol when lobster PPO was used. For white shrimp PPO, the K_i values of L-DOPA and catechol were determined to be 0.15 and 0.18 mM, respectively. The observation of lower K_i values for grass prawn and lobster PPO than white shrimp PPO further demonstrate that kojic acid exhibited a greater inhibitory effect on the former two enzymes than the latter one (Figs. 1a, 1b, and 1c).

Effect of kojic acid on O_2 uptake by PPO reaction

O_2 consumption did not take place with kojic acid, the substrates (DL-DOPA and catechol) alone, or the kojic acid - substrate mixtures (data not shown). When PPO was added to the mixture containing substrate and buffer, O_2 consumption occurred immediately. Although O_2 uptake by the PPO - substrate mixture still took place when kojic acid was added, the percentage of O_2 consumption in these mixtures was decreased proportionally to increasing concentrations of kojic acid (Table 2).

Oxy-tyrosinase is the active species that catalyzes hydroxylation or dehydrogenation. Lerch (9) demonstrated that oxy-tyrosinase is formed through the interaction of O_2 with the binuclear copper (II) of deoxy-tyrosinase. The inhibitory effect of kojic acid on O_2 consumption by the PPO shown in this study suggests that kojic acid interferes with the interaction of oxygen molecule with deoxy-PPO, and thus prevents the formation of oxy-PPO.

Effect of kojic acid on the reduction of Cu^{2+}

The absorbance of the reaction mixture increases with increasing concentrations of kojic acid and then reaches a plateau when kojic acid exceeds 0.28 mM (Fig. 2). On the basis of molar extinction coefficient for Cu^{+}-bathocuprione disulfonate complex (2), all the Cu^{2+} present in the reaction mixture was reduced to Cu^{+} when kojic acid was present at greater than 0.28 mM. Comparing to the various reducing agents for the ability to reduce total amount of Cu^{2+} to Cu^{+}, kojic acid is rated superior to H_2O_2 (3.3 mM) and ferrocyanide (60 mM), but inferior to gallic acid, phenylhydrazine, ascorbic acid, and NH_2OH with 0.016 to 0.08 mM (1).

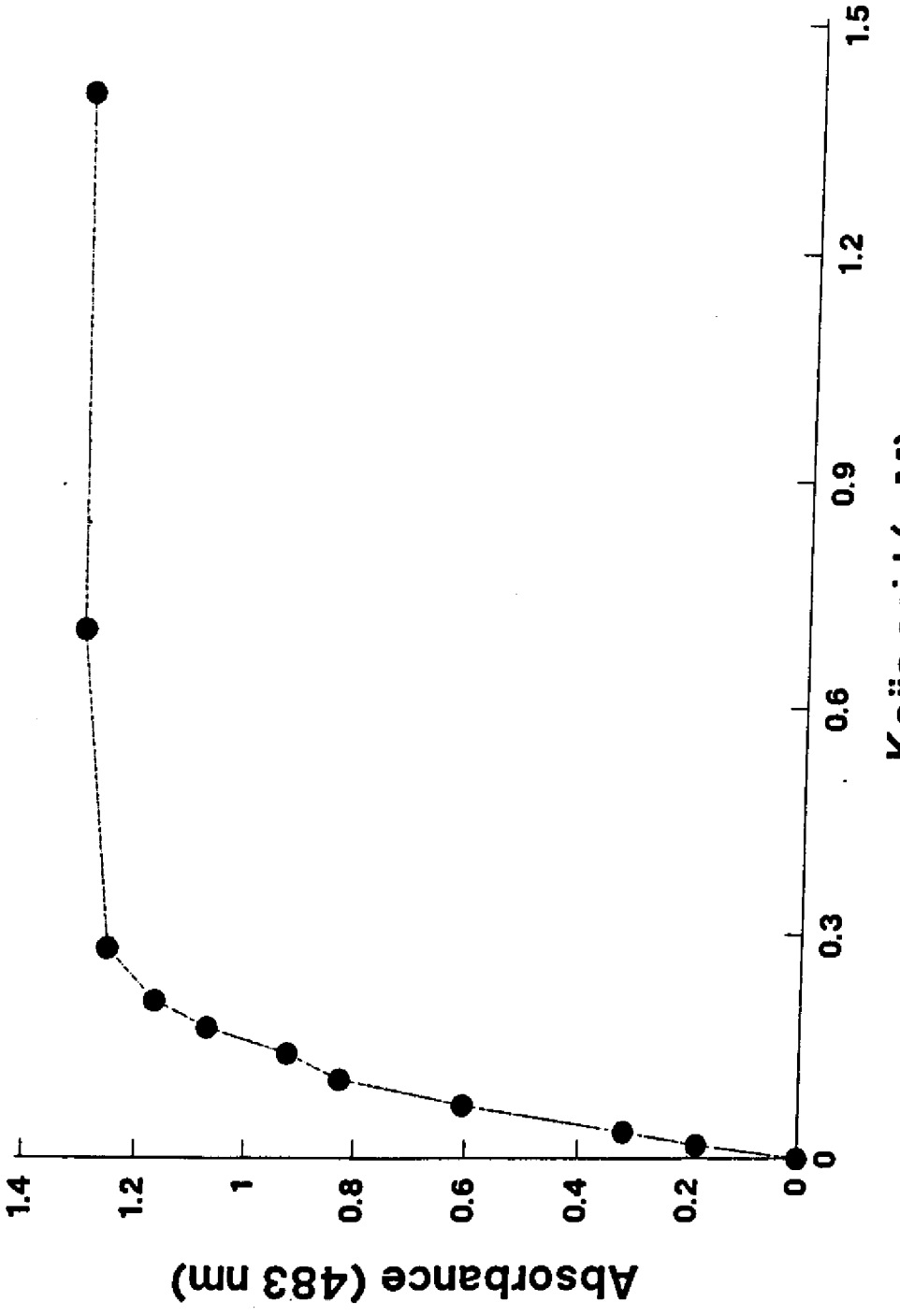

Figure 2. Effect of kojic acid on the reduction of cupuric copper.

Table 2. Inhibitory effect of kojic acid on the consumption of oxygen by spiny lobster polyphenoloxidase

Kojic acid (mM)	%Inhibition[a]	
	DL-DOPA	Catechol
0	0	0
0.28	50.2	47.4
0.56	60.3	65.0
1.06	80.3	77.5

[a] Result was an average of three observations.

Effect of kojic acid on phenolic substrates and quinone products

Lobster PPO, kojic acid, and DL-DOPA in 0.05 M sodium phosphate buffer (pH 6.5) displayed optimal absorption at 293, 297, and 288 nm, respectively (Figs. 3a, 3b, and 3c). The mixing of kojic acid with lobster PPO (Fig. 3d) or DL-DOPA (Fig. 3e) did not alter the pattern of the absorption spectra. Thus kojic acid did not interact with lobster PPO or DL-DOPA. Kojic acid was also verified not to interact with grass prawn and white shrimp PPO using the same approach.

Spectrophotometric scanning of the product generated from the reaction of DL-DOPA and lobster PPO revealed two distinct absorption peaks at 316 and 480 nm (Fig. 4a). The addition of kojic acid to this solution caused the change of the color from red-brown to violet, and the disappearance of the 480 nm peak which represents dopaquinone (6). The peak at 316 nm still remained (Fig. 4b). Similar phenomena occurred when kojic acid was added to the reaction mixture containing other crustacean PPO and DL-DOPA (data not shown). The effect of kojic acid on the spectra of products formed by the action of crustacean PPO on DL-DOPA is most likely due to the interaction of *o*-quinones with kojic acid to yield a complex. Kahn and Andrawis (7) proposed the formation of monooxime and dioxime from the reaction of *o*-quinones with NH_2OH.

CONCLUSION

The mode of inhibition of kojic acid on polyphenoloxidase is thus through the following actions: (1) by reducing the enzyme Cu^{2+} to Cu^{+}, rendering the enzyme inactive and unavailable for O_2 binding, (2) by complexing with quinone compounds to prevent melanin formation via polymerization, and/or (3) the combination of the above two actions.

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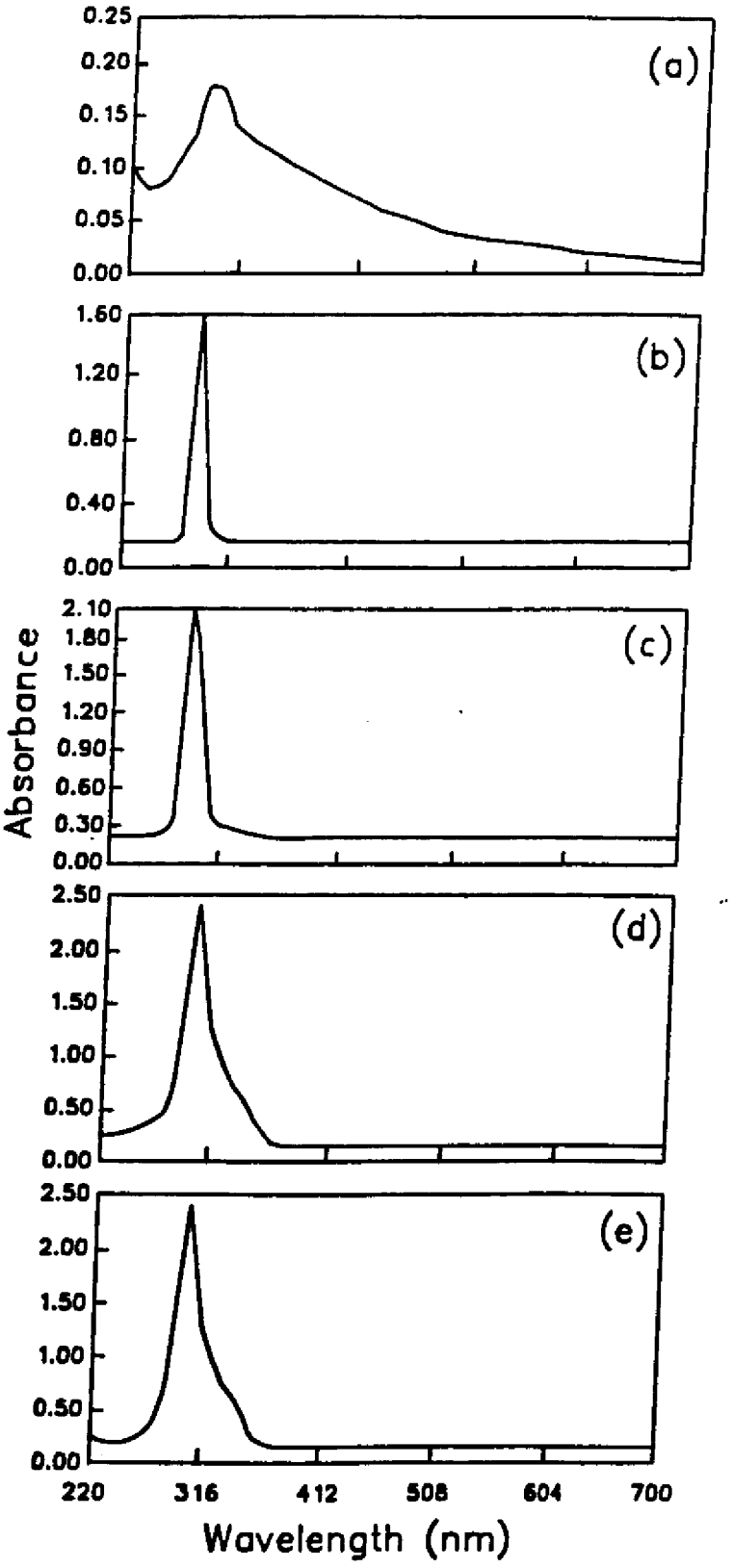

Figure 3. Scanning spectra of (a) lobster polyphenoloxidase (PPO), (b) kojic acid, (c) DL-DOPA, (d) kojic acid plus lobster PPO, and (e) kojic acid plus DL-DOPA.

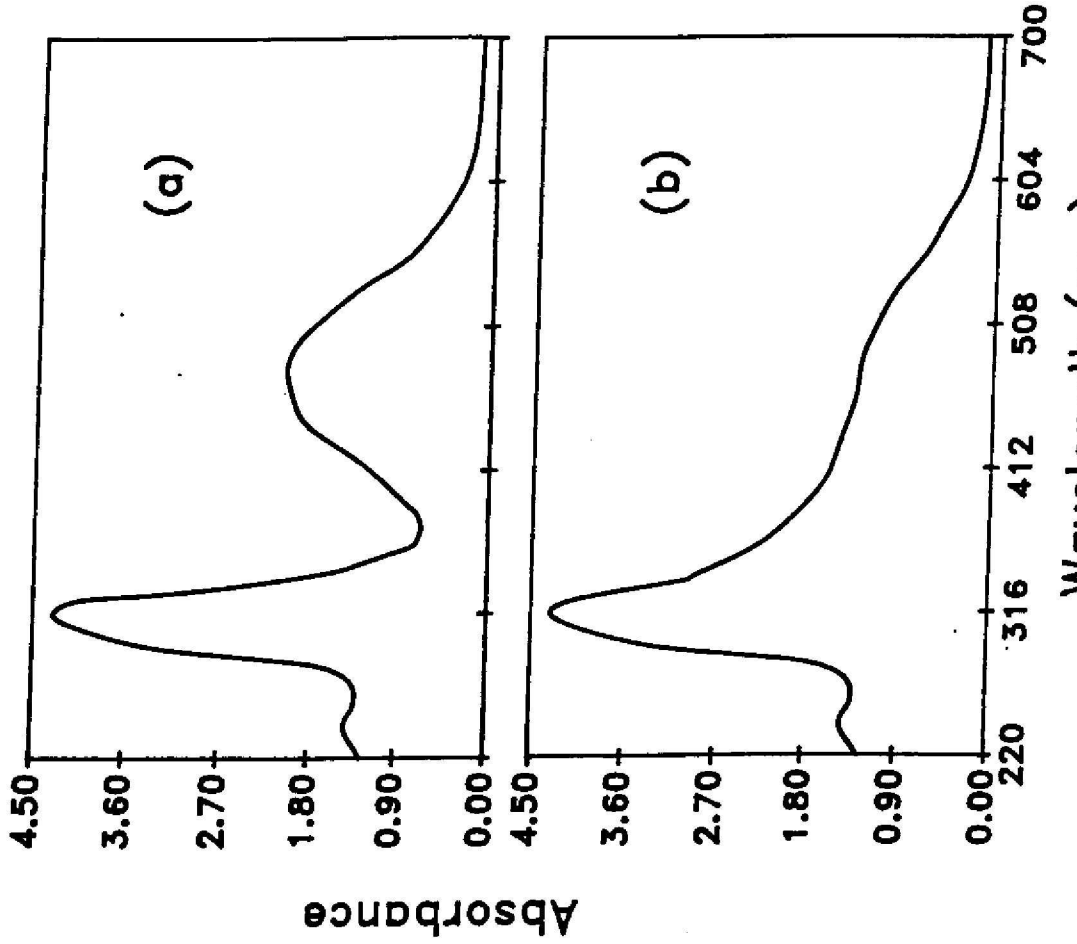

Figure 4. Scanning spectra of (a) dopaquinone and (b) dopaquinone plus kojic acid.

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APPLICATION OF COMPUTER VISION TO SEAFOOD QUALITY EVALUATION.

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INTRODUCTION

Importance of seafood

U.S. Consumption of seafood has enjoyed a steady increase. In 1987, the per capita consumption was 15.4 pounds (NMFS, 1988). Seafood is perceived as "healthy" compared to other protein sources of animal origin.

However, an increase in the demand and consumption of seafood has also created an increased level of interest in the role of seafood in the transmission of food borne diseases (Garrett, 1988). Some sectors of the population perceive seafood as having more health risks. These perceived risks are used towards regulations regarding mandatory seafood inspection.

HACCP

An important concept of food safety is that all components of a food system can be planned to control or eliminate product hazards. The goal is to integrate all parts of a food production sequence to prevent microbial, physical and chemical hazards in the final product. Labeled as Hazard Analysis and Critical Control Point (HACCP), this system is being accepted worldwide for the management of preventive food safety in an increasingly complex food production technology. HACCP concept works as follows :

1 - Assess hazards associated with growing, harvesting, processing and manufacturing, distribution, marketing, preparation, use of a raw material or food.

2 - Determine or identify critical control points required to control these hazards.

3 - Establish and develop procedures to monitor these critical points.

HACCP is emerging as the key element of the strategy of U.S. regulatory agencies for consumer protection. Food industry in general, and seafood industry in particular are developing HACCP programs that apply to their particular operation, in cooperation with regulatory agencies.

In 1989, in an attempt to address perceived needs for more seafood inspection, raw shrimp HACCP standards were developed with the collaboration of National Fisheries Institute, National Fisheries Education and Research Foundation, shrimp industry representatives, and academia. Table 1 lists the critical raw shrimp processing steps, the hazards associated with these steps, monitoring necessary, and records to be kept (National Fisheries Institute, 1989). The process model described in this manner was successfully tested in a number of processing operations. It can be seen that virtually every monitoring step in the proposed HACCP program involves visual observation and evaluation. In addition, several critical steps require sensory evaluation (smelling and evaluating the texture), chemical and/or microbiological analysis as appropriate, and determination of size count. These requirements pose difficult and sometimes impractical requirements on many sectors of the processing industry. One solution may be the integration of automatic processes for monitoring by a computer (Newell et al., 1986; Plossl,

1973). For example, the visual evaluation of melanosis and other quality attributes, and size count could be accomplished by machine vision (Pau and Olafson, 1991). Chemical analysis (e.g. ammonia, trimethylamine, sulfites, other additives etc..) may be performed with probes as they become commercially available for headspace sampling, and texture analysis could be accomplished by Instron type force-deformation instruments, all integrated into a system under computer control. Such computer systems could gather this information and "index judgement" on the final product quality and potential safety. This expert system would allow for automated operation, objective evaluations, rapid and flexible response, and good record-keeping, all necessary for successful implementation of HACCP guidelines.

Table I. Raw shrimp operations critical control points.

STEP	HAZARD	MONITORING	RECORDS
Unload Receive	Mislabeling Decomposition Excessive additives Pathogens Filth T abuse Contaminants	Visual and sensory evaluation Chemical, microbial analysis, Net weight, Size count	Product buy ticket Frozen Product Receiving Report NUOCA
Thaw	Microbial contamination T abuse, Foreign materials, Water quality, Unsanitary equipment Overcapacity	Visual observation	Log of NUOCA
Exami-nation	microbial filth T abuse Human error	Supervisor checks of operations	Log of NUOCA
Peeling deveining	Microbial contamination T abuse Chemical contamination Foreign materials	Visual observations, supervisory checks, Time temperature checks	Log of NUOCA
Deheading	T abuse, Microbial contamination, Filth	Visual operations Supervisory checks	Log of NUOCA
Re-icing	T abuse, Microbial contamination Foreign materials Water quality unsanitary equipment	Visual observation	Log of NUOCA
Optional dip	Additive abuse T abuse Contaminated dip	Visual observation Additive determination	Log of additive usage, Log of NUOCA
Glaze (IQF only)	Over or underglaze microbial contamination Foreign materials Unsanitary equipment	Measure glaze percent Visual observation	Log of NUOCA
Packaging weighing	Defective packaging, filth, incorrect labeling, foreign material, T abuse, human error, microbial contamination	Visual observation Supervisory checks Daily checks of scales	Scale calibration report, log of NUOCA

The objective of this project was to design and develop a computer-based expert system for quality evaluation of shrimp. User-friendliness and ease of used were emphasized to allow computer novices to operate the system. With the imminent Federal regulation of seafood inspection, this system would offer shrimp processors complete documentation for legal purposes.

MATERIALS AND METHODS

A hand-held scanner (Scanman Plus, Logitech Inc., Fremont, CA.) was connected to a microchannel based IBM PS/2 computer. An alternative version will run on a portable computer with an ISA bus architecture. The "programming toolkit" interface for the scanner was provided by Logitech Inc. to develop a custom computer program written in Turbo C language. The cost of the hardware, including the computer is less than $2000. The scanner itself costs about $200. The Turbo C compiler costs nearly $100, and is not required once the developed program is completed in our lab.

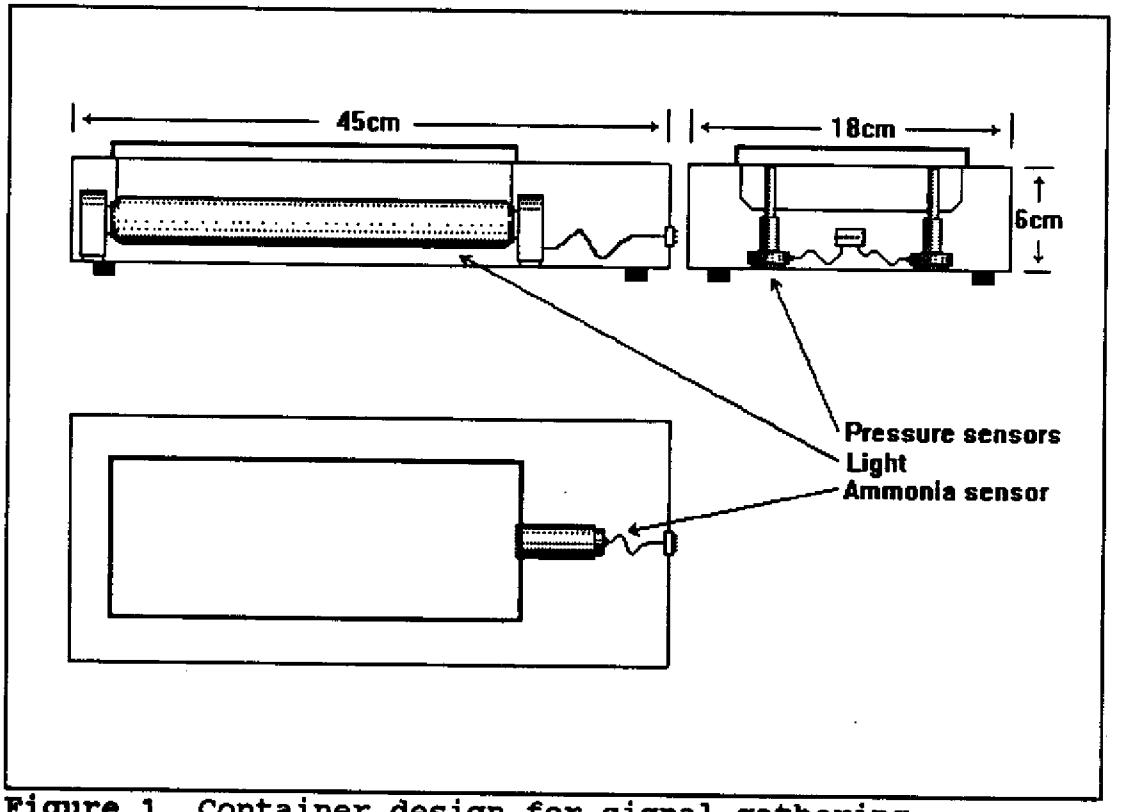

Figure 1. Container design for signal gathering.

The equipment concept was to let the system accept inputs from three devices (Figure 1). The image from a scanner is input to determine discoloration, a signal from a chemical sensor measuring the ammonia (Finne, 1982) emitted by the sample, and a signal from a pressure sensor measuring force-deformation data (texture) of the shrimp (Figure 2). These devices would account for the color, smell, and texture attributes currently considered by human experts. The chemical and texture sensors will transmit their signals to the PC through an on-board analog input card. The input from these sensors is optional and is activated by the operator. The details of the sensor design are currently being worked on.

The operator will select a sample consisting of a number of shrimp. After examining the sample s/he picks out three shrimp one of which s/he considers to be the best, one to be the worst, and one to be an average shrimp. On a specially developed form the operator marks the discolorations of all three shrimp. S/he then places this form in the scanning fixture and places the scanner on the top glass. S/he selects SCAN IMAGE from the main menu of the program, s/he is prompted for supplier name and sample number, and whether the analog sensors should be activated. Then, the scanning screen is displayed, and the operator moves the scanner over

the fixture until it hits the end-stops. The results of the evaluation are then displayed on the screen. The operator has the option to save the image to disk for future reference, and to read from disk an existing image to evaluate it (Figure 3).

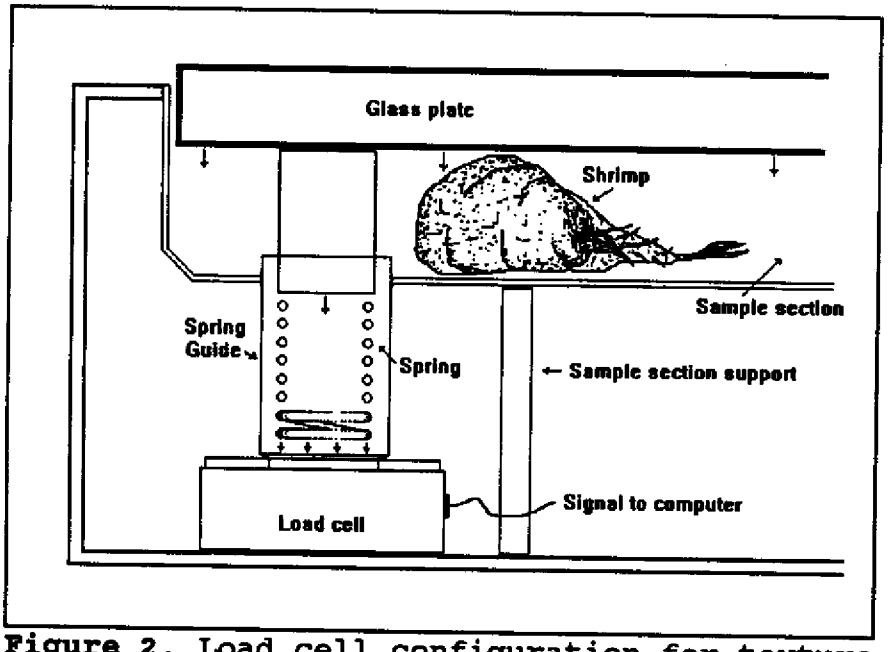

Figure 2. Load cell configuration for texture evaluation.

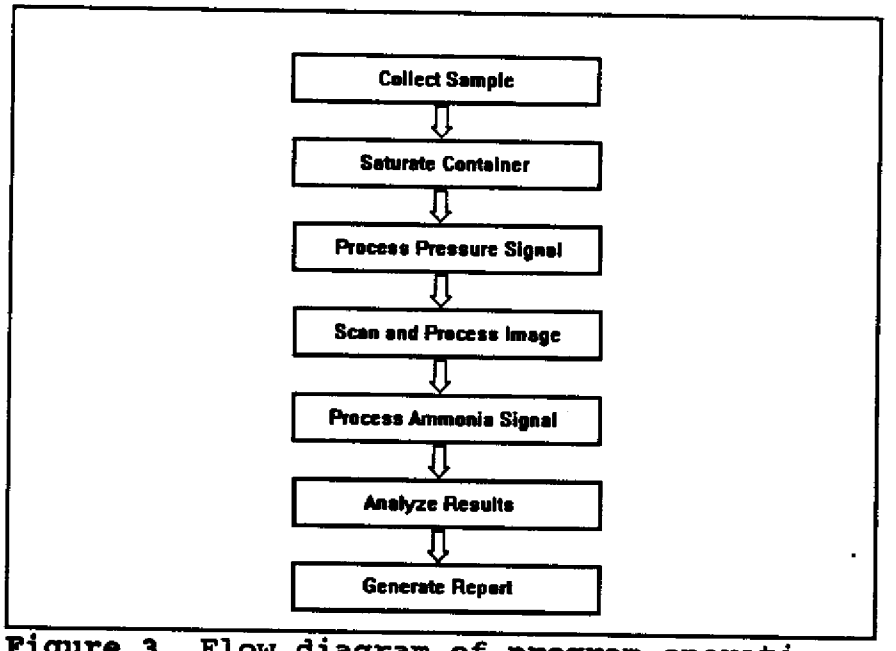

Figure 3. Flow diagram of program operation.

The operator selects INPUT SHIPMENT INFORMATION from the main menu to enter information on the shipment for reporting purposes. A report can be viewed by selecting EVALUATION AND REPORT. The report is stored to disk, and the operator can select to print out

a hard-copy or transmit a copy via a modem. The main menu allows the operator to view supplier perfor-mance graphically with histograms and trend graphs. These graphs are constructed from data in a database being accumulated with system use.

The accuracy of the scanner hardware was tested by scanning the same black and white picture repeatedly (Figure 4). White pixels were counted. Mean average percent error (MAPE), calculated as follows, was used in determining the accuracy of the readings. First, the mean response is calculated for all trials: $\mu = 1/n \, \Sigma \, d_i$; for i = 1..n; the deviation of each term from the mean is then calculated: $e = d_i - \mu$; for i = 1..n; Then the sum of all absolute values of 'e', the deviation each time, deviated by d, the observation each time, is multiplied by 100/n: $MAPE = 100/n * \Sigma \mid e / d_i \mid$; for i = 1..n; Scanning area had to be maintained constant to maintain accuracy, therefore, a special fixture was developed to place the form. Guides along the length ensure longitudinal consistency, and end-stops ensure a consistent total scan area. The moving speed of the scanner is important. The scanner has a speed indicating light which blinks if the scanner movement is too fast.

To determine individual differences in the coloring of pictures, 15 people were asked to color the same 3 shrimps. Forms were scanned and MAPE, mean and standard deviation calculated.

Finally, one person was asked to color in the same three shrimp on 15 different days, to evaluate day-to-day differences in evaluation by the same person. These were scanned in and MAPE, mean and standard deviation calculated.

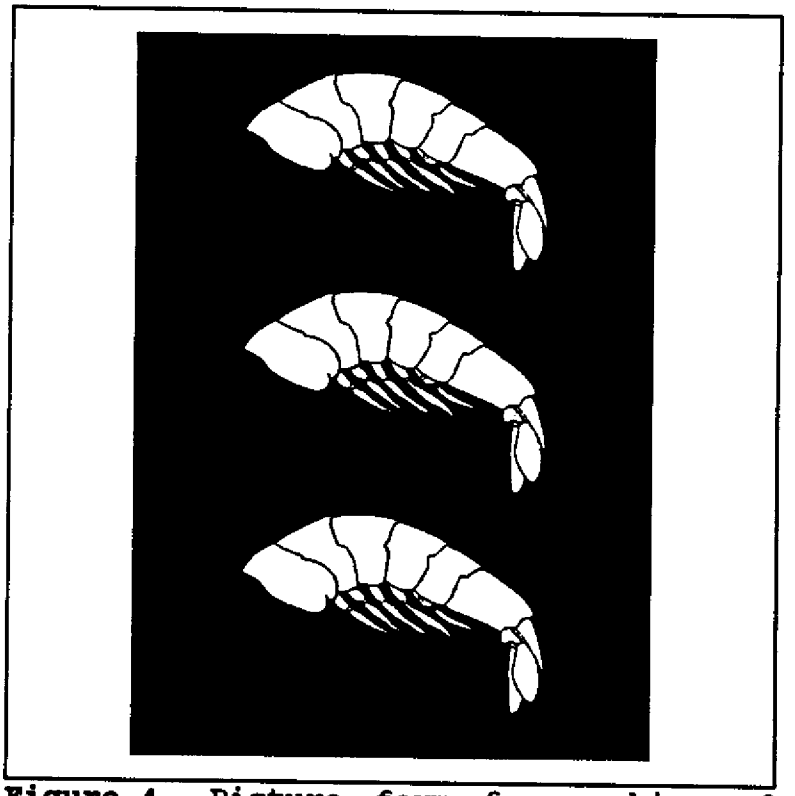

Figure 4. Picture form for marking of melanosis.

Table II. Results of hardware accuracy of the hand-held scanner. Scanning same picture 30 times.

Scan number	Absolute deviation from the mean
1	54
2	124
3	68
4	173
5	529
6	16
7	164
8	107
9	217
10	292
11	331
12	49
13	433
14	95
15	22
16	381
17	106
18	174
19	28
20	397
21	23
22	414
23	193
24	5
25	38
26	334
27	98
28	430
29	46
30	256

Mean:	**93603**
Standard Deviation:	**223**
High Deviation:	**529**
Low Deviation:	**5**
MAPE:	**0.2%**

RESULTS

Mean average % error (MAPE) for the hardware accuracy was calculated, for all settings of the scanner, giving an error of 0.2% as shown in Table 2. The low MAPE value indicates that the scanner hardware has excellent accuracy when used for this purpose.

Results of experiments to deter-mine the consistency of different people to judge same shrimp sample are shown in Table 3. Results showed that if diffe-rent people with no experience color in the pictures, we can expect a MAPE error of 11%. In this case, the MAPE value is large. However, the judges were not trained before experiments. Prior training would reduce the MAPE value.

Results of evaluation of accuracy of the same person to judge the same shrimp sample on different days are shown in Table 4. The results (MAPE=7%) indicate that less deviation is expected in evaluations if one person is assigned the task of operating this system. The person who performed the evaluations was not trained before the evaluations. Prior training would reduce MAPE values.

Table III. Results of evaluation accuracy of the same picture by different persons.

Judge no.	Absolute deviation
1	7559
2	1676
3	12056
4	3443
5	7140
6	2930
7	15798
8	3097
9	2416
10	4787
11	10569
12	13060
13	2420
14	14317
15	5928
Mean:	68882
Standard Deviation:	8535
High Deviation:	15798
Low Deviation:	1676
MAPE:	10.5%

Table IV. Results of scanning the same picture by the same person on different days.

Picture no.	Absolute deviation
1	3919
2	4924
3	506
4	798
5	3638
6	8205
7	3619
8	4979
9	3208
10	1294
11	5186
12	4305
13	4497
14	2147
15	11823
Mean:	57869
Standard Deviation:	5039
High Deviation:	11823
Low Deviation:	506
MAPE:	7.1%

FUTURE APPLICATIONS

This system will be improved by the elimination of the subjective evaluation step by an individual. Cameras or other optical devices connected to a computer will replace the scanner, and will directly gather size, color, species and defect data. When the sample in the container is scanned the shrimp are displayed in the image as dark clusters, consisting of one or more shrimp. The number of these clusters can be easily determined by use of established image processing techniques. To determine total number of shrimp in the container, it is first necessary to find the number of shrimp in each cluster. The following approach has been developed and is being tested. Let k be the number of clusters in the image. (known); p an estimate of pixels per shrimp (known); S the number of pixels per shrimp in the container (unknown); n_i be the number of shrimp in cluster i; for i = 1,2,...,k (unknown); a_i be the number of pixels in cluster i; for i = 1,2,...,k (known); T be the total number of shrimp in the container (unknown). The objective is to find S in the interval $\{p, \max(a_i)\}$, such that the sum of the squared errors (SSE) is minimized, where SSE is calculated as follows: $SSE = \Sigma (a_i - S\, n_i)^2$, i = 1 to k. As SSE is not a convex function, it is necessary to try all S in the interval $\{p, \max(a_i)\}$.

Then, $S_1 = p$ and $S_j = S_{j-1} + 1$, for j = 2 to $(\max(a_i) - p)$. For each value assigned to S, the n_i's are calculated as: $n_i = \text{round}(a_i/S)$ (returning an integer value for n_i)

For each S the sum of the squared errors (SSE) is calculated. For the optimal S corresponding to the minimum SSE, T equals the sum of the n_i's, for i = 1,2,...,k.

The system will be compatible with chemical and physical sensors that may optionally be attached to it. There is ongoing research on the implementation of the analog sensors and improvement on the image input of the actual shrimp. We are working towards building a sample container equipped with the necessary sensors. The sample will be placed in the container and allowed to equilibrate for a few minutes before the evaluation is performed. This will eliminate the deviations associated with the coloring of the pictures, giving more accurate results.

A related project, currently in its proposal stages, is the continuous inspection and evaluation of shrimp flowing on a conveyor belt, with a computer vision system. Portions of the work performed during the current project will be applicable in this continuous inspection.

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THE ANTIMICROBIAL EFFECTS OF LACTIC ACID, ACETIC ACID, AND SODIUM LACTATE ON STORAGE STABILITY OF FRESH BLACK SEA BASS AND MULLET FILLETS STORED UNDER SIMULATED RETAIL CONDITIONS

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Like meat, fish and other seafood may be spoiled by autolysis, oxidation, or bacteria activity, or most commonly by combinations of these. Most fish flesh, however, is considered more perishable than meat because of more rapid autolysis by the fish enzymes, and because of the less acid reaction of fish flesh that favors microbial growth (4).

In an effort to retard spoilage in food systems, the use of organic acids as antimicrobial agents has been extensively studied (1, 2, 3, 5). However, in many instances the interaction between the organic acids and the food to be preserved results in undesirable chemical and physical changes in the finished product.

Except for seafood, the food industry relies heavily upon the use of antimicrobial agents to extend shelf life and preserve freshness of products. It is therefore essential that objective measurement devices be utilized in the evaluation of foods. These devices will assist researchers and quality control personnel in making the most accurate decisions concerning the utilization of antimicrobial agents in food products and, their (antimicrobial agents) effect on the quality of the food at different intervals during storage.

This study was undertaken to determine the antimicrobial effects of lactic acid, acetic acid, and sodium lactate on fresh fish fillets stored under simulated retail conditions; to compare the color changes that occur in the fillets; and to evaluate the effectiveness of the COLORMET spectrum analyzer in providing objective color measurements.

MATERIALS AND METHODS

Sample Preparation

Fresh skinless fillets of Florida Mullet (Mugil cephalus) and Black Sea Bass (Centropristis striata) were purchased from a local seafood supplier in Gainesville, Florida. The fish were harvested twenty hours prior to filleting. The fillets were immediately rinsed in an ice water bath (50:50, tap water:crushed ice). For each fish, the rinsed fillets were divided into six groups and subjected to six treatments, including: control, no acid added; 1.0 % lactic acid; combination of 0.5 % lactic acid and 0.5 % acetic acid; 1.0 % acetic acid; 1.0 % sodium lactate and; 2.0 % sodium lactate (PURAC Inc., Arlington Hts., IL). The treatments were applied by immersion of the fillets in the solutions for 60 seconds.

The treated fillets were stored on crushed ice for eight hours each day at approximately 33-35 F. After eight hours storage, the fillets were rinsed in an ice water bath. The fillets were packed in layers in crushed ice, and stored overnight at 33-35 F. On the following morning, the fillets were removed from the crushed ice, and displayed for eight hours on crushed ice (33-35 F). This procedure continued through 6 days.

The fillets were analyzed after 0, 3, and 6 days storage at 33-35 F. The analyses included color measurements and aerobic plate counts. The pH values of the treatment solutions were as presented below:

Table 1. pH Values for Treatment Solutions

Treatment	pH Value
Control	6.41
1.0 % Lactic acid	2.15
0.5 % Lactic acid: 0.5 % acetic acid	2.24
1.0 % Acetic acid	2.72
1.0 % Sodium lactate	5.67
2.0 % Sodium Lactate	5.71

Color Measurements

The color measurements were determined with a hand-held spectrum analyzer referred to as COLORMET (Instrumar LTD, St. John's, NF). It provided readings of spectral reflectance, L (degree of whiteness), a (degree of redness), and b (degree of yellow/brown) color values. Duplicate 3-centimeter diameter areas of each fillet were measured for color. Subjective observations concerning the color changes in the fillets during storage were also recorded by the researcher, where a score of 1 = extreme off-color; 3 = fair, acceptable color; and 5 = excellent color (i.e. fillet exhibited characteristic fresh color).

Microbiological Analysis

The aerobic plate count method was employed as instructed by Busta et al (6).

RESULTS AND DISCUSSION

The objective, as well as subjective evaluation of the fillets revealed that as storage time increased, the color of the fillets deteriorated (Tables 2, 3, and 4). In addition, color changes were observed as a result of the acid treatments. Three hours after acid treatment of the fillets, color changes were observed. The 'L' color values, which measured the degree of lightness exhibited by the mullet fillets, revealed that treatment with the acids resulted in a bleaching of the characteristic red oxymyoglobin pigment associated with fresh mullet. The 'a' color values, which measured the degree of redness in the mullet fillets, were significantly (observed) lower for all acid treated fillets when compared to the control fillets. The color loss, as determined by the COLORMET and subjective color evaluations, occurred at a slower rate for the mullet fillets treated with sodium lactate through 6 days storage.

Similar results were reported for the Black Sea Bass fillets. Except for the fillets treated with a combination of lactic and acetic acid, all fillets resulted in color changes due to the initial treatment (i.e. Day 0) of the fillets prior to storage. The acid (lactic and acetic acid) treated fillets exhibited a cooked appearance, that was evident by the elevated 'L' values when compared to the control fillets.

Table 2. Subjective Color Measurements

Day	Treatment	Mullet	Black Sea Bass
0	Control	5	5
3		4	4
6		4	4
0	1.0 % Lactic acid	3	4
3		2	3
6		2	2
0	0.5 % Lactic acid 0.5 % Acetic acid	4	4
3		3	3
6		2	3
0	1.0 % Acetic acid	3	4
3		2	3
6		2	3
0	1.0 % Sodium lactate	5	5
3		4	4
6		4	4
0	2.0 % Sodium	4	4
3		4	4
6		3	4

* A score of 1 = extreme off-color, 3 = Fair, acceptable color, and 5 = excellent color.

Table 3. COLORMET Color Values for Mullet

Day	Treatment	Color Values L	a	b
0	Control	44.2	2.9	6.4
3		53.9	2.6	8.9
6		59.0	0.9	10.7
0	1.0 % Lactic acid	63.4	-1.2	13.9
3		65.9	-0.2	8.7
6		53.9	-0.5	7.1
0	0.5 % Lactic acid 0.5 % Acetic acid	74.8	-1.4	7.8
3		65.4	-0.3	12.7
6		61.4	-0.5	10.7
0	1.0 % Acetic acid	63.2	0.7	9.5
3		75.2	-0.7	9.6
6		67.9	-1.2	9.8
0	1.0 % Sodium lactate	44.7	5.3	8.8
3		65.5	1.5	9.4
6		56.9	1.6	10.0
0	2.0 % Sodium lactate	64.4	-1.1	8.2
3		56.0	2.9	10.5
6		59.3	0.8	9.7

Table 4. COLORMET Color Values for Black Sea Bass

Day	Treatment	Color Values L	a	b
0	Control	67.6	-1.2	7.0
3		73.2	-1.8	6.6
6		70.4	-2.4	8.1
0	1.0 % Lactic acid	75.1	-1.9	7.8
3		72.2	-2.5	3.6
6		74.8	-2.2	3.8
0	0.5 % Lactic acid 0.5 % Acetic acid	60.0	0.3	8.6
3		78.7	-1.8	5.6
6		76.0	-1.9	5.6
0	1.0 % Acetic acid	78.0	-1.4	5.5
3		79.3	-1.4	5.2
6		75.2	-2.2	2.5
0	1.0 % Sodium lactate	63.8	-1.4	8.1
3		72.5	-1.8	6.4
6		70.0	-2.4	10.1
0	2.0 % Sodium lactate	64.4	-1.1	8.2
3		70.0	-1.8	5.9
6		68.3	-2.5	7.0

Table 5. Aerobic Plate Count

Day	Treatment	37 C Aerobic Plate (count per gram) Mullet	Black Sea Bass
0	Control	8E3	4E3
3		2E4	15E3
6		1E6	6E5
0	1.0 % Lactic acid	13E3	1E4
3		4E3	3E4
6		17E3	12E3
0	0.5 % Lactic acid 0.5 % Acetic acid	4E5	68E3
3		1E5	3E4
6		12E4	2E6
0	1.0 % Acetic acid	14E3	15E3
3		9E4	8E4
6		7E4	55E3
0	1.0 % Sodium lactate	6E4	1E5
3		4E5	1E5
6		1E5	4E5
0	2.0 % Sodium lactate	4E3	1E5
3		1E4	1E4
6		5E4	2E4

Except for the Black Sea Bass fillets on day 6, the total microbial population of all acid-treated fillets remained less than one-million through 6 days storage (Table 5).

CONCLUSIONS

1. The color of all fillets deteriorated as storage time increased. This was evident from the data collected with the COLORMET, as well as subjective color measurements.

2. Treatment of the fillets (Black Sea Bass and Mullet) with 1.0 % lactic acid, 1.0 % acetic acid and a combination of 0.5 % lactic acid and 0.5 % acetic acid resulted in accelerated loss of color.

3. Treatment of the fillets (Black Sea Bass and Mullet) with sodium lactate resulted in color stability through 3 days for the fillets treated with 2.0 % sodium lactate, and through 6 days for the fillets treated with 1.0 % sodium lactate.

4. Except for the control fillets, and the fillets treated with sodium lactate, all treatments resulted in soft (undesirable) textures.

5. The total microbial population of all acid-treated fillets remained less than one-million through 6 days storage.

6. The color values recorded for COLORMET revealed a positive correlation to visual color differences observed by the researcher.

7. The COLORMET provided objective measurements for the evaluation of color changes in the fish fillets during simulated retail supermarket storage.

8. The COLORMET provided a rapid, easy and convenient method for determining the effects of the acids on color stability during storage of the fillets.

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AN ECONOMIC REVIEW OF THE SOUTHEASTERN SHRIMP PROCESSING INDUSTRY

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Emerging issues necessitate a more complete economic evaluation of the seafood processing industry. Included among these issues are (i) mandatory inspection of seafood, (ii) creation of regional and/or species specific seafood marketing councils, and (iii) an increasing concern about seafood processing discharges and calls for their reduction.

The nation's production of processed shrimp equalled $1.175 billion in 1988. Seventy-nine percent, or $932 million, of this processing activity occurred in the Southeast (defined as the coastal states of North Carolina through Texas). Shrimp processing activities typically account for about a quarter of the nation's and upwards of two-thirds of the Southeast's total seafood processing activities by value and represent the largest seafood processing component at both the regional and national level.

OBJECTIVES AND METHODS

The focus of this paper is the Southeast shrimp processing industry. Such a limitation is justified based on the significance of this industry. Specifically, the paper will present an economic analysis of the Southeast shrimp processing industry with emphasis on product form. This analysis covers the 1973-88 period and is based on the latest available secondary data from the National Marine Fisheries Service (NMFS), Fisheries Statistics Division.

The processing data used in this analysis represents voluntary dealer responses to annual end-of-year surveys conducted by NMFS personnel. These surveys represent a census of all seafood processing facilities and elicit information pertaining to the production (pounds and value) of a variety of value-added seafood products and associated employment. The data represent detailed processing information on each processing facility through time and is by far the most complete set of information available on the Southeast seafood processing industry. For purposes of this analysis, all quantities of processed shrimp have been converted to a headless shell-on basis by using the conversion factors developed by NMFS. These conversions are: 1.00 for headless shell-on shrimp, 1.28 for peeled raw shrimp, 1.97 for peeled cooked shrimp, 0.63 for breaded shrimp (raw and cooked), and either 4.83, 13.63, or 29.76 for canned and specialty shrimp products; depending upon the type. Thus, for instance, peeled cooked shrimp was converted to a headless shell-on basis by multiplying the product weight of the peeled cooked shrimp by 1.97. The conversion allows for more accurate aggregation across product forms. Also, the conversion process allows for a more direct comparison among product types and facilitates discussion.

Use of the conversion procedure means that all prices discussed throughout the paper, even those related to specific product forms, are presented on a headless shell-on basis. This is because the values reported by NMFS were divided by the converted weights, i.e., headless shell-on weights. Thus, for example, the calculated price per pound of peeled raw shrimp will be lower than that price which occurred at the time of sale by 22% (i.e., value/(pounds * 1.28)) while the price per pound of breaded shrimp will be higher by about 59% (i.e., value/(pounds * 0.63)).

SOUTHEASTERN SHRIMP PROCESSING ACTIVITIES

Southeastern shrimp processing activities are examined in this section of the paper. To do so, the industry in its aggregate is first examined. Second, attention is given to analysis of the primary shrimp products produced by the industry. Then, production of these primary shrimp products are compared amongst one another and to the aggregate. Finally, a summary is provided.

Aggregate Shrimp Processing Activities

Shrimp processing activities occur in all the Southeastern states with the exception of South Carolina in some years. The total number of plants engaged in these activities, as indicated in Table 1, has gradually been declining when evaluated on a four-year basis and this decline has been particularly evident during the 1984-88 period. The 151 plants engaged in shrimp processing activities on an annual basis during 1985-88 represent a reduction of 14 (about 9%) when compared to the 165 plants operating during 1981-84 and a reduction of 22 (about 13%) when compared to the average of 173 plants engaged in shrimp processing activities annually during the 1973-76 period.

Table 1. Selected Statistics Pertaining to Aggregate Southeast Shrimp Processing Activities for Selected Time Periods, 1973-88

TIME PERIOD	NO. OF PLANT	PROCESSED POUNDS[a]	PROCESSED VALUE		PROCESSED PRICE	
			Current	Deflated[b]	Current	Deflated
	#	1,000s	—— $1,000s ——		– $/lb –	
1973-76 avg.	173	185,388	396,284	917,773	2.14	4.95
1977-80 avg.	172	223,012	699,827	1,187,893	3.14	5.33
1980-84 avg.	165	230,259	909,819	1,099,628	3.95	4.78
1985-88 avg.	151	268,443	996,937	1,054,636	3.71	3.93

[a] processed pounds are expressed on a headless shell-on basis

[b] the deflated processed value and price are based on the 1988 Consumer Price Index, i.e., 1988 = 100

While the number of Southeast shrimp processing plants has been declining, the quantity of shrimp being processed has been steadily increasing (Table 1). For instance, the 230.3 million pounds being processed annually during 1980-84 represents about a 24% increase over annual processing activities of 185.4 million pounds during 1973-76. By 1985-88, average annual processing activities had increased to 268.4 million pounds, or about a 45% increase above the shrimp processing activities observed annually during 1973-76. Roberts et al. (1) have traced these increasing processing activities to a greater utilization of imported shrimp; needed in light of insufficient domestic landings. Relevant findings are found elsewhere in these Proceedings (Keithly et al.).

Increasing processing activities in conjunction with a decline in processing plants indicate that production per plant is increasing. This is clearly shown in Table 2. Overall, average annual shrimp production per plant during 1985-88, 1.78 million pounds, is 27% above that observed during 1981-84 (1.40 million pounds) and about 66% above that observed during 1973-76 (1.07 million pounds).

Table 2. Average Aggregate Shrimp Processing Activities per Plant in the Southeast for Selected Time Periods, 1973-88.

TIME PERIOD	PROCESSED POUNDS PER PLANT[a]	PROCESSED VALUE PER PLANT	
		Current	Deflated
	1,000s	----- $1,000s -------	
1973-76 avg.	1,071.6	2,290.7	5,305.0
1977-80 avg.	1,296.6	4,068.8	6,906.4
1981-84 avg.	1,395.5	5,514.1	6,664.4
1985-88 avg.	1,777.8	6,602.2	6,984.3

Source: Compiled from information in Table 1.

[a] processed pounds are expressed on a headless shell-on basis

The total value of Southeastern shrimp processing activities has increased steadily during the 1973-88 period (Table 1) with the average being one billion dollars annually during 1985-88. This increasing value is the result of two factors. First, as noted, the quantity being processed has been increasing. Second, the price of the processed product has, until recently, been increasing (Table 1). For instance, processors received an average of $3.95 per pound for their product (expressed on a headless shell-on basis) during 1981-84 compared to only $2.14 during 1973-76. The average price received during 1985-88, $3.71 per pound, however, is below the 1980-84 average price by about $0.24 per pound.

Much of the increasing price and, hence, value is attributable to inflation. To remove these inflationary effects, annual prices and values were adjusted to the 1988 dollars by dividing the current figures by the 1988 Consumer Price Index. Such a procedure resulted in estimates of deflated, or real, prices and values which are given in Table 1. As indicated, the deflated value of Southeast shrimp processing activities peaked at about $1.19 billion annually during 1977-80 and has since fallen by about 11% to $1.054 billion annually during 1985-88. This decline has come in spite of large increases in the quantity processed, indicating a decline in the deflated price of the processed product. This decline is identified in Table 1, with the average annual deflated price falling from $5.33 per pound during 1977-80 to $3.93 per pound during 1985-88, and is largely the result of increasing imports. Discussion of these increasing imports and their impact on prices is presented elsewhere in this proceedings issue (Keithly et al.).

When examined on a per plant basis, the current annual value of processing activities has almost tripled between 1973-76 and 1985-88; from $2.29 million to $6.60 million (Table 2). After adjusting for inflation, however, per plant shrimp processing activities have remained virtually unchanged since the 1977-80 period. This finding, in conjunction with the fact that the quantity of shrimp processed per plant has increased by more than a third during the same period, would tend to indicate that industry real profitability may be declining unless one of the following conditions hold: (i) plants are realizing economies to scale in their production processes, (ii) there has been significant technological change within the production process, (iii) input costs are declining, or (iv) some amalgam of the above factors is occurring. Of these different factors, the imput cost of raw shrimp, the largest component of variable costs, has declined in recent years. Whether the decline in shrimp imput costs has been sufficient such that plants have realized no loss in real profitability, or whether other changes have occurred to the extent that plants have maintained previous levels of real profitability, is unknown without collection and analysis of primary data.

Processing Activities By Product Form

For discussion purposes, Southeast shrimp processing activities were evaluated on the basis of four, all inclusive, product forms: (i) raw headless, (ii) peeled, both raw and cooked, (iii) breaded, and (iv) "other". This "other" category consists of canned shrimp and specialty products such as gumbos and dried shrimp.

As indicated in Table 3, many of the Southeast shrimp processing plants produce more than one of the aforementioned product forms. During 1973-76, for example, an average of 27 Southeastern shrimp processing plants produced two product forms on an annual basis while another 15 plants produced at least three different types of processed shrimp. Altogether, an average of 42 of the 173 plants engaged in processing activities during 1973-76, or 24% of the total, produced more than one processed shrimp product on an annual basis.

Table 3. Average Number of Shrimp Products Produced by Southeast Shrimp Processing Plants, for Selected Time Periods, 1973-88.

TIME PERIOD	NO. OF PLANTS	NUMBER OF PRODUCTS PRODUCED		
		one	two	3 or 4
1973-76 avg.	173	130	27	15
1977-80 avg.	172	118	38	15
1981-84 avg.	165	110	40	15
1985-88 avg.	151	90	42	18

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

Though the total number of Southeast shrimp processing plants had declined to an average of 151 by 1985-88, the number producing more than one shrimp product increased to 60; or 40% of the total. The number of plants processing three or more shrimp product forms, 18, constitutes 11.9% of the total and is about three percentage points above the 8.7% (15 of 173) observed during 1973-76.

In general, there is a clear trend in the percentage of Southeast shrimp plants processing more than one shrimp product. Several explanations can be given for the apparent increase in diversification. First, increased imports may have allowed for more continuous year-round plant operations and, hence, an increased possibility for diversification. Second, increased imports may have provided additional sources of those specific size count shrimp needed to produce the more value-added products. Third, diversification may have been undertaken by Southeast shrimp plants as a means of reducing risk associated with the production of only one product form. Finally, consumer demand for the different product forms may have increased and plants have diversified as a means of "capturing" this demand.

Analysis of the four different shrimp product forms is provided below. The information used for this analysis is given in Tables 4 and 5.

Raw headless shrimp processing. The number of Southeast shrimp plants processing a raw headless shrimp product consistently exceeds one-hundred (Table 4) and there appears to be no long-run trend towards either an increasing or decreasing number of plants producing this product when evaluated on a four-year basis. The production of raw headless shrimp on an annual basis has averaged about 100 million pounds since the 1977-80 period like the number of plants, there was no trend towards either increasing or decreasing production. This likely

reflects the fact that Southeast shrimp landings have remained relatively constant, when examined on a long-run basis, since the mid 1970s. Domestic landings, rather than imports, represent the core input product for raw headless processing activities.

While the current value of raw headless shrimp processing activities has consistently increased, from an average of $166.7 million annually during 1973-76 to $438.3 million during 1985-88, the deflated value of these activities has fallen sharply since its peak during 1977-80 (Table 4). The annual deflated value of raw headless shrimp processing activities during the 1985-88 period, $464.6 million, was only about 80% of the $582.8 million annual activities observed during 1977-80; despite the fact that the annual pounds processed during 1985-88, 105.2 million, was almost 7% above that occurring during 1977-80.

The reason for the decline in the deflated value of raw headless shrimp processing activities since the 1977-80 period is, as indicated in Table 4, the result of a significant decline in the deflated product price. Overall, the calculated deflated price of $4.41 per pound during 1985-88 was only 75% of the peak 1977-80 deflated price of $5.90 per pound.

Raw headless shrimp processing activities per plant, evaluated on a four year period, have ranged from a low of 685.6 thousand pounds annually during 1973-76 to a high of 915.1 thousand per plant during 1985-88 (Table 5). The current value of raw headless processing activities per plant has more than doubled between the 1973-76 and the 1985-88 periods from $1.57 million to $3.81 million. When expressed on a deflated basis, however, raw headless shrimp processing activities per plant fell sharply since the 1977-80 period and currently average about 80% of those observed during the peak period ($4.04 million annually during 1985-88 compared to $5.07 million during 1977-80).

Peeled shrimp (raw and cooked) processing. The number of Southeast shrimp processing plants engaged in peeling activities has been increasing consistently during the 1973-88 period when evaluated on a four year basis (Table 4). For instance, the 59 plants engaged in peeling activities on an annual basis during 1977-80 represent a 25% increase above the 47 reported annually during 1973-76. The 69 plants reported annually during 1985-88 represent a 17% increase above the 59 reported annually during 1977-80 and almost a 50% increase above the 1973-76 average of 47 plants.

As with the number of plants, the total production of peeled shrimp has been increasing consistently during the 1973-88 period when evaluated on a four year basis (Table 4). Production of 72.3 million pounds annually during the 1981-84 period was more than a third greater than the 52.5 million pounds annually produced during 1977-80 and was more than double the 1973-76 annual production of 35.2 million pounds. Annual production during 1985-88 was just shy of 100 million pounds.

The production of peeled shrimp per plant increased consistently during the 1973-88 period when evaluated on a four year basis (Table 5). It has not expanded as greatly as the total production of peeled shrimp, however, due to the increasing number of plants engaged in peeling activities. The 1.4 million pounds of peeled shrimp per plant annually during 1985-88 does represent about a 90% increase above the 748.9 thousand pounds reported annually during 1973-76 (Table 5).

Both the current and deflated values of total peeled shrimp processing activities increased during the 1973-88 period (Table 4). The sharp decline in the deflated price per pound of the processed peeled shrimp since 1977-80 (from $3.95 to $2.63 during 1985-88) indicates that the deflated value trended up because of the increased quantity processed. On a per plant basis, the deflated value of peeled shrimp processing activities has shown only minimal growth between the 1977-80 and 1985-88 periods, from $3.52 million to $3.72 million

(Table 5), even though the processed quantity per plant has expanded by close to 60% for the period. The relatively stable deflated value of peeled processing activities per plant in conjunction with large quantity increases indicates profitability per unit of output, i.e., pound of peeled shrimp, could have decreased. This would be the case if significant decreases in real input costs per unit of output did not occur.

Table 4. Selected Statistics Pertaining to Southeast Shrimp Processing Activities by Product Form for Selected Time Periods, 1973-88.

PRODUCT FORM & TIME PERIOD	NO. OF PLANTS	PROCESSED POUNDS[a]	PROCESSED VALUE		PROCESSED PRICE	
			Current	Deflated	Current	Deflated
	#	1,000 lbs	----- $1,000s -----		--- $/lb ---	
Raw Headlesss						
1973-76 avg.	106	72,677	166,726	382,465	2.29	5.26
1977-80 avg.	115	98,621	340,817	582,794	3.46	5.90
1980-84 avg.	125	94,344	403,520	488,035	4.28	5.17
1985-88 avg.	115	105,231	438,262	464,624	4.16	4.41
Peeled (Raw & Cooked)						
1973-76 avg.	47	35,197	57,993	134,036	1.65	3.81
1977-80 avg.	59	52,522	124,333	207,397	2.37	3.95
1981-84 avg.	61	72,259	193,836	234,455	2.68	3.24
1985-88 avg.	69	97,464	242,566	256,422	2.49	2.63
Breaded (Raw & Cooked)						
1973-76 avg.	45	52,327	139,817	326,570	2.67	6.24
1977-80 avg.	33	48,393	189,341	321,108	3.91	6.64
1981-84 avg.	26	48,209	275,021	331,633	5.70	6.87
1985-88 avg.	26	56,065	293,135	310,655	5.23	5.54
"Other"						
1973-76 avg.	37	25,142	31,862	75,164	1.27	2.99
1977-80 avg.	31	23,476	45,362	76,638	1.93	3.26
1981-84 avg.	24	15,388	37,583	45,673	2.44	2.97
1985-88 avg.	20	9,683	21,745	22,956	2.25	2.37

Source: Compiled from unpublished data provided by the National Marine Fisheries Service, Fisheries Statistics Division.

[a] processed pounds are expressed on a headless shell-on basis

[b] the deflated processed values and prices are based on the 1988 Consumer Price Index

Table 5. Average Shrimp Processing Activities Per Plant in the Southeast by Product Form for Selected Time Periods, 1973-88.

PRODUCT FORM & TIME	PROCESSED LBS. PER PLANT[a,b]	PROCESSED VALUE PER PLANT PERIOD	
		Current	Deflated
	1,000 lbs	----- $1,000s -----	
Raw Headless			
1973-76 avg.	685.6	1,572.9	3,608.2
1977-80 avg.	857.6	2,963.6	5,067.8
1981-84 avg.	754.8	3,228.2	3,904.3
1985-88 avg.	915.1	3,811.0	4,040.2
Peeled (Raw & Cooked)			
1973-76 avg.	748.9	1,233.9	2,851.8
1977-80 avg.	890.2	2,107.3	3,515.2
1981-84 avg.	1,184.6	3,177.6	3,843.5
1985-88 avg.	1,412.5	3,515.4	3,716.3
Breaded (Raw & Cooked)			
1973-76 avg.	1,163.8	3,107.0	7,257.1
1977-80 avg.	1,466.5	5,737.6	9,730.5
1981-84 avg.	1,854.2	10,577.7	12,755.1
1985-88 avg.	2,156.3	11,274.4	11,948.3
"Other"			
1973-76 avg.	679.5	861.1	2,031.5
1977-80 avg.	757.3	1,463.3	2,472.2
1981-84 avg.	641.2	1,566.0	1,903.0
1985-88 avg.	484.2	1,087.3	1,147.8

Source: Compiled from data in Table 4.

[a] processed pounds are expressed on a headless shell-on basis

[b] processed pounds and value per plant relate only to those plants engaged in that particular processing activity

Breaded shrimp processing. The number of Southeastern shrimp processing plants engaged in breading activities has significantly decreased during the 1973-88 period, when evaluated on a four year basis (Table 4). The largest decline appears to have occurred during the 1970s, especially during the earlier years, with little apparent change after 1980. Though not shown in the table, much of the initial decline in breaders occurred in Texas and is believed to be the result of increased restriction of U.S. shrimp boats in Mexican waters.

Production of breaded shrimp in the Southeast region was relatively stable when evaluated on a four year basis. The annual peak production of 56.1 million pounds during 1985-88 exceeded the 1981-84 low production of 48.2 million pounds by only about 15%

(Table 4). Due to the decline in the number of shrimp processing plants engaged in breading activities, however, breading activities per plant increased significantly during 1973-88 (Table 5). For example, annual breading activities per plant during 1985-88, averaging 2.16 million pounds. This exceeded the 1973-76 annual activities of 1.16 million pounds by one million pounds (85%).

The value of Southeastern shrimp breading activities consistently increased when evaluated on a four year basis (Table 4) while the value per plant increased even more sharply (Table 5). The decreasing number of plants was responsible. On a deflated basis, however, the total value of shrimp breading activities has remained quite constant; except for a slight decline during 1985-88 which occurred in spite of increased production. This decline (Table 4) was the result of falling deflated prices of the processed breaded shrimp which averaged $5.54 per pound during 1985-88 compared to $6.87 per pound during 1981-84.

After increasing rapidly through the 1981-84 period, the deflated value of shrimp breading activities per plant decreased during the latest four year period (Table 5); even though production per plant increased. This decline was due to falling breaded shrimp prices.

"Other" shrimp processing

The number of Southeastern shrimp processing plants conducting "other" shrimp processing activities has been declining (Table 4). Overall, only slightly more than one-half of the number of shrimp plants engaged in "other" shrimp processing activities during 1973-76 were active during 1985-88. This decline largely reflects the demise of the shrimp canning industry in the Southeast and may also reflect a reduction in the number of plants producing specialty products such as gumbos.

The quantity produced of "other" shrimp products also underwent a significant decline (Table 5). Overall, annual production during 1985-88, averaging 9.7 million pounds, was less than 40% of the 25.1 million pounds produced annually during 1973-76. The production per plant, after peaking at 757.3 thousand pounds annually during 1977-80, dropped to only 484.2 thousand pounds annually during 1985-88 (Table 5); a reduction of almost 40%.

After peaking during the 1977-80 period, both the current and deflated values of "other" shrimp processing activities in the Southeast experienced sharp declines. For instance, the current value of these activities decreased, from $45.4 million annually to $21.7 million (48%). The deflated value fell from $76.6 million to $23.0 million (70%), Table 4. The deflated value of other shrimp production per plant during the same period fell from $2.5 million during 1977-80 to $1.1 million during 1985-88 (55%), Table 5.

DISCUSSION

A number of observations can be made by comparing the aggregate Southeastern shrimp processing information with that provided for the different product forms. Among these observations is that increasing processing activities, as measured by the percentage of total processing plants engaged in the production of the different product types, is primarily centered around the raw and peeled product. This is evident from the information contained in Table 6. With respect to the raw headless product, 61.3% of the Southeast shrimp processing plants were engaged in the production of this product, on average, during the 1973-76 period. By the 1985-88 period, an average of 76.2% of the Southeast shrimp processing plants were engaged in this activity. The percentage of plants engaged in peeling activities increased by about two-thirds during the same period; from 27.2% to 45.7%. The percentage of plants engaged in breading and "other" activities exhibited, on the other hand, relatively large declines. The percentage of plants engaged in breading activities fell from 26.0% during 1973-76 to less than 16% during 1981-84 before marginally increasing to 17.2%

during 1985-88. The percentage of plants producing "other" products fell from 21.4% during 1973-76 to 13.2% in 1985-88, or by about 40%. Based on this information, it would appear that most diversification is into peeling activities.

Table 6. Percentage of Southeastern Shrimp Processing Plants Engaged in Production Activities by Product Form for Selected Time Periods, 1973-88.

TIME PERIOD	PRODUCTION ACTIVITY			
	Raw Headless	Peeling	Breading	"Other"
	%			
1973-76 avg.	61.3	27.2	26.0	21.4
1977-80 avg.	66.9	34.3	19.2	18.0
1981-84 avg.	75.8	37.0	15.8	14.6
1985-88 avg.	76.2	45.7	17.2	13.2

Source: Compiled from data in Tables 1 and 4.

Note: Horizontal summation of percentages in a given time period will exceed 100 because some plants produce more than one product.

Though the percentage of Southeast shrimp plants producing a raw headless product has been increasing during 1973-78, the production of this product, when evaluated as a percentage of total Southeastern shrimp processing activities, has remained relatively stable. This is the case in terms of both pounds and value (Table 7). In terms of pounds, raw headless shrimp production consistently averaged from about 39% to 44% of total shrimp processing activities in the Southeast region when evaluated on a four year basis. In value terms, the percentage runs about three to five points higher.

Table 7. Percentage of Aggregate Southeast Shrimp Processing Activities, in Pounds and Value, Represented by Different Product Forms for Selected Time Periods, 1973-88.

TIME PERIOD	PRODUCT FORM							
	Raw Headless		Peeled		Breaded		"Other"	
	Lbs.	Value	Lbs.	Value	Lbs.	Value	Lbs.	Value
	% of Aggregate Activities							
1973-76 avg.	39.2	42.1	19.0	14.6	28.2	35.3	13.6	8.0
1977-80 avg.	44.2	48.7	23.6	17.8	21.7	27.1	10.5	6.5
1981-84 avg.	41.0	44.4	31.4	21.3	20.9	30.2	6.7	4.1
1985-88 avg.	39.2	44.0	36.3	24.3	20.9	29.4	3.6	2.2

Peeled shrimp accounted for 19% of the Southeast shrimp processing activities by poundage and 14.6% by value during the 1973-76 period (Table 7). By the 1985-88 period, its percentage share had nearly doubled to 36.3% while its share by value had increased two-thirds to 24.3%. Breaded and "other" activities as a percentage of total Southeast shrimp processing activities have exhibited significant declines during the 1973-88 period. Overall, the majority of growth in the Southeast shrimp processing industry, as measured in terms of either pounds or value, appears to be in the peeled category.

Finally, some observations on processed shrimp prices are in order. As indicated in Table 4, the price of breaded shrimp is consistently the highest among all product forms, followed by raw headless, peeled, and other. Recall, however, that these prices have been calculated after all products have been converted to a headless shell-on basis. All product prices, whether expressed in current or deflated terms, have declined during the 1985-88 period when compared to the previous four year period and in most cases, the deflated price has been declining since the 1977-80 period. The largest percentage decline in the deflated price has been observed in the peeled category where price has declined by a third since its peak ($2.63 in 1985-88 compared to $3.95 during 1977-80), followed by "other" shrimp (1 27% decline), raw headless (25% decline), and breaded (20% decline).

SUMMARY

The Southeast shrimp processing industry has been examined from an economic perspective. Results show that while the number of plants decreased during the 1973-78 period, the production activities, as measured by total pounds, increased. Most of this increased activity was due to peeled product. Though processing activities in terms of pounds being processed has been increasing, the deflated value of these processing activities has exhibited no growth since the mid 1970s due to a sharp decline in the deflated price of all forms of processed shrimp, i.e., raw headless, peeled, breaded, and "others".

ACKNOWLEDGEMENTS

The project was partially supported through Saltonstall-Kennedy funds, project title "The Southeastern Seafood Processing Industry: An Economic Assessment for Private and Public Management Decision Making".

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COMPARISON OF SURIMI GEL STRENGTH MEASUREMENT TECHNIQUES

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It is well understood that surimi is a sol comprised of washed and refined fish myofibrillar proteins combined with cryoprotectants for cold storage stability (2,7). During the production of food products, such as kamaboko, the surimi sol combined with NaCl, flavor additives and texture enhancers is set into a gel through the application of heat as the actomyosin forms a three dimensional crosslinked structure. The gel forming process may be enhanced by using temperature setting histories which include a preset period below 50°C during which myofibrils form a more isotropic matrix.

Rheologically, surimi gels exhibit viscoelastic behavior, possessing solid-like elastic and fluid-like viscous properties simultaneously. Currently utilized physical testing procedures to evaluate surimi quality range in nature from empirical to fundamental (Figure 1). Torsion and puncture-probe tests have been used widely to evaluate the strength and functionality of surimi gels, but there is little information on their relationship to fundamental viscoelastic parameters. This work compares data from several large deformation test methods (puncture, using spherical, flat, rounded and cone- shaped end geometries; torsion; Kramer shear/compression; failure compression; and compression resulting in biaxial extension) as well as viscoelastic properties determined from small amplitude oscillatory shear testing. Comparisons are made for different grades and mixtures of Alaskan pollock and silver hake surimis.

MATERIAL AND METHODS

Test Products

Three surimis were used in these studies: Grade B and C Alaskan pollock surimi produced at a shore-based processing facility (Alaska Fisheries Development Foundation, Anchorage, AK) and silver hake surimi produced at sea off Nova Scotia, Canada on a Japanese research vessel. The B and C grade pollock surimis were downgraded due to moisture content and color, respectively. In addition, tests were also performed on three mixtures formed

PHYSICAL TESTING PROCEDURES

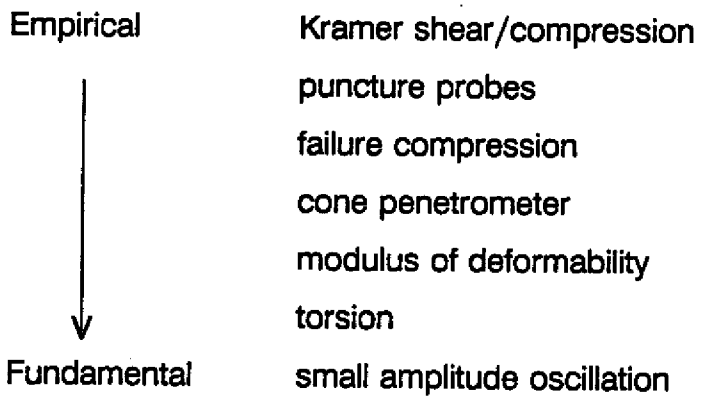

Figure 1. Classification of testing procedures used in this study

by combining 50% of one surimi with 50% of a second. In all cases, gels were produced by combining surimi, tempered at -5°C for 16-18 h, with 2% NaCl under vacuum in a cutter/mixer (Stephan VCM12, A. Stephan u. Sohne GmbH & Co., Hameln, FRG). Sample tubes were filled using a sausage stuffer and heated at 40°C for 15, 30 or 40 min, then at 90°C for 15, 20 or 36 min for tubes of 20, 25 and 50 mm diameter, respectively. After heat setting, the gels were cooled in ice water and stored at approximately 20°C for 16-18 h prior to testing. Proximate analyses of the gels are reported in Table 1.

Table 1. Proximate analyses of the surimis tested

SAMPLE	MOISTURE, % (s.d.)	PROTEIN, % (s.d.)	FAT, % (s.d.)
GR B Pollock	78.7 (0.06)	13.0 (0.14)	0.2 (0.02)
GR C Pollock	75.1 (0.10)	15.9 (0.08)	0.5 (0.08)
Silver Hake	76.9 (0.10)	13.6 (0.09)	1.5 (0.03)

Test Procedures

Puncture and compressive tests were completed using an Instron Model TM (Instron Corporation, Canton, MA) physical testing machine with operational settings as recorded in Table 2 using vertically oriented cylindrical samples, 25 mm high by 30 mm in diameter.

Table 2. Operating conditions for Instron testing

FIXTURE	CROSSHEAD SPEED (mm/min)	CHART SPEED (mm/min)	FULL SCALE FORCE (kg)
Puncture Probes	50	200	1-2
Flat and Round Probes	10	50	2
Cone Penetrometer	50	200	2-5
Kramer Shear/Compression	50	200	50
Failure Compression	50	200	100-200
Elastic Compression	50	200	10-20

Three puncture probe geometries were studied: 5 mm diameter spherical-ended probe, 7.9 mm (5/16 in) flat-ended probe and 7.9 mm (5/16 in) round- ended probe. The results of these tests were analyzed for maximum force at failure. As the force-deformation responses were essentially linear, the modulus to failure was calculated as the force at failure divided by the penetration to failure; and the compressive energy was derived from the area under the force deformation curve, calculated as one-half of the force at failure multiplied by the deformation to failure.

Gels were further characterized on the basis of compressive force at failure as a measure of the cohesiveness of the gel (3). Samples were compressed between parallel platens with 2 sheets of filter paper (#1001 070, Whatman International Ltd., Maidstone, UK) positioned under the lower surface. From the moisture collected, a measure of the expressible moisture could be derived.

Kramer shear/compression tests were completed using a cell with the sample cavity volume reduced by spacers inserted in the cell to reduce the number of blades to 2 compared to the standard 10. Gels were cut into approximately 3 mm cubes and 50 g samples were placed in the cell with a minimum of compression. The maximum recorded force during the shear/compression test was used to characterize the sample.

Cone penetrometer tests were completed using a circular cone made of polished brass with an internal cone angle of 60 degrees. The penetration stress at a vertical penetration of 10 mm was calculated for each sample using the relationship reported by Tanaka *et al.* (8):

$$\sigma_p = F \cos(\theta/2) \cot(\theta/2) / \pi h^2$$

Where: σ_p is the penetration stress, F is the applied force, θ is the internal cone angle and h is the penetration depth.

Samples for torsion tests were formed in dumbbell shapes with a minimum diameter of 10 mm at the horizontal midplane using a milling machine (North Carolina State University, Raleigh, NC). Plates, glued with cyanoacrylate adhesive to the ends of samples, held the specimens during milling and when mounted in the torsion fixture (North Carolina State University, Raleigh, NC) mounted on a Brookfield 5xHBTD viscometer (Brookfield Engineering Laboratories, Inc. Stoughton, MA). Before testing, the viscometer was calibrated for deviations resulting from design of the viscometer. In these instruments the torque on the sample was applied through a spring and the torque reading was the sum of the torsional deformation of the sample as well as the spring. The spring deformation was therefore determined and subtracted from the observed reading to obtain accurate measurements of torque at failure and sample strain. The viscometer was operated at 2.5 rpm with the output recorded on a stripchart recorder. From the data, the maximum stress and strain were calculated using the relationships proposed by Hamann (1) and the rigidity was derived by dividing the stress by the strain at failure.

The modulus of deformability (5) was calculated from the stress/strain response observed during compression of samples between lubricated teflon platens. Measurements of sample diameter before testing and in the compressed state taken with digital calipers indicated conservation of volume for strains up to 20%, thereby implying a Poisson's ratio approaching 0.5. Based on this observation the true stress/strain behavior was determined from the force/deformation data and linear regression analyses of these data were used to determine the slope of the stress/strain curves or the apparent modulus of deformability for each sample. The term deformability modulus was used instead of elastic or Young's modulus; since, during testing, energy was dissipated as heat due to the viscous flow component observed during deformation. The stress/strain curve observed upon removal of the deforming stress did not coincide with the curve recorded for compression; thus, the material was not truly elastic. In addition, the term apparent was used since the stress/strain curve was dependent on sample configuration and rate of deformation. This was analogous to the measurement of apparent viscosity of non-Newtonian fluids where viscosity changes with rate of shear and with shear stress.

Small deformation oscillatory shear testing was completed using a Carri-Med CS500 controlled stress rheometer (Carri-Med Ltd., Dorking, UK). Samples 5 mm in height cut from a 50 mm diameter cylinder of gel were initially subjected to a torque sweep at a frequency of 6.28 s^{-1} (1 Hz) to determine a linear viscoelastic range. Applying a stress within that range, fresh samples were then subjected to a frequency sweep from 0.063 to 62.8 s^{-1} (0.01-10.0 Hz). From those data the storage modulus (G'), dynamic viscosity (η') and loss tangent (tan δ), were calculated at a frequency of 5.34 s^{-1} (0.85 Hz) using the rheometer software. These dynamic shear properties were then employed in comparisons with results obtained by other test procedures.

RESULTS AND DISCUSSION

Figures 2-5 are photographs showing the sample deformation during the execution of some of the test procedures used in this study. The left-hand sequence of photographs in Figure 2 shows the compression of a cylindrical gel sample between lubricated teflon platens. The vertical walls of the sample remained essentially parallel during the test indicating the magnitude of the friction force acting on the contact surfaces was small. As friction at the contact surfaces increased, the sample became barrel-shaped during compression. Frames 1 and 2 in the right-hand sequence in Figure 2 show failure compression between parallel platens with filter paper positioned on the lower platen to absorb expressed moisture. Frame 3 shows the sample after compression, to illustrate the barrelled shape and fracture in the sidewall of the sample.

A typical Kramer shear/compression test is depicted by the sequence of photographs in Figure 3. The sample was compressed below the leading edge of the downward moving blades, while at the same time sample located between the blades and between the blades and the cell walls imparted a shear stress force on the vertical faces of the blades.

The sequence of photographs in Figure 4 shows the polished brass cone penetrating a sample. The sample deformation observed in Frame 4 suggested that sample size may affect results calculated at this depth of penetration. In this study, the penetration stress was calculated when the cone penetration was approximately as shown in Frame 3 to minimize the effect.

Figure 5 shows the deformation of a sample during torsion testing where the sample approached a full revolution of strain (Frames 7 and 8) before failing. Note the sample fracture on a 45° plane and the deformation in the unmilled end-pieces.

Data collected from the different test methods were compared in two ways using Systat statistics software (9): 1) an analysis of variance of individual tests and 2) a comparison of the linear correlations between tests as reported in Tables 3 and 4, respectively. The first set of analyses compared the sensitivity of individual test procedures to differentiate between the different gels. To facilitate the comparison, test results were normalized by dividing each result by the maximum result such that all data fell between 0 and 1. Mitchell (4) reported that ranking of gels using rupture forces and small deformations will not necessarily correspond. Also, the application of the results should be considered when choosing a test procedure. For example, if sensory acceptance is of interest then a test more sensitive than the ability of panelists to differentiate or rank a series of gels may have no practical significance. Assuming differences in gel structure resulting from molecular interactions was reflected in changes in the storage modulus (G'), of the other test procedures, differentiation between gels was best achieved from the modulus of deformation, apparent stress from the cone penetrometer and maximum force from Kramer shear/compression tests. Other procedures were less effective or incapable of differentiating between gels.

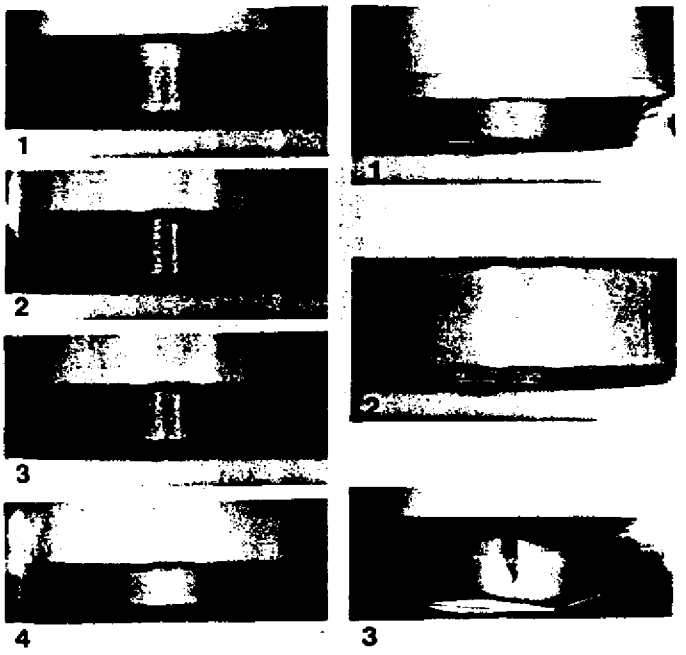

Figure 2. Compression testing showing compression between lubricated surfaces in the left-hand sequence and failure compression in the right-hand sequence with filter paper on the lower platen to collect expressible moisture

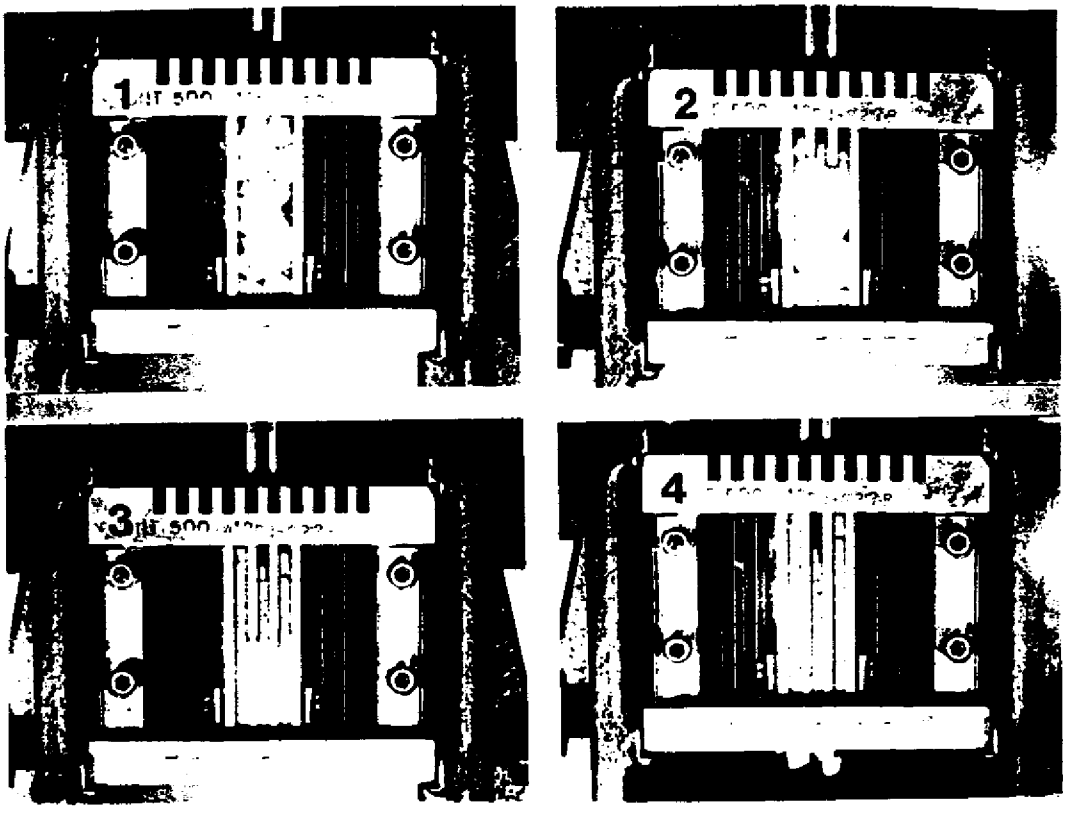

Figure 3. A sequence of photographs showing a typical Kramer shear/compression test

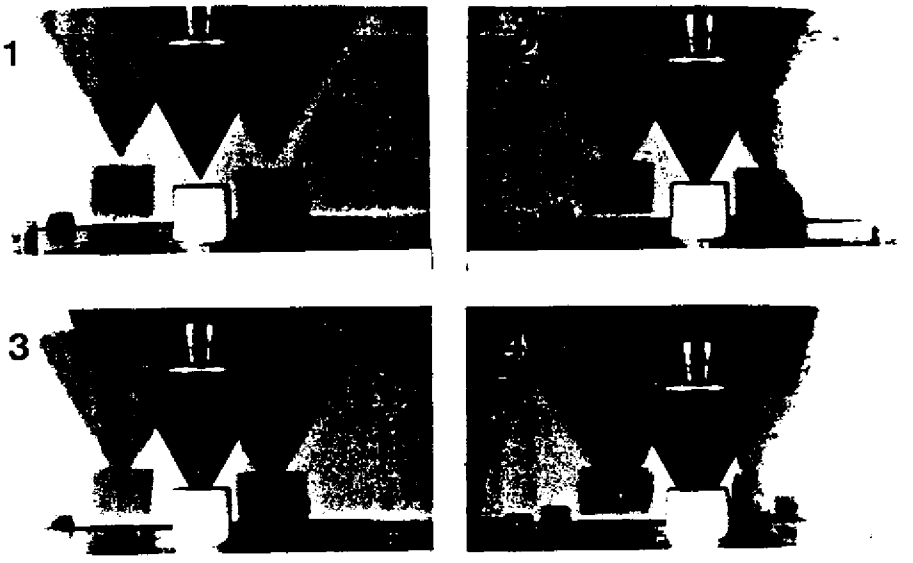

Figure 4. A sequence of photographs showing a typical cone penetrometer test

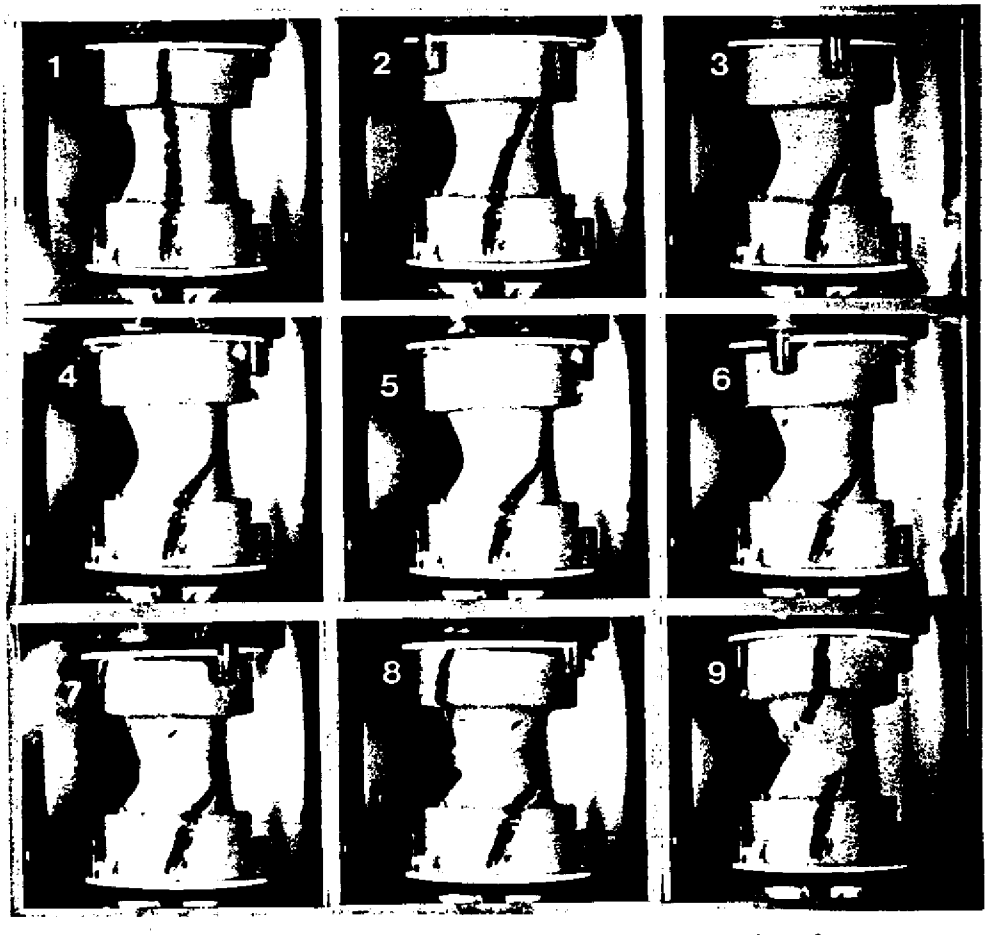

Figure 5. A sequence of photographs showing a typical torsion test

Table 3. Normalized Mean Test Results Using C-Grade Pollock (CP), B-Grade Pollock (BP) and Silver Hake (SH)

TEST	SURIMI					
	CP	BP+CP	CP+SH	SH	BP	BP+SH
Storage Modulus	1.00[a]	0.55[b]	0.46[c]	0.40[cd]	0.34[de]	0.27[e]
	(6.1)	(7.2)	(17)	(12)	(7.1)	(17)
Dynamic Viscosity	1.00[a]	0.56[b]	0.45[c]	0.37[d]	0.33[de]	0.27[e]
	(5.5)	(5.4)	(15)	(12)	(5.3)	(11)
Loss Tangent	0.94[a]	1.00[a]	0.96[a]	0.90[b]	0.96[a]	0.99[a]
	(1.3)	(2.9)	(1.7)	(3.6)	(2.9)	(7.9)
Torsion Stress	1.00[a]	0.68[c]	0.87[b]	0.81[b]	0.41[e]	0.60[d]
	(7.0)	(7.2)	(13)	(13)	(20)	(12)
Torsion Strain	0.78[d]	0.87[c]	0.94[b]	1.00[a]	0.84[c]	0.97[ab]
	(6.4)	(5.4)	(4.6)	(9.9)	(12)	(8.0)
Torsion Rigidity	1.00[a]	0.61[c]	0.72[b]	0.63[c]	0.38[e]	0.49[d]
	(7.6)	(7.5)	(14)	(15)	(13)	(11)
Modulus of Deformability	1.00[a]	0.66[b]	0.69[b]	0.56[c]	0.44[d]	0.43[d]
	(14)	(8.7)	(7.8)	(12)	(5.4)	(9.2)
Cone Penetrometer	1.00[a]	0.70[b]	0.72[b]	0.69[b]	0.50[c]	0.54[c]
	(8.5)	(5.1)	(12)	(8.3)	(7.5)	(9.3)
Kramer Shear/Compression	1.00[a]	0.80[b]	0.85[b]	0.84[b]	0.64[c]	0.67[c]
	(10)	(4.8)	(8.2)	(5.4)	(14)	(5.5)
Failure Deformation	1.00[a]	0.45[c]	0.46[c]	0.45[c]	0.68[b]	0.34[d]
	(14)	(10)	(7.6)	(3.9)	(11)	(11)
Spherical Probe Force	0.95[ab]	0.84[bc]	0.90[ab]	1.00[a]	0.91[ab]	0.78[c]
	(5.9)	(9.9)	(10)	(15)	(17)	(12)
Spherical Probe Energy	0.76[c]	0.77[c]	0.81[bc]	0.98[ab]	1.00[a]	0.81[bc]
	(12)	(15)	(17)	(21)	(23)	(19)
Spherical Probe Modulus	1.00[a]	0.77[c]	0.84[b]	0.85[b]	0.70[d]	0.63[e]
	(3.4)	(6.7)	(4.4)	(9.5)	(13)	(5.8)
Round Probe Force	0.95[a]	0.93[a]	0.97[a]	1.00[a]	0.87[ab]	0.77[b]
	(9.4)	(13)	(12)	(17)	(18)	(13)
Round Probe Energy	0.84[ab]	0.93[ab]	0.96[ab]	1.00[a]	0.92[ab]	0.80[b]
	(15)	(17)	(17)	(23)	(23)	(18)
Round Probe Modulus	1.00[a]	0.85[b]	0.92[b]	0.93[ab]	0.76[c]	0.69[c]
	(4.8)	(8.4)	(6.6)	(11)	(14)	(7.9)
Flat Probe Force	0.97[a]	0.84[bc]	0.91[ab]	1.00[a]	1.00[a]	0.78[c]
	(9.1)	(6.4)	(7.3)	(8.5)	(18)	(16)
Flat Probe Energy	0.83[bc]	0.74[c]	0.80[bc]	0.91[ab]	1.00[a]	0.76[bc]
	(16)	(12)	(11)	(14)	(25)	(22)
Flat Probe Modulus	1.00[a]	0.84[d]	0.91[bc]	0.97[ab]	0.87[cd]	0.71[e]
	(3.6)	(4.6)	(3.8)	(5.9)	(13)	(9.7)

The results are mean values with the coefficient of variation (%) associated with each mean in parentheses. Values in a row with the same letter are not significantly different ($p>0.05$).

Table 4. Correlation Matrix of the Test Results

TEST	STORAGE MODULUS	DYNAMIC VISCOSITY	LOSS TANGENT	TORSION			MODULUS OF DEFORMABILITY	CONE PENETROMETER
				Stress	Strain	Rigidity		
Storage Modulus	1							
Dynamic Viscosity	1[b]	1						
Loss Tangent	0.20	0.26	1					
Torsion Stress	0.70[b]	0.68[b]	-0.13	1				
Torsion Strain	0.66[b]	0.68[b]	-0.45	-0.07	1			
Torsion Rigidity	0.87[b]	0.86[b]	0.05	0.95[b]	-0.38	1		
Modulus of Deformability	0.90[b]	0.90[b]	0.20	0.80[b]	-0.52[a]	0.92[b]	1	
Cone Penetrometer	0.91[b]	0.90[b]	0.07	0.85[b]	-0.44	0.94[b]	0.95[b]	1
Kramer Shear/Compression	0.78[b]	0.76[b]	-0.15	0.90[b]	-0.24	0.92[b]	0.89[b]	0.93[b]
Failure Deformation	0.79[b]	0.79[b]	0.07	0.30	-0.72[b]	0.52[a]	0.61[b]	0.62[b]
Spherical Probe Force	0.32	0.29	-0.56[a]	0.38	-0.06	0.36	0.27	0.39
Spherical Probe Energy	-0.44	-0.47[a]	-0.60[b]	-0.39	0.23	0.44	-0.54[a]	-0.43
Spherical Probe Modulus	0.82[b]	0.80[b]	-0.18	0.81[b]	-0.32	0.86[b]	0.83[b]	0.89[b]
Round Probe Force	0.30	0.28	-0.29	0.42	0.04	0.38	0.39	0.44
Round Probe Energy	-0.14	-0.15	-0.29	-0.01	0.23	-0.08	-0.05	-0.02
Round Probe Modulus	0.67[b]	0.65[b]	-0.22	0.74[b]	-0.18	0.74[b]	0.74[b]	0.79[b]
Flat Probe Force	0.23	0.20	-0.54[a]	0.14	0.11	0.17	0.15	0.23
Flat Probe Energy	-0.13	-0.15	-0.49[a]	-0.25	0.01	-0.23	-0.24	-0.17
Flat Probe Modulus	0.60[b]	0.57[a]	-0.43	0.57[a]	-0.23	0.59[b]	0.58[b]	0.65[b]

[a] Significant, $p<0.05$

[b] Highly significant, $p<0.01$

Table 4 cont.

TEST	KRAMER SHEAR/ COMPRESS.	FAILURE DEFORMATION	SPHERICAL PROBE			ROUND PROBE			FLAT PROBE		
			Force	Energy	Modulus	Force	Energy	Modulus	Force	Energy	Modulus
Storage Modulus											
Dynamic Viscosity											
Loss Tangent											
Torsion Stress											
Torsion Strain											
Torsion Rigidity											
Modulus of Deformability											
Cone Penetrometer											
Kramer Shear/Compression	1										
Failure Deformation	0.45	1									
Spherical Probe Force	0.47[a]	0.48[a]	1								
Spherical Probe Energy	-0.32	-0.01	0.62[b]	1							
Spherical Probe Modulus	0.88[b]	0.65[b]	0.72[b]	-0.09	1						
Round Probe Force	0.51[a]	0.29	0.81[b]	0.33	0.72[b]	1					
Round Probe Energy	0.08	0.01	0.67[b]	0.57[a]	0.32	0.88[b]	1				
Round Probe Modulus	0.82[b]	0.52[a]	0.77[b]	0.02	0.96[b]	0.88[b]	0.55[a]	1			
Flat Probe Force	0.32	0.56[a]	0.89[b]	0.68[b]	0.54[a]	0.64[b]	0.55[a]	0.58[b]	1		
Flat Probe Energy	-0.07	0.35	0.65[b]	0.82[b]	0.11	0.33	0.43	0.16	0.89[b]	1	
Flat Probe Modulus	0.70[b]	0.65[b]	0.91[b]	0.29	0.90[b]	0.81[b]	0.52[a]	0.90[b]	0.83[b]	0.48[a]	1

[a] Significant, $p<0.05$

[b] Highly significant, $p<0.01$

The linear correlation analyses provided an indication of the validity of using a given test procedure to predict viscoelastic responses which reflect the fundamental interactions of the gel matrix. The results were plotted on linear coordinates to examine the data for curvilinear responses and the data conformed visually to the assumption of linearity. There were highly significant linear correlations ($p<0.01$) between the viscoelastic parameters of storage modulus and dynamic viscosity and the other tests procedures with the exception of the loss tangent as well as the force and energy measurements derived from puncture tests. Puncture test results did show the modulus to failure provided better correlations with other test procedures than the force to failure or compressive energy derived from the same data. Oscillatory testing data indicated the loss tangent was essentially constant for frequencies from 0.063 to 62.8 s^{-1}, which was an indication of stable cross-linking in the gel structure. In addition, the phase angle was approximately 10^{o} which confirmed the highly elastic nature of the gels. The phase angle for a purely elastic material would be 0^{o} whereas a purely viscous material would have a phase angle of 90^{o}. Although the variability was greater, this elastic nature was also observed in the relatively uniform response in torsion strain and the linear nature of force/ deformation and stress/strain curves for the surimi gels tested in this study.

ACKNOWLEDGEMENT

Financial support for these studies from a Strategic Operating Grant from the Natural Sciences and Engineering Research Council of Canada is greatly appreciated.

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CONSUMER ACCEPTANCE ON PASTA SUPPLEMENTED WITH SURIMI

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Surimi, a bland fish mince, has been used in our laboratory for past several years to incorporate into pasta formulation (Huang, 1988). A new surimi pasta was developed and its production does not require the major change of processing equipment. In general, the consumption of acceptable surimi pasta could increase the fish consuming population in this country. It will deliver the health benefits of eating fish to those who are not regular seafood consumers. Although the physical, chemical, sensory and nutritional qualities of surimi pasta were studied (Huang, 1988; Kim et al., 1990), the consumer perception through sensory evaluation needs to be evaluated before the value of surimi supplementation can be determined.

The objectives of this study were to determine consumer preference to levels of surimi in pasta and purchase intention for surimi pasta formulation; and to analyze the effect of demographic characteristics of panelists on consumer acceptance of surimi pasta.

METHODOLOGY

A. Preparation of pasta sample

Pasta sample was made by incorporating Alaska pollock surimi (Unisea Corp., Redmond, WA) into formulations at 0, 10, 20 and 30% levels. The base material for pasta was Durum semolina. Pasta made from 100% Durum semolina were served as control. A laboratory pasta machine, Pasta Mark V (The Bonnot Co., Kent, OH), was used to extrude pasta in the form of Rotelle. The optimum processing conditions for producing pasta was according to the report of Huang (1988). Duplicate batches of fresh pasta in each formulation were produced.

B. Demographic characteristics of consumer panel

One hundred thirteen consumer panelists from metropolitan Atlanta, Georgia were recruited from the data base to participate the testing. Panelists were selected on the basis of availability and experience on the pasta. Demographic profile of panelists had been established in a data base. The panel was composed 98 females and 15 males. Among them, there were 98 white, 15 black and 4 others. The panelists included 22 people under 25 years old, 19 between 35 and 44, 24 between 45 and 54, 13 between 55 and 64, while 35 over 64. The distribution of yearly household income of the panel were including 17 people under $9,999 per year, 17 between $10,000 and $19,999, 17 between $20,000 and $29,999, 28 between $30,000 and $39,999, 12 between $40,000 and $49,999, 10 between $50,000 and $59,999, and 10 above $60,000 (Table 1). In addition to those data, other information including education, family size, occupation, marital status and nutritional knowledge were also available.

C. Consumer panel testing

Questionnaires were designed to include six sensory attributes, acceptance of sale price and frequency of buying this product compare to other pasta products. Sensory attributes included color, appearance, flavor, off-flavor, mouthfeel and overall acceptability. A 9-point hedonic scale, with 1 being dislike extremely and 9 being like extremely, was used for evaluating color, appearance, flavor, mouthfeel and overall acceptability; and a 5-point hedonic scale, with 1 being very strong and 5 being not detectable, was used for evaluating the off-flavor.

Table 1. Demographic characteristics of consumer panel

Group Characteristics	Total sample[a] (%) (n=113)
Age:	
< 34	20
35-44	17
45-54	21
55-64	12
> 64	31
Sex:	
Male	13
Female	87
Yearly income:	
< 9,999	15
10,000-19,999	15
20,000-29,999	15
30,000-39,999	25
40,000-49,999	11
50,000-59,999	9
> 60,000	9
Race:	
White	83
Black	13
Others	4

[a] Percent may not be equal to 100% due to rounding of numbers.

Pasta samples were cooked in boiling water for 2 min, placed on disposable cups, given a random three-digit number and evaluated immediately. Only one cooked pasta sample with a formula was presented to the panelist for evaluation at a time. Every session was conducted for maximum 10 panelists. The order of sample presentation was designed differently for each session in order to eliminate the bias. Panelists were asked to rate each of the eight samples (duplicate samples of four formulations) for all attributes in the same session. Responses to sensory questionnaires were collected after the session was completed. A cash of $10.00 was provided as an incentive. Sessions were conducted at the sensory evaluation facilities of the Department of Food Science and Technology, the University of Georgia Experiment Station in Griffin, GA. The entire testing session took two whole days.

D. Analysis of data

Data were analyzed using Analysis of various procedures of the Statistical Analysis System (SAS, 1987). Duncan's Multiple Range Test at levels of 0.05 was used to find significant differences among samples.

RESULTS AND DISCUSSION

Means scores of the consumer panel for color, appearance, flavor, off-flavor, mouthfeel and overall acceptability of cooked fresh pasta are presented in Table 2 . No significant difference ($p<0.05$) in all attribute for pasta with up to 20% substitution of surimi was found. Responses for all sensory attributes of pasta with 30% of surimi addition were significantly different. However, these scores were still slightly higher than the middle point (3 correspond to "slight" for off-flavor, 5 correspond to "neither like nor dislike" for the other attributes). No significant differences in two replications was found.

Table 3 showed that consumer's age was a significant factor to determine the score of sensory attributes. Panelists with age of between 55 and 64 and older than 65 years old gave the highest scores for surimi added pasta, while age group between 35 and 44 gave the lowest scores. Results of this study revealed that older people would be a potential market for surimi pasta.

Table 2. Consumer perception of surimi pasta

Surimi content	Color[c]	Appearance	Flavor	Off-flavor	Mouthfeel	Acceptance
0%	6.32a	6.32a	5.93a	3.90a	5.85a	5.82a
10%	6.29a	6.32a	6.04a	4.06a	5.84a	6.00a
20%	6.27a	6.25a	5.92a	3.99a	5.71a	5.81a
30%	5.85b	5.89b	5.10b	3.47b	5.02b	4.93b

[a,b] Means (n=226) with the same letter in a column are not significantly different at level of 0.05

[c] Color, appearance, flavor, and mouthfeel were rated using a 9-point hedonic scale with 9 being like extremely and 1 being dislike extremely; off-flavor was using a 5-point hedonic scale with 1 being very strong and 5 being not detectable.

The incomes of panelists did not significantly affect scores for color of all the pasta samples (Table 4). It did affect scores of flavor and mouthfeel, off-flavor, appearance and flavor, and flavor and mouthfeel for the control, 10%, 20% and 30% surimi supplemented pasta, respectively. However, no significant association between the income of panelists and the score of sensory attributes was found.

No significant differences in scores of all attributes for all formulations regardless of sex (Table 5) and race (Table 6) were found in this study. Purchase frequency scores of 1.16 to 1.24 on a 7-point scale correspond to "once a month" and "twice a month" for up to 20% surimi added pasta.

Table 3. Effect of age on consumer perception of surimi pasta

(A) Control pasta

Age	Color	Appearance	Flavor	Off-flavor	Mouthfeel	Acceptance
>34	6.36ab	6.21ab	6.05a	4.05a	6.00a	6.00a
35-44	5.71b	5.74b	5.18b	3.71a	5.47a	5.21a
45-54	6.38ab	6.52a	6.02a	3.73a	5.83a	6.00a
55-64	6.42ab	6.54a	6.17a	4.00a	6.13a	5.92a
<65	6.54a	6.50a	6.07a	3.99a	5.86a	5.89a

(B) Pasta with 10% surimi addition

Age	Color	Appearance	Flavor	Off-flavor	Mouthfeel	Acceptance
>34	6.46ab	6.41ab	6.43a	4.32a	6.23a	6.34a
35-44	6.03ab	6.08b	6.05a	4.24a	5.82a	5.90a
45-54	5.79b	5.94b	5.69a	3.63b	5.50a	5.77a
55-64	6.75a	6.83a	6.33a	4.38a	6.08a	5.33a
<65	6.50ab	6.47ab	5.92a	3.99ab	5.76a	5.90a

(C) Pasta with 20% surimi addition

Age	Color	Appearance	Flavor	Off-flavor	Mouthfeel	Acceptance
>34	6.46a	6.16ab	6.02a	3.91a	5.91a	6.02a
35-44	5.34a	6.61a	5.32a	3.95a	5.21a	5.29a
45-54	6.10a	6.08ab	5.88a	3.85a	5.54a	5.63a
55-64	6.33a	6.50a	6.17a	4.21a	5.67a	5.83a
<65	6.72a	6.67a	6.11a	4.08a	5.99a	6.07a

(D) Pasta with 30% surimi addition

Age	Color	Appearance	Flavor	Off-flavor	Mouthfeel	Acceptance
>34	5.80ab	5.46c	4.98ab	3.43bc	4.84ab	4.62bc
35-44	5.21b	5.40c	4.26b	2.95c	4.58b	4.08c
45-54	5.48b	5.58bc	4.65b	3.04c	4.56b	4.50c
55-64	6.29a	6.21ab	5.79a	4.04a	5.42ab	5.46ab
<65	6.32a	6.50a	5.68a	3.85ab	5.53a	5.68a

[a,b,c] Means with the same letter in a column are not significantly different at level of 0.05

Table 4. Effect of sex on consumer perception of surimi pasta

Surimi content	Sex	Color	Appearance	Flavor	Off-flavor	Mouthfeel	Acceptance
0%	M	6.30	6.47	6.27	3.90	6.10	6.03
	F	6.32	6.30	5.87	3.90	5.81	5.79
10%	M	6.63	6.60	6.60	3.97	6.23	6.43
	F	6.24	6.28	5.95	4.07	5.78	5.94
20%	M	6.37	6.67	6.33	3.90	6.27	6.27
	F	6.25	6.18	5.85	4.00	5.63	5.74
30%	M	5.87	6.17	5.37	3.70	4.97	5.00
	F	5.85	5.84	5.06	3.42	5.03	4.92

Table 5. Effect of incomes on the consumer perception of surimi pasta

(A) Control pasta

Income	Color	Appearance	Flavor	Off-Flavor	Mouthfeel	Acceptance
<10,000	6.45a	6.22a	6.03abc	3.81a	5.88abc	6.16a
10,000-	6.47a	6.68a	6.00abc	4.09a	6.03abc	6.06a
20,000-	5.97a	6.09a	5.35bc	3.74a	5.15c	4.91b
30,000-	6.27a	6.16a	5.97abc	4.10a	5.73abc	5.79ab
40,000-	6.54a	6.58a	6.65a	3.58a	6.62a	6.62a
50,000-	5.90a	6.10a	5.05c	3.50a	5.50bc	4.90b
>60,000	6.75a	6.65a	6.40ab	4.10a	6.20ab	6.30a

(B) Pasta with 10% surimi addition

Income	Color	Appearance	Flavor	Off-Flavor	Mouthfeel	Acceptance
<10,000	6.06a	6.00a	5.53a	3.59b	5.47a	5.75a
10,000-	6.50a	6.62a	5.91a	4.06ab	5.65a	5.74a
20,000-	6.44a	6.29a	6.15a	4.12ab	5.88a	6.06a
30,000-	6.50a	6.59a	6.41a	3.36a	6.11a	6.29a
40,000-	5.92a	6.12a	6.00a	3.65a	6.04a	6.27a
50,000-	6.05a	6.00a	6.25a	4.30a	5.75a	5.90a
>60,00	6.15a	6.20a	5.70a	4.15ab	5.80a	5.75a

Table 5. cont'd

(C) Pasta with 20% surimi addition

Income	Color	Appearance	Flavor	Off-flavor	Mouthfeel	Acceptance
<10,000	6.19a	6.06a	5.81ab	3.81a	5.69a	5.94ab
10,000-	6.24a	6.27ab	5.74ab	3.88a	5.53a	5.68ab
20,000-	6.68a	6.65a	6.06ab	4.00a	5.88a	5.88ab
30,000-	6.34a	6.29ab	5.98ab	4.18a	5.73a	5.79ab
40,000-	6.04a	6.19ab	6.23a	3.89a	5.96a	6.27a
50,000-	6.00a	5.55b	5.05b	3.75a	5.05a	5.00b
>60,000	6.10a	6.40ab	6.40a	4.25a	5.95a	5.85ab

(D) Pasta with 30% surimi addition

Income	Color	Appearance	Flavor	Off-flavor	Mouthfeel	Acceptance
<10,000	6.16a	6.13a	5.91a	3.66a	6.13a	6.06a
10,000-	6.06a	6.06a	5.62ab	3.71a	5.56ab	5.53ab
20,000-	5.50a	6.00a	4.56bc	3.65a	4.27cd	4.32cd
30,000-	5.89a	5.79a	4.91abc	3.45a	4.59bcd	4.66bc
40,000-	5.46a	5.89a	4.96abc	3.08ab	5.19abc	4.92bc
50,000-	5.70a	5.20a	3.96c	2.75b	3.95d	3.45d
>60,000	6.10a	5.85a	5.60ab	3.75a	5.45ab	5.15abc

[a,b,c,d] Means with the same letter in a column are not significantly different at level of 0.05

Table 6. Effect of race on the consumer perception of surimi pasta

Surimi content	Race	Color	Appearance	Flavor	Off-flavor	Mouthfeel	Acceptance
0%	W	6.30a	6.31a	5.89a	3.87a	5.87a	5.79a
	B	6.57a	6.46a	6.27a	3.93a	5.85a	6.00a
	O	6.00a	6.13a	5.75a	4.50a	5.38a	6.00a
10%	W	6.28ab	6.32a	6.01a	4.07ab	5.81a	5.97a
	B	6.58a	6.35a	6.27a	3.77b	6.12a	6.31a
	O	5.50b	6.13a	6.00a	4.63a	5.75a	5.88a
20%	W	6.25a	6.25a	5.92a	3.99a	5.69a	5.81a
	B	6.62a	6.42a	6.15a	3.89a	6.00a	5.96a
	O	5.63a	5.75a	5.13a	4.38a	5.38a	5.38a
30%	W	5.86a	5.90a	5.01a	3.42a	4.95a	4.84a
	B	5.89a	5.73a	5.81a	3.69a	5.50a	5.54a
	O	5.50a	6.00a	5.00a	3.88a	5.00a	5.13a

[a,b] Means with the same letter in a column are not significantly different at level of 0.05

CONCLUSION

Results of this study indicate the potential for the development of a surimi-supplemented pasta. Sensory evaluation by 113 consumers showed that supplement of up to 20% of surimi resulted in pasta judged to have favorable sensory qualities. Results also showed that 30% of surimi addition was acceptable. Sex, race and incomes of consumers would not be the factors to affect the sensory perception of the surimi pasta. However, older consumers gave higher scores for pasta with higher percent of surimi supplementation than the younger ones did, indicating that surimi pasta was more acceptable by the old people (> 55 years old).

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FLESH RECOVERY FROM FILLETED FISH FRAMES

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INTRODUCTION

Development of fish byproducts and processes to economically support the fishing industry is a complex task with the need for production of many types of products. Atpresent in the main, fish fillets and 'gurry' as solid waste have been the only products. At present, the disposal of solid wastes from this industry is costly and a nuisance.

For example, five years ago, the Gloucester Fish 0Protein company paid fish processors $20.00 per ton for this waste to be converted to dehydrated fish meal, fish oils and fish 'solubles'. These byproducts were sold as ingredients for poultry feed, largely in the New England area in competition with other sources of protein such as soybeán meal. However, with the high cost of energy and a process which was obsolete, the plant was shut down permanently in 1985 because of environmental and economic problems. Since much of New England's fishing industry was served by this plant, the disposal of fish wastes then became a problem and the Commonwealth of Massachusetts agreed to share equally the $20.00 cost of loading barges with this waste and dumping at sea for a limited time until the practice was discontinued in 1986.

This created a problem of crisis proportions and threatened the continued existence of the entire fishing industry in New England. Since that time, disposal of fish waste has been sporadic and uncertain, utilizing such facilities as mink farms, lobster bait suppliers and land-fills. Costs to the operators now up to $50.00 per ton are common, dependent on the location of the filleting operation. The industry is in dire need of new approaches and new products which will turn a costly problem into a profit-able source of income, especially in light of an increased demand for fish and the higher cost of the landings to the processor.

There has been some progress in the development of composting fish wastes for the bedding industry and the product-ion of hydrolysates by acidification as a fertilizer substitute for cranberry production. Another hydrolysate using enzymatic action has been developed to produce a high protein product useful in the weaning of piglets. While these processes show promise for partial alleviation of the waste disposal problem, the byproducts are of an economic value at least one order of magnitude less than before.

Clearly, what is needed are new products which can utilize fish morsels and which are of food grade. Two such products are surimi (now manufactured in California and sold as crab leg substitutes) and fish/rice extrusions (under development at U Mass by Clayton) which can be flavored as high protein snack foods. Other such products are Omega III fish oils (recently disputed as a deterent to heart failure) and whole fish flour which is a dehydrated product, but not cleared for domestic use by the FDA.

Herring processed as sardines have diminished in popularity in the marketplace with a huge percentage of plant capacity abandoned in New England. Many 'old world' and oriental fish forms such as marinated herring, sushi, squid, ad infinitum are in demand overseas, but any such exports are far outweighed by imports of frozen blocks of fish fillets for the breading industry which constitute one of the largest imports into the US.

The need to which this concept is addressed is to better utilize that portion of the fish catch which currently goes to low-valued fish products or creates a problem for disposal. The advantage of better utilizing fish flesh which cannot be removed as fillets for human food is a greater economic return for a larger yield of a highly nutritious foodstuff for the general public which has an increased awareness of the benefits of fish products in their diet. Although recovery of fish flesh by the proposed process will not of itself completely solve the disposal problem and in fact represents a relatively small portion of those wastes, it nevertheless could provide an important income to the fishing industry which would serve to offset other costs and aspects of fish waste disposal.

In terms of economic impact, consider one annual total fish landings in Massachusetts alone during a recent year which were valued at $250 million. If half of these landings are processed as fillets (with as much as 500,000 pounds of gurry in a single week), and if the process described within can lead to a 10% increase in yield, than an increase of $12.5 million can be realized to the processors. This will go a long way towards paying the costs incurred in the current disposal of gurry.

FILLET FISH PROCESS:

Some fish to be filleted are 'gutted and headed' prior to the filleting process and except for cod cheeks, the rejected gurry has little potential as human food. This represents at least 40% of the total weight, somewhat dependent on size and species. Some cod heads alone have weighed more than 20 lbs for the larger of the species. Again dependent on size and species, the fish may be filleted in the whole as for cod or haddock scrod.

Typical fillet yield may be as high as 30%, but for some species such as whiting or large herring, yields as low as 15% have been measured. The remainder of the fish is comprised of bone structure (called frames) with attached flesh which remains after the filleting operation. Some blood vessels and organs such as the liver cling to the underside of the backbone and can discharge undesirable color and odor producing matter. At least 10% of edible, food grade flesh remains attached to discarded frames.

For the most part, cutting is still done by humans wielding sharp knives with great speed and skill such that the fine bones are avoided in the final product from the nape to the tail. Skinning of the fillets is often part of the same operation although mechanical skinners are in widespread use. The skins become part of the waste stream. There are some commercial filleting machines available which pull the fish by the tail between two rotating knives which are automatically spaced according to the diameter of the body of a fish, as sensed and adjusted by a miniprocessor (computer) control system (Baader).

In any case, the incidence of any bones in fillets is undesirable from a consumer safety (choking) standpoint. Thus filleting operations tend to err on the side of safety leaving more flesh attached to the frames than might appear necessary. Inspection of fillets often follows the filleting operation either by the naked eye or with 'candling' tables to detect off-grade product with a need for cutting out the offending portions. A method to 'candle' white fish fillets electronically and to automatically eject bones has been researched at U Mass (Whitney et.al.) but has yet to be commerc-ially developed.

NON-FILLET FISH PROCESSES:

There are commercial machines available to remove fish flesh from filleted frames utilizing a pressure belt against a perforated steel drum. However, the consistency of the product can best be described as mince, well suited for further processing as fish 'flour'or fish cakes.

Frugal cooks have long known that by cooking fish frames, either by steam or in boiling water, partially cooked flesh can be easily detached with a gentle slipping motion with their fingers. We have demostrated that cooked flesh may be blown away by a jet of compressed air or high pressure steam. However, if the frames are cooked too long, the flesh becomes 'rubbery' and clings to the bones.

Further, any remaining blood or organs within the frame become congealed during the cooking process, rendering them stable and fixing them to the discarded frames. From the organ-aleptical standpoint, the ensuimg product is superb with no off-colors or flavors to a white fish product. Flesh is gleaned as white chunks and has the mouthfeel and texture which is consistent with the needs for culinary concoctions such as fish chowder or other fish recipes.

Such products have been canned or frozen as ingredients for soups, chowders and fish dishes which are delectable and highly reliished in many homes and restaurants in the N.E. region. With this potential as an economic spur, we have tried to develop a concept and unit operation to duplicate a practice which has been long in use in many N.E. households. Another potential use for such partially cooked chunks may be as feedback inclusion into fish blocks before freezing in order to increase yields. But the 'bone-free' requirement of the breading industry must be carefully observed.

Typically, these frames have thicknesses of 1/4" or less such that the heat transfer time requirements are of the order of 1-3 minutes. As an example, since the time required for a body to attain a desired temperature by the penetration of heat varies as the square of the least dimension, a typical 1/4" thick fish frame can cook in 1/16 the time required to cook a 1" thick fish fillet.

MACHINE CONCEPT:

The first iteration of such a machine is essentially a continuous conveyor within a steam tunnel which cooks the fish frames during a short time period using steam jets which impinge on both sides of thin frame sections. Other methods of heating such as by infrared or microwave energy may be more acceptable to processors since the need for a source of steam may be eliminated. However, this has yet to be investigated.

Our initial design of a machine incorporated a steam injection principle so that a short tunnel but 8 feet long with a typical processing time of approximately one minute was provided. This method partially cooked the fish flesh to a condition where it could easily slip off bone structures without dislodging rib bones from vertebra. Fine bones from the nape, fins and tail tended to become dislodged and will require further study. The fish chunks that emerged were moist and tender, well suited for chowder stock or other such uses.

As a prototype steam tunnel, the shell of a commercial continuous dishwasher was altered by reducing the speed to approximately 8 feet per minute. A variable speed gear (DC) motor with a chain and sprocket final drive completed the transform-ation. The typical open chain conveyor was fitted with a removable tray which was pushed along the path by conventional attachment links so that easy access to and from the tunnel was possible for our initial pilot studies. A wire grill was mounted vertically on the tray so that its plane was aligned with the direction of travel. Fish frames were attached to the wire grill so that high pressure culinary steam could impinge on both sides. In a future iteration, the open conveyor and sliding pan could be replaced by a horizontal wire conveyor belt on which the fish frames would lay flat as in the sketch of Figure 1.

Two banks of four 1/2" stainless steel tubes were plumbed as manifolds, one on each side of the longitudinal path so that jets (1/64" holes, 2" o.c.) of live steam at 30 psi cooked the fish flesh. The tubes were spaced vertically at 2 inch intervals. Cooking juices and condensate were recovered in the tray as a potential broth constituent for a final product. Excess steam escaped to the environment, perhaps unacceptable in a commercial process.

Future models could have heat sources (either steam jets or other forms of energy) above and below horizontally conveyed frames. In lieu of steam jets, culinary compressed air can be utilized to separate flesh by downward blowing of partially cooked chunks from frames. Electronic inspection for bones may be required to insure a consumer safe product, somewhat dependent on species. Also, it may be necessary to remove the unskinned tail section as part of the cutting process to eliminate the incidence of attached skin or other unusable parts of the frame in the final product.

FLOW CHART:

This machine concept is a 'low-tech' approach with minimal investment and energy requirements. Incorporation of such a machine into typical filleting process lines is illustrated by the flowchart in Figure 1.

The outputs from typical filleting operations are fish fillets and gurry. In a redesigned process, we propose that heads, viscera (and perhaps the tail) be separated from the fillet producing section, either before or after filleting and directed to the stream of gurry much as before. The fillets would be directed to the washing and packing operations as the product of primary importance as is the usual practice.

However, an altered process would divert the filleted frames with attached fish morsels to the proposed machine where the partially cooked chunks of fish would emerge as a secondary food grade stream for either on-site packaging as a frozen or canned product. We envision freezing as the better option because less machinery would be involved for what will be a relatively low volume --perhaps 10% of the fillet output.

Finally, the spent, stripped fish frames would be directed to the main gurry stream for further processing as fish hydroysates, as compost or rendered into fish meal by state-of-the-art dehydration plants. Current high costs of energy and capital outlay may preclude the last option.

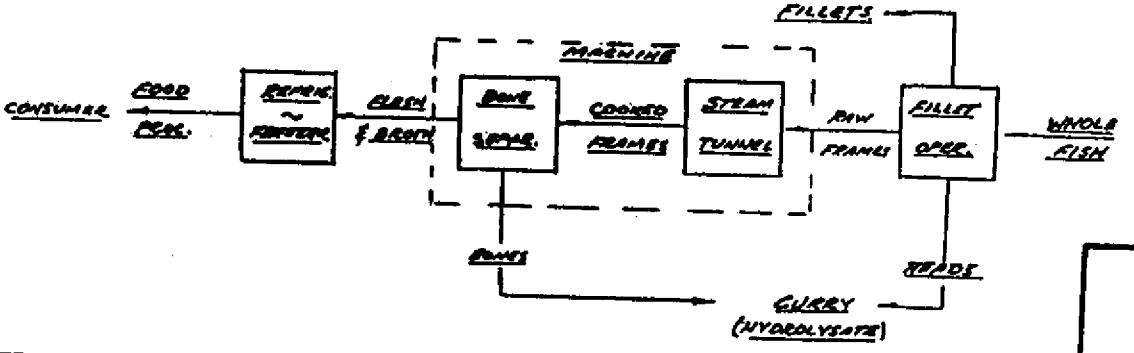
If sufficient numbers of processors are within close proximity of each other, then it may well be that collection of individual outputs can be made by a local (cooperative) canning facility which could market either fish chunks for chowder, or fish chowder as a finished product--much as for the existing clam/shellfish chowder market. Such a scheme would fit in well for large concentrations of independent processors as at Gloucester, Ma.

CONCLUSIONS:

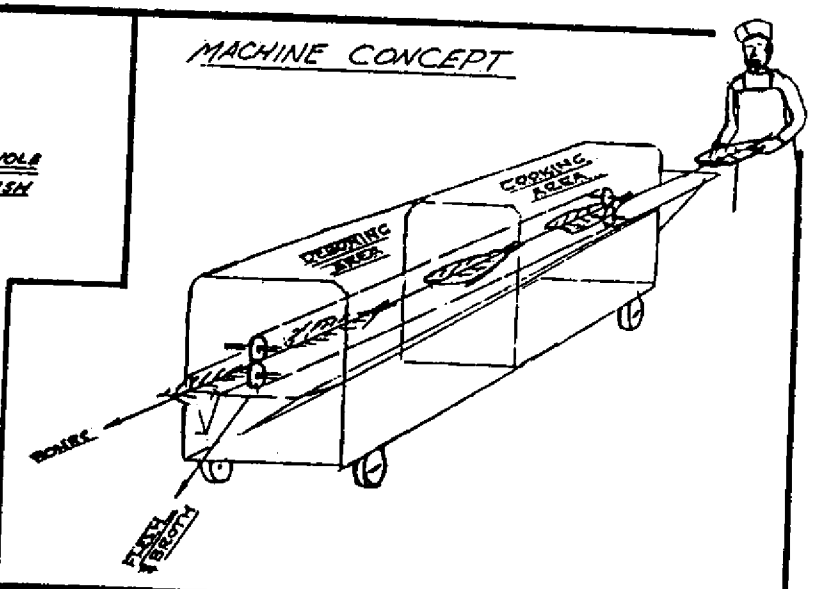
While this concept has not been researched sufficiently to produce conclusive results which would lead to immediate adoption by the industry, preliminary investigations with a prototype machine are sufficiently encouraging to warrant investigation by others for specific species of fish and process conditions.

Evaluations of the proposed process by potential processors and purveyors indicated promise for successful adoption. One outlet, a large food bank, was even willing to produce such food grade fish products themselves in order to supply a badly needed source of protein to supplement most food donations which tended to be higher in carbohydrates and fat than in proteins. Thus, consumer groups and processors alike have indicated sufficient interest to warrant further study.

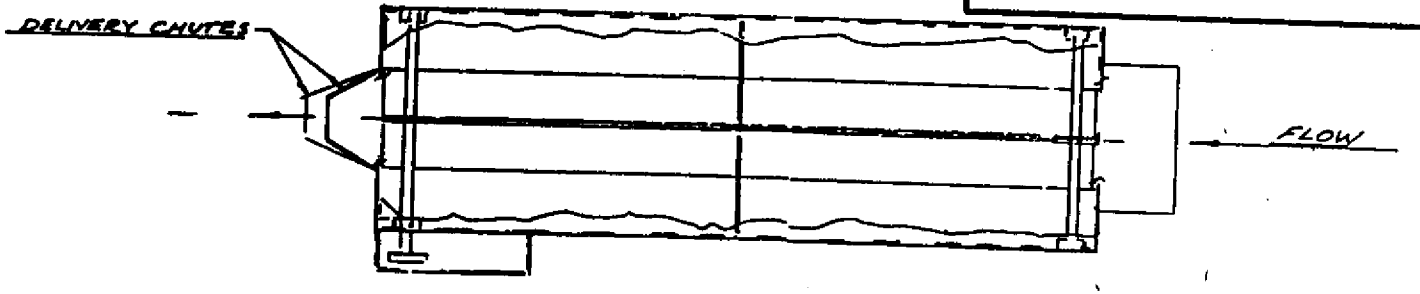
FLOW DIAGRAM

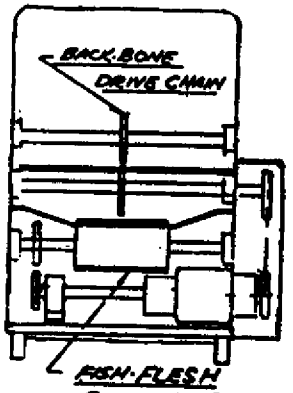


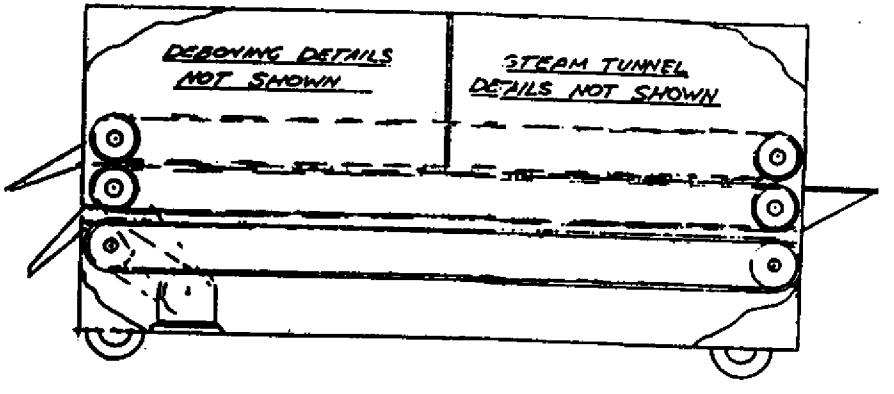
MACHINE CONCEPT



PROPOSED MACHINE DESIGN (SIMPLIFIED)







COOPERATIVE EXTENSION WORK IN AGRICULTURE AND HOME ECONOMICS
FOOD ENGINEERING DEPARTMENT
UNIVERSITY OF MASSACHUSETTS
UNITED STATES DEPARTMENT OF AGRICULTURE COOPERATING

NON-FILLET FISH FLESH AS VALUABLE BY-PRODUCT

4-6-87	PROJECT	SHEET 1 OF 1
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THE EFFECT OF pH ON SALTED AND NO-SALT SURIMI GELS

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INTRODUCTION

Engineered seafood products based on surimi have found an important niche in the American marketplace. Fish mince of various species of fish, once washed and cryostabilized, have the ability to form protein gels that can be structured to make products that successfully imitate shellfish foods (Lee, 1984). A key element in the cryostabilization is the addition of cryoprotectants to protect the proteins from damage during frozen storage. Salt is also added in varying amounts (1.5 to 3%) before the formation of the gel to help solubilize the proteins (Samejima et al., 1969; Chang-Lee et al., 1990). Although there is a significant body of literature showing the effects of various types of starches and different cryoprotectant formulations (Kim and Lee, 1987; Wu et al., 1985; Park et al., 1988), there is little information concerning the formation of surimi-based protein gels under no-salt conditions. Recent work (Trevino et al., 1989) demonstrated that interaction of salt and pH is important in the formation of a surimi-based gel. The purpose of this current work was to show the effect of pH on surimi gelation with and without salt.

MATERIALS AND METHODS

Materials

Red Hake (Urophycis chuss) were harvested off the coast of Rhode Island and purchased from the Point Judith Fishermen's Co-op (Narragansett, RI). It was re-packed in ice and transferred to the Department of Food Science and Nutrition Research Center pilot plant at The University of Rhode Island where they were headed and gutted the following day.

Preparation of Surimi

Mince was prepared from the headed and gutted fish with a Baader deboner (Model 694) with a drum having 5 mm-diameter perforations. The mince was washed twice with chilled (8-10°C) water at a ratio of 4:1 (w/w) water to mince. The excess water was drained after the first washing by gravity through a cheese cloth. After the last wash with 0.2% salt solution, the resulting slurry was passed through a Bibun strainer (Model SUM 420) and then dewatered by passing through a Bibun screw dehydrator (Model SR 1000).

Sucrose (4%), sorbitol (4%) and tripolyphosphate (0.3%) was added to the mince as cryoprotectants using a 5 quart Hobart silent cutter (Model 84142) with a chopping time of 3 min. The comminuted surimi was then vacuum packed in cryobags and stored at -20°C.

Surimi gel formation

After storage, surimi was thawed to -2°C and chopped in a Hobart silent cutter. One batch was made with 1.5% NaCl while another batch was made without salt. The paste was extruded into 30 mm-diameter cellulose casings using an 8 lb. capacity hydraulic press extruder (Hubert Food Service Suppliers, Cincinnati, OH) and steam cooked at 90°C for 15 minutes. The gels were then cooled in an ice-water bath and stored overnight at 4°C.

pH determination

The pH was adjusted during the chopping phase of gel formation with 1.0 M NaOH or 1.0 N HCl solutions. Measurements of pH were done by adding 10 g of chopped surimi in 90 ml of deionoized water, homogenized with a stirring bar and measured directly with a pH meter.

Evaluation of gel properties

The Instron Universal Testing Machine (Model 1122) was employed in the assessment of the mechanical properties of surimi gels. The gels were cut in 25 mm lengths and measured for hardness and elasticity according to procedures of texture profile analysis (Montejano et al., 1985). Compression was measured at 50% of the gel at 100 mm/min head speed and 50 mm/min chart speed.

RESULTS AND DISCUSSION

Figure 1 shows the effects of pH on the hardness of the surimi gel prepared with 1.5% and 0% salt. The 1.5% salt gel showed typical characteristics of surimi-based gels with a peak hardness between pH 6-7 and then diminishing values on the alkaline side of the pH. For the surimi gel without salt lower gel hardness values were displayed on the acidic pH side while peak values, which slightly exceeded the salted surimi gel, were found near pH 9.

As shown in Figure 2, the rise in pH had a positive effect on the elasticity of the no-salted surimi gel while the salted gel appears to peak early at neutral pH and then stabilized.

These results compare favorably with the previous results of Trevino et al. (1989) in their study on the effects of ionic strength in sardine surimi. This previous investigation showed peak values on the alkaline side of the no-salt surimi when measuring values such as protein solubility, water holding capacity and cohesiveness. Burgarella et al. (1985) also found thermal gelation of surimi sensitive to NaCl concentration showing a decrease in the rigidity modulus as NaCl concentration increased from 0% to 3%. Earlier work by Ishiroshi et al. (1979) with purified rabbit myosin gels showed a much higher shear modulus (rigidity) at lower salt concentrations at pH 6, however this effect was lost with storage. Wicker et al. (1986) also demonstrated that low salt surimi had an effect on the gel by promoting an earlier setting time.

There are points of interest in the formation of an alkaline pH no-salt surimi gel. The first issue concerns the physical-chemical interactions that are involved in the strengthening of the gels without salt and the converse weakening of the salted gels at higher pH's. A probable answer lies in the interaction of the cationic sodium ions at the higher pH with the abundant ionic groups of fish proteins. The negation of charge through their interaction may actually hinder the openness of the gel network and its interaction with water in the salted gels. In the absence of salt and at alkaline pH, the anionic carboxyl groups of the fish proteins may be forcing an openness to the gel and promoting strong interaction with water molecules and good gel forming properties. Consequently, pH alterations can have profound effects on surimi gel attributes, even by raising the system 1 pH unit.

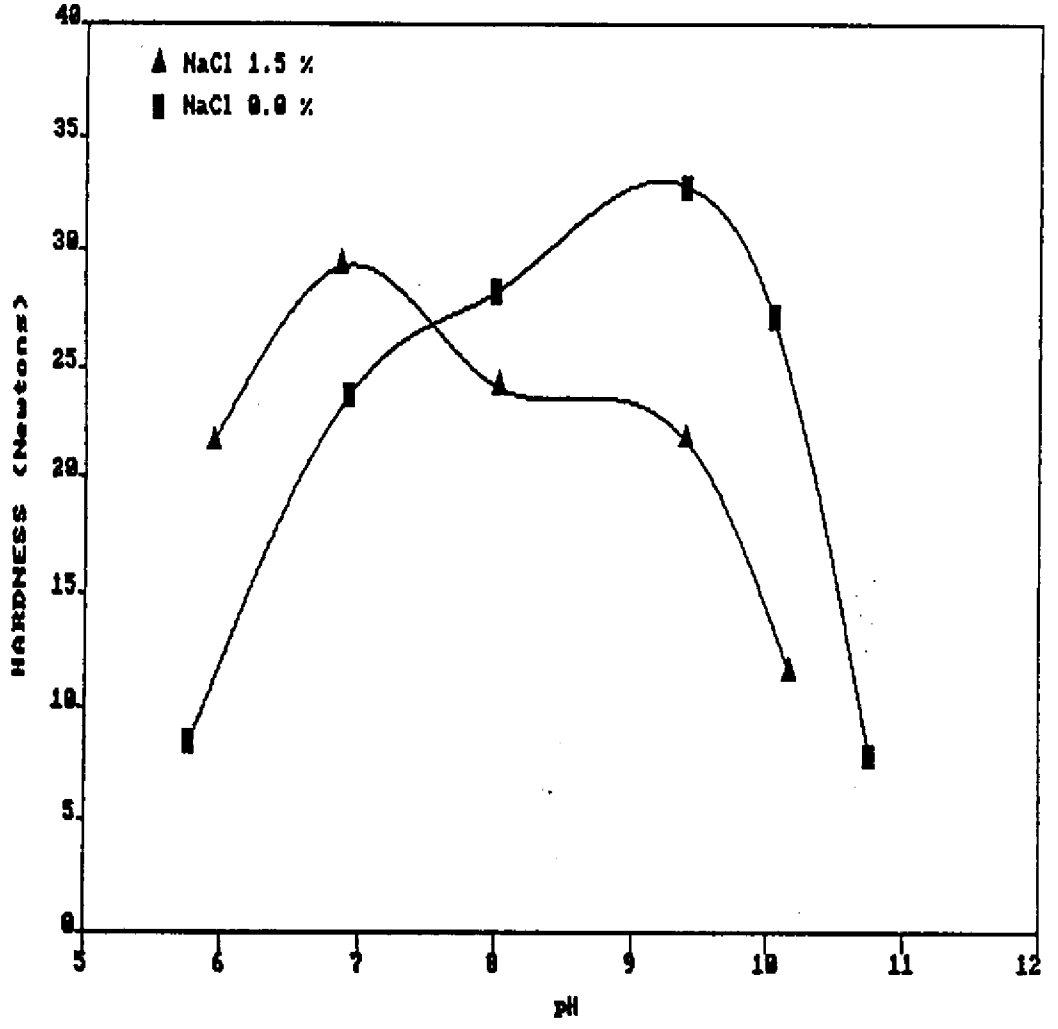

Fig.1.-Effect of pH on hardness for salted and non-salted surimi.

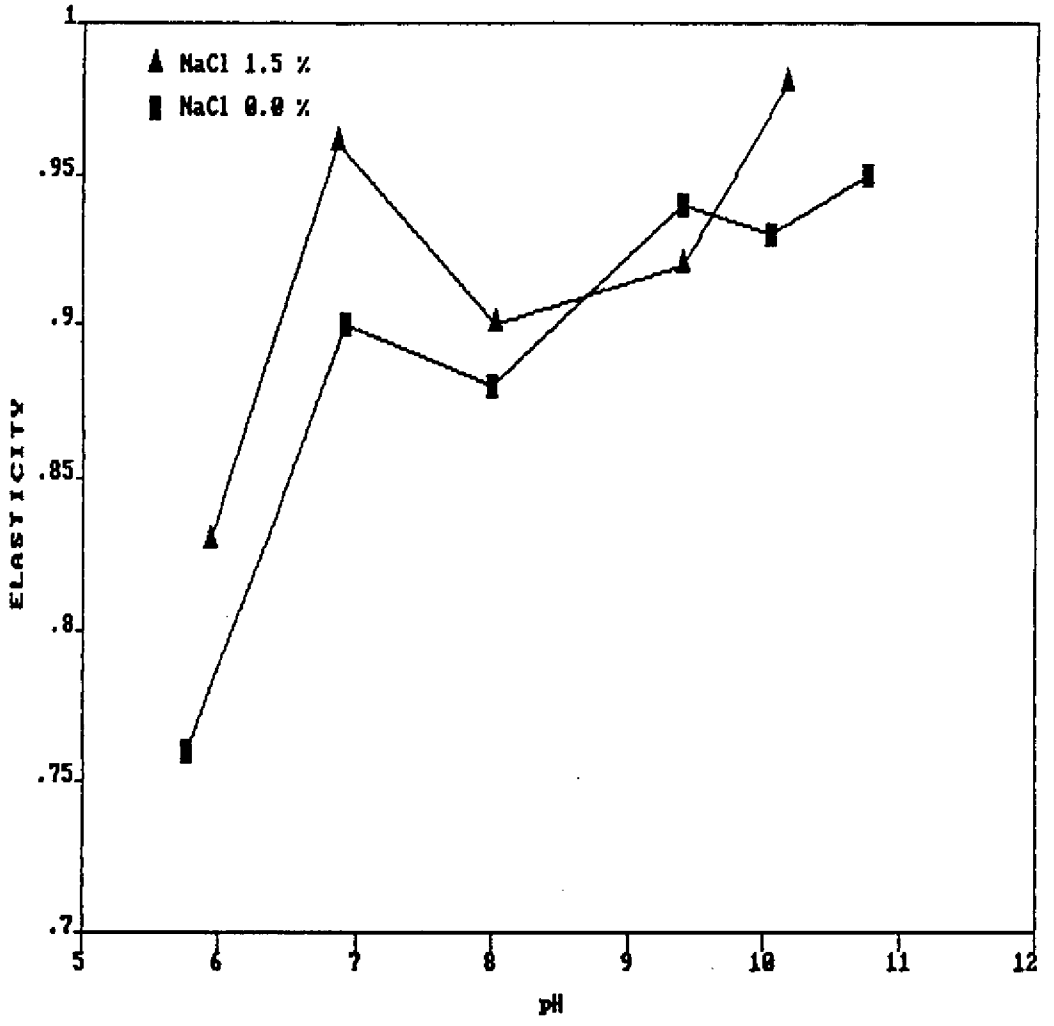

Fig.2.-Effect of pH on elasticity for salted and non-salted surimi.

ACKNOWLEDGEMENT

The authors would like to thank Dr. Chong Lee for his assistance and advice and the Department of Food Science and Nutrition at The University of Rhode Island for the use of their facilities. We also acknowledge CONACYT of Mexico for their support of B. Trevino during graduate studies.

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SILVER HAKE SURIMI TRIALS ON BOARD THE "SHINKAI MARU"

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Silver hake (*Merluccius bilinearis*) has been targeted as one of the most promising species for development in Atlantic Canada. The fish is a small gadoid (averaging 22 to 35 cm in length) closely related to the European blue whiting (*Micromesistius poutassou*). Large concentrations of silver hake are found in the deep waters off Nova Scotia and in the deep basins and channels of the Gulf of Maine, which comprise major fishing zones for this stock (2). Sustainable harvests of silver hake are estimated at approximately 135,000 to 200,000 metric tonnes (t) annually; however, historically there has been little Canadian involvement in the fishery. The majority of silver hake taken from Canadian waters over the past 25 years has been harvested primarily by foreign factory trawlers for processing into frozen whole fish blocks, frozen headed and gutted (H&G) fish and fish meal. Generally, these products have been sold domestically in the Soviet Union and Cuba with only a small portion exported to other foreign markets (2).

Recently, the Japanese Marine Fishery Resource Research Center (JAMARC), with assistance from the Canadian Department of Fisheries and Oceans (DFO), tested the potential of manufacturing silver hake surimi at sea in NAFO fishing divisions 4W-L and 4W-J. An initial trial conducted by the "Shinkai Maru" in April 17-22, 1989, investigated the feasibility of harvesting and utilizing silver hake as raw material for surimi. Results from that work led to a second study, carried out May 14-29, 1990, which included intensive fishing and processing experiments aimed at assessing the potential of the resource for the production of high quality kamaboko products. The "Shinkai Maru" is one of several research vessels owned by JAMARC that works primarily in the Atlantic Ocean, and has its home port located in Las Palmas, Canary Islands, Spain. Specifications for the 3,395 t stern trawler included: horse power-5,000 PS, overall length-100.9 meters, width-16.0 meters, height-10.0 meters; with maximum processing capacity estimated at 200 t of fish per day. The ship was equipped with numerous plate freezers, a large frozen storage hold, fish meal and surimi manufacturing plants. Maximum surimi production was between 7 to 9 t per day for large fish, and 5 t daily for smaller species such as silver hake. Maximum fresh water output totalled 90 t daily, i.e., 30 t from an engine room evaporator and 24 t, 24 t, and 12 t from each of three reverse osmosis filtration units. The ship's crew consisted of 39 men, many of whom worked as deck hands as well as machine operators in the surimi plant.

PROCESSING TECHNOLOGY

The primary objective of the research was to evaluate the feasibility of using silver hake as a raw material for surimi. As such, the investigation focused strictly on the harvesting and handling of this species, while processing of the by-catch was treated as a secondary concern. Bottom and off-bottom trawls with either 60, 70 or 120 mesh gear in the cod end were used at fishing depths ranging from 138 to 307 meters; towing times ranged from 2.7 to 7.5 hours.

Throughout the investigation, surimi was produced by a semi-continuous batch process as shown in Figure 1. In the 1989 trial, preprocessing of the silver hake was carried out by manually feeding the fish into a double blade circular saw, such that the fish was cut behind the gill region (in order to remove the head portion) and also at mid-body which exposed the gut cavity of the animal. The head portion was subsequently used for the production of fish meal, and the middle body and tail portions were washed and then conveyed to a deboner for mincing. A Bibun, model SDX-16, deboner equipped with a 5 mm orifice drum was operated with light pressure and fresh water to prepare a mince/water slurry which was then pumped through several meters of pipe to the first dewatering screen. Subsequently, the dewatered mince was combined with additional fresh water in the first wash sink and then mixed for several minutes in a tank to extract undesired compounds, e.g., water soluble proteins, pigments and lipids, from the muscle proteins. The second mince/wash water slurry was dewatered via a second rotary dewatering screen and then dehydrated in a three meter long single screw press. The total quantity of fresh water used was estimated at approximately six times the weight of deboned mince. Finally, the tissues were refined through a meat strainer with 2 mm holes, blended with stabilizing ingredients, packaged in polyethylene bags (10 kg) and frozen in a plate freezer. Minimum residence time in the freezer was six hours, after which the surimi was removed from the freezer trays, packaged in paperboard cartons (20 kg) and loaded into the ship's frozen storage hold.

Generally, the production of surimi was carried out by a similar protocol during the 1990 experiment with some improvements made to the processing operations. A revolving drum fish washing machine, which was used to wash the whole fish prior to cutting, had been added to the line. Also, a filleting machine which was not used in 1989 was employed to dress (butterfly fillet) larger fish that had been separated out through a size-sorting machine. Fish from the filleter (estimated at approximately 30% of the total weight of fish for surimi) was combined with product from the two cutting lines (approximately 70% of the fish for processing) and fed into the deboner. The typical, small size and shape of silver hake, as well as the limited processing capacity of the filleting line restricted the amount of raw material that could be filleted efficiently. Fish from the cutting lines usually had much of the gut contents attached, which consequently gave a lower quality starting material than is generally recommended for surimi processing.

During the 1990 trial, various processing parameters were monitored and manipulated in an effort to maximize the quality of surimi produced. Refrigeration was used to lower the temperature of the plant, a saline solution was added to the second wash cycle to improve dewatering, adjustments were made to the screw press, and the refiner was modified to decrease the number of impurities, i.e., small pieces of skin or black belly lining in the product. The stabilizing ingredients added to all of the 1989 production (on a w/w basis) were: 5% sorbitol, 4% sucrose, 0.3% sodium tripolyphosphate/tetrasodium pyrophosphate (Erco-54, Albright & Wilson Americas, Division of Tenneco Canada Inc., Islington, Ontario), which were combined with the refined mince by blending in a silent cutter for 3 to 4 min. Various specialized cryoprotectants were tested in 1990. In most cases, 5% TP-5330-N1 (a patented ingredient produced by Ueno Seiyaka Company Limited, Osaka, Japan) and 4% sucrose were added to the washed and dewatered mince; however some batches also contained 0.5% added egg albumin powder.

Silver hake were processed as efficiently as possible after harvesting. The longest holding period prior to usage was estimated at 30 h after harvest in the 1989 trial and 20 h post-mortem in 1990. Temperatures were monitored and controlled throughout the manufacturing operation with processing water chilled to 2°C and the production areas and equipment generally kept at less than 10°C. The maximum temperature of the fish/surimi prior to freezing was consistently less than 10°C in 1989. Ambient temperatures both inside and outside the plant were somewhat higher in 1990, consequently, temperatures of the surimi made during the second year averaged 13°C, ranging from 11°C to 15°C at the time of packaging.

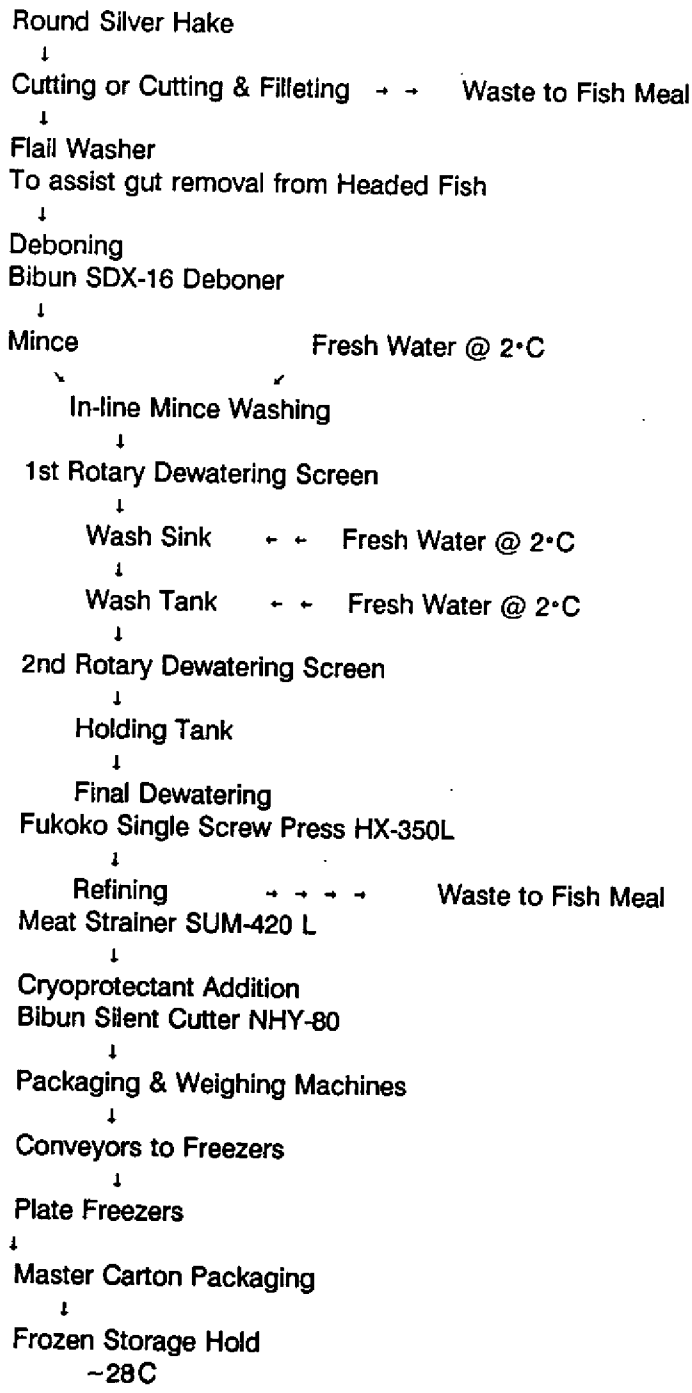

Figure 1. Surimi Process On Board the "Shinkai Maru"

PRODUCT EVALUATION

Throughout the investigation, variables which may have affected the product attributes, including "freshness" and quality of the raw materials, were closely monitored and documented. Samples of the experimental products were tested daily on board the vessel for quality attributes including: moisture content, number of impurities, color, gel-forming ability and sensory characteristics. Specific details regarding the samples are discussed later in this report. Representative samples of the production were analyzed at the Canadian Institute of Fisheries Technology (CIFT) for proximate composition and pH by standard laboratory techniques (11). Functional properties of the surimi were tested by the procedures of Lee (6), as well as those recommended by the Technical Subcommittee of the National Fisheries Institute Surimi Committee (7). Gel preparations were made with partially frozen (tempered) surimi and 2% NaCl in a vacuum chopper (VCM-12, Stephan Machinery Canada Limited, Mississauga, Ontario). The resultant surimi-salt sols were extruded into 30 and 21 mm (i.d.) tubes which were sealed and then allowed to "set," i.e., gel, at various time/temperature regimes. The gel-setting treatments for the 30 mm tubes, which were used in determining the fold score (10), gel strength by the Instron punch test (6), cohesiveness by axial compression (3), Kramer shear force, cone force at 1.0 cm deformation and viscoelastic properties by Carri-Med controlled stress rheometry were: "90" - 90°C for 30 min; "40/90" - 40°C for 30 min followed by 90°C for 20 min. Torsional stress, strain and rigidity (5) were measured on 21 mm diameter specimens given the following heat treatments: "90" - 90°C for 15 min; "40/90" - 40°C for 15 min followed by 90°C for 15 min. Cooking at 40°C and 90°C was carried out in water baths, after which the gels were immersed in ice water, chilled and analyzed in triplicate within 48 h. Color of the cooked surimi gels was measured with a Colormet Spectrophotometer (Metron Instruments Inc., St. John's NF) as recommended by Martin (7). Sensory characteristics of the products were assessed by noting the odor of rheological test specimens.

RESULTS AND DISCUSSION

An estimated 212,600 kg of fish, including 192,800 kg of silver hake, were hauled in 18 sets during the 1989 trial. Production included 24,620 kg (1231 cases) of silver hake surimi, 14,350 kg (410 bags) of fish meal and 10,120 kg of dressed fish from the by-catch. Yields of surimi and fish meal were estimated at 12.8% and 8.0%, respectively, based on the weight of the whole fish.

In 1990, an estimated 334 t of fish consisting of 92.6% silver hake were harvested in 46 sets hauled over the 14 days. Products manufactured included 36,700 kg of surimi, 10,360 kg of stabilized mince, 29,435 kg of fish meal and 13,466 kg of frozen fish products from the approved by-catch species. Recovery of surimi was estimated at 13.1%, while the yield of fish meal from the undersized silver hake, silver hake offal and other species was calculated at 10.6%. The predominant by-catch species in 1990 were squid (*Illex illecebrosus*) and Atlantic pollock (*Pollachius virens*), which amounted to 4.1% and 1.2% of the total catch, respectively.

Key parameters in the utilization of silver hake include the identification of successful techniques for harvesting and handling the species. Lanier (4) concluded that silver hake, as well as red hake, had excellent potential as raw material for surimi production when high-quality fish were used as the starting material. Silver hake frozen at sea by Cuban factory trawlers was considered unacceptable for reprocessing into surimi due to the low gel-forming ability of the resultant products (8). Initial trials conducted in the food processing pilot plant at CIFT, showed that H&G silver hake could be used to prepare surimi with excellent color and sensory characteristics, if the fish was processed within a few days of harvesting (9). Further research aimed at optimizing the processing methods and gel-forming ability of silver hake surimi showed that skin-on and skin-off fillets from fish that had been held in refrigerated sea water (RSW) or ice for two to three days made surimi comparable in quality to commercially manufactured Alaska pollock (*Theragra chalcogramma*) surimi.

The majority of silver hake caught during the trials were ~26cm in length, which was smaller than the average size reported by DFO, i.e., 30-34 cm. Equipment limitations, as well as the small size of the fish, made it impossible to efficiently fillet all of the silver hake; consequently, head-off, partially gutted fish were used as the starting material in surimi production. The Japanese indicated that ideally, fillets should have been used and expressed keen interest in accessing filleting equipment that could handle silver hake.

During the 1990 trial, an experiment was undertaken to estimate losses of fish at various stages of the surimi manufacturing operation. As is shown in Table 1, most significant losses of fish were found at the heading, deboning, dewatering, pressing and refining steps. Much of the wasted fish could have been recovered and added back to the surimi or fish meal production lines with little effort; however the Japanese stated that their primary goal was to optimize product quality and admitted that product yields were knowingly sacrificed. An accurate assessment of product yield is necessary in order to evaluate the commercial feasibility of manufacturing silver hake surimi.

Analyses of samples taken during the study indicated that silver hake has strong potential as a resource for high quality surimi. Functional properties of the product were comparable to those exhibited by Alaska pollock, which is currently the predominant resource species for both the Japanese and North American surimi industries. Moisture content of the surimi averaged 77.3% in 1989, which is 2% greater than the amount recommended by Martin (7). Protein content of the 1989 production was slightly low ranging from 13.1% to 14.1%, while the fat content averaged 1.4%.

Surimi samples from the 1990 sea trials had moisture contents ranging from 72.8% to 77.0%, protein content ranged from 16.8% to 18.7%, and lipids varied from 1.0% to 1.5%. The effort directed toward maximizing the quality and minimizing moisture content of the products was considered successful, in that, functional characteristics of the surimi made during the second year were far superior to those exhibited by the 1989 product.

Gel-forming ability of surimi from silver hake processed within 30 hours after harvesting was considered acceptable for the production of Japanese surimi-based products. In comparison to Alaska pollock surimi from shore-based processing plants, silver hake surimi displayed marginally lower gel strength, but equivalent resiliency (as measured by the fold test) and firmness or puncture force resistance (see Table 2). Torsional true strain values at failure, which are a measurement of gel cohesiveness, showed that silver hake surimi was superior to typical Alaska pollock samples in this characteristic. Torsional shear stress values at failure indicated that products of 1990 sea trials gave gels that were as strong as those from sea-processed Alaska pollock surimi. Overall, there was no obvious difference; i.e., in composition, functionality or color, between surimi samples made with or without egg powder, although inclusion of the third additive may have improved cohesiveness and shear strength of the product.

The sensory attributes of silver hake surimi were rated as highly acceptable to excellent. Cooked samples were light in color and had a mild seafood flavor without off flavors or aromas. Product appearance was very good, even though some samples had an excessive number of impurities that were not removed during refining. Whiteness or brightness of the silver hake gels, as measured by the color L values, was slightly lower than that reported for Alaska pollock gels. Results to date indicate that silver hake surimi is stable in frozen storage. Sensory quality and functionality of experimental samples retested after four months storage at -30°C were unchanged from when the product was first evaluated (see Figures 2-7).

Table 1. Estimated Amounts of Fish Recovered or Lost During Processing

PROCESSING STEP	FISH AVAILABLE FOR SURIMI	(%)	WASTE & LOST FISH	(%)	FISH FOR REDUCTION TO MEAL	(%)
Sorting	whole fish	100				
Cutting/Filleting	head-off fish	57	guts, roe	5	heads, frames	38
Deboning/Mincing	mince	31	skin, bones, fat	26		
Conveyors, lost in transport	cut fish, mince	30	cut fish, mince	1		
Washing/Dewatering	washed mince	27	blood, pigments	3		
Screw Press Dehydration	pressed mince	22	mince lost thru screens	5		
Straining	refined mince	12	dark meat, skin, bones	10		
Add Cryoprotectants @ ~9.3% (w/w) of fish	surimi	13				
Fish & Offal Reduction					fish meal	11
Finished Production	Surimi Yield: 13%		Unused & Lost Fish: 50%		Meal Yield: 11%	

Table 2. Functional Properties of Alaska Pollock Surimi and Silver Hake Surimi

	ALASKA POLLOCK SURIMI			"SHINKAI MARU" SILVER HAKE SURIMI		
Product from:	Japan[1]	Alaska[1]	Japan[2]	4/17/89[3]	5/28/90[3]	5/21/90[4]
Moisture (%)	76.1 ± 0.2	76.7 ± 1.7	73.1 ± 0.1	77.6 ± 0.2	75.0 ± 0.1	72.8 ± 0.1
Fold Score (1-6)	6	6	6	6	6	6
Punch Force (g)	930 ± 19	440 ± 87	853 ± 83	453 ± 71	492 ± 47	712 ± 116
Punch Deformation (mm)	16.9 ± 0.5	13.9 ± 1.4	10.8 ± 0.1	13.1 ± 2.2	10.4 ± 1.1	11.2 ± 0.1
Gel Strength (g.cm)	1564 ± 85	617 ± 153	927 ± 151	602 ± 198	514 ± 101	811 ± 213
Gel Stiffness (g/cm)	542 ± 57	317 ± 56	789 ± 26	347 ± 16	475 ± 16	631 ± 32
Shear Stress (kPa)	76.5 ± 6.8	49.4 ± 11.5	158 ± 0.0*	64.8 ± 7.4	127 ± 9.4	158 ± 0.0*
True Strain	2.3 ± 0.5	2.2 ± 0.3	2.0 ± 0.1*	2.8 ± 0.2	2.5 ± 0.1	2.4 ± 0.1*
Rigidity (kPa)	33.8 ± 4.2	22.9 ± 5.6	81.0 ± 2.0*	23.3 ± 1.8	50.4 ± 1.2	67.0 ± 2.3*
Color L Value	82.1 ± 0.1	77.3 ± 1.1	73.5 ± 0.6	74.2 ± 1.3	72.5 ± 0.7	72.0 ± 2.9
Cohesiveness (kg)			166 ± 27		185 ± 10	273 ± 3.6
Cone Stress (kg)			49.9 ± 1.2		29.9 ± 1.9	42.4 ± 0.7
Shear Force (kg)			47.2 ± 3.3		20.4 ± 1.3	31.2 ± 1.2
Compression (slope)			172 ± 3.1		120 ± 3.4	144 ± 6.7
Carri-Med G' (η'/m^2)			77783 ± 4434		55660 ± 2818	83587 ± 4058
Carrimed η' (Pa.s)			2652 ± 145		1939 ± 98	2837 ± 132

[1]Data taken from Anonymous (1).
[2]AAAA Grade Alaska pollock surimi tested at CIFT, gels cooked @ 40°C followed by 90°C.
[3]Surimi made with standard cryoprotectants, gels cooked @ 40°C followed by 90°C.
[4]Surimi containing egg albumin added along with the standard cryoprotectants, gels cooked @ 40°C/90°C.
*Gel samples did not fail at the maximum limit of the torsion test. Stress values are reported as the maximum measurable number. Strain and rigidity are calculated at the maximum measurable stress.

CONCLUSIONS

Silver hake surimi processing trials conducted at sea, on board the "Shinkai Maru" gave promising results. Analysis of the Japanese product indicated that silver hake has strong potential as a resource for high quality surimi, although punch test scores of the experimental samples were somewhat lower than those found in top grade Alaska pollock surimi. The Japanese speculated that preprocessing of the fish into fillets prior to deboning would probably have improved the product quality. Fish post-mortem age and processing were important factors influencing the gel-forming ability of silver hake surimi. Further research and collaboration with JAMARC was planned for the 1991 silver hake fishing season.

ACKNOWLEDGEMENTS

The financial support provided by the Fisheries Development and Fishermen's Services Division, Department of Fisheries and Oceans, and the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged. Appreciation is also expressed to JAMARC, D. Singer, J. Kell and K. Wright for their assistance in the execution and completion of this work.

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SEAL MEAT: THE ULTIMATE IN SURIMI TECHNOLOGY

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Harp seal (Phoca groenlandica) is found in abundance in the southern Labrador and in the Gulf of St. Lawrence. Their population is estimated at 2.5 millions in this area. The total allowable catch of harp seals at the present time is 186,000 heads annually. However, in recent years only 60,000 - 70,000 heads have been harvested (7). Hunting of pups is banned by regulation.

The major primary products of seal include seal oil rendered from blubber and seal pelts. The use of seal meat in food products is limited mainly to flippers and even this is confined to Atlantic Canada and particularly Newfoundland. Some seal meat is also canned for human consumption. The rest may either be reduced to low-grade silage for animal feed or dumped.

Underutilization of seal meat is perhaps due to several factors. The major one being related to the unusually dark color of the flesh and its intense flavor. Lack of information on the nutritional quality of seal muscle proteins is another major contributor.

As part of a program to investigate the chemical and nutritional quality of seal muscle proteins and lipids we have studied the effect of aqueous and saline washings on the chemical and nutritional compositions. Color and flavor quality of washed seal meat separated by mechanical means is also reported in this contribution.

MATERIALS AND METHODS

Seal meat was separated by mechanical means from carcass using a POSS deboner (Poss Limited, Model PDE 500, Toronto, Ontario, Canada). Small portion of comminuted seal meat were vacuum packed and kept frozen at -20°C until use.

The mechanically separated seal meat (MSSM) was washed 3 times, sequentially with water using a meat to water ratio of 1:3 (w/v). Other samples were washed with water and then with 0.3% NaCl solution both at a meat to solvent ratio of 1:3 (w/v). Washings were carried out at 2°C for approximately 10 min. The washed meats were then collected and possibly pressed to reach an acceptable moisture level.

Moisture content was determined by oven drying of the sample at 105°C to a constant weight. Crude protein was calculated as the total nitrogen (N) determined by Kjeldahl, multiplied by 6.25 (1). The total lipids were extracted and quantified according to Bligh and Dyer (3). The ash content was determined by AOAC (1) procedure.

The individual amino acids of proteins and fatty acids of lipids were determined as described elsewhere (7).

Subjective evaluation of flavor and color intensity of unwashed and washed meat was performed using a 10-point scale system. In this system 1 refers to the least and 10 to the most intense attributes.

The tristimulus color parameters were measured on the top surfaces of meat samples using an XL-20 colorimeter (Gardner Laboratory, Inc., Bethesda, MD). Standard plate number XL20-167C with Hunter L (light, 100; dark, 0) value of 92.0, a (red, +; green, -) value of -1.1 and b (yellow, +; blue, -) value of 0.7 was used as reference.

Total hemoproteins were determined according to Hornsey's (5) method by extraction of hemin with acidified 80% acetone in water solution. The amount of hemoproteins was calculated according to the equation:

$$\text{Total pigment } (mg/g) = \frac{\text{Total heme } (mg/g) * \text{Molecular weight of}}{\text{Molecular weight of hemin}}$$

The cholesterol content was determined according to Rudel and Morris (6) using o-phthalaldehyde reagent (Sigma Chemical Corporation). The absorbance of the samples at λ = 550 nm was measured using a Beckman DU-8 spectrophotometer. The standard curve was prepared using a cholesterol standard (Fisher Scientific Co., New Jersey, U.S.A.) in concentrations from 5 to 40 μg of cholesterol per sample (3 ml solution).

RESULT AND DISCUSSION

The yield of meat from beaters (seals of less than one year old) was over three times higher for mechanically separated seal meat (MSSM) as compared to manually separated products (7). Thus a yield of 82 ± 3% was obtained for MSSM.

The chemical composition of mechanically separated seal meat and as affected by washing is summarized in Table 1.

Table 1. Chemical Composition of Seal Meat as Affected by Washing in Surimi Production.[a]

COMPOSITION	MSSM	WASHED MSSM[b]
Moisture	70.8 ± 0.1	78.9 ± 0.1
Crude protein, N x 6.25	23.2 ± 0.1	16.4 ± 0.0
Lipid, total	3.7 ± 0.1	2.6 ± 0.1
Ash	2.0 ± 0.0	1.6 ± 0.0
Total hemoprotein		
Pigments	5.9 ± 0.1	1.2 ± 0.0
Cholesterol, mg%	98.8 ± 5.8	145.7 ± 8.9

[a]Results are mean values of 3 to 6 replicates ± standard deviation.

[b]Washed sequentially with water and 0.3% NaCl solution.

The MSSM had a crude protein content of about 23.2%. The protein content of seal meat generally exceeded those of beef, pork, cod and herring (4, 7, 8). Its fat content only exceeded that of cod, but was much less than those of beef, pork and herring. The essential amino acids composition (Table 2) was comparable with those of beef and pork (8). Lipid composition of MSSM is summarized in Table 3. The fatty acids of lipids from MSSM contained 17.6% saturated and 81.1% unsaturated fatty acids. The amount of mono-, and polyunsaturated fatty acids from

MSSM were higher than those in beef and pork. The presence of ω-3 fatty acids has nutritional significance because they are probably effective in lowering of serum blood cholesterol (2). Results presented indicate that seal meat is an important source of protein with a well-balanced amino acids composition. However, its dark color and intense flavor has thwarted its unrestricted use. Large amounts of hemoprotein pigments (about 5.9%) are present in seal muscles and these are responsible for its unusually intense dark color, and these may also influence the developing of off-flavor in the meat. Application of surimi technology to seal meat afforded a product which had a low content of hemoproteins. The washings in the preparation of seal surimi removed up to 79% of hemoprotein pigments present in MSSM (Table 1) and brought about a significant improvement in the color of meat. The Hunter lightness 'L' increased from 17.2 in unwashed MSSM to 36.1 in meat washed 3-times with water. The washings also resulted in higher Hunter 'a' and 'b' values of MSSM (Table 4).

Table 2. Essential Amino Acid Composition of MSSM and Seal Surimi.[a]

AMINO ACID	MSSM	WASHED MSSM
Histidine	5.0 ± 0.1	2.7 ± 0.0
Isoleucine	4.6 ± 0.0	4.2 ± 0.1
Leucine	7.4 ± 0.0	6.0 ± 0.1
Lysine	8.7 ± 0.1	7.6 ± 0.1
Methionine	1.7 ± 0.1	1.7 ± 0.0
Phenylalanine	4.6 ± 0.0	4.0 ± 0.1
Threonine	4.5 ± 0.0	4.2 ± 0.0
Tryptophan	1.2 ± 0.0	1.0 ± 0.0
Valine	5.8 ± 0.1	5.3 ± 0.0

[a]Results are mean values of 3 replicates ± standard deviation.

Table 3. Lipid Composition of MSSM and as Affected by Washing.

TYPE OF FATTY ACIDS	MSSM	WASHED MSSM	PORK[a]	BEEF[a]
Saturated (S)	17.6	19.9	37.0	46.7
Monounsaturated (MU)	56.5	54.1	52.6	47.1
Polyunsaturated (PU)	24.6	17.5	10.4	6.2
Long-chain ω-3	14.6	10.6	-	-
S/(MU + PU)	0.22	0.27	0.58	0.87

[a]From Schewigert, (8).

Table 4. Total Hemoprotein Content of MSSM and as Affected by Washing.

MEAT	TOTAL HEMOPROTEINS, %
Unwashed MSSM	5.93 ± 0.14
Washed: 1 x H_2O	2.99 ± 0.05
2 x H_2O	2.07 ± 0.01
3 x H_2O	1.93 ± 0.03
1 x H_2O then 0.3% NaCl	1.26 ± 0.02

The flavor intensity of seal meat was determined by sensory panel. Results of this analysis are summarized in Table 5. The data indicates only a little improvement of the flavor characteristics of MSSM as a result of its extraction with water or with 0.3% NaCl solution. However, washing with 3% bicarbonate solution resulted in a definite flavor change from a "sealy" to a "fishy" note.

Table 5. Effect of Washing on Color and Flavor Intensity of MSSM and as Affected by Washings

MEAT/WASHINGS	FLAVOR INTENSITY	COLOR INTENSITY	HUNTER VALUE		
			L	a	b
MSSM	7.0	9.0	17.25	5.25	2.11
3 x H_2O	5.0	5.0	36.12	8.73	8.20
1 x H_2O and then 1 x 0.3% NaCl	5.0	5.2	34.45	8.87	8.43
1 x 3% Bicarbonate and then 1 x H_2O	3.0 - 3.5	-	-	-	-

The texture parameters of MSSM, and as affected by washing were also assessed sensorially (Table 6). The unwashed MSSM had a very "sticky" texture. However, it was found to be less fibrous and quite soft. Washing had a definite influence in decreasing the cohesiveness of MSSM. The texture of washed meat was modified to a more fibrous and a harder product.

Table 6. Sensory evaluation of textural properties of unwashed MSSM and MSSM after washings (1:3 w/v, 10 min. 5°C).

SOURCE	SCORES[a]		
	Hardness	Cohesiveness	Fibrousness
Unwashed MSSM	4.0	7.8	3.0
MSSM washed:			
1 x H_2O	5.8	4.0	5.0
2 x H_2O	6.0	3.9	5.2
3 x H_2O	6.0	4.0	5.3
1 x H_2O then 1 x 0.3% NaCl	5.8	3.7	5.5

[a]1, Extremely low; 10, Extremely high.

Washing of seal meat with water and 0.3% NaCl solution enhanced its moisture content. Ash content was also increased from 6.85 to 7.58%, on dry basis, and fat content was reduced by about 40.2% of its initial amount (Table 1). Of particular interest is the reduction in the lipids content as it might have a direct influence in reducing the development of the flavor intensity of washed seal meat. However, washing with salt solution decreased the level of nutritionally important eicosapentaenoic (C22:5ω3) and docosahexaenoic (C22:6ω3) fatty acids by about 32.4 and 23.7%, respectively (Table 3). As given in Table 2, washing also decreased the content of some of essential amino acids, namely histidine, leucine, lysine and phenylalanine.

Manually separated seal meat contained about 66 mg% of cholesterol (Table 1). Mechanical separation of seal meat resulted in the enhancement of its cholesterol content and indeed this value ranged between 116 and 121 mg% (Table 1). This is not unexpected since other

sources of muscle foods show a similar trend when meat is separated by mechanical means or residual flesh is scraped from the bones. Washing with 0.3% NaCl solution enhanced the cholesterol content of the samples from 98.8 to 145.7 mg% (Table 1).

CONCLUSIONS

The present results indicate that seal meat is an excellent source of proteins with a well-balanced essential amino acids composition. Its dark color was improved by the application of surimi technology which afforded a product with low content of hemoproteins. Seal meat had a high content of long-chain omega-3 fatty acids which are recognized for their beneficial health effects. However, washing somewhat decreased their content in the meat.

ACKNOWLEDGEMENTS

This work was financially supported by a NIFDA grant through the Department of Fisheries of Newfoundland and Labrador.

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WHOLE MARINE SHRIMP USAGE AND PREFERENCES IN THE U.S. WHOLESALE MARKET SECTOR

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In 1988, 766,776 million pounds (3) entered U.S. market channels. Much of the domestic and imported marine shrimp, mainly Penaeus spp., entering the wholesale market channels in the U.S. was sold in various frozen headless product forms. Past marketing studies have generally focused on various U.S. markets for headless (heads-off) marine shrimp. In contrast, there is a paucity of data on U.S. markets for whole marine shrimp, especially at the wholesale level.[1] Moreover, cultured marine shrimp producers in the U.S. (e.g., South Carolina, Texas, etc.) and other countries (e.g., Ecuador, Panama, etc.) have attempted to identify and develop whole (heads-on) shrimp market segments in the U.S and Europe (1).

The objective of this research was (a) to describe existing marine shrimp preferences in the wholesale market and (b) to identify critical product attributes in the wholesale market when selling heads-on marine shrimp. Use and preference data was collected on U.S. seafood wholesalers and distributors based on a 1989 mail survey. Data for a conjoint analysis of buyer preferences was also gathered during 1990 telephone interviews.

MATERIAL AND METHODS

Mail Survey

A two-page questionnaire was designed and tested for a mail survey of marine shrimp usage and preferences by U.S. wholesalers. The survey questions consisted of the respondent mailing address, shrimp product preference, and current purchases of shrimp. Dun & Bradstreets Marketing Service in Parsippany, New Jersey, was the source used for the seafood wholesalers mailing list. Companies listed under the Standard Industrial Classification (SIC) Code of 514699 "Fish & Seafoods, nec" were selected for the survey's mailing list.

In May 1989, thirty-three firms were randomly selected for the seafood wholesalers survey pretest. A week after the mail-out of questionnaires, a telephone follow-up was conducted to see if the firms received a questionnaire and to encourage them to return their survey. Eight (24%) of the thirty-three firms returned their questionnaire and four were returned due to insufficient addresses.

In June 1989, 6,021 remaining questionnaires were mailed to seafood wholesalers, distributors and other buyers in the U.S. The highest percent of firms had mailing addresses in the Northeast region, followed by the West region, the South and the Midwest (Table 1).

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[1] Wholesalers and/or distributors usually buy shrimp directly from importers, processors and/or "handlers" (assemblers). Shrimp handlers in the Southeastern U.S., often purchase and/or broker heads-on and heads-off shrimp directly from producers (e.g. shrimp trawler operators) and sell the shrimp to wholesalers or processors.

Table 1. The Seafood Wholesaler Mailing List Addresses by Region, 1989.

Region	Mailings	
Northeast[1]	2,232	(36.9%)
West[2]	1,671	(27.6%)
South[3]	1,645	(27.2%)
Midwest[4]	506	(8.3%)
Total	6,054	(100.0%)
Returned Mailings[5]	58	(1.0%)
Total Completed Mailings	5,996	(99.0%)

[1]CT, ME, MA, NH, RI, VT, DE, DC, MD, NJ, NY, PA, VA, WV
[2]AZ, CO, ID, NV, UT, WY, AK, CA, HI, OR, WA, NM, OK, TX
[3]AL, FL, GA, NC, SC, AR, KY, LA, MS, MO, TN
[4]IA, KS, MN, MT, ND, SD, NE, IL, IN, MI, OH, WI
[5]Insufficient address, forwarding address expired or no forwarding address

Note: The U.S. Bureau of the Census' Divisions and Regions were not the source used for this study.

A microcomputer data entry program, MPA Version 3.0 by Detail Technologies Inc., was used to enter responses for each questionnaire. A cross tabulation or crosstabs microcomputer software, A-cross by Strawberry Software, was used to analyze the questionnaire data.

Telephone Interviews and Conjoint Analysis

A conjoint analysis software, Sawtooth's Adaptive Conjoint Analysis (ACA), Version 3, was used in conducting in-depth telephone interviews (8) for those firms interested in purchasing heads-on marine (whole) shrimp. Those firms interested in purchasing heads-on shrimp were identified in the 1989 wholesalers survey. Conjoint analysis (7) implies the study of "joint" or combined effects of multiple attribute variables (often product characteristics) on the order of preference or choice for an overall product. This technique calculates the relative importance of selected product attributes based on the trade-offs respondents have made (2).

Forty-six firms expressing interest in purchasing heads-on shrimp based on the 1989 mail survey were mailed background information about the interview procedure. After mailing these exhibits, the respondents were contacted to schedule interviewing times. Representatives of six companies were interviewed in-person at seafood trade shows in 1990. Respondents were asked to give preferences for the following attributes of heads-on shrimp: shrimp source (i.e. wild vs. farmed), product form (e.g. IQF vs. fresh), availability, count (size), quantities per order and price (Table 2). The interview lasted about 20-25 minutes, and a total of 20 (43%) respondents completed the telephone or in-person trade show interviews. The data was analyzed using the ACA software including the simulation of preferences and likelihood of purchase.

RESULTS AND DISCUSSION

Mail Survey Results

A total of 393 (6.4%) usable questionnaires and six (1.5%) unusable questionnaires were returned. Other marketing researchers have reported response rates under 10% for mail surveys of U.S. seafood wholesalers and brokers. For example, Harvey, et al. (3) reported

Table 2. Summary of Heads-on Shrimp Attributes Used in the 1990 Telephone Survey.

Product Form:	1. Fresh, Never Frozen (Heads-on)
	2. Quality IQF (Heads-on)
	3. Quality Block Frozen (Heads-on)
	4. Live Shrimp
Quantities Purchased Per Order:	1. We would want to order about 1,000 to 5,000 Lbs. (Heads-on)
	2. 5,000 to 10,000 Lbs. (Heads-on)
	3. 10,000 to 20,000 lbs. (Heads-on)
	4. 20,000 to 30,000 lbs. (Heads-on)
	5. We would want to order a full trailer load of heads-on shrimp.
Price per Pound:	(Heads-on, FOB in South Carolina)
	1. $2.20/lb (Heads-on)
	2. $2.50/lb (Heads-on)
	3. $2.80/lb (Heads-on)
	4. $3.10/lb (Heads-on)
	5. $3.40/lb (Heads-on)
Count:	(Number of Whole Shrimp per Pound)
	1. 28-30 Count/lb (Heads-on)
	2. 25-27 Count/lb (Heads-on)
	3. 22-24 Count/lb (Heads-on)
Availability:	1. We want to be able to order S.C shrimp at anytime.
	2. in Jan. thru March.
	3. in April thru June.
	4. in July thru Sept.
	5. in Oct. thru Dec.
Shrimp Species Source:	1. Wild S.C. White Shrimp (trawler caught)
	2. Quality, Farmed S.C. White Shrimp

that out of 920 wholesalers mailed questionnaires in the Mid-Atlantic states, only 65 (7%) responded to their 1989 mail survey regarding cultured hybrid striped bass.

The highest percent of respondents had mailing addresses in the Northeast region (34.4%) followed by the South (31.0%), the West (23.4%) and the Midwest (11.2%) (Fig. 1). The number of responses was generally consistent with the number of mailings by region (see Table 1).

When asked to classify their business, 45.2% chose wholesaler/distributor, 12.7% processor, 10.2% broker, 10.0% importer, 8.6% retailer, 6.8% trader, 4.9% exporter and 1.6% others (e.g. restaurants, producers) (Table 3). Since none of the businesses used in the mailing list were given specific classifications by the list vendor, it is difficult to comment on the response rate by different type of businesses. The highest number of "processor" classifications was found in the South region. In 1987, shrimp processors in the South Atlantic and Gulf states represented 73% of reported 329 million pounds U.S. frozen processed shrimp products (9).

Of those firms 381, responding to the question regarding their interest in purchasing marine white shrimp farmed (cultured) in the U.S., 45.9% said "maybe", 42.5% said "yes" and 11.5% said "no" (Table 4a). Midwest respondents had the highest response percentage for the "yes" category (60.5%) while Northeast buyers had the lowest response (Table 4a). "Traders" and "Brokers" had the highest percentages relative to interest (i.e. a "yes" response) while "Other" and "Exporters" had the lowest response rates to "yes" (Table 4b). In comparison, Wirth (10) reported that 87% of the responding Mid-Atlantic seafood wholesalers were willing to purchase U.S. cultured hybrid striped bass. The availability of both domestic and imported shrimp products compared to other aquaculture species (e.g. hybrid striped bass, etc.) may have accounted for the lack of interest in U.S. farmed shrimp.

As might be expected, heads-off shrimp products dominated the general purchases and reported preferences of seafood wholesalers and others in the market channel. When asked to indicate the raw shell-on shrimp products purchased in 1988, 54.2% of those responding indicated that they purchased heads-off frozen shrimp including individually quick frozen (IQF) shrimp, 18.1% heads-off fresh, 13.6% heads-on fresh, 11.9% heads-on frozen and IQF, and 2.2% indicated live shrimp. Based upon shell-on shrimp product forms, the most commonly desired (requested) product was heads-off frozen shrimp (28.1%), heads-off IQF (20.3%), heads-off fresh (16.9%), heads-on fresh (9.8%), heads-on IQF (8.3%), heads-on frozen (6.4%), other products (5.5%) and live (4.7%) (Fig. 2).

The desired count sizes (i.e. number of individual shrimp per pound) for selected heads-on (i.e. fresh, frozen and IQF) shrimp was the following: 16-30 counts (31.1%), all major counts (28.3%), U-15 counts (20.6%), 31-50 counts (16.7%), counts greater than 50 (3.3%) (Table 5). Responses suggest that heads-on shrimp buyers are generally not interested in purchasing shrimp counting fifty or more per pound. In addition, the highest percentage for four count range was in the 16-30 count range (Table 5). The highest number of responses regardless of count size was recorded for fresh heads-on shrimp (Table 5). IQF shrimp products had second highest number of total responses (Table 5).

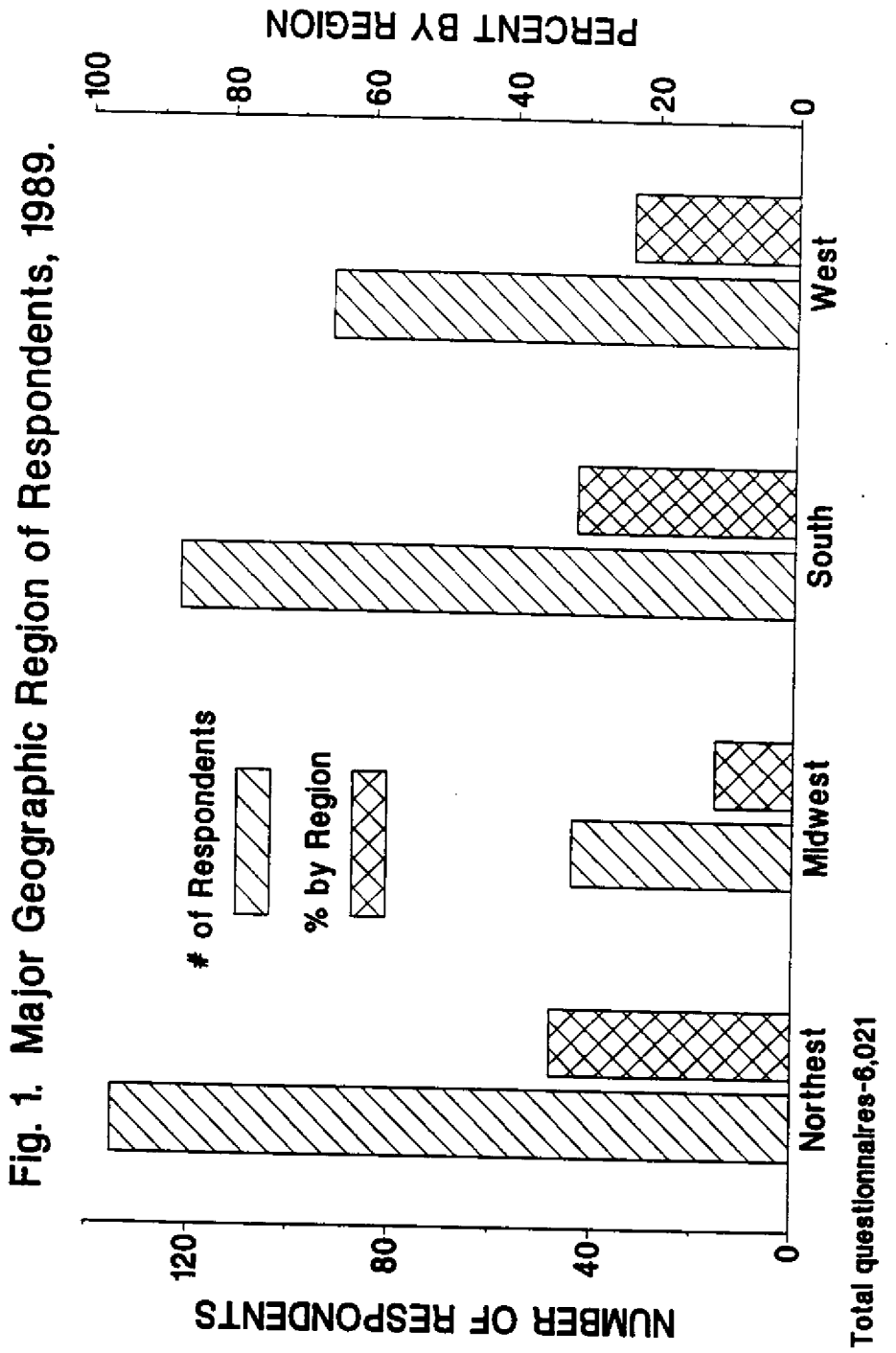


Fig. 1. Major Geographic Region of Respondents, 1989.

Table 3. Classification of Business by Regions of the Respondents, 1989.

How would you best describe your firms seafood marketing activities?										
Business Classification	Northeast		Midwest		South		West		All Regions	
Broker	18	(9.6%)	4	(6.3%)	20	(9.4%)	22	(13.3%)	64	(10.2%)
Exporter	4	(2.1%)	1	(1.6%)	11	(5.2%)	15	(9.0%)	31	(4.9%)
W/D[1]	100	(53.3%)	36	(56.3%)	93	(43.9%)	55	(33.1%)	284	(45.2%)
Processor	13	(7.0%)	4	(6.3%)	42	(19.8%)	21	(12.7%)	80	(12.7%)
Importer	17	(9.1%)	4	(6.3%)	15	(7.1%)	27	(16.3%)	63	(10.0%)
Trader	12	(6.4%)	3	(4.7%)	11	(10.2%)	17	(10.2%)	43	(6.8%)
Retailer	20	(10.7%)	12	(18.8%)	17	(8.0%)	5	(3.0%)	54	(8.6%)
Others	3	(1.6%)	0	(0.0%)	3	(1.4%)	4	(1.2%)	10	(1.6%)
Totals	187	(29.7%)	64	(10.2%)	212	(33.7%)	166	(26.4%)	629	(100.0%)

[1]Wholesaler/Distributor

Note: There were multiple responses to this question. Percents were calculated based upon totals for a given column except for "Totals" category. These percentages are not consistent with percentages in Fig. 1 due to multiple responses to the business classification question.

Table 4a. Respondents' Willingness to Purchase Marine White Shrimp Cultured in the U.S., 1989.

Region	Are you interest in... shrimp farmed in the U.S.? "Yes"		"No"		"Maybe"		N/M[1]	
Northeast	48	(36.1%)	21	(15.8%)	64	(48.1%)	85	(63.9%)
Midwest	26	(60.5%)	1	(2.3%)	16	(37.2%)	17	(39.5%)
South	53	(44.9%)	10	(8.5%)	55	(46.6%)	65	(55.1%)
West	35	(40.2%)	12	(13.8%)	40	(46.0%)	52	(59.8%)
Total	162	(42.5%)	44	(11.5%)	175	(45.9%)	219	(57.5%)

Table 4b. Responses by Business Classification, 1989.

	Are you interested in ... shrimp farmed in the U.S.? "Yes"		"No"		"Maybe"		N/M[1]	
Broker	38	(60.3%)	4	(6.3%)	21	(33.3%)	25	(39.7%)
Exporter	12	(41.4%)	3	(10.3%)	14	(48.3%)	17	(58.6%)
W/D[2]	124	(44.6%)	26	(9.4%)	128	(46.0%)	154	(55.4%)
Processor	35	(44.9%)	11	(14.1%)	32	(41.0%)	43	(55.1%)
Importer	28	(45.2%)	10	(16.1%)	24	(16.1%)	34	(54.8%)
Trader	31	(73.8%)	2	(4.8%)	9	(4.8%)	11	(26.2%)
Retailer	17	(32.7%)	5	(9.6%)	30	(57.7%)	35	(67.3%)
Other	2	(15.4%)	5	(38.5%)	6	(46.2%)	11	(84.6%)
Total	287	(46.5%)	66	(10.7%)	264	(42.8%)	330	(53.5%)

[1]No or Maybe (combined)
[2]Wholesaler/Distributor
Note: There were multiple responses to this question and business classification question. Percentages in Table 4b are not consistent with percentages in Table 4a due to multiple responses to the business classification question.

Table 5. Desired Count Sizes of Selected Heads-on Shrimp Products from White Shrimp Cultured in the U.S.

Count Sizes	Responses Regardless of Business Classification Heads-On Product Form IQF	Fresh	Frozen	Total
U-15	9 (16.1%)	20 (25.3%)	8 (17.4%)	37 (20.6%)
16-30	16 (28.6%)	26 (32.9%)	14 (30.4%)	56 (31.1%)
31-50	10 (17.9%)	12 (15.2%)	8 (17.4%)	30 (16.7%)
>50	2 (3.6%)	2 (2.5%)	2 (4.3%)	6 (3.3%)
"All"[1]	19 (33.9%)	19 (24.1%)	13 (28.3%)	51 (28.3%)
Total[2]	56 (31.1%)	79 (43.9%)	45 (25.0%)	180 (100.0%)

[1]All major counts wanted.
[2]The totals in Table 5 are not consistent with totals in Table 6, due to multiple business classification responses and/or non-responses to a given question. Note: There were multiple responses to this question.

"Wholesalers/distributors", the largest group responding to this survey, had the highest percentage response rate in the 16-30 count range (excluding the "All Major Counts Wanted" category) (Table 6). For wholesalers/distributors, fresh heads-on shrimp had the highest total response rate, 41.8%, followed by IQF whole shrimp (Table 6).

When asked to indicated desired monthly quantities (pounds) of heads-on shrimp, 40.7% chose fresh, 40.0% IQF and 19.3% frozen (Table 7). Average monthly quantities desired were the highest for IQF shrimp (Fig. 3). Buyers in the South Region had the highest response rate to this question and the highest mean quantity desired (Table 7). Some of the firms in the South Region responding to this question were probably shrimp handlers ("dealer") in the Gulf and South Atlantic States willing to pay only ex-vessel prices.

The mail survey results are generally consistent with recent observations on the marketing of cultured marine shrimp in South Carolina. For example in 1990, the major buyer of S.C. cultured shrimp was a Gulf wholesaler purchasing fresh, heads-on, mainly 16-30 count shrimp (heads-on) in 20,000 to 40,000 pound quantities.

Conjoint Analysis

Most of the firms interviewed (90%) for the conjoint analysis (of heads-on shrimp product attributes) functioned as wholesalers and/or distributors. As might be expected, purchase price was the most "important" attribute in the preference rating followed by product form (Fig. 4). IQF shrimp had the highest average utility value as a level in the product form attribute.

Seven shrimp products (heads-on) which might be marketed by S.C. shrimp farmers were selected (Table 8) for buyer preference simulation. Using a share of preference model with an adjustment for product similarity (5), a preliminary conjoint analysis simulation assigned the two highest preference rankings (percentage) by buyers to 25-27 count shrimp as fresh or IQF products selling for $2.50 and $2.80 per pound (F.O.B., South Carolina), respectively (Table 8). The average likelihood of purchase for these selected products exceeded 60% (Table 8). The conjoint analysis results are considered inconclusive due to the lack of statistical differences between preference and purchase likelihood percentages for these products. Regardless, the conjoint analysis results suggest that there might be a limited latent demand for IQF, 22 to 30 count (heads-on) marine shrimp in the U.S. wholesale sector. Consequently, South Carolina

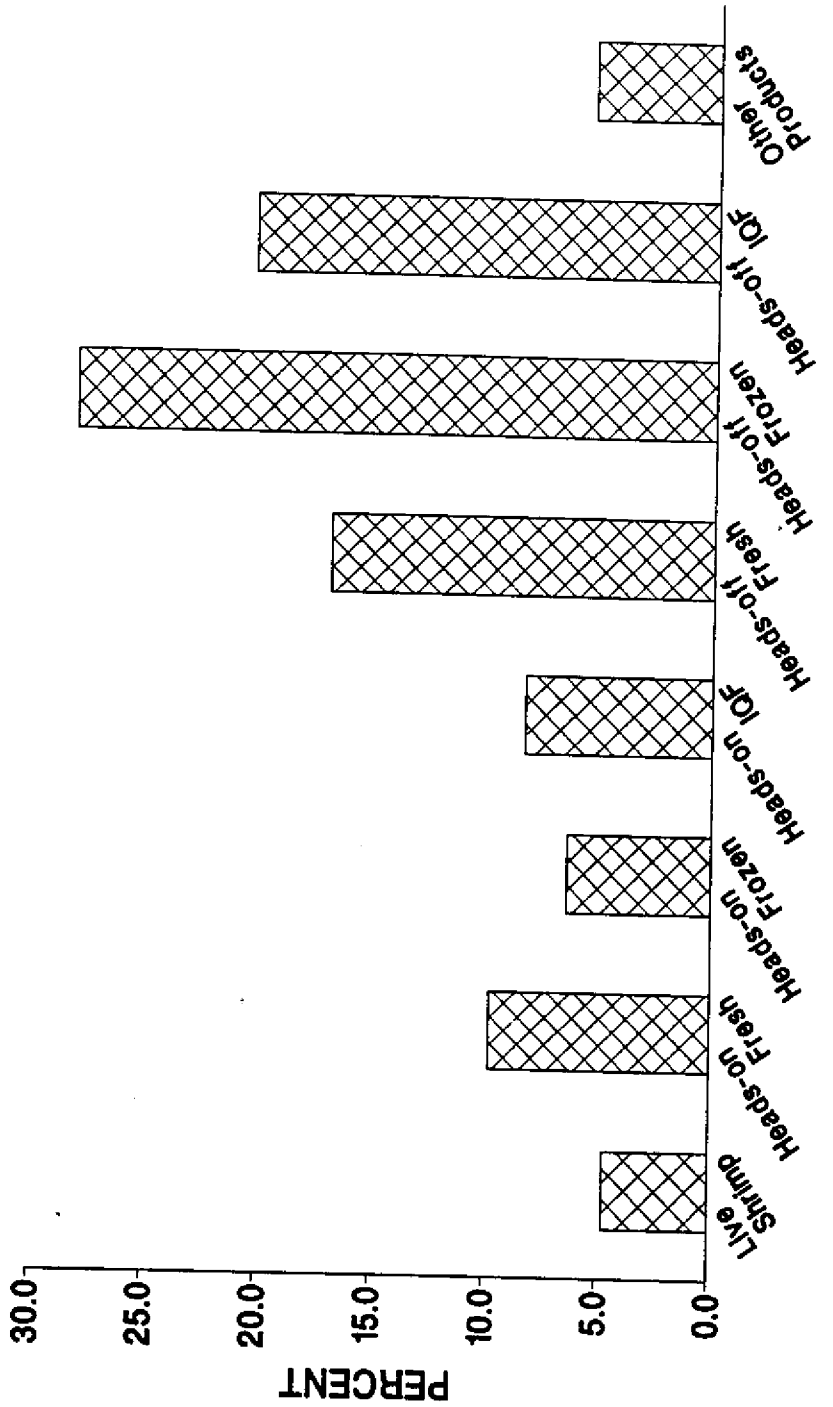


Fig. 2. U.S. Farmed Marine Shrimp Products Desired by Respondents, 1989.

Table 6. Count Size of Preferences for Selected Heads-on Marine Shrimp Products, 1989.

If you are interested in white shrimp culture in the U.S., indicate desired counts? Business Classification	U-15	16-30	31-50	>50	All Major Counts Want	Total Responses[4]
Heads-on, IQF:						
W/D[1]	9 (19.6%)	14 (30.4%)	9 (19.6%)	2 (4.3%)	12 (26.1%)	46 (15.5%)
Processor	0 (0.0%)	1 (10.0%)	2 (20.0%)	0 (0.0%)	7 (70.0%)	10 (3.4%)
Retailer	2 (25.0%)	1 (12.5%)	1 (12.5%)	0 (0.0%)	4 (50.0%)	8 (2.7%)
"Other"[2]	3 (11.5%)	4 (15.4%)	3 (11.5%)	0 (0.0%)	16 (61.5%)	26 (8.7%)
Total	14 (15.6%)	20 (22.2%)	15 (16.7%)	2 (2.2%)	39 (43.3%)	90 (30.3%)
Heads-on, Fresh:						
W/D[1]	18 (26.1%)	23 (33.3%)	11 (15.9%)	2 (2.9%)	15 (21.7%)	69 (23.2%)
Processor	2 (16.7%)	3 (25.0%)	0 (0.0%)	0 (0.0%)	7 (58.3%)	12 (4.0%)
Retailer	3 (23.1%)	5 (38.5%)	2 (15.4%)	0 (0.0%)	3 (23.1%)	13 (4.4%)
"Other"[2]	5 (16.7%)	10 (33.3%)	4 (13.3%)	0 (0.0%)	11 (36.7%)	30 (10.1%)
Total	28 (22.6%)	41 (33.1%)	17 (13.7%)	2 (1.6%)	36 (29.0%)	124 (41.8%)
Heads-on, Frozen:						
W/D[1]	7 (18.4%)	11 (28.9%)	8 (21.1%)	3 (7.9%)	9 (23.7%)	38 (12.8%)
Processor	1 (10.0%)	1 (10.0%)	1 (10.0%)	0 (0.0%)	7 (70.0%)	10 (3.4%)
Retailer	1 (33.3%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	1 (33.3%)	3 (1.0%)
"Other"[2]	2 (6.3%)	10 (31.3%)	6 (18.8%)	0 (0.0%)	14 (43.8%)	32 (10.8%)
Total	11 (13.3%)	23 (27.7%)	15 (18.1%)	3 (3.6%)	31 (46.2%)	83 (27.9%)
Grand Total[3]	53 (17.8%)	84 (28.3%)	47 (15.8%)	7 (2.4%)	106 (35.7%)	297 (100.0%)

[1]Wholesaler/Distributor
[2]Brokers, Exporters, Importers, Traders and Others
[3]Combined totals from Heads-on IQF, fresh and frozen
[4]Column percentage based on "Grand Total" for this column, "Total Responses".
Note: There were multiple responses to these questions.

Table 7. Desired Monthly Quantities of Selected U.S. Cultured Heads-on White Shrimp Products by Region, 1989.

If interested in heads-on shrimp... quantities desired?	Fresh	Frozen	IQF
Northeast:			
Number	6	1	1
Percent[1]	35.4%	3.1%	61.5%
Mean	958	500	10,000
Total	5,750	500	10,000
Midwest:			
Number	5	5	3
Percent[1]	33.6%	48.9%	17.6%
Mean	880	1,280	767
Total	4,400	6,400	2,300
South:			
Number	20	8	19
Percent[1]	43.8%	13.3%	42.8%
Mean	43,970	33,437	45,200
Total	879,400	267,500	858,800
West:			
Number	15	12	9
Percent[1]	25.3%	48.8%	25.9%
Mean	6,843	16,467	11,667
Total	102,650	197,600	105,000
Grand Total[2]			
Number	46	26	32
Percent[3]	40.7%	19.3%	40.0%
Mean	21,569	18,154	30,503
Total	992,200	472,000	976,100

[1]Percentages of desired quantities of heads-on white shrimp products in each region.
[2]Combined totals from the Northeast, Midwest, South and West regions.
[3]Percentages of desired quantities of heads-on white shrimp products from the "Grand Total"

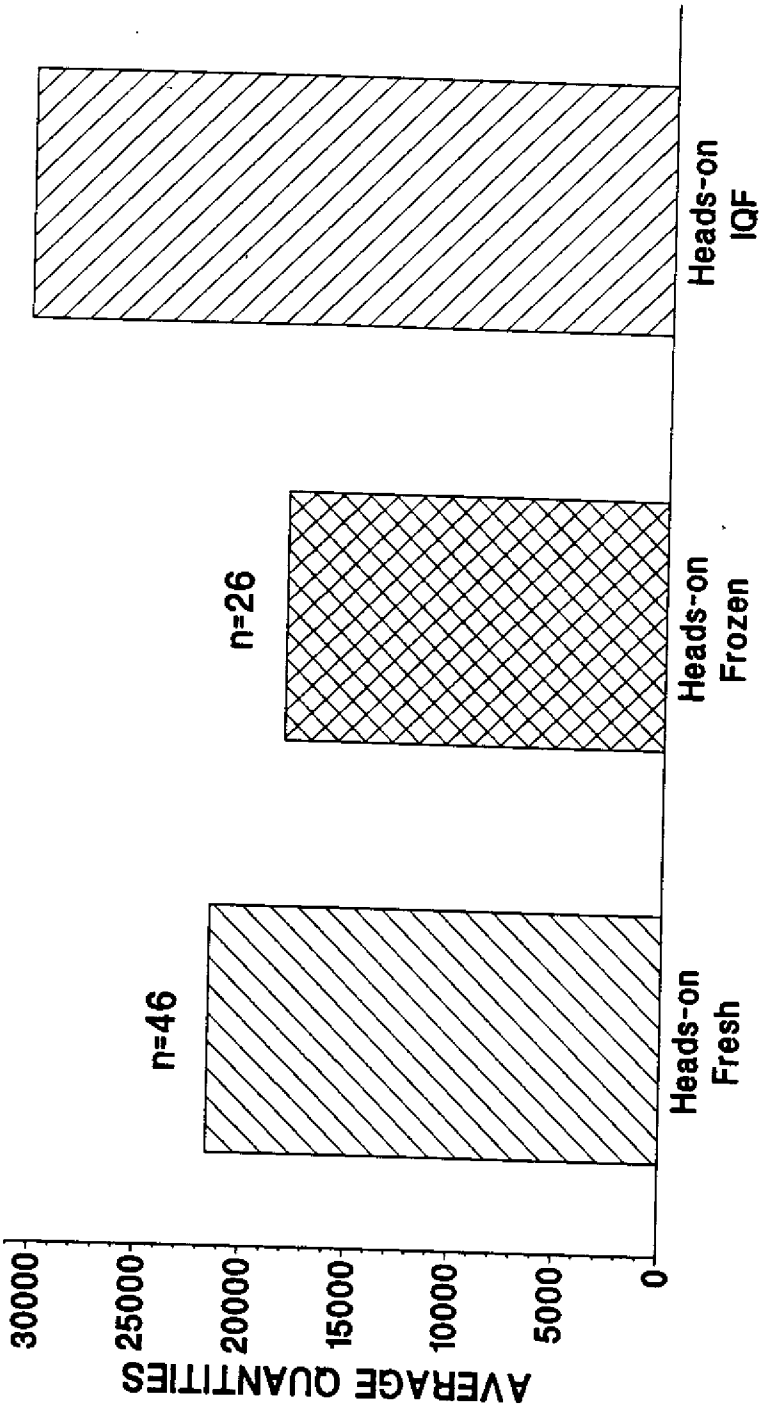
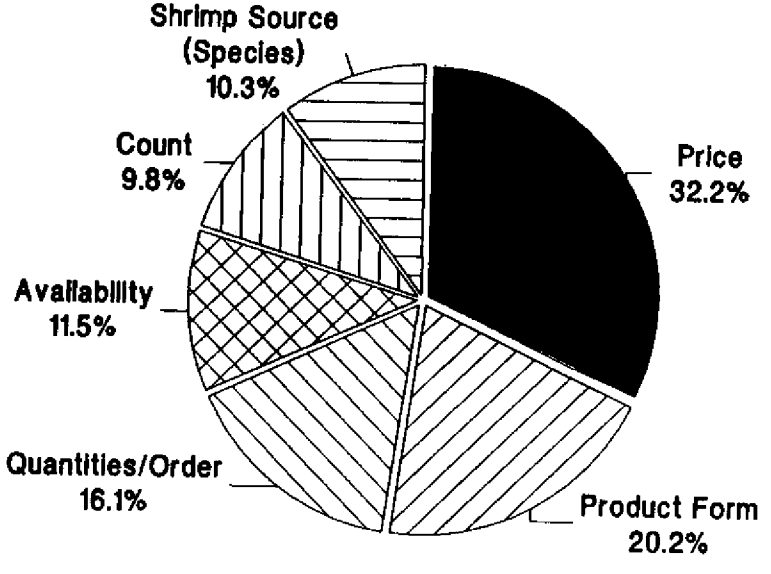


Fig. 3. Reported Monthly Quantities (pounds) of U.S. Farmed Shrimp Desired by Respondents, 1989

Fig. 4. U.S. Wholesaler Telephone Survey



n=20 Respondents
Relative Importance of Whole Shrimp Products Attributes

marine shrimp farmers may want to consider test marketing of IQF heads-on marine shrimp.

Table 8. Preferences and Purchase Likelihood Percentages, for Selected Whole (Heads-on) Cultured Marine Shrimp (N=20 Respondents), 1990.

Count	Form	Availability	Price	Share of Preference[1]	Likelihood of Purchase
28-30	Fresh	Summer	$2.20	12.4%	65.1%
28-30	Fresh	Fall	$2.20	11.8%	66.5%
25-27	Fresh	Fall	$2.50	16.4%	68.1%
28-30	Frozen	All Year	$2.50	14.5%	65.7%
25-27	Frozen	All Year	$2.80	11.6%	66.2%
28-30	IQF	All Year	$2.50	15.9%	73.6%
25-27	IQF	All Year	$2.80	17.5%	72.5%

1 Based upon a share of preference model adjusted for product similarity.

Note: Purchase quantities were held constant at less than 5,000 pounds per order.

CONCLUSION

Although heads-off marine shrimp is one of the preferred product forms in the U.S, there appears to be some willingness in the wholesale seafood sector to purchase various heads-on products. Due to low response rates, the authors are reluctant to estimate magnitude of whole shrimp usage at various levels in the wholesale sector. The conjoint analysis results suggest that product form, not size, may be the next important attribute for heads-on shrimp in the 22-30 count range after price.

Marketing experience by S.C. shrimp farmers (6) is consistent with the apparent latent demand for heads-on marine shrimp identified in this research. Additional research on the various heads-on market segments in the U.S. is needed including shell-on IQF shrimp.

ACKNOWLEDGEMENTS

This research was partially funded through the Cooperative State Research Service, United States Department of Agriculture. Mention of product names does not imply endorsement by the State of South Carolina or the Cooperative State Research Service, U.S. Department of Agriculture.

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MUTAGENICITY STUDIES OF KOJIC ACID

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INTRODUCTION

Kojic acid (5-hydroxy-2-hydroxymethyl-*r*-pyrone), an antibacterial and antifungal metabolite (13) produced by many species of *Aspergillus* and *Penicillium* (14), is believed to be widely present in Japanese fermented foodstuffs because many fungal strains isolated from these foodstuffs were shown to produce this compound (8). Kojic acid inhibits mushroom tyrosinase (15), and the polyphenoloxidase (PPO) purified from potato, Florida spiny lobster, white shrimp, and grass prawn (J.S. Chen, personal communication). A Japanese product containing kojic acid, ascorbic acid, and citric acid is used as an inhibitor for tyrosinase in foods.

The use of kojic acid as a food additive to prevent enzymatic browing should not ignore the need to investigate the safety of this compound. Only limited information is available related to kojic acid toxicity. The compound was reported to have a minimal lethal dose (MLD) of 30 mg in 17-g mice when given intraperitoneally (13). The LD_{100} of the compound for 12-day old chick embryos is 12 mg/egg (10). Dogs receiveing kojic acid intravenously at 150 mg/kg body weight showed signs of intoxication, and died at 1 g/kg (7). Kojic acid was shown to cause DNA breaks and clastogenic effect in cultured rat liver cells (8), and to induce gene mutation in *Salmonella typhimurium* (4, 16, 19). In addition, this compound was shown to induce teratogenic effect in chick embryos (8). However, kojic acid was shown not to induce "Rec effect" with *Bacillus subtilis* (5) and forward mutation in cultured Chinese hamster cells (16). The compound also gave negative results in inducing dominant lethal effect in mice (Shibuya et al., 1982). Auffray and Boutibonnes (3) showed that kojic acid was unable to induce SOS in *Escherichia coli.*

Due to these inconsistent and controversial results on kojic acid toxicity, this study was undertaken to assess the clastogenic activity of the compound in inducing chromosomal aberrations (CAb) in Chinese hamster ovary (CHO) cells. CAb is used routinely to assess the potencies of chemical carcinogens/mutagens (1). In addition, the Ames *Salmonella*/microsome assay was performed to confirm the mutagenic activity of kojic acid using both plate-incorporation and preincubation methods.

MATERIALS AND METHODS

Chinese hamster ovary cells

Chinese hamster ovary cells (CHO-K1) were grown as monolayers in medium 199 (M-199) supplemented with 10% fetal calf serum (FCS) and 1% antibiotic-antimycotic. The cultures were maintained at 37°C in a 5% CO_2, humidified atmosphere. Mycoplasma-free cultures at about 4 x 10^5 cells per T25-cm^2 flask were maintained in 5 ml medium for 24 hr before addition of test compounds.

Cytotoxicity test

Exponentially growing cells in Corning 60 x 15 mm culture dishes were incubated for 24 hr with different concentrations of kojic acid (1.5 -12 mg/ml) in M-199 in order to establish the 50% toxic concentration (TC_{50}) in this system. At the end of the exposure period, cultures were

examined for cytopathic effects (CPE) using a phase contrast microscope (200 x magnification). Following morphological examiantion and removal of the medium, the cells were washed twice with 0.1 M phosphate buffered saline (PBS, pH 7.4) and then hydrolyzed with 0.2 N NaOH. Protein determination was conducted using the method of Lowry et al. (11). After the percentage decrease of total cell protein at each test concentration was determined, the TC_{50} for kojic acid in CHO cells was calculated.

Determination of chromosomal aberrations (CAb)

One day after culture initiation, cells in T25-cm^2 flasks were incubated for 2 hr with kojic acid at 3, 4.5 and 6 mg/ml or controls in the presence or absence of the S9 mix. Following washing with PBS, the cells were incubated for another 22 hr in fresh medium containing 10% FCS; and colchicine (0.1 μg/ml) was added for the last 3 hr of cultivation. The rounding-up cells were harvested by mitotic shake-off (18), then centrifuged and treated for 20 min with a hypotonic solution (75 mM KCl). Cells were then fixed twice for 20 min each with a methanol-glacial acetic acid (3:1, v/v) fixative, dropped onto clean cold slides and air dried. The slides were stained in 4% Giemsa in Gurr buffer and permanently mounted. At least one hundred metaphases per flask were scored for each dose; individual types of aberrations including gaps and endoreduplications were scored.

M-199 was used as the solvent control, and freshly prepared cyclophosphamide (CP) and triethylenemelamine (TEM) as the positive controls in the presence or absence of the S9 mix, respectively. The rat hepatic S9 mix was prepared following the recommendations of Ames et al. (2). For those flasks containing S9 mix, the culture medium was replaced with serum-free medium containing 10% S9 mix (9) 24 hr after culture initiation.

Ames mutagenicity assay

Mutagenicities of kojic acid was determined using the standard plate-incorporation (2, 12) and preincubation assays (20) applying *Salmonella typhimurium* tester strains TA98 and TA100. The assays were performed in the presence or absence of the liver S9 mix prepared from rats pretreated with Aroclor 1254. The plate-incorporation assay was performed by adding 0.1 ml of the overnight cultures with 0.2 ml of aqueous kojic acid solutions and 0.5 ml of S9 mix or phosphate buffer into 3.5 ml of top agar containing small quantities of histidine and biotin. After votexing, the mixture was poured onto a bottom agar plate. For preincubation assay, 0.2 ml of the aqueous kojic acid solution and 0.5 ml of the S9 mix or phosphate buffer were added to test tubes containing 0.1 ml of tester culture. After preincubation at 37°C for 20 min in a water bath, the mixture was mixed with 3.5 ml top agar at 45°C and poured onto bottom agar plates. Concurrent positive and negative controls were included in all assays; 2-aminofluorene (2-AF) was used for both strains in the presence of the S9 mix. Methyl methanesulfonate (MMS) was used for strain TA 100, and 2-nitrofluorene (2-NF) for TA98 when the S9 mix was absent. The plates were incubated at 37°C for two days before the colonies were counted. The background bacterial lawn was examined microscopically to monitor the toxicity of kojic acid: a sparse bacterial lawn with pointed colonies indicated toxic levels of kojic acid (2, 6).

RESULTS AND DISCUSSION

Kojic acid at high doses was toxic to CHO cells; morphological cytopathic effects observed included rounding of cells and loss of nucleolar definition. From the loss of cellular proteins, the TC_{50} level of kojic acid in CHO cell system was determined to be 10.86 ± 3.86 (standard deviation) mg/ml of the culture medium.

Kojic acid showed a dose-related increase in inducing chromosomal aberrations in CHO cells in the presence or absence of the rat S9 mix (Tables 1 and 2). Except for rings, all

Table 1. Chromosomal Aberrations Induced by Kojic Acid in Chinese Hamster Ovary Cells in the Absence of the Hepatic Activation System

Treatment	Dose (μg/ml)	Aberrant cells (%)	Number of aberrations per 100 metaphases[a]						
			Gaps	Breaks	Deletions	Exchanges	Rings	Dicentrics	Total Aberrations
Control	0	29	19	0	3	10	1	3	36
TEM[b]	0.25	56	36	6	7	14	6	11	80
Kojic acid	3000	38	27	4	5	12	2	5	55
	4500	49	29	7	8	20	3	7	74
	6000	63	39	9	16	20	1	10	95

[a]One hundred cells were scored for each duplicate flask.
[b]TEM = Triethylenemelamine.

Table 2. Chromosomal Aberrations Induced by Kojic Acid in Chinese Hamster Ovary Cells in the Presence of the Hepatic Activation System

Treatment	Dose (μg/ml)	Aberrant cells (%)	Number of aberrations per 100 metaphases[a]						
			Gaps	Breaks	Deletions	Exchanges	Rings	Dicentrics	Total Aberrations
Control	0	28	8	4	2	7	3	11	35
CP[b]	5	58	44	9	6	11	11	9	90
Kojic acid	3000	50	32	10	5	8	8	3	66
	4500	59	40	4	3	12	13	9	81
	6000	67	48	13	5	19	17	7	109

[a]One hundred cells were scored for each duplicate flask.
[b]CP = Cyclophosphamide.

categories of CAb increased with increased doses of kojic acid in the absence of the S9 mix (Table 1). Kinosita et al. (8) found asymmetric mitosis, and chromosome stickiness and breaks in cultured rat liver cells after treatment with kojic acid.

Kojic acid was mutagenic to strains TA98 and TA100 in the presence and absence of the S9 mix (Table 3). The compound showed a higher mutagenic activity in the presence of the S9 mix than in the absence. When the S9 mix was not added, kojic acid was more toxic to strain TA98 than to TA100. The results of this study were in agreement with those of Shibuya et al. (16) who also showed that kojic acid was mutagenic to strains TA98 and TA100. Kojic acid was shown by Wehner et al. (19) to be mutagenic only to strain TA98 in the presence of the S9 mix, and by Bjeldanes and Chew (4) to be mutagenic only to TA100.

Studies with preincubation assay further confirmed that kojic acid was mutagenic to strains TA98 and TA100 in the presence or absence of the liver S9 mix (Table 3). The preincubation assay is more sensitive than plate-incorporation assay in detecting the mutagenic activity of many compounds.

CONCLUSION

Kojic acid is capable of inducing CAb in cultured CHO cells and shows mutagenic activity in *S. typhimurium*. Based on the results of this study and literature reports on the potential toxicity of this compound, it becomes evident that kojic acid could not be approved for use as a food additive at this time. Further studies should be conducted to elucidate the mutagenic and clastogenic activity and the potential carcinogenic activity of this compound in order to verify its safe use as a food additive.

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Table 3. Results of Mutagenicity Tests of Kojic Acid Using *Salmonella typhimurium* Strains TA98 and TA100[a]

		Plate-incorporation assay				Preincubation assay			
		TA98		TA100		TA98		TA100	
Sample	Dose/plate	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Bacteria		26± 6	20± 2	122±23	128±10	29± 5	23± 3	131± 9	138± 4
Bacteria + DMSO	25 μL	25± 5	23± 5	113±11	117± 8	25± 3	23± 3	133±13	143±11
Kojic acid	100 μg	70± 9	NT[b]	NT	NT	70±10	NT	NT	NT
	250 μg	94±15	NT	NT	NT	105±19	NT	NT	NT
	500 μg	89±26	26± 3	162±20	155±20	47± 8	27± 4	195±20	156±10
	750 μg	64±27	NT	NT	NT	T[c]	NT	NT	NT
	1 mg	T	29± 5	298±24	212±13	T	36± 4	296±60	201±28
	2 mg	T	48± 9	412±85	323±18	T	70±11	379±33	442±36
	4 mg	T	94±13	584±60	552±55	T	136±18	330±23	795±94
	6 mg	T	159±15	478±24	1045±214	T	184±20	T	981±87

[a]Values are mean numbers of revertants/plate ± standard deviations of duplicate runs, each with 4 plates/dose.
[b]NT, not tested.
[c]T, toxic.

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COMPARISON OF KOJIC ACID PRODUCTION IN THREE CULTURE MEDIA

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INTRODUCTION

Kojic acid (5-hydroxy-2-hydroxymethyl-*r*-pyrone), a fungal metabolite produced by many species of *Aspergillus* and *Penicillium* (6), has recently been shown to inhibit polyphenoloxidase (PPO) activity of mushroom (7), potato, apple, and crustaceans including white shrimp, grass prawn, and Florida spiny lobster (J.S. Chen, personal communication). A Japanese product containing kojic acid, ascorbic acid, and citric acid has been used as an inhibitor for tyrosinase in foods. In addition, kojic acid is widely produced by many fungal strains isolated from fermented Japanese foodstuffs (5). Since kojic acid may have a potential application in preventing browning, it is important to explore the cultivation methods to produce kojic acid in large quantities. Thus the objective of this study was to compare kojic acid production by *Aspergillus candidus* in three culture media. The effect of agitation on kojic acid production was also compared with stationary culture.

MATERIALS AND METHODS

Culture medium

A modified Czapek-Dox liquid medium (2) containing 0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, 0.1% yeast extract, and 10% glucose was designated as medium A in this study. The medium of Tadera et al. (8) consisting of 5% glucose, 0.6% peptone, 0.1% KH_2PO_4, 0.1% $MgSO_4 \cdot 7H_2O$, 0.001% $CaCl_2$ and 0.001% $FeCl_3$ was designated as medium B. Yeast extract/sucrose medium (4) containing 2% yeast extract and 20% sucrose was medium YES.

Effect of shaking on kojic acid production

One hundred 50-ml Erlenmeyer flasks containing 15 ml of medium A were used to evaluate agitation on time-related changes in kojic acid production, medium pH, and fungal cell mass (dry weight). The flasks were wrapped with Perfection Dubl-Chem-Faced milk filters and Whatman filter paper #4. After the medium was autoclaved at 121°C for 15 min, 0.1 ml of *Aspergillus candidus* ATCC 44054 spore suspension (1.4×10^7 spores/ml) was inoculated into each flask. Fifty flasks were incubated without agitation
in the dark at room temperature (25 ± 2°C) and the other fifty at 25°C in a water bath shaker at a shaking speed of 100 rpm.

Three flasks from each group were removed every three days. The flask contents were filtered through a 0.45-μm filter. Following pH measurement of the filtrates, the mycelial mass was scraped onto a preweighed aluminum pan, dried in an oven for 2 d at 80°C and then reweighed to determine dry weight fungal mass. The entire experiment was repeated once.

Kojic acid production in three liquid culture media under stationary conditions

Fifty 125-ml Erlenmeyer flasks containing 25 ml liquid medium (A, B, or YES) were used to determine time-related changes in kojic acid production, medium pH, and fungal cell mass. The flasks were prepared and the media inoculated with the spore suspension as previously described. Following stationary incubation of the cultures at room temperature (25 ± 2°C) in the dark, three flasks were removed every three days and samples handled as previously described. The experiment was performed twice.

Quantitation of kojic acid in culture media

Kojic acid production in culture medium was estimated by reacting the properly diluted samples with 1% ferric chloride in 0.1 N HCl (2). The absorbance at 505 nm was read in a Beckman DU 40 spectrophotometer and compared with a kojic acid standard curve prepared using the respective medium.

Detection of aflatoxin

At each sampling interval, 5 ml aliquot of the media was extracted three times with an equal volume of chloroform-methanol (9:1, v/v) in a separatory funnel. After the solvent extracts were pooled and the solvent removed by rotary evaporation, the residue was dissolved in 500 μl benzene-acetonitrile (98:2, v/v). Samples were spotted along with aflatoxin B_1 standard on analytical thin-layer chromatographic plates and developed with a chloroform-acetone-isopropanol (CAI; 85:15:2.5, v/v/v) solvent system. The plates were examined for aflatoxin fluorescence under long-wavelength ultraviolet light in a Chromato-Vue Cabinet.

RESULTS AND DISCUSSION

The only approach to accurately quantitate kojic acid in medium A, B, or YES applying the method of Bentley (2) was to use the dose-related absorbance standard curve, prepared by dissolving kojic acid in the respective medium at 0.05 to 0.75 mg/ml. The use of the dose-related absorbance standard curve derived from aqueous kojic acid solutions failed to provide accurate quantitation for spiked kojic acid in various medium systems (data not shown).

Agitation of *A. candidus* during cultivation did not enhance kojic acid production; stationary cultures produced more kojic acid than those shaken at 100 rpm (Fig. 1). Maximum production of kojic acid occurred after 12-15 days of cultivation for stationary cultures, and after 18-24 days for those agitated. More mycelial mass (at least 2-fold more) was produced by stationary cultures than shaken. As with the other fungal secondary metabolites, most production of kojic acid occurred after the accelerated increase in fungal mass (Fig. 1).

A comparison of the three media A, B, and YES revealed that medium YES was superior in supporting kojic acid production by *A. candidus* (Fig. 2). Maximal production at approximately 60 mg/ml occurred on day 9-12 of cultivation in YES, which was about three times more than in medium B. Medium YES has been reported to be the most favorable for kojic acid production; Bajpai et al. (1) showed that kojic acid at 81.5 mg/ml was produced by *A. flavus* in this medium. Maximal kojic acid production at about 40 mg/ml occurred on day 12-15 in medium A. Medium A was better than medium B in supporting kojic acid production by *A. candidus*.

Kojic acid disappeared almost completely from YES and medium B after 27 days of cultivation. Although kojic acid was still present in medium A at this time, the compound disappeared eventually on more prolonged cultivation (2). The old fungal mycelia may play a role in causing the disappearance of kojic acid in these media.

The mycelial mass collected from YES on day 12 or 15 weighed about three times more than those from medium A or B (Fig. 2). All three media had a decrease in pH values following fungal growth. As time proceeded, the pH increased above initial values (Fig. 2).

Unlike many strains of *Aspergillus flavus* and *A. parasiticus* which produce aflatoxin, *A. candidus* did not produce aflatoxin in any of the three media throughout the entire cultivation period. The chloroform-methanol extracts of the culture media failed to shown any fluorescence under UV light; TLC analysis of the solvent extracts on silica plates also failed to show any fluorescent spots corresponding to aflatoxin standard.

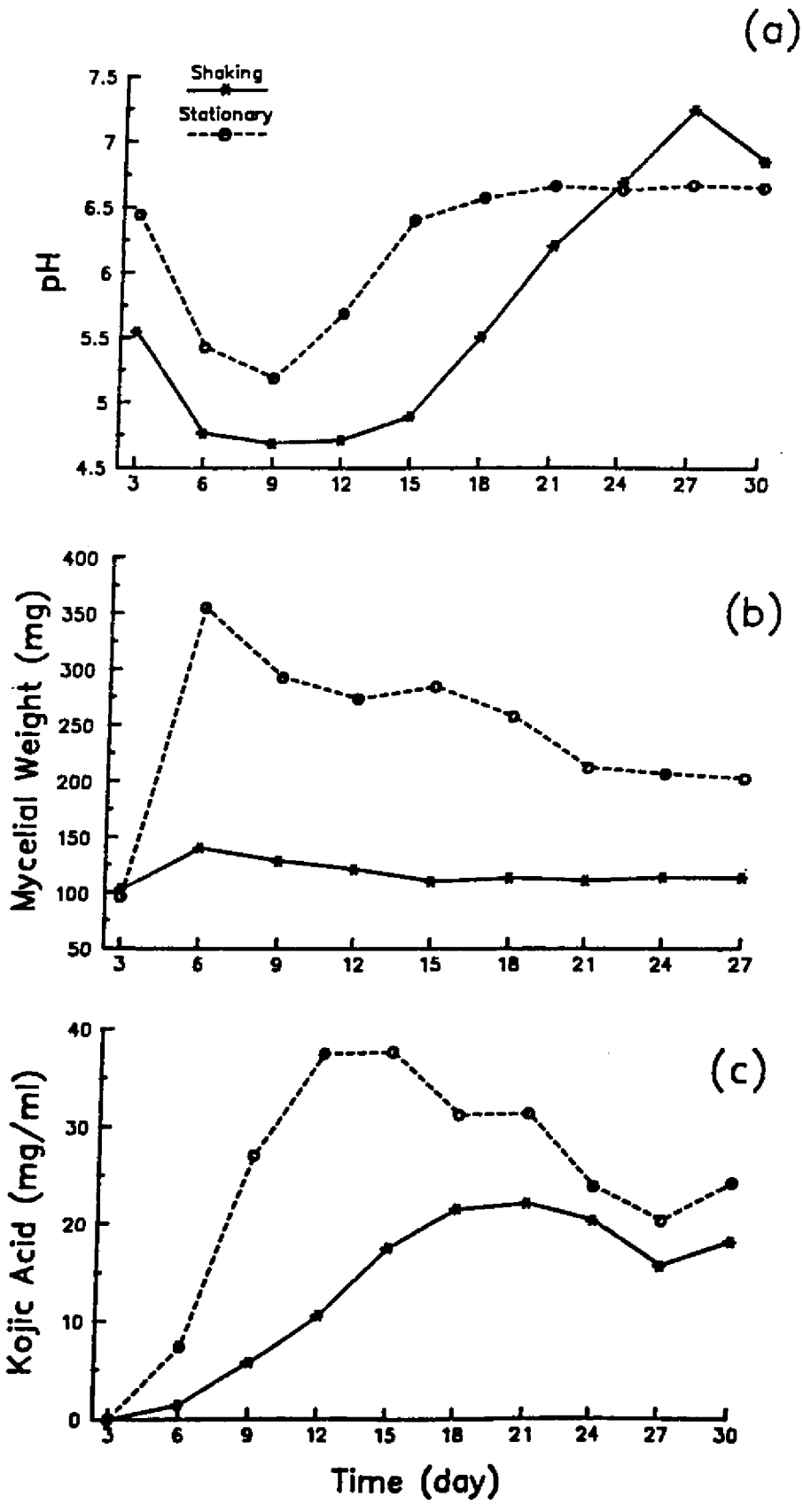

Figure 1. A comparison of shaking versus stationary culture condition on time-related changes of (a) medium pH, (b) mycelial weight and (c) kojic acid production by *Aspergillus candidus* grown in medium A.

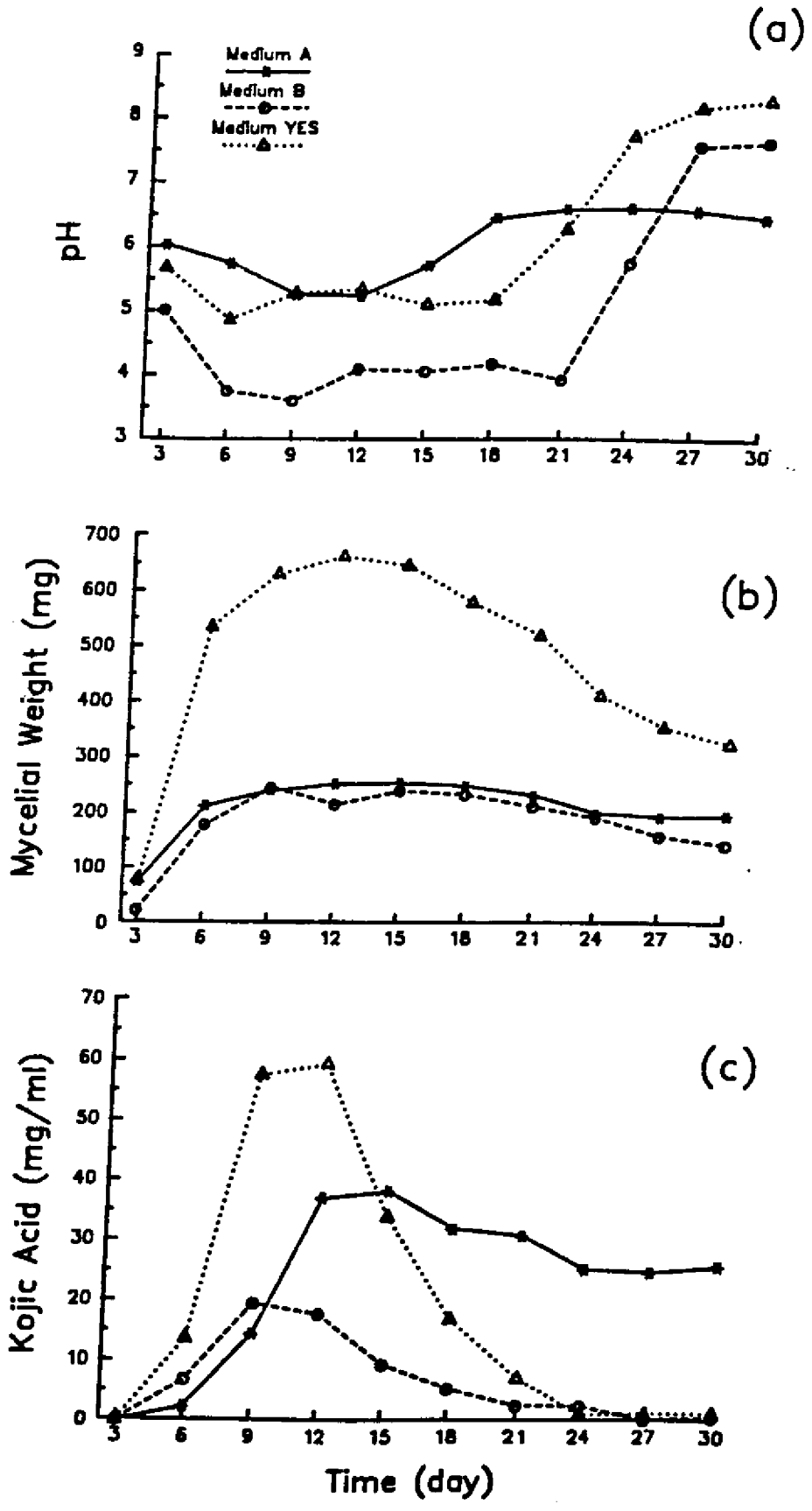

Figure 2. Time-related changes of (a) medium pH, (b) mycelial weight and (c) kojic acid production due to *Aspergillus candidus* growth in media A, B, and YES. The cultures were maintained at stationary position throughout the cultivation period.

CONCLUSION

YES is a suitable medium for kojic acid production by *A. candidus*. Since kojic acid production can be affected by several factors, it is worthwhile to explore such possibilities to enhance its production. Tadera et al. (8) demonstrated that the addition of cycasin in culture medium increased kojic acid production by *A. oryzae*, *A. flavus* or *A. tamarii*. Formation of kojic acid occurs most readily in highly acidic substrates (approximately pH 2-3) containing about 10% glucose or xylose and high levels of phosphate, but lacking nitrogen (3). Therefore modification of the YES medium following these principles may enhance kojic acid production by *A. candidus*.

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A TECHNOLOGY ANALYSIS OF U.S. ATLANTIC BLUE CRAB (Callinectes sapidus) PROCESSING INDUSTRY: PART I DEVELOPMENT OF CRABMEAT YIELD COMPUTER PROGRAM

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INTRODUCTION

Although blue crabs (Callinectes sapidus) are a warm water crab with wide geographical disbtribution, the United Stes catches over 95 percent of the world's total production of blue crabs (3). The technology used in processing blue crabs has changed little since fresh crabmeat was first marketed in the late 1800s. Currently, a substantial amount of the knowledge on blue crab processing is primarily based upon the experience of plant owners and managers and the limited research information produced by trade associations and academic institutions.

The major concerns and problems in the processing of crabmeat are attainments of maximum yield and production of high microbial quality products. Especially, meat yield is one of the major factors affecting profitability of a crab processing firm (14). However very little information is available about the effect of traditional and alternative processing operations on the yield of blue crabs and the effect of processing operations on the yield of blue crabs and the effect of traditional and alternative processing operations on the yield of blue crabs and the effet of processing variables on microbiological quality. Ulmer (13) and Tinker and Learson (11) found that some natural and processing variables and biological factors have a distinct influence on yield although their data were inadequate to show that any definite yield pattern was produced by these variables. Furthermore, many important technological factors were not considered in their research such as the number of pickers and

replications, picker's variation on yield, the type (season, size, physiological state) and sex of crabs used. Also most of their studies were performed in laboratory or pilot plant scale which showed different results from those of commercial scale operations. Therefore, their results and findings could not give a significant recommendation on methods that would improve crab processing procedures.

Ulmer (13) concluded that steaming crabs at 121°C (250°F) for 10 minutes produced higher yields than did pressure steaming for either longer or shorter periods and steam boiling crabs at 100°C (212°F) in tap water for 10 to 15 minutes produced a higher yield than did pressure steaming at 121°C for 10 minutes. However, in contrast to the conclusion of Ulmer (13), Tinkers and Learson (11) believed crabs cooked for 8 minutes in steam at 100°C resulted in a greater meat yield than a 10 minute steam cook at 121°C.

Dressel et al. (3) noted several points concerning the blue crab yields in their review study. Processing yields vary within fullness of the crab and picking technique employed. Fat cabs, those which have not molted recently and are full of meat, yield approximately 14 percent (live crab weight basis).

The main objectives of this study were to determine the effect of processing variables (cooking time and methods, storage time, picking methods) and biological variables (season, crab sex, size, and sexual maturity) on the total and differential (lump, top flake, bottom flake, and claw) meat yield and to develope a computer model which can predict and calculate the final meat yield before the process begins in any combination of biological and processing variables. Picker variations (between pickers and trials of each picker) in total and differential meat yields were also examined in this study.

MATERIALS AND METHODS

Raw Materials

Blue crabs (Callinectes sapidus) used in this research were harvested by potting in summer and dredging in winter during 1988, 1989, and 1990. The crabs were harvested in the lower Chesapeake Bay and its estuaries and landed in the Hampton, Virginia area.

Blue Crab Processing

All the blue crab unit operational and yield studies were performed during both the summer and winter in a commercial crab processing plant located in Hampton, VA. Live crabs (less than six hours after harvesting) from boats or trucks were washed in a reel washer to remove soil, seaweed, and other extraneous material on the crab shell. The crabs were then sorted by sex, type (sexual maturity; adhering egg mass), and/or size (less than 13 cm and larger than 15 cm) and weighed in a metal retort basket (rim) (91 cm, diameter X 48 cm, height) before each cooking so that the yield could be obtained on the basis of live crab weight.

After weighing, the crabs were cooked in a commercial steam retort for 8, 10, and 12 minutes after the temperature reached

121°C with a corresponding pressure of 15 pounds psig. and in boiling water for 10 minutes after the water returned to a rolling boil. For the retort process, a three basket (rim) process was performed as the normal commercial cooking procedure. About 540 kg (1190 lb) of live crabs were cooked in each batch for the studies. The cooked crabs were allowed to cool initially at ambient temperature under fans for approximately 2 hours and then stored at 3°C in a cooler until picked. The crabs were stored for 24, 48, 72, and 96 hours to determine the effect of low temperature storage on various meat yields.

Hand Picking Process

Prior to the hand picking process, the crabs in the rims were reweighed and placed in several plastic baskets. Each basket contained about 18 kg (live crab weight basis) of crabs (about 100 - 160 crabs). Duplicating baskets were weighed and given to seven professional pickers having 2 - 40 years of picking experience. All the hand picking processes were conducted in the picking room of the commercial crab processing plant. The picked meat was separated into four categories (types): lump; top flake; bottom flake; and claw meat. The picked meat from each basket, picker, and cooking process were separately weighed and packed. The percent meat yield of the hand-picking operation was calculated on the live whole crab weight basis for each basket, picker, crab type, and processing method.

Machine Picking Processes

For the Harris machine process, crab claws were placed into several plastic containers (27 kg capacity) and weighed. The claws were stored at 3°C in a cooler for 24, 48, 72, and 96 hours prior to processing. The claws were reweighed immediately prior to processing and the results recorded on a barrel basis. A nineteen percent (by weight per volume) salt solution (71.7 percent saturation at 15.6 °C) was used to separate the meat from the shell. The picked claw meat was collected from the conveyer belt and again weighed. Excess water was extracted from the meat by a metal squeezer and the product was reweighed. The percent yield of the claws was calculated on the basis of the 0 day cooked claw weight.

For the Quik-Pik process, the crab claws and sponges (egg masses) were removed by hand from the crab bodies prior to the picking process. To determine the effect of initial crab temperature (before the steam tunnel treatment) on meat yield, both hot crabs (average internal temperature of 55°C) and cold crabs (average internal temperature of 18°C) were machine picked. Twenty-four crab bodies were weighed and picked in each batch (24 crabs per tray). The temperature of the steam tunnel was set to 90.6°C. Air pressure and motor rpm. of the Quik-Pik machine was adjusted to 680.4 g and 3,525, respectively. Tray clearance and shaking time was set to 1.59 mm and 4 - 5 seconds. Finally, the meat produced by the machine was recovered from the conveyor belt and weighed. The percent meat yield was calculated on a whole

live crab weight basis and live crab body (without claws) weight basis.

Development of a Computer Program, VTBCP

Linear equations which were the correlations of cooking time (between 8 and 12 minutes) and crabmeat yield were developed using the SAS Linear Regression Analysis Program (9). Separate equations were obtained for: each type of crabmeat (lump, top flake, bottom flake, total flake, claw, and total meat yield); each season; and for different sexes and physiological states (male, clean female, and sponge female). Based on the equations and other yield data obtained from the study, a computer program, Virginia Tech Blue Crab Program (VTBCP), was developed using the Turbo Pascal Programming Language (version 4.0). The program VTBCP was designed to predict the final meat yield based on factors as total weight (up to 1,000,000 pounds), size, and sex of the live crab. The computer program was also developed to include all the natural and processing variables (season, cooking methods and time, storage time before and after the cook, and picking methods) in the calculation of final meat yield.

RESULTS AND DISCUSSION

Blue Crab Yield

1. Hand Picking Process

The total and differential meat (lump, top flake, bottom flake, and claw) yield of crabs by the hand picking process were determined during winter and summer and are presented in Tables 1 and 2. The percent meat yield in the tables was the average of 14 replications from 7 professional pickers and calculated on the live whole crab weight basis.

The results show that cooking time, crab sex and type, and season affect total and partial meat yield significantly From the results, it is also noted that season is an important variable affecting meat yield.

Picker variations (between pickers and trials of each picker) in total and differential meat yields were determined for the first time in this study. During summer, picker variations in total meat yield was 22.1 ± 3.2 % (12.1 ± 2.4 % during winter) between pickers and 3.7 ± 1.6 (2.3 ± 1.4 during winter) between pickers' trials. Depending on the pickers, the differential meat yields varied significantly. Although two pickers picked the same amount of total meat, one picker produced more lump, the most expensive product, and less flake meat than the other. This finding can affect the current wage system in which pickers are paid by the total meat (pound) they produce regardless of meat type.

The yield of top flake meat was almost the same, or even higher, than that of bottom flake meat regardless of processing variables. Considering the fact that many pickers normally discard the top part of the crab core because it has been believed that not much meat is contained in the top, this finding can be used to develope a new processing policy in the crab processing

Table 1. Percent meat yield[ab] and standard deviaton of winter pressure cooked[c] crabs by hand-picking process. (18 hrs. after cook)

Crab Sex & Type	Cook Time (min)	Lump	Top Flake	Bottom Flake	Claw	Total Yield
clean female	8	4.8 +0.49	3.4 ±0.58	4.3 ±0.52	3.0 ±0.41	15.5 ±1.19
clean female	10	5.0 ±0.39	3.4 ±0.48	4.0 ±0.54	2.6 ±0.39	15.0 ±0.89
clean female	12	4.7 ±0.32	3.2 ±0.45	3.7 ±0.43	2.4 ±0.40	14.0 ±0.74
male	8	6.3 ±0.58	4.7 ±0.50	3.3 ±0.43	4.0 ±0.47	18.3 ±1.07
male	10	6.3 ±0.51	4.3 ±0.38	3.3 ±0.42	4.0 ±0.39	17.9 ±0.99
male	12	5.9 ±0.44	4.2 ±0.32	3.2 ±0.40	4.0 ±0.29	17.3 ±1.01

[a] uncooked (live) crab wt. basis
[b] Each meat yield is the average of 14 observation from 7 pickers.
[c] cooked at 250 F (121 C), 15 psi.

industry.

Effect of Crab Sex and Type on Meat Yield

Tables 3 showes the effect of crab sex and type on total and partial meat yield during summer and winter. During summer, male crabs yielded significantly ($p \leq 0.05$) more lump, claw, and total meat than female crabs. Clean female crabs yielded more lump, top flake, and total meat (13.4±1.27 %) than sponge female crabs (total yield, 11.9±1.32) when retort cooked for 10 minutes. However, the yield of bottom flake meat was not affected by crab sex and type during the summer season.

All types of meat (lump, top flake, bottom flake, claw, and total meat) were produced in a significantly greater ($p \leq 0.05$) quantity from male crabs (total yield 17.9±0.99) than female crabs (total yield, 15.0±0.89) during winter after 10 minutes of retort cook. Male crabs yielded about 35 - 42 percent more claw meat than female crabs during both seasons.

It was found that male crabs always yielded more top flake meat than bottom flake meat regardless of season variable. During the summer season, clean female crabs also produced more top flake

Table 2. Percent meat yield[ab] and standard deviation of summer pressure cooked[c] crabs by hand-picking process. (18 hrs. after cook)

Crab Sex & Type	Cook Time (min)	Lump	Top Flake	Bottom Flake	Claw	Total Yield
sponge female	8	4.1 ±0.32	2.9 ±1.00	3.6 ±0.92	2.5 ±0.47	13.1 ±1.32
sponge female	10	3.9 ±0.31	2.8 ±0.81	2.9 ±0.80	2.3 ±0.42	11.9 ±1.32
sponge female	12	3.7 ±0.21	2.7 ±0.77	2.7 ±0.73	2.2 ±0.19	11.3 ±1.24
clean female	8	4.5 ±0.58	3.8 ±0.96	3.0 ±0.48	3.0 ±0.42	14.3 ±1.05
clean female	10	4.4 ±0.47	3.6 ±0.77	2.9 ±0.59	2.5 ±0.53	13.4 ±1.27
clean female	12	4.2 ±0.42	3.2 ±0.77	2.6 ±0.44	2.6 ±0.37	12.6 ±1.16
male	8	5.8 ±0.82	4.3 ±0.91	3.2 ±0.61	4.0 ±0.58	17.3 ±1.32
male	10	5.4 ±0.57	4.0 ±0.87	3.0 ±0.46	4.0 ±0.41	16.4 ±0.99
male	12	5.0 ±0.58	3.6 ±0.83	2.7 ±0.44	3.9 ±0.51	15.2 ±1.48

[a] uncooked (live) crab wt. basis
[b] Each meat yield is the average of 14 observation from 7 pickers.
[c] cooked at 250 F (121 C), 15 psi.

Table 3. Effect of crab type and sex on the % meat yield[xyz].

Season	Crab Type and Sex	Lump (%)	Top Flake (%)	Bottom Flake (%)	Claw (%)	Total Yield (%)
Summer[p]	sponge female	3.9 a ±0.31	2.8 d ±0.81	2.9 f ±0.80	2.3 g ±0.42	11.9 i ±1.32
	clean female	4.4 b ±0.47	3.6 e ±0.77	2.9 f ±0.59	2.5 g ±0.53	13.4 j ±1.27
	male	5.4 c ±0.57	4.0 e ±0.87	3.0 f ±0.46	4.0 h ±0.41	16.4 k ±0.99
Winter[q]	clean female	5.0 a ±0.39	3.4 c ±0.48	4.0 e ±0.54	2.6 g ±0.39	15.0 i ±0.89
	male	6.3 b ±0.51	4.3 d ±0.38	3.3 f ±0.42	4.0 h ±0.39	17.9 j ±0.99

[x] uncooked (live) crab wt. basis.
[y] % meat yields followed by same letter within columns in each season are not significantly different ($p \leq 0.05$).
[z] Crabs were steam pressure cooked at 250 F (121 C) for 10 min. and the meat was picked 18 hrs. after the cook.
[p] July, 1989
[q] February, 1989

meat than bottom flake meat while sponge female crabs yielded more bottom flake meat than top. However, during the winter, more flake meat was produced from the bottom part than the top in clean female crabs.

Effect of Crab Size on Meat Yield

Table 4 summarize the effect of crab size on the total and differential meat yield. Crabs which are 6 inches or larger (distance from the end of the carapace to the other end) yielded significantly ($p \leq 0.05$) more total and all differential meats than smaller crabs (size, 5.25 inches or smaller).

This difference may be due to the unique characteristic of crab picking process. Hand picking with a crab knife, makes it extremely difficult to remove meat pieces in the tiny crab chambers of small size crabs.

Effect of Season on Meat Yield

Table 5 summarizes the effect of season on meat yield of crabs for both sexes. Crabs harvested during winter yielded significantly ($p \leq 0.05$) more lump and total meat than those processed during summer for both male and female crabs. The yield

Table 4. Effect of crab size on the % meat yield[xyz].

Crab Size	Lump	Top	Bottom	Claw	Total Yield
	(%)	Flake (%)		(%)	(%)
Large (≥ 6")	5.2 a ±0.36	3.5 c ±0.44	4.0 e ±0.51	2.7 g ±0.36	15.4 i ±0.84
small (≤ 5.25")	4.9 b ±0.38	3.2 d ±0.53	3.8 f ±0.39	2.2 h ±0.44	14.1 j ±0.97

[x] uncooked (live) crab wt. basis.
[y] % meat yields followed by same letter within columns are not significantly different ($p \leq 0.05$).
[z] Crabs were steam pressure cooked at 250 F (121 C) for 10 minutes during winter (Feb., 1989) and the meat was picked 18 hrs. after the cook.

Table 5. Effect of season on the % meat yield[xy] of 10 min. pressure cooked crabs. (18 hrs. after cook)

Sex	Season[z]	Lump	Top	Bottom	Claw	Total Yield
		(%)	Flake (%)		(%)	(%)
male	summer	5.4 a ±0.57	4.0 c ±0.87	3.0 d ±0.46	4.0 f ±0.41	16.4 g ±0.99
	winter	6.3 b ±0.31	4.3 c ±0.81	3.3 e ±0.80	4.0 f ±0.42	17.9 h ±1.32
clean female	summer	4.4 a ±0.47	3.6 c ±0.77	3.9 d ±0.59	2.5 e ±0.53	13.4 f ±1.27
	winter	5.0 b ±0.31	3.4 c ±0.81	4.0 d ±0.80	2.6 e ±0.42	15.0 g ±1.32

[x] Results are the averages of 14 observations from 7 pickers.
[y] % meat yields followed by same letter within columns in each sex are not significantly different ($p \leq 0.05$).
[z] Summer crabs were harvested in July, 1989 and winter crabs in January, 1989.

of crabs harvested during the fall season (early November), just before the semi-hibernation, was also investigated. Crabs harvested in the late fall yielded as much as the winter crabs in total and differential meat.

The effect of season on crab meat yield of crabs may be explained by their different proximate composition (7), especially fat and protein contents, during both seasons and their biological characteristics. During winter, crabs are in semi-hibernating condition. All physical and physiological activities of the crabs are at a minimum and plenty of energy is accumulated in the form of fat and protein during that time. However, during summer, the most energy consuming activities of crabs, mating and shedding, are actively performed. These biological and physiological factors are believed to be a main reason for the yield difference.

Effect of Cooking Method on Meat Yield

The comparisons of retort cook and boil cook with respect to the meat yield of male, clean female, and sponge female crabs are presented in Tables 6, 7 and 8, respectively.

When the male crabs were picked within one hour after the cooking process, the boil cooked crabs yielded significantly ($p \leq 0.05$) more total and differential meats with the exception of the top flake meat, in comparison with the retort cooked crabs. Ulmer (13) found that the meat from the boiled crabs contained more moisture than meat from the steamed crabs. This result can be also explained by the fact that pressure steaming dries the crabmeat during the cooking process.

However, after 24 hours of cold storage, the yields of boiled and retort cooked crabs were not significantly ($p \leq 0.05$) different except those of claw meat. The yield of steamed crabs increased while that of boiled crabs decreased during cold storage for 24 hours. These new findings were not surprising considering the results of the dehydration rate of crabs cooked by both methods. A higher dehydration rate of boiled crabs occurred during first 24 hour storage period. In the case of claw meat, however, the boiled meat is protected from dehydration by the surrounding claw shell. These results should be also interpreted with the effects of cold storage on meat yield which is discussed at the end of the this section.

Clean and sponge female crabs processed by both cooking methods showed very similar results in yield as the male crabs.

Effect of Cooking Time on Meat Yield

The effects of cooking time on the meat yield of male, clean female, and sponge female crabs are shown in Figure 1. From the results, it is noted that male crabs cooked for 8 minutes during summer showed the greatest total yield, 17.3 ± 1.32 percent (live crab weight basis), in comparison with 16.5 ± 0.99 percent for 10 minute cooked crabs and 15.2 ± 1.48 percent for 12 minute cooked crabs. Male crabs cooked for 8 minutes yielded significantly more total meat than those processed for 12 minutes ($p \leq 0.05$) and 10 minutes ($p \leq 0.10$). However, the yield of claw meat yield was not

Table 6. The effect of cooking method[x] on the percent meat yield[yz] of male crabs.

Picking Time (after cook)	Meat Type	Retort Cook (10 min.)	Boil Cook (10 min.)
1 hour after cook	lump (%)	5.5±0.80a	6.3±0.82b
	top flake (%)	3.5±0.70c	3.6±0.78c
	bottom flake (%)	2.6±0.41d	3.0±0.49e
	claw (%)	3.6±0.74f	4.1±0.56g
	total yield (%)	15.2±1.40h	17.0±1.62i
25 hours after cook	lump (%)	5.4±0.53a	5.7±0.59a
	top flake (%)	4.0±0.85b	3.3±0.80c
	bottom flake (%)	2.9±0.46d	3.0±0.43d
	claw (%)	4.0±0.41e	4.4±0.48f
	total yield (%)	16.3±1.00g	16.4±1.52g

[x] Retort cook: The crabs were cooked at 250 F (121 C) with a retort pressure of 15 psig. during summer season.
Boil cook: The crabs were cooked in boiling water at 212 F (100 C) for 10 minutes after the rolling boil of water.

[y] The percentage meat yield was calculated on the live (uncooked) crab weight basis.

[z] % meat yields followed by same letter within rows are not significantly different ($p \leq 0.05$).

Table 7. The effect of cooking method[x] on the percent meat yield[yz] of clean female crabs.

Picking Time (after cook)	Meat Type	Retort Cook (10 min.)	Boil Cook (10 min.)
1 hour after cook	lump (%)	4.4±0.51a	5.4±0.52b
	top flake (%)	3.3±0.81c	3.1±0.84c
	bottom flake (%)	3.0±0.55d	3.3±0.75d
	claw (%)	2.1±0.74e	3.2±0.54f
	total yield (%)	12.8±1.41g	15.0±1.61h
25 hours after cook	lump (%)	4.4±0.46a	4.3±0.56a
	top flake (%)	3.5±0.75b	3.4±0.76b
	bottom flake (%)	3.0±0.61c	3.2±0.69c
	claw (%)	2.5±0.53d	3.5±0.63e
	total yield (%)	13.4±1.29f	14.4±1.59f

[x] Retort cook: The crabs were cooked at 250 F (121 C) with a retort pressure of 15 psig. during summer season.
Boil cook: The crabs were cooked in boiling water at 212 F (100 C) for 10 minutes after the rolling boil of water.

[y] The percentage meat yield was calculated on the live (uncooked) crab weight basis.

[z] % meat yields followed by same letter within rows are not significantly different ($p \leq 0.05$).

Table 8. The effect of cooking method[x] on the percent meat yield[yz] of sponge female crabs.

Picking Time (after cook)	Meat Type	Retort Cook (10 min.)	Boil Cook (10 min.)
1 hour after cook	lump (%)	4.1±0.40a	4.4±0.41b
	top flake (%)	2.6±0.88c	3.5±0.77d
	bottom flake (%)	2.5±0.62e	3.0±0.71f
	claw (%)	1.9±0.44g	2.2±0.64g
	total yield (%)	11.1±1.30h	13.1±1.40i
25 hours after cook	lump (%)	3.9±0.36a	4.0±0.44a
	top flake (%)	2.8±0.84b	3.0±0.83b
	bottom flake (%)	2.8±0.82c	2.8±0.72c
	claw (%)	2.4±0.41d	2.5±0.51d
	total yield (%)	11.9±1.32e	12.3±1.52e

[x] Retort cook: The crabs were cooked at 250 F (121 C) with a retort pressure of 15 psig. during summer season.
Boil cook: The crabs were cooked in boiling water at 212 F (100 C) for 10 minutes after the rolling boil of water.

[y] The percentage meat yield was calculated on the live (uncooked) crab weight basis.

[z] % meat yields followed by same letter within rows are not significantly different ($p \leq 0.05$).

affected by the cooking time significantly dislike those of the other types of meat.

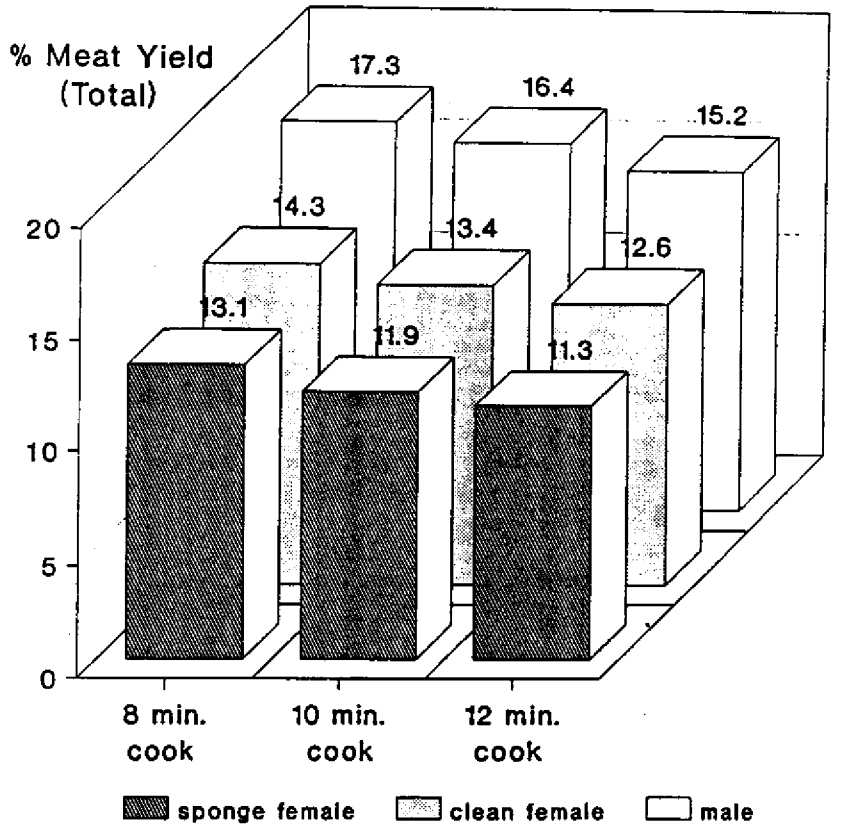
Clean female and sponge female crabs showed the same results as male crabs with respect to the effect of cooking time on meat yield. When clean female crabs were cooked for 8 minutes, they yielded about 14 percent more meat than than crabs cooked for 12 minutes.

From the results, it is obvious that the shorter the cooking time, the greater the crabmeat yielded in the cooking time range of 8 to 12 minutes. However, too short a cook time, such as 5 minutes, caused a lower yield than a normal cooking (8 to 10 minute cook) process.

Our findings are in contrast to the conclusions of Ulmer (13) in which higher yields were obtained after a 10 minute cook than with either a longer or a shorter cooking time. However, his research did not include an 8 or 9 minute cook study as well as an 11 or 12 minute.

The yield loss in a long cook time (12 minutes) process may be the result of moisture loss from the edible tissues. Short cook times cause less drying of the meat and therefore produce greater yield.

Figure 1. Effect of cooking time on the total meat yield of crabs.



Effect of Storage Days on Meat Yield

Tables 9 - 12 present the effect of cold cooked crab storage on the total and differential meat yield. The results from the retort cook study show cooked crabs stored for 24 hours in a cooler yield more total meat in comparison with those which were just cooked or stored for only a few hours without refrigeration (hot or warm crabs). However, storage days in the cooler was not an important factor for the meat yield after the first 24 hours of storage even though about a 3 - 5 percent weight loss was observed in cooked crab during the 4 day cold storage period. The possible reasons for this may be explained as follows: The 3 - 5 percent weight loss of cooked crab is not from the crab meat but mainly

Table 9. The effect of storage days on the % meat yield[ab] of 10-minute retort cooked[c] male crabs.

Storage Time	Lump	Top	Bottom	Claw	Total Yield
	(%)	flake (%)		(%)	(%)
0 day (1 hour after cook)	5.5 ±0.80	3.5 ±0.70	2.6 ±0.41	3.6 ±0.74	15.2 ±1.40
1st day (25 hours)	5.4 ±0.53	4.0 ±0.85	2.9 ±0.46	4.0 ±0.41	16.3 ±1.00
2nd day (49 hours)	5.6 ±0.53	3.9 ±0.78	3.0 ±0.54	4.0 ±0.40	16.5 ±1.28
3rd day (73 hours)	5.6 ±0.62	4.4 ±0.83	2.7 ±0.67	4.0 ±0.38	16.7 ±1.37
4th day (97 hours)	5.0 ±0.70	3.5 ±0.76	2.5 ±0.38	4.2 ±0.52	15.2 ±1.67

[a] The percentage meat yield was calculated on the live (uncooked) crab weight basis.

[b] The percentage yield was the average of 10 observations of 5 different pickers.

[c] The crabs were cooked at 250 F (121 C)with a retort pressure of 15 psig. during the summer season.

Table 10. The effect of storage days on the % meat yield[ab] of 10-minute retort cooked[c] female crabs.

Crab Type	Storage Time	Lump (%)	Top flake (%)	Bottom (%)	Claw (%)	Total Yield (%)
Sponge Female	0 day (1 hr.)	4.1 ±0.40	2.6 ±0.88	2.5 ±0.62	1.9 ±0.44	11.1 ±1.30
	1st day (25 hrs.)	3.9 ±0.36	2.8 ±0.84	2.8 ±0.82	2.4 ±0.41	11.9 ±1.32
	2nd day (49 hrs.)	4.1 ±0.44	2.9 ±0.79	2.9 ±0.72	2.1 ±0.67	12.0 ±1.45
	3rd day (73 hrs.)	3.9 ±0.49	2.4 ±0.81	2.3 ±0.80	2.3 ±0.61	10.9 ±1.51
	4th day (97 hrs.)	3.9 ±0.45	2.8 ±0.77	2.7 ±0.81	2.2 ±0.51	11.6 ±1.42
Clean Female	0 day (1 hr.)	4.4 ±0.51	3.3 ±0.81	3.0 ±0.55	2.1 ±0.44	12.8 ±1.41
	1st day (25 hrs.)	4.4 ±0.46	3.5 ±0.75	3.0 ±0.61	2.5 ±0.53	13.4 ±1.29
	2nd day (49 hrs.)	4.6 ±0.50	3.2 ±0.76	2.9 ±0.43	2.6 ±0.58	13.3 ±1.40
	3rd day (73 hrs.)	4.3 ±0.49	3.4 ±0.88	2.6 ±0.61	2.5 ±0.59	12.8 ±1.45
	4th day (97 hrs.)	4.6 ±0.59	3.0 ±0.79	2.7 ±0.52	2.3 ±0.49	12.6 ±1.58

[a] The percentage meat yield was calculated on the live (uncooked) crab weight basis.

[b] The percentage yield was the average of 10 observations of 5 different pickers.

[c] The crabs were cooked at 250 F (121 C) with a retort pressure of 15 psig. during the summer season.

Table 11. The effect of storage days on the % meat yield[ab] of 10-minute boil cooked[c] male crabs.

Storage Time	Lump (%)	Top Flake (%)	Bottom Flake (%)	Claw (%)	Total Yield (%)
0 day (1 hour after cook)	6.3 ±0.82	3.6 ±0.78	3.0 ±0.49	4.1 ±0.56	17.0 ±1.62
1st day (25 hours)	5.7 ±0.59	3.3 ±0.80	3.0 ±0.43	4.4 ±0.48	16.4 ±1.52
2nd day (49 hours)	5.6 ±0.63	3.5 ±0.79	3.0 ±0.51	4.2 ±0.44	16.3 ±1.48
3rd day (73 hours)	5.6 ±0.60	3.7 ±0.86	2.8 ±0.62	4.1 ±0.39	16.2 ±1.46
4th day (97 hours)	5.7 ±0.68	3.4 ±0.86	2.6 ±0.58	4.2 ±0.42	15.9 ±1.64

[a] The percentage meat yield was calculated on the live (uncooked) crab weight basis.

[b] The percentage yield was the average of 10 observations of 5 different pickers.

[c] The crabs were cooked in boiling water at 212 F (100 C) for 10 minutes after the rolling boil of water.

Table 12. The effect of storage days on the % meat yield[ab] of 10-minute boil cooked[c] female crabs.

Crab Type	Storage Time	Lump (%)	Top flake (%)	Bottom (%)	Claw (%)	Total Yield (%)
Sponge Female	0 day (1 hr.)	4.4 ±0.41	3.5 ±0.77	3.0 ±0.71	2.2 ±0.64	13.1 ±1.40
	1st day (25 hrs.)	4.0 ±0.44	3.0 ±0.83	2.8 ±0.72	2.5 ±0.51	12.3 ±1.52
	2nd day (49 hrs.)	4.1 ±0.41	3.0 ±0.78	2.8 ±0.70	2.3 ±0.57	12.2 ±1.55
	3rd day (73 hrs.)	4.0 ±0.39	2.8 ±0.80	2.6 ±0.73	2.6 ±0.61	12.0 ±1.49
Clean Female	0 day (1 hr.)	5.4 ±0.52	3.1 ±0.84	3.3 ±0.75	3.2 ±0.54	15.0 ±1.61
	1st day (25 hrs.)	4.3 ±0.56	3.4 ±0.76	3.2 ±0.69	3.5 ±0.63	14.4 ±1.59
	2nd day (49 hrs.)	4.4 ±0.47	3.1 ±0.70	2.9 ±0.53	3.0 ±0.57	13.5 ±1.46
	3rd day (73 hrs.)	4.3 ±0.49	3.1 ±0.80	2.7 ±0.71	2.9 ±0.59	13.0 ±1.55

[a] The percentage meat yield was calculated on the live (uncooked) crab weight basis.

[b] The percentage yield was the average of 10 observations of 5 different pickers.

[c] The crabs were cooked in boiling water at 212 F (100 C) for 10 minutes after the rolling boil of water.

Table 13. Percent meat yield[a] and weight loss of crab claws by Harris machine process[b] as affected by cold storage

Storage[c] Time	Wt. Loss[d] (%)	Yield[e] (%)
0 hour	0.0	25.6±1.1 a
24 hours	1.6±0.2	29.4±1.1 b
48 hours	2.6±0.2	29.5±1.1 b
72 hours	3.1±0.1	31.7±0.9 c
96 hours	3.5±0.2	29.8±0.7 b

[a] - The crab claws were steam pressure cooked (at 250 F (121 C), 15 psig.) for 10 min.
- 0 day cooked claw weight basis.
- The yield was calculated after the squeezing process.

[b] 70 % (w/v) saturated salt solution was used for the meat separation process.

[c] The temperature of the cooler was 36 F (2.2 C).

[d] 60 pound (27.2 kg) capacity plastic containers were used for the dehydration study.

[e] % meat yields followed by same letter within columns are not significantly different ($p \leq 0.05$).

from the inedible parts of crab such as the dead man's finger (gills). Although the weight loss is from the crab meat, the meat yield can be maintained at the same level because the meat is more easily picked from the shell as storage days increased (some physical reactions between the crab shell and meat may occur).

In the case of boil cooking, the hot or warm crabs which were just cooked and cooled in atmospheric temperature produced more meat than crabs stored in a cooler over 24 hours. This is in contrast to the result obtained from of retort cooked crabs.

The percent meat yield of each crab meat type (lump, claw, top flake, and bottom flake meat) was calculated to determine which part has the greatest weight loss (moisture loss) during cold storage. However, the results show that there were no significant differences in weight loss between the crab meat types cooked crabs.

2. Harris-machine Picking Process

Determination of Meat Yield During Cold Storage

The percent meat yield of crab claws produced by the Harris machine precess was determined with respect to the effect of cold storage on weight loss and meat yield (Table 13). In the Harris machine process, claws stored at 1.7 - 2.2°C (35 - 36°F) for 24 hours yielded significantly ($p \leq 0.05$) more meat (29.4±1.1 %, cooked claw wt. basis) than warm (0 hour storage) claws (25.6±1.1 %). These results are very comparable with those of the hand pick yield test. Highest yield obtained by the Harris machine occurred on crab claws stored for 96 hours (3 days). The reason for this result is not clear. The plausible reason can be that some type of physical reaction occur between the meat and shell which loosens the bond between the meat and shell during the storage period.

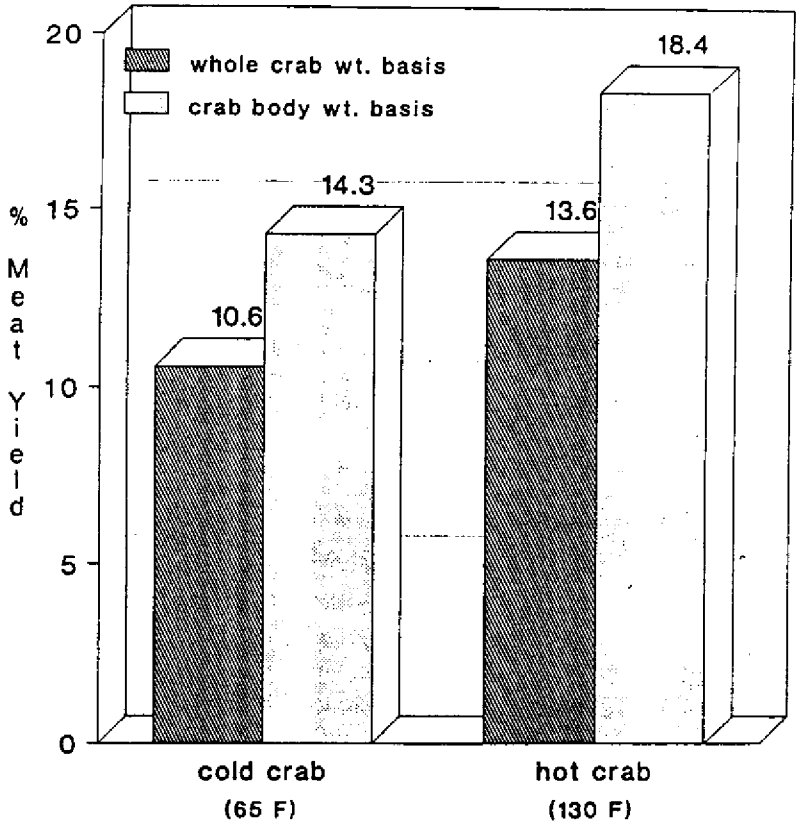
3. Quik-Pik Process

Determination of Yield

The meat yield of Quik-Pik process crabs was evaluated and the effect of crab temperature before the steam tunnel treatment on meat yield was also examined (Figure 2). The yields of sponge female crabs, which is the most common crab type for the Quik-Pik process, were determined (10.6±0.23 %, whole live crab weight basis) for cold crabs (internal temperature of 18.3°C, 65°F) and for hot (13.6±0.36 %) crabs (54.4°C, 130°F). In the Quik-Pik process, crab body weight (without claws) should be also considered as the basis for yield calculation because crab claws are not utilized in the process. In this case, the yields of the sponge female crabs were 14.3±0.32 % for the cold crabs and 18.4±0.47 % for the hot crabs.

In comparison with normal hand picking process, the Quik-Pik process produced about 13 percent more meat in the case of cold

Figure 2. Percent meat yield of blue crab by Quik-Pik process.



(18.3°C) sponge female crabs, and about 32 percent more meat in hot (54.4°C) crabs (yield, whole live crab weight basis).

Effect of Crab Temperature on Yield

The hot crabs (54.4°C, 130°F) cooked immediately prior to the Quik-Pik process yielded significantly ($p \leq 0.05$) more meat than the cold (18.3°C, 65°F) crabs in both yield calculation methods.

In this study, hot crabs produced about 22 percent more meat than cold crabs. This result may be due to the unique characteristics of the mechanical extraction (vibration) process. Fish and Stewart (4) also reported that temperatures ranging from 54.4 to 62.8°C (130 to 145°F) are the optimum operating range for the extraction process. Another plausible reason for the result may be explained by the fact that the moisture content of crabmeat from the hot crabs was higher (77.7±0.15) than that from the cold crabs (76.3±0.12 %).

Development of VTBCP

Separate prediction equations which were the correlation of

Table 14. Prediction equations of blue crab yield during summer. (cooking time: 8 - 12 minutes)

crab sex	meat type	predicted equations
sponge female	lump	$Y^a = -0.0983 \times CT^b + 4.8878$
"	top flake	$Y = -0.0500 \times CT + 3.3000$
"	bottom flake	$Y = -0.2250 \times CT + 5.3167$
"	total flake	$Y = -0.3000 \times CT + 8.9333$
"	claw	$Y = -0.0750 \times CT + 3.0833$
"	total	$Y = -0.4500 \times CT + 16.700$
clean female	lump	$Y = -0.0750 \times CT + 5.1167$
"	top flake	$Y = -0.1500 \times CT + 5.0333$
"	bottom flake	$Y = -0.1000 \times CT + 3.8333$
"	total flake	$Y = -0.2500 \times CT + 8.8667$
"	claw	$Y = -0.1000 \times CT + 3.7000$
"	total	$Y = -0.4000 \times CT + 17.333$
male	lump	$Y = -0.2000 \times CT + 7.4000$
"	top flake	$Y = -0.1750 \times CT + 5.7167$
"	bottom flake	$Y = -0.1250 \times CT + 4.2167$
"	total flake	$Y = -0.3000 \times CT + 9.9667$
"	claw	$Y = -0.1083 \times CT + 4.9389$
"	total	$Y = -0.5000 \times CT + 21.300$

[a] : Y = Yield in percent (live crab wt. basis).
[b] : CT = Cooking Time in minutes.

Table 15. Prediction equations of blue crab yield during winter. (cooking time: 8 - 12 minutes)

crab sex	meat type	predicted equations
clean female	lump	$Y^a = -0.0250 \times CT^b + 5.0833$
"	top flake	$Y = -0.0500 \times CT + 3.8333$
"	bottom flake	$Y = -0.1500 \times CT + 5.5000$
"	total flake	$Y = -0.1750 \times CT + 9.0167$
"	claw	$Y = -0.1500 \times CT + 4.1667$
"	total	$Y = -0.3500 \times CT + 18.267$
male	lump	$Y = -0.1000 \times CT + 7.1667$
"	top flake	$Y = -0.1250 \times CT + 5.6500$
"	bottom flake	$Y = -0.0250 \times CT + 3.5167$
"	total flake	$Y = -0.1750 \times CT + 9.3833$
"	claw	$Y = 0.0009 \times CT + 3.8961$
"	total	$Y = -0.3000 \times CT + 20.867$

[a] : Y = Yield in percent (live crab wt. basis).
[b] : CT = Cooking Time in minutes.

cooking time (between 8 and 12 minutes) and yield were developed for each type of crabmeat (lump, top flake, bottom flake, total flake, claw, and total meat yield), each season, and for different sexes (male, clean female, and sponge female) (Tables 14 and 15). From those equations, the effect of cooking time on the meat yield could be predicted regardless of the natural and processing variables.

Based on the equations and other data from the yield study, a computer program, Virginia Tech Blue Crab Program (VTBCP), was developed using Turbo Pascal Programming Language.

With the use of VTBCP, a processor can predict and calculate the final meat yield (total and differential meat) before the beginning of crab processing or purchasing of crabs regardless of season, crab sex and type, crab size, cooking time, cooking method, and storage temperature and period.

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ACKNOWLEDGEMENT

This work is a result of research sponsored in part by NOAA Office of Sea Grant, U. S. Department of Commerce, under Grant No. NA86AA-D-SG042 to the Virginia Graduate Marine Scince Consortium and the Virginia Sea Grant College Program. The U. S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon.

HEAT TOLERANCE OF SPOILAGE ORGANISMS IN PASTEURIZED CRABMEAT

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INTRODUCTION

Within the shellfish industry, blue crabs are second only to shrimp in dollar value of harvested product (1). Landings of blue crabs, which are found from Massachusetts to Texas, have been increasing within the last 10 years. In the Chesapeake Bay alone, over 70 million pounds of live crabs are caught annually (5).

Due to the seasonality of the fresh blue crab industry, prices and employment are unstable. Many processors pasteurize crabmeat during times of abundance to sell during times of scarcity to help steady these inconsistencies. The National Blue Crab Industry Association (NBCIA) recommends a pasteurization process with an $^{1}F_{185}$ of 31 minutes. This should extend product shelf life to one year or longer (7). However, recently, many processors had excessive losses, due to reduced shelf life, in meat that was pasteurized according to NBCIA recommendations.

The purpose of this study was to identify the cause of this reduced shelf life and to assess the suitability of recommended pasteurization schedules.

MATERIALS AND METHODS

Isolation

Spoiled pasteurized crabmeat was obtained from several east coast processors. Spoilage organisms were enumerated both aerobically and anaerobically on Brain Heart Infusion agar (BHIA) (BBL) and modified Brain Heart Infusion agar (mBHIA) (Brain Heart Infusion (BBL)-3.7 g., Granulated Agar (BBL)-0.55 g./roll tube, distilled water-100 ml., hemin solution-1.0 ml., resazurin solution-0.4 ml., Vitamin K-0.02 ml., cysteine hydrochloride-0.05 g.) (3). To determine if spores were present, the crabmeat was also subjected to a heat treatment of 60°C and 80°C for 10 minutes prior to plating. Plates and roll tubes were incubated at 20°C, 30°C, and 45°C for 24-48 hrs. Colonies of differing morphology were isolated and stored on Trypticase Soy agar (TSA) (BBL) slants or in Chopped Meat Glucose broth (3).

Can Seam Measurement

Following enumeration, the spoiled crabmeat cans were broken down and seam measurements taken using a Starrett Can Seam Micrometer. These measurements were then compared to can specifications to determine if spoilage was due to faulty can seams.

Thermal Inactivation

Isolated organisms were grown in BHI (BBL) or pre-reduced Peptone-Yeast-Glucose broth (PYG) (3) and tested for heat tolerance when cultures were approximately 18 hrs. old. Spores were produced by allowing cultures to grow, without transfer, in BHI or PYG for at least two weeks. The presence of spores was ascertained by spore staining with malachite green. Eight borosilicate glass reaction vials

with teflon septa (Fisherbrand, 40 ml.), each containing 1.5 ml. of culture, were submerged in a Lauda WB-20/R circulating water bath heated to 185°F (85°C). One vial was removed from the water bath at time=0, 15, 20, 25, 32, 40, 60, and 85 minutes, and immediately placed in an ice bath. Surviving microorganisms were enumerated on BHIA and mBHIA and incubated at 30°C for 24-48 hrs. A total of 25 isolates were screened for heat tolerance in this manner. Organisms, or spores, surviving longer than 31 minutes were identified to species level by their gram reaction, spore test and biochemical reactions, and retested for heat tolerance at least three more times following the above procedure. D-values were determined from survivor curves.

RESULTS

Can seam measurements indicated that spoilage from one processor was most likely due to faulty can seams. The length of the seam on the company end of cans from this processor exceeded specifications. In properly sealed cans, spoilage was due to the outgrowth of heat resistant sporeforming microorganisms.

Spores of two organisms isolated from spoiled pasteurized crabmeat survived a heat treatment of 31 minutes or longer at 185°F. Vegetative cells of these organisms were unable to survive for longer than 15 minutes. (See figures 1 and 2).

One of the heat tolerant organisms was a gram positive, facultative anaerobe which sporulated only under aerobic conditions and was identified as a Bacillus sp. The other organism was a gram positive, spore forming, strict anaerobe that was provided by Dr. Wayne Segner (Continental Can Company). This organism was isolated from spoiled pasteurized crabmeat and identified as a Clostridium sp. This isolate grew to temperatures as low as 2°C. This same Clostridium sp. was isolated in our laboratory and it appears not to have been previously identified. D-values obtained for spores of the Bacillus sp. was 34 minutes and 9 minutes for the Clostridium sp.

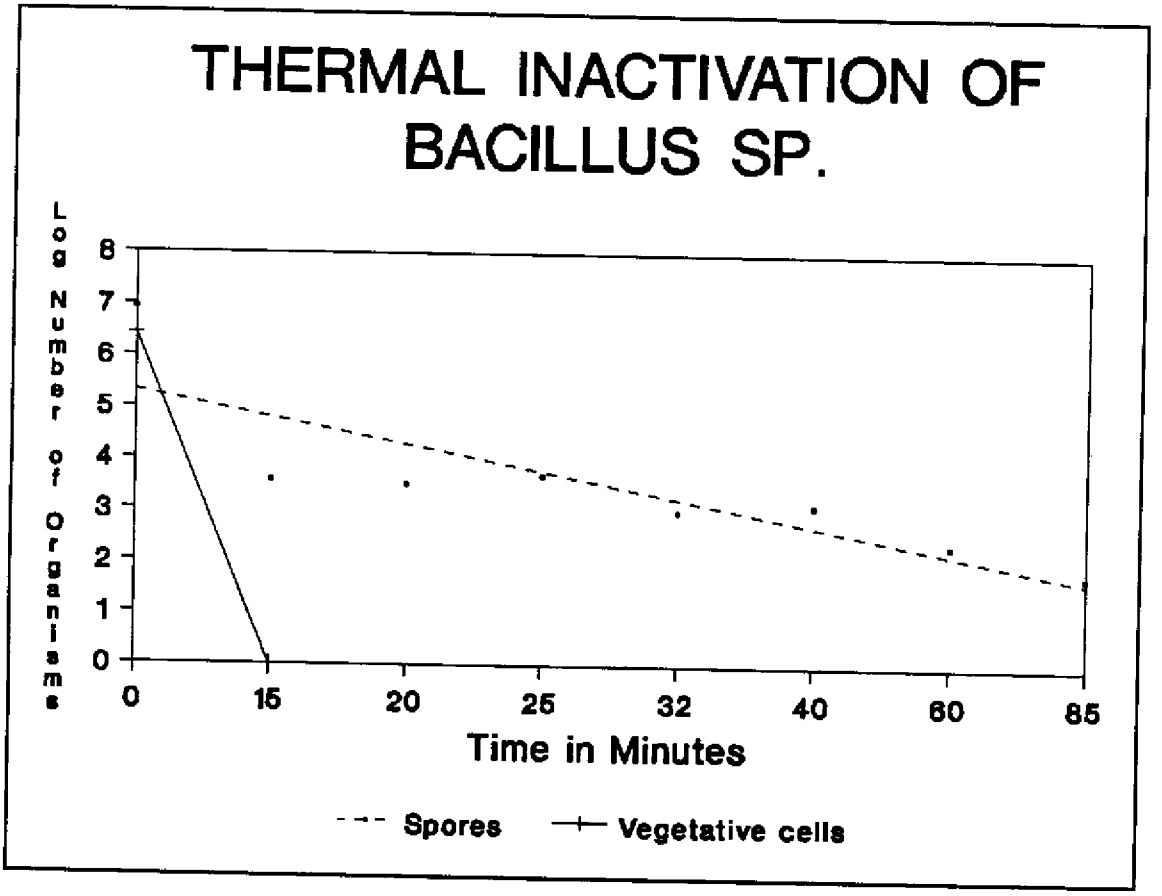
DISCUSSION

Despite 20 years of research, the blue crab industry is still occasionally confronted with unexpectedly shortened shelf life of pasteurized crabmeat due to spoilage. The most recent example of this was in 1989 and it affected processors throughout the United States.

Blue crabs, as compared with other crab species, are harvested from estuarine and coastal areas and, hence, are very susceptible to environmental factors (1). Qualitative and quantitative differences in microbial flora are seen depending upon the season, the water quality and the geographic location in which the crabs were caught (6). This may have played a role in the most recent spoilage episode. Spoilage was first noticed after hurricane Hugo struck the east coast of the United States in September of 1989. It is postulated that the habitat of the isolated Clostridium sp. is marine sediments. The storm could have disturbed deeply buried sediments and allowed this organism to inoculate the water column.

When processors receive live crabs, they cook, cool and refrigerate them overnight. The crabs are picked the following morning. After cooking, the crabs should be sterile. However, many researchers report a lack of uniformity in the cooking parameters amongst processors (1). Therefore, there could be significant numbers of bacteria surviving cooking. If only a few cells of the Clostridium sp. were to survive the cooking step, appreciable numbers of spores could be present in the crabmeat before pasteurization since this organism is psychrotrophic.

Figure 1



The D-value for spores of the Clostridium sp. was 9 minutes at 185°F in PYG. If the D-value is the same for crabmeat, as few as 6 spores/gram in a one pound container of crabmeat would be sufficient to allow a few spores to survive the NBCIA recommended pasteurization process. These spores could then outgrow and cause significant losses due to spoilage. This appears to be quite feasible, especially since this organism is psychrotrophic and could grow to high levels before and after pasteurization.

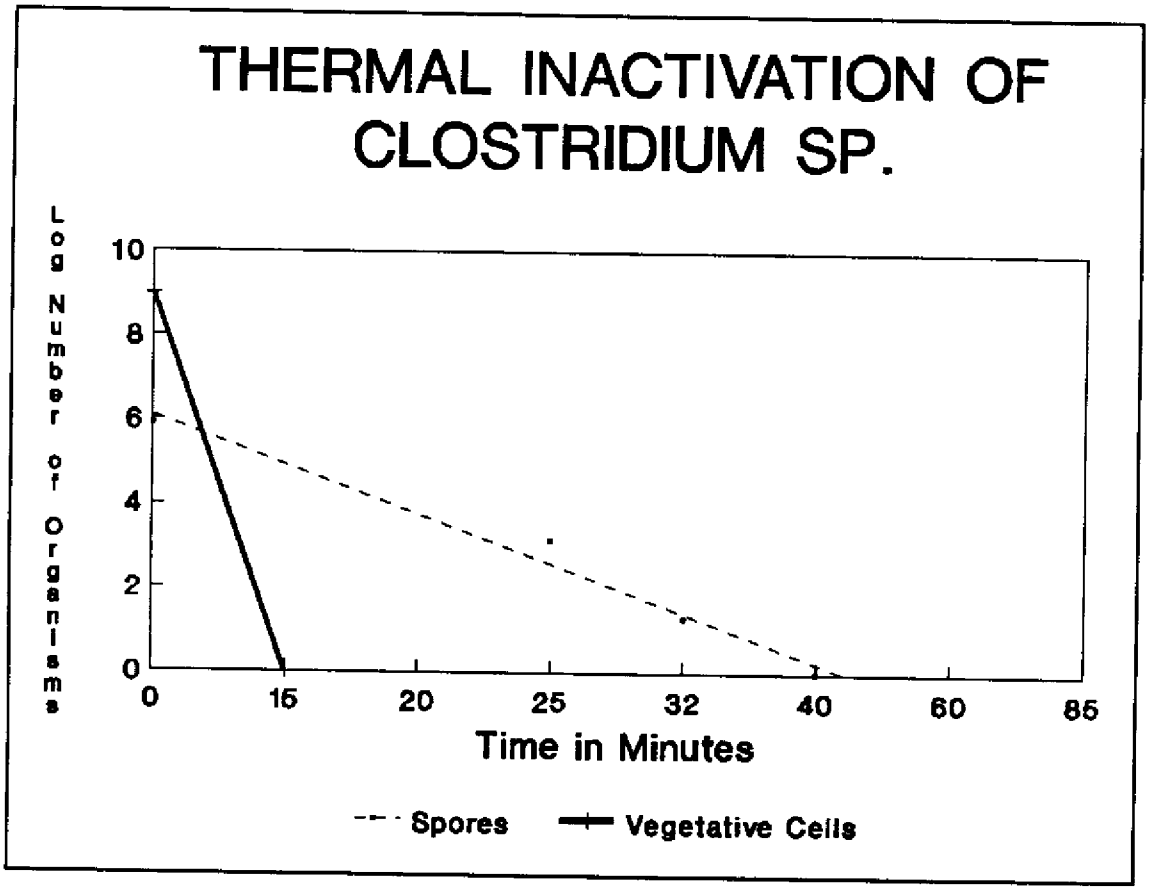
A Bacillus sp., possibly Bacillus pasteurii, was also isolated from one spoiled can along with the Clostridium sp. Its spores were found to have a D-value of 34 minutes in BHI. In 1927, Fellers (2) reported a non-gaseous spoilage prevalent in pasteurized crabmeat that was caused by Bacillus cereus and Bacillus mesentericus. Loaharanu and Lopez (4) found that Bacillus, Micrococcus, and Alcaligenes survived a pasteurization process of 85-87°C for 110 minutes.

Although this Bacillus sp. undoubtedly survived the pasteurization process, it is not felt that it contributed greatly to spoilage. The predominant spoilage was gaseous and had a rotten, putrid odor. The Clostridium sp., grown in broth, had the same odor as the spoiled crabmeat and it produced gas. The Bacillus sp. did not have that characteristic odor and did not produce gas. Also, the Bacillus sp. was only isolated from one properly sealed can, whereas the Clostridium sp. was isolated from all properly sealed cans.

CONCLUSION

The finding of a Clostridium sp. and a Bacillus sp. whose spores survive the NBCIA recommended pasteurization process clearly shows that this recommendation is inadequate. These findings are especially significant because the Clostridium sp. could grow at refrigeration temperatures and significantly reduce the shelf life of the product.

Figure 2



It is essential for the economic development of the blue crab industry to have a pasteurization process that will ensure a reasonable shelf life for their product. This process must also reliably destroy foodborne pathogens so that consumer safety is assured. Without these measures, the growth of the blue crab industry may be hindered.

Future work will focus on use of these spore formers as reference organisms for the pasteurization of crabmeat and more work will be done on characterizing the heat tolerant isolates, especially the Clostridium sp. A pasteurization schedule will be developed that will ensure a reasonable shelf life while continuing to maintain quality sensory characteristics.

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THE SEA SCALLOP FISHERY: A CASE FOR FREEZING AT SEA

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INTRODUCTION

The fishery for scallops is a valuable part of Atlantic Canada's fish industry; the annual landed value exceeds CAN$ 60 million. The sea scallop (*Placopecten magellanicus*) is the main species and evidently there are under-exploited stocks of Icelandic scallop (*Chlamys islandica*) which is smaller and relatively difficult to shuck. In order to derive the maximum return from the fishery and ensure that the industry's reputation for good fish products is maintained and enhanced if possible, handling and processing practices must be kept under review. In one major area of activity, operations on board the scallop dragger, there have been few changes over the past thirty years or so. While the fleet has been successful, the following factors have prompted review of the design and operation of vessels.

1) Much of the fleet consists of older vessels that will have to be replaced within a few years.
2) There is an increased demand for products of consistently high quality.
3) Catch quotas have been introduced and have prompted greater emphasis on handling and preservation, as opposed to catching, in order to maximize returns.
4) The work of the crew is arduous and difficult.

Practically all aspects of the work on board need to be examined; shooting and hauling of the gear, culling of the catch, shucking of scallops, preservation of scallop meats, etc. Increased mechanization would enable the crew to devote more attention to activities concerned with improved quality and increased yield. Recovery of roes and rims (mantles) would bring an increase; the weight of roe by itself varies with season from 25 to 65 per cent of the weight of scallop meat. Loss of meat (adductor muscle) on manual shucking is substantial. Naidu (1) estimated the amount of lost meat, which remains on the shell and is discarded, to be 11 per cent for the sea scallop and 23 per cent for the Icelandic scallop. Also, of course there may be losses incurred during subsequent handling and processing, depending on the amount of spoilage and the methods employed.

MATERIALS AND METHODS

Much of the supply of scallops comes from the Georges Bank fishery which involves voyages of about 12 days. The meats are stored in cotton bags, which each hold 15 kg, surrounded by melting freshwater ice in the hold. Storage life under these conditions is roughly 18 days. In 1969 Varga and Blackwood (2) reported rates of cooling of meats in the bag. They observed

cooling times of about 20 h and proposed chilling of individual meats prior to bagging. This suggestion was taken up recently, when a special chilling system (chilled sea water) was installed in the dragger 'Cape Rouge' on an experimental basis.

In work done at the Canadian Institute of Fisheries Technology, which included seagoing observations on the 'Cape Rouge' and 'Cape Keltic' in October 1987, quality and weight losses in scallop meats frozen at sea were examined. The equivalent of 16 bags of meats was frozen on board and stored for later examination ashore. Particular attention was paid to conditions prior to freezing.

The sequence of handling operations on board, shown in Figure 1, was typical of the fleet except for the special chilling system in the holding tank in the 'Cape Rouge'. Chilling was found to give good results and extend the storage life of the meats by 2 days.

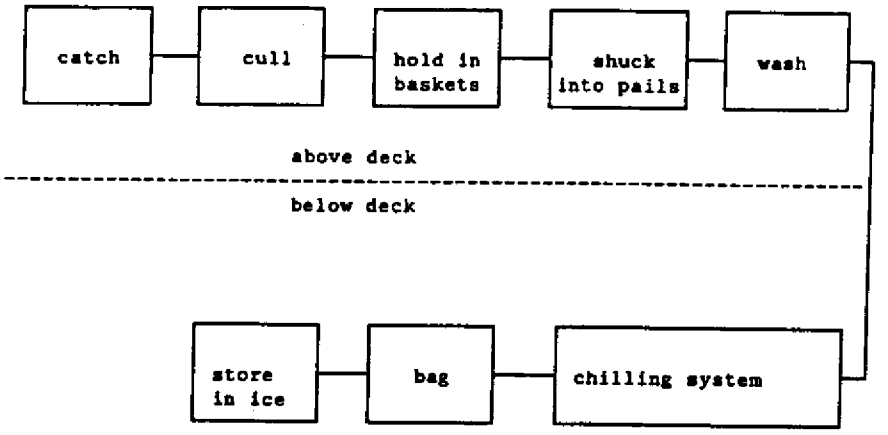

Figure 1. Handling operations on 'Cape Rouge'

The time interval between catching and bagging normally ranges up to 6 h, the length of a watch. It is known, particularly from work on the freezing at sea of various species of fish, that delays of a few hours at high temperature (10•C for example) can result in loss of quality, notably toughening of texture and loss of water-holding capacity. These changes are associated with the phenomenon of *rigor mortis* in muscle. There are, however, few data available on scallops.

Scallops for freezing were taken at intervals up to 6 h before bagging. Some were frozen immediately and others were frozen after periods of up to 10 days storage in ice, in partially-full bags. All were frozen individually, in about 6 h, in a domestic storage cabinet at minus 18•C. Ambient temperatures were about 10•C and the observed temperature of the scallop meats was 10•C prior to chilling.

A 'K-Tron' KS-1 scale (K-Tron Arizona Inc) was used to weigh labelled full bags of scallop meats on stowage in ice and at the point of landing. It had an averaging feature which enabled precise measurement at sea.

After landing but after 11 days in the cabinet in all cases, the frozen scallops were transferred to cold storage at minus 30•C. The work on shore included assessments of quality for up to 6 months in storage. Taste panel results were correlated with instrumental measurement of texture and drip (weight loss) was measured on thawing and cooking.

Drip was measured according to three procedures, i.e., in three stages; free thaw drip, expressible drip and cook drip. In each case the measurement was carried out four times. To

measure thaw drip, four frozen meats, about 50 g, were placed on a wire gauze on top of a plastic cup, all enclosed in a plastic bag to prevent evaporation and placed in a room at 5°C for 17 h. Expressible drip was measured after the application of a compressive force to the same four meats, in a perforated cup and plunger assembly. The magnitude of the force was not sufficient to cause much physical damage. Cook drip was measured after placing the pressed meats in a polyethylene bag and plunging them into boiling water for 6 minutes. After cooking, the meats were left to drain on a wire gauze and cup, enclosed at room temperature. The meats were wiped carefully and reweighed after each procedure.

RESULTS AND DISCUSSION

Total drip (the sum of the three losses) was found to give a good indication of quality, with the lower losses from scallops of higher quality. It correlated closely with taste panel and texture measurements.

Table 1. Typical values of drip

Storage in ice (Days)	Thaw drip (percent)	Total drip (percent)
0	0.73 ± 0.13	18.6 ± 1.12
1	1.81 ± 0.33	24.4 ± 1.41
4	3.79 ± 1.83	34.0 ± 2.66
6	8.34 ± 2.79	41.8 ± 3.51
10	8.18 ± 6.62	39.0 ± 6.99

The amount of drip was much dependent on days in ice, as shown by the results (with standard deviations) in Table 1. Time up to 6 h in the chilling system and time up to 6 months in cold storage at minus 30°C were of less significance. The values in Table 1 are for scallops which were removed from the chilling system after not more than 5 minutes and stored at minus 30°C for 6 months. They are typical of the results overall. It is likely, however, that the measured amounts of drip in the experimental meats from the partially-full bags were higher than values that would have been obtained with full bags. Moisture contents in the frozen samples were measured by drying the muscle in an oven at 103°C until a constant weight was reached. They exhibited large increases with days in ice, although there would have been some losses, not measured, in freezing and cold storage. The increases correspond to a gain in bag weight of about 12 per cent at 10 days in ice and 3 per cent at 6 days. According to the recorded weights of 12 bags on stowage and landing, on the other hand, the gain at 10 days is between 3 and 9 per cent. This discrepancy and considerable variability observed cannot be explained altogether but will be associated with the tendency of the meats to absorb water and swell in the bag (some anaerobic spoilage occurs as a result). Possibly weight increase is influenced by the degree of 'tightness' reached on swelling and is unevenly distributed with much of the gain at the outsides where there will be greater exposure to meltwater. By the same token, meats in the partially-full bags destined for freezing would have been exposed to more meltwater than those in full bags.

The amount of drip will depend to some extent on the method of measurement and will vary with season and possibly other factors; more observations are needed. Nevertheless, although no adjustment has been made in Table 1 for the discrepancy between measured moisture content and weight increase, it is clear that the amount for the consumer's plate will be increased by freezing at sea.

CONCLUSION

According to the results, frozen-at-sea scallop meats are markedly superior to meats stowed in ice in the conventional way before freezing. The meats frozen at sea retained much of the original sweet flavour and tender texture characteristic of very fresh scallops. Similarly, on thawing and cooking, they retained more of the original weight. The differences became greater with increased number of days in ice before freezing. The results indicate that an increase of more than 15 per cent in yield of cooked meats could be realized.

Freezing at sea would appear to be a worthwhile option in the Georges Bank scallop fishery. Classic elements in favour of the technique are present; with icing as practised, the draggers are returning to port with holds only partly full and the freezing of meats on board gives superior quality and increased yield. Freezing on board also might facilitate the recovery of rims and roes.

ACKNOWLEDGEMENTS

The work described was carried out with support from the Department of Fisheries and Oceans and the International Centre for Ocean Development and with the cooperation of Scotia Trawler Equipment Limited, National Sea Products Limited and A. Orlic & Associates Limited. Clearwater Fine Foods Inc., Comeau Sea Foods Limited and Fishery Resource Development Limited also provided assistance.

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CONGELATION EXPERIMENTALE DE PETONCLES GEANTS, *Placopecten magellanicus*, A BORD D'UN BATEAU

RESUME: La pourcentage de perte d'eau (perte de poids) encourut durant la decongelation et la cuisson de la chair (muscle adducteur) du petoncle geant, *Placopecten magellanicus*, demontre que la qualite est dependente en grande partie des conditions de manutention avant la congelation. Les chairs congelees aussitot apres la capture exhibent un taux d'egouttement minime et une meilleur qualite que les chairs congelees apres un ou plusieurs jours sous glace. La pratique courante de la peche sur le banc Georges est de conserver sous glace les chairs de petoncle pour une duree pouvant aller jusqu'a 12 jours avant le retour au port.

La congelation a bord resulterait en un accroissement du rendement en chair cuite pouvant atteindre, selon nos resultats, plus de 15 pourcent. Le recorvrement des oeufs (rave) et autres parties du petoncle pourrait aussi etre envisager.

THE EFFECT OF STOCKING PROCEDURE AND PRODUCT SAFETY ON CONSUMPTION OF SHELF LIFE IN REFRIGERATED SEAFOODS DISPLAYED IN REFRIGERATED DISPLAY CASES AND ICE-ONLY FREESTANDING GONDOLAS*

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INTRODUCTION

The Food Marketing Institute (1987) reported that shrinkage in the refrigerated seafood line accounted for 10%-15% of (departmental) sales. A direct cost, shrinkage proportionally reduces departmental contributions to store overhead. As well, the factors which manifest excessive shrinkage may raise customer concerns about the safety, freshness, and time available to use seafood purchases. These concerns could affect store loyalty, thereby eroding a source of competitive advantage.

There are two sources of shrinkage: i) paying for products not received (i.e. inconsistencies between invoices and deliveries) and ii) spoilage. Insuring that orders match deliveries can eliminate the first source of shrinkage. This is best achieved by designing detailed, measurable product specifications, communicating them to vendors, and evaluating incoming deliveries against these criteria. Much has been written about proper receiving practices for food retailers (2,4,6,7,9).

With all perishable products, some spoilage is inevitable since the time required to sell them may exceed remaining shelf life. This situation is exacerbated with seafoods because, in many instances, a significant amount of shelf life has been consumed prior to retail receipt. In fact, retailers often exert management control over no more than the last 20--25% of remaining shelf life for many species (approximately 70--80 clock hours).

However, shrinkage due to rapid consumption of remaining shelf life occurs because of high product temperatures which speed microbial action, inadvertent contamination or cross contamination which increases the abundance of spoilage organisms (also reducing the time required to putrefy the product), or interaction between these conditions. And given the limited

* This paper is excerpted from a monograph due to be released in Spring 1991 by the Sea Grant College Program at Texas A&M University. It is the culmination of a multi-year project designed to enumerate, evaluate and recommend comprehensive, stepwise procedures by which the retail seafood quality process could be improved. Several organizations have financially supported this work including Texas A&M University (both the Sea Grant College Program and the Texas Agricultural Extension Service), the Texas Parks and Wildlife Department through annual, interagency contracts with the Texas Agricultural Extension Service, the Gulf and South Atlantic Fisheries Development Foundation, Inc. (contract 32-15-18160/15660), and the Behmann Brothers Foundation.

amount of shelf life remaining upon receipt, methods which do not insure that products with the least amount of shelf life are sold first may also account for a significant proportion of avoidable shrinkage.

Shrinkage resulting from spoilage and improper rotational sequences can be sharply reduced by correcting deficiencies in the retail quality management process. This process includes in-store protocols used at each step in the retail inventory cycle (Figure 1), as well as the employee handling required to move products from one step in the cycle to another. Improving the quality process is particularly important within food retailing since providing consumers with safe, fresh, long lasting products is linked to management programs; not technologies.

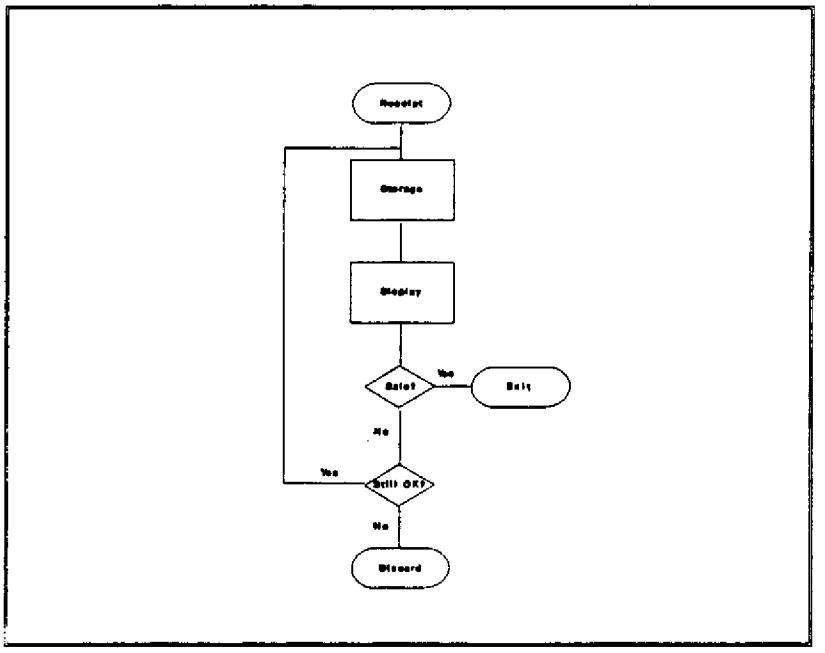


Figure 1. The Retail Inventory Cycle for Refrigerated Seafoods

Diminution in quality cannot be recaptured. Therefore to reduce shrinkage and pass along fresh, long lasting seafoods to consumers, work plans, methods, protocols, etc. must simultaneously maintain low product temperatures, employ good sanitation practices, and respect stock rotation sequences throughout the inventory cycle. If all of these criteria are not satisfied at each step within the cycle, remaining shelf life can be rapidly consumed.

Product display is often overlooked for its effect on spoilage. This step can significantly contribute to rapid spoilage if products remain on display for long periods prior to sale (e.g. 10-14 hours between initial stocking and sale) and the stocking procedure employed cannot maintain optimally low product temperatures. With many products having minimal shelf life upon receipt,

maintenance of low product temperature in the display step can mean the difference between a sale or a discard.

Since stocking procedures are the means of achieving display management objectives, they should be consistent with the amount of time products remain on display prior to sale. Thus, if displayed product turns over every 4 or 5 hours, stocking procedures should focus on maximizing eye appeal at the expense of shelf life since the practical effect of higher product temperatures is offset by rapid inventory turnover. Conversely, longer case residence times suggest using stocking procedures which also maintain low product temperatures.

While retail management intuitively recognizes the need to maintain cold product temperatures in all but the fastest turning situations, two factors complicate effective management of the display step. First is the mistaken assumption that display equipment per se' is capable of maintaining low product temperatures, regardless of procedures used to stock the case. But, maintaining low product temperatures is determined by stocking procedure (i.e. the manner that product and ice are combined).[1] The second impediment to effective display management is the lack of performance data which measures the amount of shelf life consumed when different stocking procedures are used.

To this end, the paper i) outlines the quantitative impacts that different product temperatures have on shelf life and ii) measures how various stocking procedures commonly used by the food retailing sector affect product temperature and thus the rate of decomposition. By comparing the shelf life consumed under a variety of stocking procedures with expected case residence time, retail management can select those stocking procedures which best balance the objectives of eye appeal and maintenance of shelf life.

THE INFLUENCE OF PRODUCT TEMPERATURE ON SHELF LIFE

Holding product temperature constant, the progression high protein foods make from fastidiously fresh to completely putrid is inevitable and predictable (1). However, when product temperature is increased, the rate of spoilage accelerates (5). Specifically, cod (Gadus mohura) held at 32° F since death is of acceptable quality for about 336 hours (14 days) (Figure 2). At a holding temperature of 40° F, the same product is acceptable for only 168 hours (7 days). The difference is not the amount of shelf life available, but the rate at which it is consumed.

Peters (1986) used this relationship to compute the amount of shelf life lost per elapsed hour (i.e. the rate of spoilage) (Figure 3). Therefore, by knowing product temperature and length of holding period, the amount of shelf life consumed throughout any step in the retail inventory cycle can be estimated.

Evaluating the Influence of Stocking Procedure Upon Product Temperature

Food retailers may use a number of different stocking procedures. Additionally, these different stocking procedures may be combined with various case door opening regimens, different ambient case airspace temperatures and numerous models of equipment thereby creating hundreds of unique combinations; all of which impact product temperature. Evaluating, let alone enumerating, each of these unique combinations is not practical. Instead, the approach taken here is to focus on those elements which are considered critical in determining product temperature. Those elements are different ambient case temperatures and specific stocking procedures. By evaluating combinations of these two considerations, most of the differences in product temperature can be addressed.

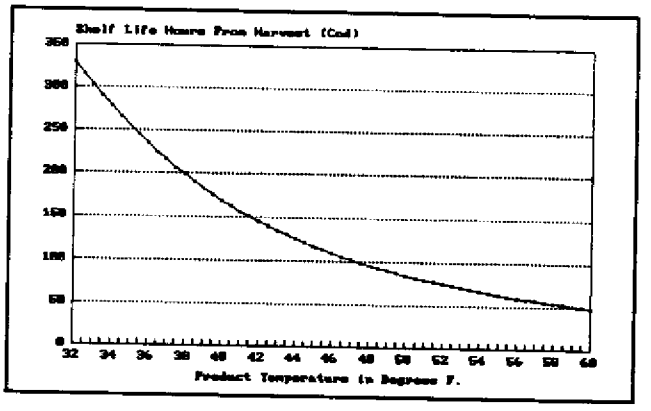


Figure 2. Hours The Product is Acceptable as Determined by Product Temperature

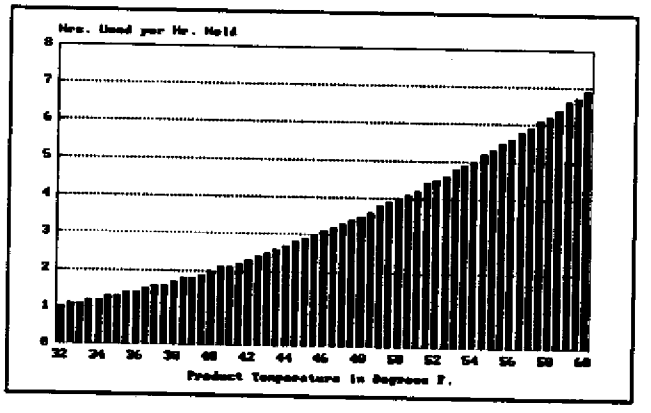


Figure 3. Hourly Shelf Life Consumption Rates Determined by Product Temperature

All stocking procedures were conducted under ambient case airspace temperatures of 40° F, 50° F, and 60° F. Evaluating stocking procedure performance at different temperature settings is important for several reasons. First, the physical placement of the thermistor within the case may vary by model as well as from store to store; thereby leading to inaccurate assessments of air space temperature above the product. Second, periodic opening and closing of case doors also affects air temperature above the product. Third, operating characteristics of refrigeration systems suggest that variability in case air temperatures over time are a normal occurrence. For example, the preselected case temperatures were achieved based on an average computed over each ten hour trial. However, mechanical refrigeration equipment can create dramatic variations in case airspace temperature as the compressor cycles; particularly at lower settings (Figure 4).

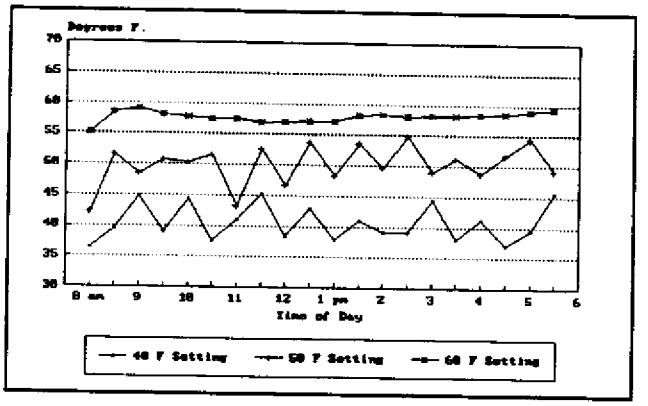


Figure 4. Ambient Case Airspace Temperature Histories When Set at 40° F, 50° F, and 60° F

All trials were carried out using a full service display case which relied on ice and supplemental refrigeration for chilling. Prior to stocking product in the case, a four inch layer of flaked ice was added. Perforated, half size stainless steel steam table pans were used in all stocking procedures with fillets.

Four stocking procedures were evaluated using fillets. These included: i) pans of fillets placed on bed ice, ii) pans of fillets embedded in ice, iii) pans of fillets embedded in ice with light top icing, and iv) pans embedded in ice with fillets separated from the pan bottom by a 1 inch airspace created with 2 inverted 2S foam meat trays. A stack of three fillets were used in the first three treatments while two fillets were used in the false bottom stocking procedure.

Thermocouples were attached in the approximate geometric center of each fillet (the warmest location). Fillet temperatures were recorded every 30 seconds, and averaged into container values over the hypothetical 9.5 hour sales day. Using spoilage rates which correspond to average container temperatures (Figure 3), the total amount of shelf life consumed under each stocking procedure was computed.

RESULTS

Skinless Fillets

On ice stocking procedure. This stocking procedure uses the ice bed as a platform on which to display inventory. Due to pan placement, 44% of the container surface is ineffective as a heat exchanger. And because pans are placed on the bed of ice rather than being embedded in it, there is no way that the cold air generated at the air/ice interface can insulate product from higher ambient temperatures.

Products placed on ice were quite sensitive to ambient case temperature settings between 40° F and 50° F, but exhibited similar changes in average container temperature over time between 50° F and 60° F (Figure 5). Computed average container temperatures were 36.2° F at the 40° F ambient case setting and 40.2° F at both 50° F and 60° F settings. Over a 9.5 hour hypothetical sales day, the estimated consumption of shelf life ranged from 13.3 hours (40° F setting) to 19 hours (both 50° F and 60° F settings).

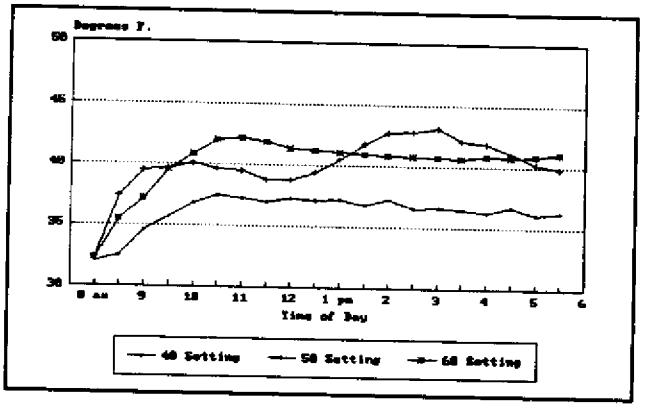


Figure 5. Pans of Fillets Placed On Ice Using a Refrigerated Case Set at 40°F, 50°F, and 60°F

In ice stocking procedure. Pans were placed in carved depressions in the bed of ice such that a) all five container surfaces were completely contacted by ice and b) the lip of the pan was even with the top of the ice bed. By embedding pans in ice, this stocking procedure maximizes the opportunity for ice to conduct heat away from the product through all container surfaces. Also, physical placement of pans in the ice bed allows cold, dense air to settle into pans which, in part, insulates fillets from higher ambient temperatures.

Products gained heat within the first 90 minutes on display at all case settings but stabilized at 35° F, 38° F and 39° F depending upon case airspace temperature (Figure 6). Average container temperatures at the three ambient case airspace settings were 34.3° F, 37.7° F and 38° F respectively. Based on these average container temperatures, estimated shelf life consumption was 12.4 hours (40° F setting), 15.2 hours (50° F setting) and 16.2 hours (60° F setting).

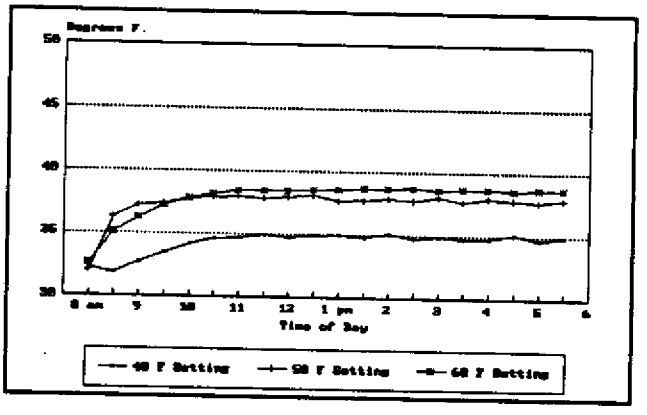


Figure 6. Pans of Fillets Placed In Ice Using a Refrigerated Case Set at 40°F, 50°F,and 60°F

Pans placed in ice with periodic top icing. Once the pan was embedded in ice and fillets were introduced, a light periodic top icing regimen was maintained over the 9.5 hour trial. Cube ice was used as a top dressing such that the product was visible (not buried), and melting ice was allowed to flow over fillet surfaces.

Despite a 20°F (50%) difference in case temperature, average product temperatures in this stocking procedure exhibited minimal variation (Figure 7). However, a warmer airspace did require adjustment in the time interval between periodic replenishment of top ice; being more frequent at the higher temperature.

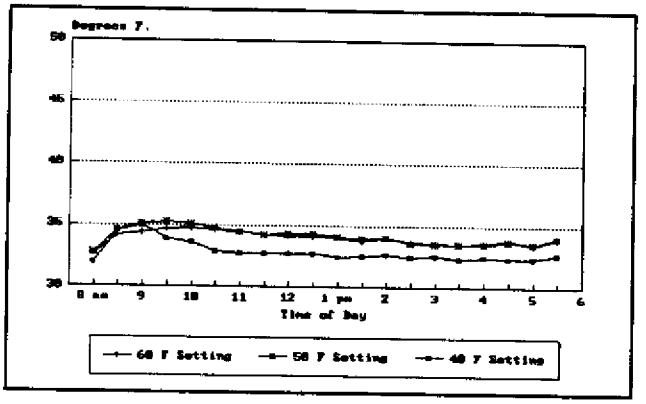


Figure 7. Pans of Fillets Placed In Ice, Periodically Top Iced In a Refrigerated Case Set at 40°F, 50°F, and 60°F

False bottoms added to pans embedded in ice. This approach focused on making half size stainless steel steam table pans more shallow thereby allowing retailers to cover the interior of the case with less product. It is sometimes used when management expects the interval between initial stocking and sale to be long. The rationale suggests that since the surface area of the case is effectively covered, the use of false bottoms provides shoppers with images of abundance, but allows the firm to display less product within a given sales day.

Unfortunately, false bottom use achieves the objective of displaying less product per sales day, but with a high cost in terms of lost shelf life. At temperatures above 40° F, products rapidly gain heat from the ambient case airspace (Figure 8). The effect on shelf life consumption was dramatic, ranging from 16 hours being lost (40° F setting) to 28.5 and 29.5 hours respectively at the 50° F and 60° F settings.

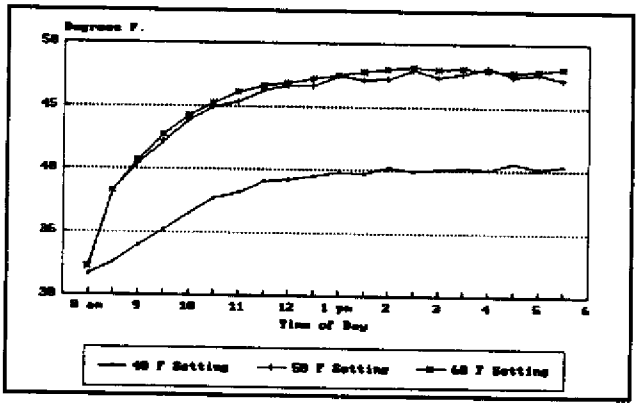


Figure 8. Pans of Fillets Placed In Ice With False Bottoms In a Refrigerated Case Set at 40°F, 50°F, and 60° F

Discussion of Skinless Fillet Trials

It is hardly surprising that products placed in cold environments and stocked such that the direct and indirect effects of ice are maximized return cold average temperatures. Thus, when stocking procedures were evaluated at the same ambient case setting, all were significantly different at the 95% level as computed using least significant difference.

When similar stocking procedures were evaluated over the range of case temperature settings, all stocking procedures which relied on indirect uses of ice were impacted by airspace temperatures. Interestingly, significant differences only exist between ambient settings of 40° F and 50° F, but not between the 50° F and 60°F settings.

What is surprising is the impact that seemingly small differences in stocking procedure have on product temperature and therefore the amount of shelf life consumed (Table 1).

Table 1. Average Container Temperature and Shelf Life Consumed over a 9.5 Hour Hypothetical Sales Day

	AVERAGE AMBIENT CASE AIR TEMPERATURE					
	40°F		50° F		60° F	
STOCKING METHOD	Avg. Temp.	Hrs. Lost	Avg. Temp.	Hrs. Lost	Avg. Temp.	Hrs. Lost
Indirect use of ice						
on ice	36.2	14.3	40.2	19.0	40.2	19.0
in ice	34.3	12.4	37.7	15.2	38.0	16.2
in ice, f.bottom	38.2	16.2	45.3	27.6	45.7	28.5
Direct & indirect use of ice in ice, top icing	33.0	10.5	33.3	10.5	33.3	10.5

So long as ice chills indirectly, product temperature will, in part, be determined by case airspace temperature. The extent to which case setting affects product temperature depends upon the particular stocking procedure. Those procedures which compromise the effectiveness of indirect chilling (e.g. on ice method or false bottom method) account for significantly warmer average temperatures and additional reductions in shelf life when compared to more effective stocking procedures.

The top icing method utilized both the direct and indirect effects of ice as a chilling mechanism. When both effects are combined in one stocking procedure, product temperature is minimized, chilling is rapid, there is almost no variation in product temperature, and products remain cold regardless of different ambient case (or room) temperatures. As well, shelf life consumed per clock hour on display is minimized thus affording food retailers the maximum amount of time to sell the product. Whereas the results of indirect uses of ice are partially dependent upon ambient temperature, the judicious, direct use of ice provides identical results regardless of ambient settings. From the standpoint of design and implementation of stocking procedures firm-wide, this is the most significant managerial benefit of direct use of ice.[2]

When compared to other stocking procedures, the false bottom method exhibits the greatest increase in shelf life consumption between 40° F and 60°F; practically doubling the

number of shelf life hours lost per actual display hour. With a slow turning inventory situation, stocking procedures are required which maximize the amount of product sales time. Ironically, the use of a false bottom rapidly reduces shelf life for those food retailers who most need to conserve it.

Shucked Oysters

Molluscan shellfish products present additional safety concerns to food retailers since these products may be consumed raw or without adequate heating. Just as with skinless fillets, the objective of these trials was to quantify temperature changes which jars of shucked meats underwent while on display. These data can then be used to evaluate how well various stocking procedures conformed to regulations which state that molluscan shellfish should be held at temperatures below 40° F.

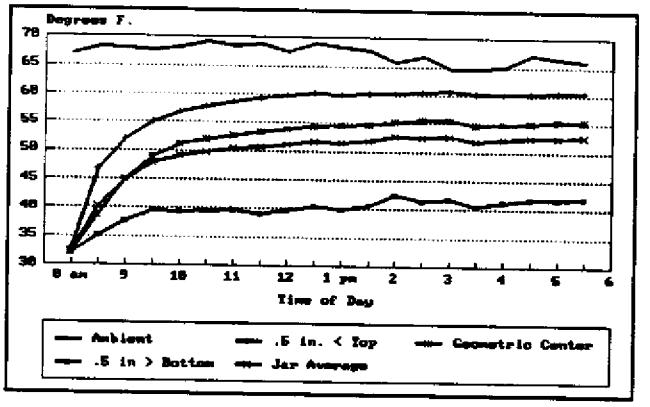
Containers of oysters are indirectly chilled through conduction, with the jar serving as a heat exchanger. However heat exchange potential is significantly compromised when ice contact is lost. Thus, physical placement in bed ice determines conduction (heat removal) efficiency and insures maintenance of low product temperatures. In addition, conduction works only upon contact with container surfaces, so as ice melts away from jar sides, the ability to remove heat is drastically reduced. Therefore the ice surrounding these jars was repacked periodically to eliminate air spaces between jars and ice.[3] The frequency of repacking and replenishing ice is dependent upon ambient room temperature. For example, ice was repacked around jars in the ice-only display case (70° F ambient room temperature) at approximate 1 hour intervals.

All trials were conducted for 9.5 hours. Since quarts, pints and smaller sized packages of shucked shellfish meats are well suited for self service, an ice- only free standing gondola was used for displaying these jarred shellfish meats.

Three stocking options with pint containers were evaluated: (i) jars placed on ice so that only the bottom contacted ice, (ii) jars embedded half in ice, and (iii) jars embedded in ice up to the bottom of the lid. The amount of total jar area contacting ice was (i) 14.5% (9 sq. inches) for jars placed on ice, (ii) 46% (30 sq. inches) for jars embedded half in ice, and (iii) 76% (50 sq. inches) for pints buried up to the bottom of the lid.

Temperature was recorded at three locations along the axis of the jar: (i) .5" below the mouth, (ii) at the geometric center, and (iii) one half inch above the bottom. Experimentation documented that chilling occurred only within that region of the jar which was level with or embedded in ice. Thus, when containers were placed on ice, significant variation was observed among top, middle, and bottom locations over the 9.5 hour trial, with top and bottom locations reflecting a 10° F difference in average values (Figure 9). Note that if average jar temperature is used, the exceptionally high temperatures from the top location are hidden. Since the entire contents are typically consumed, these "hot spots" may compromise product safety since temperatures above 40° F for extended periods of time provide the proper environment for growth of naturally occurring marine pathogens.

Figure 9. Temperature Variation of Shucked Oyster Meats Packaged in Glass Pint Jars, Placed On Ice and Stocked in an Open, Ice-Only Gondola



Regulations specify that jars must be stored upright in drained ice. To facilitate selection, then at least the container top must be visible. As such, stocking procedures should focus on ways to minimize the temperature at the top location over time. Therefore subsequent discussion of product or jar temperatures refer to readings taken at the top location.

Figure 10 presents temperature behavior of glass pints stocked under the three different approaches. When pints are merchandised in an open, ice-only gondola and are stocked such that only the jar bottom contacts ice, temperature increases are rapid. Product temperature increases about 15° F above 32° F within 30 minutes of placement; stabilizing at 60° F within 4 hours on display. By embedding containers half in ice, ice contacts three times as much surface area (46% vs. 14.6%). Despite this, product temperature increases to 50° F within 90 minutes; ultimately stabilizing around 50° F for the remainder of the trial. Even when jars are embedded in ice up to the bottom of the lid, temperature stabilizes around 40° F. Therefore, when such products are displayed in ice only environments, containers should be embedded in ice so only the top of the lid is visible.

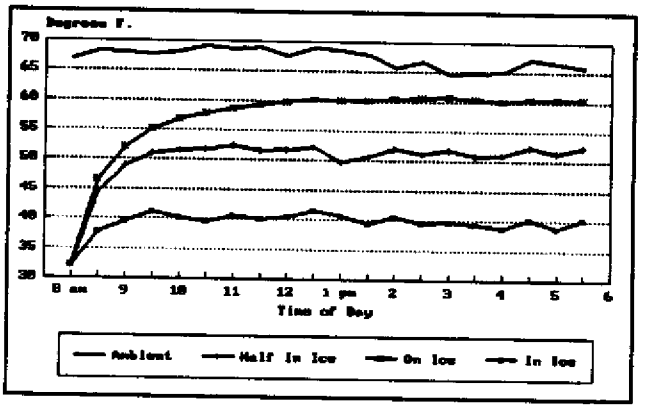


Figure 10. Variation in Jar Temperature Based on Depth in Ice When Stocked in an Ice Only Gondola

Discussion of Oyster Stocking Trials

Consumer packages of oyster meats lend themselves to self service applications regardless of the department orientation. As a result, quarts, pints and smaller sized packages are often displayed in free standing gondolas. Since this type of equipment cannot chill the airspace above the bed of ice, the difference in temperature between incoming product and air is maximized. Unless containers are fully embedded in ice, previous trials suggest that (i) rapid increases in product temperature will occur and (ii) room temperature will determine equilibrium jar temperature for the display period. Ambient room temperatures are high enough to compromise product safety in all but the fastest turning inventory situations (Table 2).

Table 2. Hours Jar Temperature Exceeds 40°F Over a 9.5 Hour Sales Day

STOCKING METHOD	HOURS JAR TEMPERATURE EXCEEDED 40° F
jars on ice	9.5
jars half in ice	9.5
jars embedded in ice	5.0

Use of ice only gondolas should not be curtailed. These vessels provide food retailers with the flexibility needed to merchandise seasonal specials, and provide the shopper with self service convenience. And product temperatures can be easily controlled by embedding jars in ice up to the top of the lid.

CONCLUSIONS

Shelf life of seafood is continuously consumed at refrigerated temperatures, with the amount lost each hour proportional to increases in product temperature. Maximizing remaining shelf life and insuring product safety are best achieved by maintaining product temperatures near 32° F.

Product temperature is ultimately influenced by the amount of heat gained from ambient conditions and the length of holding period. When the time interval between initial stocking and sale is more than 5 hours, seafoods must be insulated from warmer temperatures to control heat gain.

Depending upon case residence times, the amount of shelf life lost can be significant since displayed products typically warm up during the sales day. However, stocking procedures can insulate product from higher ambient temperatures. The four stocking procedures evaluated here fell into three categories: those that work regardless of ambient case settings, those which work under some, but not all ambient settings, and those which do not work under any ambient case settings.

Embedding pans of fillets in bed ice and periodically top icing them quickly stabilizes fillet temperature at 32° F - 33° F, regardless of ambient conditions. Of course, higher ambient temperatures require more frequent applications of ice to maintain optimal product temperatures. Sales flexibility (shelf life) is maximized with this stocking method.

At case settings of 40° F, embedding pans of fillets in ice returns average container temperatures close to products which are top iced. However the beneficial effects of indirect chilling are compromised at higher case temperatures.

The false bottom procedure is often employed by food retailers who need additional time to sell the product. While this approach provides images of abundance, it minimizes the indirect chilling effects of ice, while simultaneously maximizing product surface area exposed to warmer, ambient temperatures. The result is a rapid reduction in shelf life at all ambient settings.

Insuring the safety of shucked molluscan shellfish through proper temperature control is an important consideration for food retailers since consumption of any raw, high protein food such as molluscan shellfish carries an increased risk of food borne illness.

Jars are chilled through conduction, with the container itself facilitating the exchange of heat between product and bed ice. When heat exchange potential is compromised by improper stocking procedures, that portion of the jar not contacting ice responds quickly to higher, ambient temperatures. Thus, in all but the most rapidly turning inventory situations, jars should be embedded in ice up to the top of the lid.

Ice must physically contact the jar. When ice melts away from the jar, its effectiveness in removing heat is significantly reduced. Therefore, to insure contact with jar surfaces, periodic redistribution of ice is crucial when displaying jarred products in ice only equipment.

END NOTES

1. The effectiveness of ice is strictly determined by the manner in which it is used. Ice is most effective at removing heat when it melts over the product. Direct contact with melting ice chills products about 5 times quicker than cold air (1). Melting ice, being such an efficient heat removal mechanism, maintains constant, low product temperatures regardless of ambient air temperature.

   When product and ice are separated (i.e. products are placed in pans and then embedded in bed ice) chilling from ice occurs through conduction, and depending on stocking procedure, creation of a cold air barrier which acts to insulate products from a warmer ambient environment. There are two important considerations in the indirect use of ice. First, stocking procedure (i.e. depth of the pan in ice) determines the extent of indirect benefits. Second, ambient case airspace temperature influences the effectiveness of these indirect means since at higher ambient temperatures the beneficial effects of conduction and insulation may be substantially reduced.

2. Some workers have suggested that placing ice directly on some species of extremely fresh skinless flounder fillets may slightly alter muscle coloration.

3. Flake ice was used in all display trials. As this type of ice melts, it tends to form a solid mass which makes periodic repacking more difficult and time consuming. Since the use of free standing gondolas is predicated on limited attention by department personnel, the use of cube ice is the preferred type of ice. While cube ice can form a solid mass, breaking up these masses can be done quickly and easily so that maximum conduction can occur.

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EXPERIMENTAL SEAFOOD PROCESSING LABORATORY

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INTRODUCTION

Latent resources represent a large and potentially valuable commercial resource in the Gulf of Mexico. The latent resources program conducted by National Marine Fisheries Service (NMFS) has focused on a group of species loosely designated as coastal herrings. The coastal herrings include gulf butterfish, harvest fish, Spanish sardine, scaled sardine, thread herring, round herring, rough scad, bigeye scad, round scad, and chub mackerel. Several investigators (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14) have estimated an annual potential yield of the coastal herrings up to and possibly exceeding 5 million metric tons.

One important component of the latent resources program has included investigations into the handling and processing of coastal herrings and butterfish. Such research is vital to provide information that will enable anyone entering into a fishery for these species to produce and market high quality seafood products.

The seafood industry is the third largest contributor (oil and cars are the major contributors) to the national trade deficit. The industry contributes about $6 billion to the trade deficit. The future of the seafood industry on the Gulf coast and in the rest of the United States lies in the specialized and higher technology in order to be competitive with foreign products.

The seafood industry has had a profound effect on the Gulf coast. Generations of Gulf coast residents have made their living from the sea. With the tremendous latent resources in the Gulf, the seafood industry on the Coast needs to identify problems and opportunities, and develop the ability to further process and market the seafood caught and processed on the coast through innovative technology and strategy, in turn improving the industry trade deficit figure.

APPROACH

In order to better carry out the strategic research, a cooperative Experimental Seafood Laboratory was jointly established by the Agricultural and Forestry Experimentation Station and Cooperative Extension Service of Mississippi State University (MSU) and the NMFS, Southeast Center, Charleston and Mississippi Laboratories of the National Oceanic and Atmospheric Administration (NOAA) with financial support from Jackson County, Mississippi.

The resolution, establishing the Seafood Laboratory, was signed between MSU, NMFS and Jackson County on December 16, 1988 at the NMFS Pascagoula Laboratory in Mississippi (11).

The facility is staffed with senior scientists from NMFS and MSU. In addition to the senior scientists, technical staffs are also being assembled. Scientific and technical support is also available from the adjacent NMFS Laboratory, the Charleston NMFS Laboratory and MSU's main campus.

Research at this laboratory will focus on obtaining information on the composition, uses, handling methods, processing requirements, yields, and quality retention of Gulf of Mexico species to ensure quality and safety of seafood products. Processing and utilization work at this facility will be carried out in close coordination with resource identification, evaluation, and capture technology programs being carried out in the Gulf of Mexico by the NOAA Mississippi Laboratories using the NOAA ship Chapman and other research vessels.

Because of the small size and oily character of many of the latent resources species in the Gulf, special handling and processing techniques will need to be developed before they can be effectively marketed as domestic or export products. Initial work will be devoted to the evaluation of on-board handling techniques
and their effects on the quality of Gulf coastal herring species. Research on production of surimi and other "value-added" new products will follow. Cost analyses and economic feasibility of value-added and diversified new products will be conducted for potential domestic and international markets. The priority for choosing species for study will be established by the level of information on resource availability and harvestability of those species. When technological profiles are complete, the profiles will then be made available in forms needed for decisions by resource managers, fishermen, processors, and consumers of seafood.

The Seafood Laboratory will carry out fishery research in coordination with the emerging aquaculture industry and existing seafood industry in the region. Studies to be undertaken will include assessment of potential product diversification with farm-raised fish and optimization of processing methods to increase production, yields, and export opportunities.

RESULTS AND DISCUSSION

This laboratory is located on land north of the NMFS Pascagoula facility. Building renovations and transfer of over one-half million dollars in equipment from the Charleston laboratory are complete. The Seafood Laboratory has also procured surimi production equipment from a pilot plant located in Reedville, Virginia.

The laboratory will cooperate with the private industry for proprietary research and development by providing unique state-of-the-art equipment and competent scientists. Table 1 shows the equipment available at the Seafood Laboratory.

The Seafood Laboratory will conduct strategic research for the Gulf of Mexico seafood industry. This strategic research will provide specialized new higher technology to improve the value of seafood products landed from the Gulf coast. The research will determine the potential of the Gulf coast seafood industry to identify opportunities for new product development and to add value to processed products. This will help revitalize the Gulf coast seafood industry for better international competition.

Table 1. A list of equipment available at the Seafood Laboratory

ITEMS		
Plate Freezer - Dole	Refiner/Strainer - Ryan RE120	Sample Disintegrator (Stomacher)
Blast Freezer - Hobart	Wash Tanks, Surimi x3	Moisture and Fat Analyzer-CEM
Freezer, Storage - walk in	Dehydrator (Screw Press) - Bibun	pH Meter - Orion
Retort, Steam - Dixie	Scales, several different capacities	Color Meter (reflectance) - Hunter
Boiler/Steam Generator	Centrifuge, Decanter - Bird	Baths, Water Thermostated
Compressor, Air - Gardner	Mixer/Blender (Silent Cutter) - Ryan	Texture Unit
Sealer, Vacuum Cans	Filler, Bag (twin screw) - Ryan	- Rheo-Tex (Punch)
Sealer, Vacuum Pouches	Depositor/Extruder - Autoprod	- Instron Universal Testing Machine
Pump, Vacuum - Kinney	Patty Former - Hollymatic	- Kramer shearpress
Cooler, Storage - Kessel	Batter and Breader - Stein	Microbiology Lab Equipment
Smoker, Torry Mini-Kiln	Fryer, Batch	Freeze-Dryer - Hobart
Dryer, Humidity control	Homogenizer/Disintegrator	Bag Sealer with bag stock - Audion 420
Chiller, Fresh or Sea Water	Grinder, Dry - Reitz	Heat Sealer - Model 62B
Storage Tubs		Distillation Unit with digester and Tubes - Buchi 315
Washer, Fish - Ryan	Cutter/Mixer, Vertical - Stephan	Conductivity Meter - YSI
Sorter, Fish - Petco	Mixer, Batch - Hobart	Dynoscreen Separator
Scaler, Fish - Simco	Tables, Cutting	Freeze Dryer - Virtis
Header, Fish - Lapine	Stuffer, Casing	pH Meter - Fisher Accumet
Gutter, Fish - Lapine	Metal Detector	Pump, Sanitary - Varidrive
Filleter, Fish - Lapine	Meter - Torry Fish Quality	Pump - Alsop Centrifugal
Filleter, Fish herring		Recorder - Linseis
Washer, Fillet - Ryan		Fish Scaler - Hand Operated
Skinner, Filet - Arenco		Waterbath - Cole Parmer mD1 1095-00
Separator, Meat/Bone - Bibun 15"		Vacuum Can Tester - Dun -Rite
Separator, Meat/Bone - Bibun 13"		
Refiner/Strainer - Bibun 420		

The Seafood Laboratory will provide a fish/seafood processing facility for education, training, and technology transfer to interested industry. The facility will offer the opportunity for generation and dissemination of information resulting from the research on the marine resources in the Gulf of Mexico. These activities will be closely coordinated with those in the other areas which are being carried out by academic, government, and industry organizations in the region.

Coordination of research at the Seafood Laboratory with an overall strategic economic development plan in the region will identify problems and opportunities, and create consensus among entrepreneurs and the seafood industry. With the existence of marine based industry, new jobs and a greatly enhanced economy are possible through the coordination initiative. In order to achieve success, an approach involving all levels of government and the private sector is essential.

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ABSTRACTS

RETAIL OVERVIEW OF THE FDA/NOAA FISH AND FISHERIES PRODUCT INSPECTION PROGRAM

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FMI strongly endorses the voluntary FDA/NOAA inspection program for seafood. This initiative will encompass seafood from point of catch/harvest to the ultimate consumer, and will include retail food store operations. The inspection system will be based upon the Hazard Analysis Critical Control Point (HACCP) concept, and will include both economic and food safety/hygiene concerns. The new program will provide for the use of official seals that indicate federal compliance and can be used as an effective way to market seafood. FMI believes that this program will quickly realize consumer acceptance and assurance that fishery products purchased from the retail establishment will meet their expectations for safe, wholesome, quality seafood which is properly labeled. Nationwide coverage is anticipated.

STATUS: GULF AND SOUTH ATLANTIC FISHERIES DEVELOPMENT FOUNDATION, INC.

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(INVITED PRESENTATION)

TOXIC FRACTIONS OF MULLET (MUGIL CEPHALUS) VISCERA

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Four cases of human intoxication resulting from consumption of whole, grilled mullet occurred in California in 1990. The symptoms reported were consistent with those caused by marine polyether toxins such as ciguatoxin or brevetoxin. Fractions of mullet viscera were found toxic in a bioassay; however, the nature of the toxic component is unknown. Although the incidence is rare and limited, the consequences imply the necessity for appropriate care in procurement, distribution records, labeling and consumer education relative to whole fish marketed with viscera intact.

RECENT ADVANCES IN CIGUATERA FISH POISONING RESEARCH

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Ciguatera, an illness caused by consumption of a variety of tropical and subtropical species of fish, remains a serious public health and economic problem in many areas of the world. It has been cited as the most frequent cause of illness due to seafood toxins in the United States. Once thought to be caused by one or possibly two toxins (ciguatoxin and maitotoxin) produced by a benthic dinoflagellate (Gambierdiscus toxicus), it now appears to involve a number of distinct toxins which are elaborated by several species of dinoflagellates. Recent studies examining these toxins and species will be discussed.

GAS CHROMATOGRAPHIC DETERMINATION OF THE VOLATILE AMINES DMA AND TMA IN SEAFOOD PRODUCTS: PROBLEM AREAS AND SOLUTIONS

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The gas chromatographic method developed by Lundstrom and Racicot (JAOAC (1983) Vol. 66(5) 1158-1163) provides a rapid and easy technique for the simultaneous determination of diethylamine and trimethylamine in seafood products. Modifications have been made to the original method which enhance the method's utility, accuracy, and safety. The internal standard used in the original method (n-polyamine) reacts with formaldehyde present in some types of samples. This results in the formation of a new compound with a concomitant reduction in the peak area of the internal standard, causing erroneous quantitation of DMA and TMA. The substitution of diethylamine as the internal standard obviates these problems. Concerns over potential exposure of laboratory workers to benzene, the organic solvent used to extract the amines from the aqueous phase, and over problems in the disposal of the hazardous benzene waste led us to evaluate possible substitutes. n-Hexane and n-Amyl Alcohol have proved to be acceptable substitutes. The extraction of the amines from muscle tissue using dilute perchloric acid (6%w/v) also was of concern since handling of perchloric acid (particularly in a concentrated form) was a potential hazard that required the use of a special perchloric acid fume hood. The use of trichloroacetic acid was found to be a acceptable substitute. The utility of the original method was also extended by incorporation of a step to allow measurement of trimethylamine oxide after reduction to TMA using titanous chloride.

USE AND POTENTIAL USE OF LACTIC ACID AND LACTATES IN FISHERY PRODUCTS

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Lactic acid and/or lactates can extend shelf-life of fish products and control the growth and toxin formation of pathogenic bacteria, including Clostridium, Listeria, Pseudomonas, Staphylococcus, etc. Specific applications for lactic acid (pH <5) as a surface treatment and for sodium and potassium lactate (pH=7) as ingredients in processed and fresh fish will be reviewed.

COMPARISON OF NUTRIENTS IN FARMED AND WILDFISH AND SHELLFISH

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In order to provide additional data on fish and shellfish for tables of nutrient composition, USDA investigated the nutrient profile of wild and cultivated channel catfish, rainbow trout, coho salmon, Eastern oysters, red swamp and white river crayfish. Samples were harvested at two different seasons from the wild and at peak production from cultivated environments and were analyzed raw and cooked for proximates, cholesterol, fatty acids and ten vitamins. General findings indicated that cultivated fish had more fat than wild fish and that wild and cultivated oysters and crayfish were similar in nutrient content.

INHIBITION OF FISH HEAT-STABLE PROTEASES BY ALPHALIN

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The protein α_2-macroglobulin, commonly known as alphalin, occurs in significant quantities in blood plasma and egg albumin (ovomacroglobulin). The latter two materials are known to exhibit inhibitory activity against the heat-stable proteases occurring in several fish species, from differing sources (gut enzymes, parasitic attack, or endogenous to the muscle). This protein, although itself heat sensitive, may survive the spray drying process used to prepare certain high functionality dried egg albumin and plasma products. A semi-pure preparation of alphalin from beef plasma was found to be equally effective (at much lower usage levels) to beef plasma and egg white in preventing the breakdown of myosin in surimi of several species due to heat-stable proteases. Commercial production of alphalin, as a by-product of an existing process for production of biomedical products from bovine plasma, may yield a cost-effective source of this inhibitor for the surimi industry.

USE OF CARRAGEENAN BASED STABILIZERS FOR STORAGE, STRUCTURE AND IMPROVEMENT OF SURIMI AND FABRICATED SEAFOOD PRODUCTS

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Current market demands upon seafood stocks utilized for fabricated seafood products have placed a strain on the supply - demand quality balance of raw material (surimi) to final product (kamaboko). Alternate species and lower quality fish pastes contribute quality problems during their storage and subsequent use. Carrageenan based stabilizers can help to improve both the raw material and finished product quality.

PRELIMINARY EVALUATION OF BACTERIAL SPOILAGE OF PROCESSED AT-SEA SCALLOP MEATS, PLACTOPECTEN MAGELLANTICUS

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It is commonly noted that processed at-sea scallop meats stowed on ice may become "yellowed" by the time of off-loading. This yellowing, which is not pronounced during the summer, creates a less desirable product. Although, the cause of yellowing has not been formally examined, it has been hypothesized as the result of improper chilling and icing of bagged meats during on-board stowage. Most food scientists and industry personnel believe bacteria are implicated, but some have not ruled out the possibility of a non-bacterial enzymatic process. Our initial approach was to measure bacterial surface counts, meat ph, visual and organoleptic qualities, and meat fluorescence during iced storage. Various experimental treatments, including bag material, washes or processing aids were examined under both laboratory and commercial conditions. A simple method was developed to assay changes in surface bacterial numbers through recovery and enumeration of bacteria from known areal portions of scallops storage bags. Excised pieces of bag material were vortexed in sterile seawater, diluted, spread plated on a medium made from scallop meats, and incubated at 4°C before counting. During iced storage at 2-3°C under commercial stowage conditions, surface ph, bacterial counts and meat fluorescence reflected changes in product quality that implicate bacteria as agents of spoilage.

PRINCIPLES OF PASTEURIZATION, MINIMAL THERMAL PROCESSING AND STATUS OF SOUS VIDE

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Moderate temperature thermal processing is used to extend the refrigerated shelf-life of certain pre-packaged seafoods. The relatively mild heating conditions result in color, texture, and flavor characteristics which are similar to "fresh" products, but with greatly extended shelf-life. While almost any seafood can be moderately heat processed, until recently only blue crabmeat (Callinectes sapidus) has received significant attention. Other internal temperatures and F-values for each batch and permits operators to identify potential problems or the impact of alternative processing procedures.

DESCRIPTION OF A MICROPROCESSOR CONTROLLER AND REPORTING SYSTEM FOR THERMAL PROCESSING

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Processors and regulators require verification that minimum processing standards are attained. A thermal process controller system has been developed which assures that targeted processing parameters are achieved. This is an improved, commercial version of an earlier prototype. The desired accumulative F-value (microbial lethality) and process parameters related to heating and cooling rates are programmed into the microprocessor. If desired, each batch can be monitored in both the crabmeat and waterbaths; alerting the operator to irregularities. Since the controller is processed-based, a desired F-value can be achieved regardless of operating conditions. For example, the heating time is precisely extended to compensate for temporary loss of steam or the use of a slow heating container. The unit generates printed reports that verify time/temperature histories, lethalities and batch information.

SURVIVAL OF PATHOGENS IN MINIMALLY PROCESSED AND PASTEURIZED REFRIGERATED SEAFOODS

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Most pathogens are heat sensitive and can be destroyed by low to moderate heat. The heat resistance of various non-spore forming pathogens at 65.5°C (this represents sous vide processing temperatures) may range from 2.8 seconds for Vibrio parahaemolyticus to 50 seconds for Listeria monocytogenes. At 85°C (the pasteurization temperature of crabmeat) D values for non-spore forming pathogens is less than 0.2 seconds. D values for psychrotrophic strains of Clostridium botulinum (type E) range from 0.2 minutes at 85°C to 251 minutes at 65.5°C. Psychrotrophic pathogens will not survive crabmeat pasteurization temperatures but may survive certain sous vide processes.

FACTORS LIMITING THE SHELFLIFE OF PASTEURIZED CRABMEAT

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Despite the excellent health safety record of pasteurized crabmeat, regulatory officials have expressed concern at the diversity of methodologies currently in use. Researchers at Virginia Tech have analyzed numerous commercial systems and have observed large variations in crabmeat heat exposures. Shelf-life was found to range from 6 weeks to 24 months or more. When they occur, problems most often relate to, 1) slow cooling, 2) leaky container seams, 3) insufficient heating, and 4. high initial counts of certain bacteria. Conditions that greatly affect these factors include water bath circulation patterns, initial meat temperature, and the adoption of HACCP-like plans to mitigate contamination and seal integrity problems. Processors and regulators must understand that process lethalities define anticipated shelflife, and that due to the principles of microbial survival, irregular spoilage patterns should be expected towards the end of shelflife.

FREEZING OF BLUE CRAB MEAT - A REVIEW

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Despite nearly twenty years in developmental efforts, product market forms for blue crab remain primarily hand-picked, fresh or pasteurized. This paper reviews commercial blue crab freezing practices, efforts to market partially cooked whole and crab body parts, and research on frozen crab cores destined for hand-picking operations.

PRELIMINARY ASSESSMENT OF FROZEN BLUE CRAB MEAT WITH AND WITHOUT CRYOPROTECTANTS ADDED

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This study assesses physical, chemical and sensory qualities of frozen (liquid nitrogen) blue crab meat with either a blend of sucrose/sorbitol/phosphate or polydextrose present. Comparison is made with pasteurized crab meat and frozen product with no added cryoprotectants. Preliminary assessment is made on samples stored over four months.

ENZYMIC HYDROLYSIS OF $_k$ CARRAGEENAN

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Enzymic hydrolysis of $_k$ carrageenan was investigated. Pseudomonas carrageenovora isolate used in this study grew very well at room temperature (GT=0.80 hr), but production of the extracellular $_k$ carrageenase was low to negligible. At 6°C the growth rate was significantly slower (GT=2.61 hr), but enzyme production was consistently high. Ammonium precipitate of the cell free fraction had good activity (about 140 u/ml) and was relatively pure (0.6 mg/ml total protein content). The enzyme is somewhat stable with pH and temperature optima similar to those in the literature, but behavior of the preparation on charged columns was different. Energy of activation of the crude preparation was about 2.5 Kcal.

QUALITY ASSESSMENT OF HYBRID STRIPED BASS FOLLOWING FROZEN STORAGE

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This report is the second of a two-part study designed to assess the effects of product form and processing techniques on the quality of hybrid striped bass following refrigerated and frozen storage. It will focus on the quality assessment of frozen striped bass using objective and sensory evaluation techiques.

IMPORTANT CONSIDERATIONS IN DEVELOPING HIGH w-3 FATTY ACID CANNED MACKEREL

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The study was designed based on an idea of deliverying a high omega-fatty acid food in a more palatable form with approach of organical seasoning of high omega-fatty acid mackerel. The proper combination of brining, formulation and seasoning yielded an organoleptically superior product to the commercial ones. Important factors found were freshness and fat content of the fish, brining condition, the form of fish, and formulation of organic broth and seasonings.

PROCESSING EFFECTS ON PSP TOXICITY IN SURF CLAMS

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Surf clams (Spisula solidissima) having an initial whole animal toxicity of 2340 µg/saxitoxin-equivalents per 100g tissue were processed by standard industrial methods into strips and mince. Following processing, the strips and mince each had a toxicity of approximately 300 µg/100g tissue, while the waste material (digestive glands) assayed at 5400 µg/100g. The edible tissues were further treated by alkali dip, freezing, or canning to evaluate their effect on toxicity.

AN AUTOMATED ELISA SYSTEM FOR CROSS-REACTION TESTING OF MONOCLONAL ANTIBODIES TO COMMERCIAL SEAFOOD PRODUCTS

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Laboratory robotics are used to characterize species identification monoclonal antibodies against hundreds of commercial seafood species. A Zymark[1] microliter plate system performs all steps of the ELISA procedure testing 24 monoclonal antibodies against 96 antigens. Assay plates are read photometrically, and the data are transmitted via a serial interface to a spreadsheet. A threefold increase in throughput has been achieved over manual performance with a concomitant improvement in confidence levels of the data. Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

(66) USE OF AN ACID PROTEASE FOR HYDROLYSIS OF GROUND COD FRAMES

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Rapid acidification of fish by-products will stablize the material microbiologically. An acid protease was evaluated with protein substrates and ground fish for response to pH, temperature effects and changes induced in viscosity, soluble nitrogen, and size distribution of products. Optimal pH was 3.5; optimal temperature was 60°C.

(77) SUPPRESSION OF MELOIDOGYNE JAVANICE IN SOIL AMENDED WITH BLUE CRAB SCRAP COMPOST

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Two greenhouse pot experiments were conducted to determine the effect of compost made of blue crab (Callinectes sapidus) scrap and cypress (Taxodium distichum) chips on the reproduction of Meloidogyne javanica on tomato (Lycopersicon esculentum). Foliar plant weights and root weights were significantly higher in crab compost than in the 0% compost level. Crab compost significantly reduced number of egg masses produced by M. javanica.