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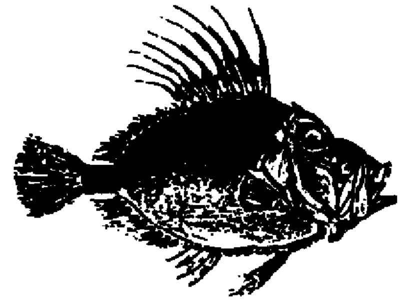

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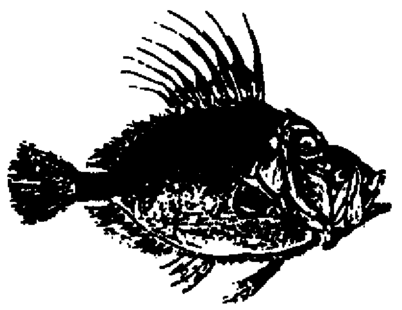

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SIXTEENTH ANNUAL CONFERENCE

TROPICAL AND SUBTROPICAL FISHERIES TECHNOLOGICAL CONFERENCE OF THE AMERICAS

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ENVIRONMENTAL QUALITY AND FISHERIES

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Fisheries resources depend upon adequate environmental quality to support their habitat and production. A majority of the species rely upon estuarine habitat for some part of their life cycle. In the southeastern United States, about 90 % of the commercial landings and about 70 % of the recreational landings require estuarine habitat during their life history.

Water quality is a major concern for estuarine habitats. The trend during the latter half of this century is for the increasing population of the United States to become more concentrated in the coastal states. Increased run-off from the estuarine watershed and outflow from increasingly larger point sources carry nutrients into estuarine ecosystems, leading to eutrophication that degrades habitats through algal blooms, anoxia and changes in primary productivity. Toxins entering estuarine habitats cause fish kills, reductions in reproduction, diseases and contamination of seafood. Changes in and use on estuarine watersheds result in non-point sources of nutrients, pathogens and organic contaminants affecting habitat and fisheries productivity. Physical changes in stream flows, water exchange and habitat morphology degrade habitat.

This paper will discuss a selected resume of environmental quality and illustrate trends as they affect fisheries productivity in the important estuarine habitat.

HYPOXIA

Hypoxic conditions (i.e., low dissolved oxygen and/or no available dissolved oxygen) occur in estuaries as the result of a complexity of conditions. In general, hypoxia occurs during times of low flow, calm meteorological situations, where a source of organic decomposition takes up the available dissolved oxygen. The upper reaches of estuaries, where the flow of river discharge is under-laid by a tongue of brackish water from the saltwater end of the system, suffer from periods of hypoxia. The resulting stratification, unbroken by physical conditions, prevents re-oxygenation from the atmosphere and the bottom water simply loses its dissolved oxygen.

Hypoxic conditions are made larger and more recalcitrant by the inflows of organic materials from upstream that sink into the quiescent estuarine waters. Algal blooms, from excess nutrient inputs, are major contributors to the process.

The impact of hypoxia is mainly two-fold. Higher aquatic organisms such as fish simply cannot live in the absence of adequate levels of dissolved oxygen--resulting in fish kills if populations of fish are "caught" in an area where hypoxia is initiated. A more insidious phenomenon is the change in oxidation-reduction potential of surface sediments during times of low dissolved oxygen, resulting in the release of organics and toxins that have been stored in the sediments. These releases can cause toxicity, diseases, mycosis and/or physiological stresses.

Apparently, hypoxic conditions have existed in estuarine headwaters for generations. The management question rests on the issue of whether or not the conditions for initiating hypoxia have become worse with the increasing trend of cultural eutrophication. Determination of possible trends is hindered by the problem of measuring hypoxia accurately enough to render judgement. Conditions leading to hypoxia can be initiated in very short time intervals (as quickly as overnight) and the situation goes largely undetected until something like a fish kill is observed--which in itself can contribute to anoxic conditions.

ALGAL BLOOMS

Blooms of certain species of algae result when excess nutrients become available and physical conditions permit. The problem is generally made worse when the process of a bloom development excludes the species of phytoplankton generally used by the estuarine food chain and favors an almost pure culture of blue-green algae. The onset of algal bloom is a complex combination of nutrient inflows and hydrodynamic conditions. When the flow of water slows as a stream enters the tidal reaches, opportunistic blue-green algae dominate the phytoplankton community--forming a bloom.

Other blooms, such as "red tide", may be the result of unique oceanographic conditions concentrating certain populations of nuisance algae in specific geographic situations. The red tide episode of 1987 in North Carolina coastal waters emphasized just how intricate the estuarine dynamics are with ocean hydrodynamics.

More recently, unique dinoflagellate blooms in estuarine habitats have been discovered. Here, under certain physical conditions, dormant toxic dinoflagellates may be released from their sediment storage and released to the water column. Instant fish kills have resulted from such situations.

The potential for estuarine algal blooms have been somewhat reduced by the management schemes of reducing nutrient inflows from point sources and run-off. However, recent increases in acid rain

along our coastline has boosted the potential of algal blooms in the coastal ocean. Acid rain, while it offers little threat of pH changes in the highly buffered salt water, contributes excess nitrogen loading in the area of the system where natural processes have removed much of the land source nutrients through biological filtering.

PATHOGEN/TOXIC CONTAMINATION AND SHELLFISH CLOSURES

One of the more insidious problems in estuarine ecosystems, the presence of pathogens and toxins present potential contamination of seafood that impacts public health. Management agencies monitor the presence of pathogens, which result in closures of contaminated areas to the harvest of seafood to protect the public health. Concentration of pathogens and toxins, usually in shellfish, can result in levels directly harmful to those who may consume the seafood. In some areas, large acreages may be closed to harvest (in North Carolina, for example, the closed areas exceed 300,000 acres), removing productive ecosystems from harvest and lowering the potential catch available to the fishing industry.

Sources of pathogens and toxins in estuaries include poorly functioning septic systems, agricultural activities, land run-off, marinas and municipal outfalls. Non-point sources are the most difficult to assess and manage--and unquestionably represent the most widespread source of contaminants. Many are attached to sediment particles and become stored in the estuary as sediments accumulate.

When seafood is contaminated and/or not harvested, considerable adverse economic impact occurs. The safety of seafood for public consumption is of high national concern. Research indicates that contaminated seafood is usually the result of contaminated environment rather than unsanitary handling and processing. Surveys confirm that sales of seafood sharply decline in areas where contamination has been reported regardless of whether the seafood is actually contaminated.

SUBMERGED AQUATIC VEGETATION

One of the most important ecosystems in coastal waters, submerged aquatic vegetation serves as an obligatory habitat for many estuarine species. The tremendous productivity, protection and the large surface area provided by grass blades render submerged aquatic vegetation a critical habitat. Destruction of these underwater meadows is a high priority concern to fisheries managers.

Water clarity is of paramount importance to the well-being of submerged aquatic vegetation. In shallow, protected embayments light penetrating to the bottom is necessary for growth of rooted aquatic vegetation. The most important problem is thought to be increases in turbidity of coastal waters. Dredging, filling and other bottom disturbances (e.g., mechanical harvest of shellfish,

trawling, etc.) increase turbidity in the estuary. Fringing wetlands act as natural filters of sediments washed from the land, but their destruction removes that natural safeguard. Increased nutrients lead to increased phytoplankton growth, which in turn decreases light penetration leading to decreased production of submerged aquatic vegetation.

DECLINE IN FISHERIES

There is a general opinion within the fishing industry and among recreational fishermen that fishing has declined in recent years. Although, fisheries statistics suffer from many forms of inaccuracy, the catch data reflect dramatic declines in certain species. Recreational fishermen declare that "fishin ain't what it used to be", with considerable surveys to back it up. The bottom line is that fishing effort has dramatically increased--with more efficient gear, larger nets and boats, more recreational fishing days per year. Overfishing has definitely affected many species, resulting in reduced stocks and declines in recruitment.

Man's activities have affected fishery stocks in many other ways as well. Environmental quality has had tremendous impact, especially in the primary nursery areas. Primary nursery areas are typically on the upper ends of estuaries where land use changes have had their largest effects. Drainage of large acreages for farming, forestry, development and the infrastructure of cities and towns have impacted large segments of our estuaries. Barriers on the streams that nurture the estuarine ecology have interfered with migration patterns of fish, inflows of nutrients and vitamins, and physical circulation of water. All of these activities have changed the basic productivity of the habitat so necessary for the recruitment of fishery stocks.

The different interests of man result in conflict for many species of fish. Often, commercial and recreational fishermen are interested in same species, resulting in conflict about allocations and management schemes. By-catch, the taking of untargeted species in some fishing activities (e.g., trawling for shrimp, pound-netting for fish, etc.), has become a tremendous social and economic conflict in most areas. Allocation of catch among fishing interests leads to considerable argument, often resulting in over-fishing some species and interfering with realistic management schemes to enhance the overall fishery.

FISH AND SHELLFISH DISEASE

There have been recent proliferation of diseases, sores and kills at many locations in estuarine systems along the coastline. Ulcerative mycosis, red sore and other septicemic infections have affected several species of fish and shellfish, rendering them unacceptable for recreational catch and sales. More recent discoveries of dinoflagellate toxicity have explained many fish kills.

All of these maladies have been attributed to declines in water quality, but the cause-and-effect relationships have so far eluded explanation. Recent experiments indicate that the causes are extremely complex and pin-pointing exact causes await further research. Even if the causes of fish and shellfish disease in natural systems can be described, it is doubtful that prophylactic relief is forthcoming.

UPTAKE AND RESUSCITATION OF VIABLE BUT NON-CULTURABLE <u>VIBRIO VULNIFICUS</u> BY <u>MERCENERIA CAMPECHIENSIS</u>

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<u>Vibrio vulnificus</u> is an autochthonous bacterium of marine and estuarine environments of temperate and tropical climates. Seawater and seafood has been implicated as vectors in the transmission of the organism causing primary septicemia and wound infections in humans. While wound infections can occur in healthy individuals upon contact with shellfish or the marine environment; septicemia primarily affects compromised hosts with underlying liver diseases after the ingestion of raw shellfish (18, 19, 8). Although isolated especially from the warmer waters of the southeastern and Gulf coasts (10, 14, 20), Vibrio vulnificus has been reported in seawater and shellfish from the East Coast (16) and the West Coast (9). During cold winter months, the occurrence of Vibrio-associated illnesses is lowest and V. vulnificus is not recoverable from estuarine environments (10). Now, it has been well established that the inability to recover these organisms on laboratory culture media is not totally due to cell death, but due to entry of the cells into the viable but nonculturable (VBNC) state (5, 12, 2). It has been shown by several researchers that V. vulnificus VBNC state could be induced upon incubation at low temperatures (2, 12, 15). These cells undergo a reduction in size and lower their metabolism to a minimum as a survival strategy against adverse environmental conditions. These cells cannot be recovered by conventional laboratory techniques but their viability could be detected through several indirect procedures, such as fluorescent antibody staining (3) and yeast extract-nalidixic acid incubation (11). In 1988, Colwell (4) reported that non-recoverable strains of V. cholerae caused typical cholera clinical symptoms when ingested by human volunteers and the organism could be recovered from their stools. This confirmed the suspicion that non-culturable cells of some pathogenic bacteria could repair themselves, proliferate, and cause disease. Birbari and Rodrick (2) were the first to show that VBNC cells of V. vulnificus are capable of self repair in artificial seawater, at room temperature to become platable again using standard methods. Other researchers later on obtained similar results (15). However, resuscitation in marine animals due to temperature increases has not been reported and needs to be experimentally demonstrated. It was the purpose of this study to determine whether VBNC cells of <u>V. vulnificus</u> could be resuscitated and recovered after uptake by the clam (Merceneria campechiensis).

MATERIALS AND METHODS

Bacterial strains and cultures

Strain CVD 713 of V. vulnificus was used throughout this study and was kindly supplied by Glenn Morris, Jr., of the Center for Vaccine Development, University of Maryland. This strain has a TnPhoA transposon insertion that codes for kanamycin resistance and production of alkaline phosphatase. This strain is recovered on regular V. vulnificus media and in addition can be selectively grown to produce blue colonies on a nutrient medium containing kanamycin and the dye 5- Bromo-4- chloro-3- indolyl phosphate (TN agar). The use of this strain allows the distinction of experimentally introduced cells from background V. vulnificus that might be present in the clams. Cultures were maintained on 1% alkaline peptone agar containing 2% NaCl at room temperature.

Preparation of VBNC V. vulnificus microcosms

Artificial seawater (ASW) microcosms were prepared as previously described (2). However, stock cultures of Vibrio vulnificus strain CVD 713 were used and incubated at 4oC. The culturability profile of this strain was monitored over time (Fig. 1) to determine whether it was different from strain 7184 that was used in the previous study. Total bacterial numbers were determined using the acridine orange direct count (AODC) (7) whereas the direct viable count (DVC) method of Kogure et al. (11) was used to measure the number of actively metabolizing cells. Culturable counts were determined by plating on the non-selective HI agar, the vibrio selective thiosulfate-citrate-bile salts-sucrose (TCBS) and the Vibrio vulnificus-selective cellobiose polymyxin colistin (CPC) agar (13) in duplicate. TN agar media was also used to detect CVD 713 V. vulnificus strain.

When the organisms became non-recoverable on solid media, after 4 weeks, three 10 ml aliquots from the microcosms were inoculated into three tubes of double strength alkaline peptone broth (DS-APB) (10 ml each, pH 8.4). When these APB tubes were all negative after 24 h at 37oC, the microcosms were presumed to be in the non-culturable state.

Preparation of clams and aquaria

Clam specimens (Merceneria campechiensis) were obtained from Apalachecola (Leavins Seafoods), Florida and transported in cooled containers. The clams were allowed to acclimatize in 150 L water tanks (aquaria) for several days. The holding tanks contained dechlorinated tap water to which seasalt was added to the required salinity (30 ppt). Seawater in each tank was recirculated by a Magnum 330 pump (Marine Aquarium Products) through a pleated cellulose filter to remove suspended particulate matter. The system was connected to a 35 W UV light chamber (Aquanetics Systems), in series, as the disinfecting agent. Clams were fed phytoplankton consisting of Chlorella and Chlamydomonas by slow drip infusion into the holding tanks at a rate of 10 ml/min. The algae were grown at 25oC in flasks containing F/2 media (6) under fluorescent lighting for 12/24 h with constant aeration.

Inoculation of clams with VBNC V. vulnificus by natural feeding

The overlaying seawater and clams were examined at the beginning of the experiment to determine background levels of V. vulnificus (onto TCBS, CPC, and TN) after streaking from positive MPN tubes (alkaline peptone broth, APB). One L of algal culture (of 2 x 106/ml) was added to three hundred ml of the VBNC V. vulnificus microcosm (3.5 x 104/ml viable cells) and divided into two equal portions. Each portion was added to one

of the two aquaria containing clams by slow drip infusion (10 ml/min.). The clams were allowed to feed for 5 h, during which the UV and recirculating systems were turned off. Following uptake of the VBNC cells, 0-time seawater and clam samples were collected from both aquaria. Experimental clams were maintained in recirculating aerated seawater, whereas control clams were maintained in recirculating aerated and UV treated seawater. Samples of clams and seawater were examined from both aquaria after 24 h, 48 h, 4 and 6 days, as described above. Vibrio-like MPNs and resuscitated V. vulnificus CVD 713 were determined from MPN positive tubes by streaking onto TCBS, CPC, and TN agar media.

Inoculation of clams with VBNC V. vulnificus by injection

Clam specimen were injected with 4 ml each of the ASW microcosm containing non-culturable V. vulnificus cells (3.5 x 104/ml viable cells), through an incision made using a file. These clams were divided into two groups, one group was stored at ambient air temperature (25oC) and the other was refrigerated (4oC). Samples were examined at 0, 24, and 48 h. The whole contents of the shell were homogenized in an equal weight/volume of 1% peptone and streaked onto TCBS, CPC, and TN agar media. The homogenate was also inoculated into MPN alkaline peptone to determine vibrio-like numbers, according to standard methods (1) and positive tubes were streaked onto selective media (TCBS, CPC, and TN agar).

It was demonstrated prior to these experiments that VBNC cells of strain CVD 713 of V. vulnificus has the ability to resuscitate similar to strain 7184 that was used earlier (2). The two resuscitation-in-the-clam experiments were started at the same time. Simultaneously, a sample from the VBNC microcosm was transferred to a separate glass tube and incubated at room temperature to examine the resuscitation ability of the batch employed.

RESULTS AND DISCUSSION

After 4 weeks of incubation at 4°C V. vunificus CVD 713 became non-culturable (VBNC) (Figure 1). The cells showed a slow decrease in viable counts (DVC) and a rapid loss of culturability on agar media (HI, TCBS, and CPC), whereas the total cell number (AODC) remained rather constant.

The VBNC microcosm sample that was incubated at room temperature resuscitated after 24 h and could be recovered on HI, TCBS, CPC, and TN agar media (table 1). This was consistent with previous studies showing resuscitation of V. vulnificus non-culturable cells upon incubation in the same ASW medium at room temperature (2, 15).

V. vulnificus CVD 713 was recovered from clam specimens that were inoculated with the VBNC organisms through natural feeding after 24 h. VBNC cells were resuscitated inside the clam tissues in the experimental group (no UV treatment) but not in the control group (with UV treatment) (table 1). The cells regained their culturability and were recovered on agar media (TCBS, CPC, and TN). However, V. vulnificus was undetectable in the overlaying seawater of both aquaria over the period of the experiment. V. vulnificus failed to be recovered later in the experiment and this was attributed to the low initial inoculum numbers.

Inoculated clams that were incubated in the UV treated aquarium (Control) did not show any resuscitation and V. vulnificus could not be recovered at any time throughout the experiment (table 1). This might have some implications favoring the use of shellfish depuration. However, this needs further study as previous reports have shown a quick depuration by shellfish of experimentally inoculated microorganisms but stable levels of

naturally occurring V. vulnificus (17). A similar finding has been made by Groubert et al. (in publication) for V. vulnificus strain CVD 713 in oysters.

Clams that were injected with VBNC cells also allowed resuscitation of the bacterium when incubated at room temperature after 48 h (table 2), but not after only 24 h. V. vulnificus could not be recovered from clams that were incubated at refrigeration temperature (4oC). Clam homogenates were positive on agar media plates (TCBS, CPC, TN) both when streaked directly and when streaked from inoculated positive MPN tubes (APB).

CONCLUSION

Viable but non-culturable cells of V. vulnificus retain their ability to resuscitate even after they are taken in by the clam. VBNC cells were introduced into the clam (Merceneria campechiensis) through natural feeding and injection and were incubated at room temperature. V. vulnificus became culturable again and were recovered from the clams after 24 and 48 h on various nutrient and differential media. These results might have serious implications on the shellfish industry storage requirements as temperature abused product might favor the resuscitation, and consequently the multiplication, of originally non-recoverable organisms. These findings might explain previous anecdotal reports by various investigators that V. vulnificus was undetectable in freshly harvested oysters, but at a later time could be found and sometimes in high numbers.

Figure 1. Survival of CVD 713 V. vulnificus in Artificial Seawater Microcosms upon Incubation at 4 °C.

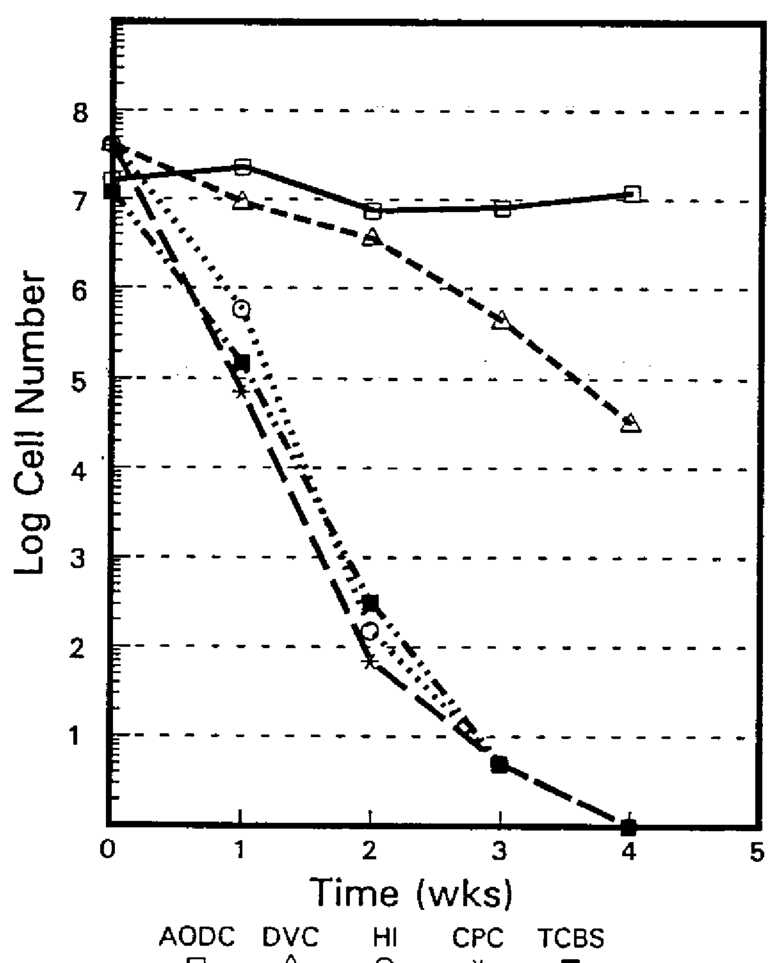

Table 1. Clams inoculated with VBNC V. vulnificus CVD 713 by natural feeding for 5 h in two ASW aquaria. Control clams were allowed to sit in filtered UV recirculating water . Experimental clams were allowed to sit in filtered recirculating water without UV treatment[a].

Treatment	V. vulnificus Recovery
Control	
0 Time	-
1 Day	-
2 Days	-
4 Days	-
6 Days	-
Experimental	
0 Time	-
1 Day	+[b]
2 Days	-
4 Days	-
6 Days	-

[a] Overlaying seawater samples remained negative on TCBS, CPC, and TN agar media throughout the experiment.

[b] Clam homogenates were positive when streaked directly onto TCBS, CPC, and TN and upon streaking of positive MPN tubes (APB) on the same media.

Table 2. Clams injected with VBNC V. vulnificus CVD 713 and incubated at ambient room temperature or 4°C.

Treatment	V. vulnificus Recovery
Refrigerated for 24 h	-
Refrigerated for 48 h	-
Incubated at RT for 24 h	-
Incubated at RT for 48 h[a]	+
Microcosms incubated at RT for 24 h[b]	+

[a] Clam homogenates were positive upon direct streaking on TCBS, CPC, and TN agar media and upon streaking from positive MPN tubes (APB) on the same media.

[b] Microcosms were streaked onto TCBS, CPC, and TN agar media.

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DISTRIBUTION OF VIBRIO VULNIFICUS IN OYSTERS (CRASSOSTREA VIRGINICA) TISSUES DURING ARTIFICIAL INOCULATION AND DEPURATION

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Oysters obtain their food by pumping large volume of water by ciliary action over the gills, which function as a sieve to remove particulate material including microorganisms. The filtered particles then become enmeshed in a mucus material which is continually secreted by the oyster during the pumping action. The mucus coated material is directed by the cilia of the gills toward the mouth of the oyster, where it is ingested or directed to the exterior and eliminated as pseudo-feces. Waste material from the alimentary tract is discharged as feces in the form of a fine, mucous thread (3, 4). Some ingested microorganisms become concentrated in the gut or other tissues, as a consequence of the filter-feeding system.

Oysters take in Vibrio vulnificus as a result of the normal filtration process (7). Commercial depuration has been shown to have little effect on the removal of this pathogen from oysters. Studies have shown that Vibrios may even multiply in depuration shellfish tank water and pumping systems (1, 2, 6). From 1975 to 1989, there have been 115 cases of shellfish-associated Vibrio vulnificus infections (9). Vibrio sp. are found in the organs of the oyster associated with the digestive tract, predominately in the digestive diverticulum and the lower intestine (8). The objective of this study was to examine the distribution of Vibrio vulnificus in oyster tissues during the inoculation and the depuration. The tissues of uninoculated oysters were compared to that of the inoculated oyster to observe any difference in the distribution during the depuration. Oysters were dissected into four tissue parts (adductor, mantle, gills and the digestive system). It was hoped that by separating the tissues and keeping the digestive system in tact, we could determine whether artificial inoculation and depuration were surface contaminations or an internal infection and removal. Scanning electron microscopy was used to examine the ultrastructure of the oyster epithelial surfaces. Micrographs were compared to the depuration studies to examine the association of Vibrio vulnificus to these surfaces.

MATERIALS/METHODS

Preparation of oysters. Oysters (30-70 cm), from Sharps, Virginia, were scrubbed with a wire brush under running cold tap water to remove surface dirt and debris. They were then placed in an immersion tank for depuration studies or in a contamination tank for artificial inoculation studies. The depuration tank was a recirculating system with a 6 watt ultraviolet light, a volume capacity of 63.7 liters, a salinity of 24 ppt and temperature of 20 ℃. The contamination tank was a large tub with a salinity of 24 ppt and temperature of 25 ℃, and aerated with air stones.

Artificial inoculation. Oysters were placed in a tank filled with artificial seawater (24 ppt, 25°C), with 10^4 cfu Vibrio vulnificus / ml. Oysters were held in the tank for 12 hours then placed in the depuration tank.

Bacterial enumeration. Ten oysters were shucked aseptically at each sampling period and dissected to remove the adductor, the mantle, the gills and the digestive system. The digestive system was inclusive of all tissues other than the adductor, the mantle and the gills. Tissues were enumerated for the presence of Vibrio vulnificus using a 3 tube MPN series; colistin polymyxin cellobiose (CPC) and thiosulfate citrate bile salt sucrose (TCBS) media; and confirmed using API rapid NFT biochemical tests.

Preparation of samples for electron microscopy. The methodology for electron microscopy was modified from procedures of Tall and Nauman (10). Oyster tissues were placed in 1% glutaraldehyde-filtered artificial seawater (pH 6.0, 14 ppt) for 24 hours at 4°C. The samples were postfixed in 1% osmium tetroxide-filtered artificial seawater for 24 hours at 25°C. After fixation the tissues were dehydrated with a graded series of ethanol and further prepared for scanning or transmission electron microscopy. For scanning electron microscopy the samples were critical point dried with CO_2 and sputter-coated with gold palladium. Specimens were viewed in a Philips 505 scanning electron microscopy. Micrographs were recorded with Polaroid film. For transmission electron microscopy the samples were dehydrated, infiltrated with graded series of Spurr low-viscosity embedding media epoxy resin, and embedded at 100% resin for 24 hours at 71°C. They were sectioned on MT-6000 microtome, stained with lead citrate and uranyl acetate, and examined in a Zeiss High-Resolution transmission electron microscope. Micrographs were recorded with Kodak electron micrograph film 4489.

RESULTS/DISCUSSION

Efficiency of ultraviolet light during depuration. The ultraviolet light in the depuration tank was an effective means of disinfection (Fig. 1). There was an initial count of 3.4 log Vibrio vulnificus/ml in the depuration tank water. Within one hour there was a reduction to <0.477 log unit of Vibrio vulnificus cells in the water.

Artificial inoculation and depuration. During the artificial inoculation of the oyster tissues the adductor and the digestive system did not increase in the number of Vibrio vulnificus (Fig. 2). There was 2 log units of increase on the gill tissues and 1 log unit on the mantle tissues. Artificially inoculated oysters when placed in the depuration tank had an initial number of Vibrio vulnificus at 6.4 logs (Fig. 3). Within the 72 hours of depuration there was a decrease to 4 log units. The reduction in the number of Vibrio vulnificus was primarily due to the decrease in the number of cells on the adductor, the mantle and the gills. The number of Vibrio vulnificus did not decrease significantly in the digestive system.

Depuration of naturally infected oysters. In the distribution of Vibrio vulnificus in uninoculated oysters (Fig. 4) there was a 1 log unit decrease in the cell number during the 72 hour depuration. Oysters have a high initial count of Vibrio vulnificus at 3 to 4 log units. These oysters were harvested during the warm months of July, August and September, where there is a high prevalence of Vibrio vulnificus in the warm water. During depuration only the mantle showed a reduction in the number of Vibrio vulnificus. The adductor, the gills and the digestive system remained relatively constant in the number of Vibrio vulnificus throughout the depuration period. This may explain why the uninoculated oysters only had a 1 log unit decrease in the cell number as oppose to a decrease of 2 log units in inoculated oysters.

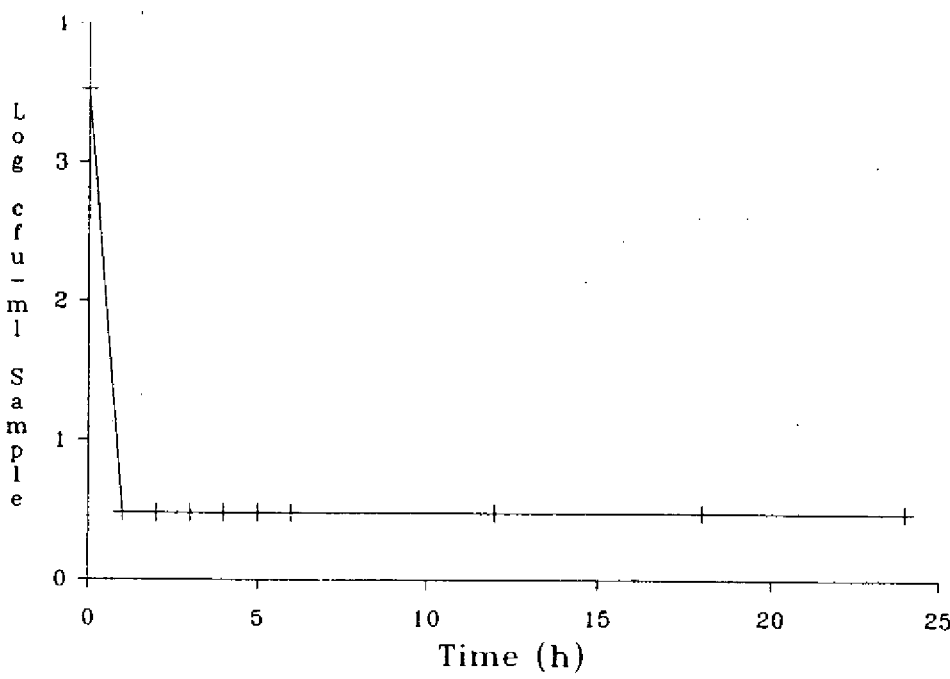

FIG 1. Efficiency of ultraviolet light during depuration.

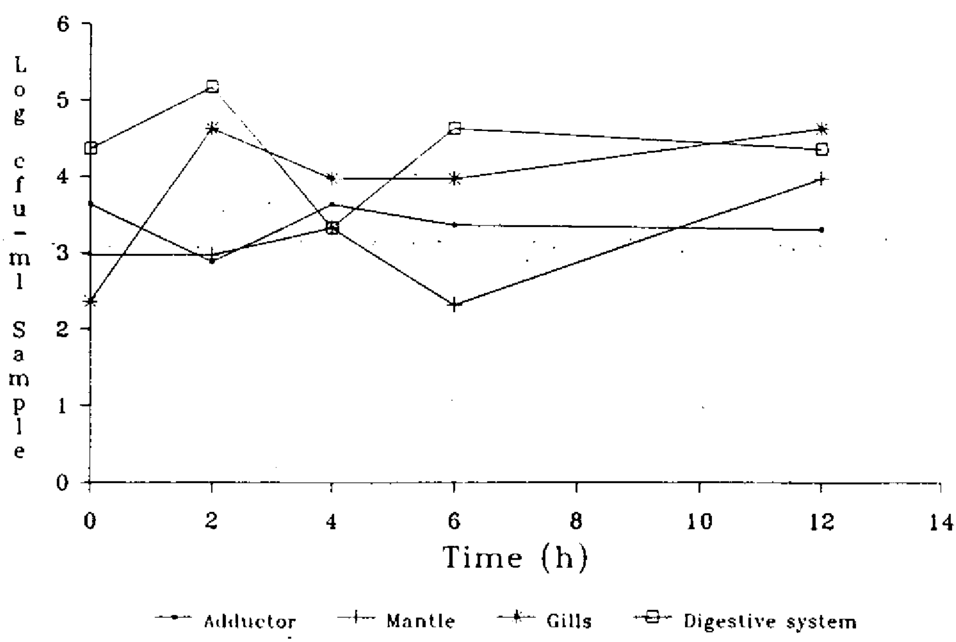

FIG 2. Distribution of Vibrio vulnificus in oyster tissues during artificial inoculation.

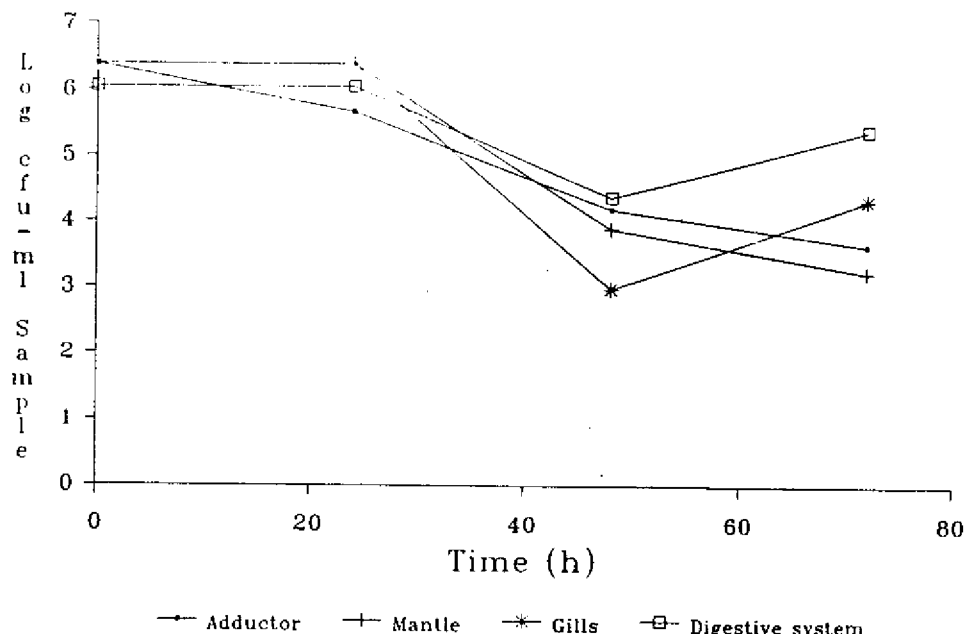

FIG 3. Distribution of Vibrio vulnificus in tissues of artificially infected oysters during depuration.

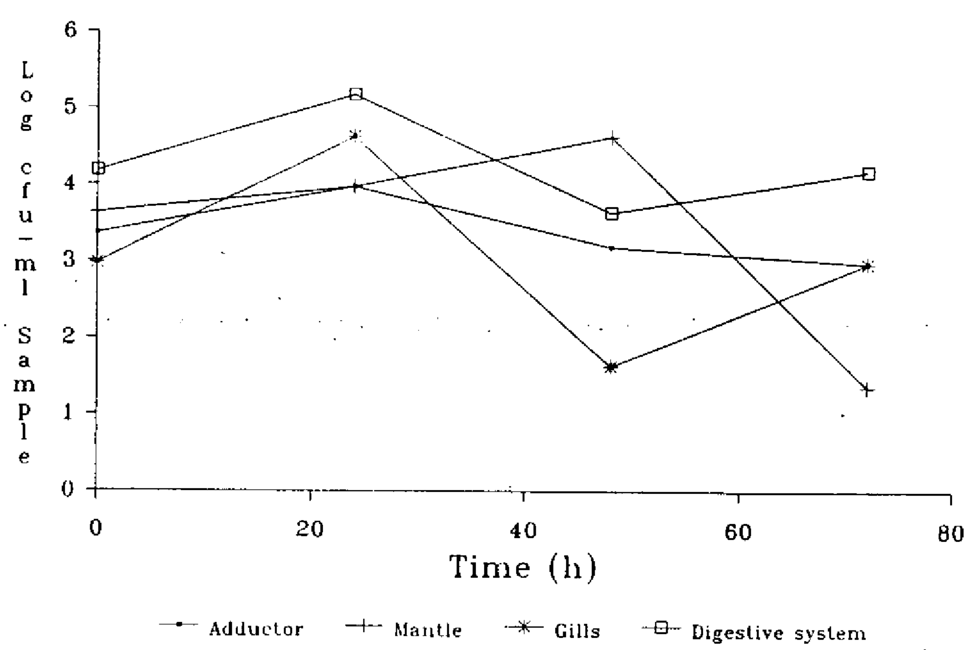

FIG 4. Distribution of Vibrio vulnificus in tissues of naturally infected oysters during depuration.

Scanning and transmission electron microscopy of oyster tissues. The adductor, the mantle and the gills were examined for surface-associated material (Figs. 5 to 7, respectively). The adductor and the mantle were nonciliated surfaces, while the gill surface was ciliated. The structural unit of the gill was a tubular filament of ciliated epithelium. All the surfaces were covered predominately with mucus and mucocytes. Similar observations were seen by Garland et al. (5). A transmission electron micrograph of the gill tissues showed the secretion of mucocytes through the cilated surface (Fig. 8).

Oyster tissues were artificially inoculated with Vibrio vulnificus. Micrograph of the vibrioid shape of this organism can be seen on figure 9. On the epithelial surfaces of the adductor, the mantle and the gills, vibrioid organisms were associated with the mucus on the surfaces (Figs. 10 to 12, respectively). There was an abundance of mucus covering a large part of the surfaces. On the gill tissues clusters of vibrioid organisms were to the right of the cilia (Fig. 12). The intestine and the stomach of oysters were also ciliated surfaces mostly covered with mucus with vibrioid organisms entrapped in the mucus cluster (micrographs not shown).

CONCLUSIONS

The design of the depuration tank was effective in disinfecting the tank water from Vibrio vulnificus. But when oysters were placed in the tank, the disinfecting unit appeared ineffective. The inability of the oysters to depurate may be due to the association of Vibrio vulnificus with the oyster tissues and not with the tank system itself. When the tissue parts were examined during the inoculation process, only the gills showed a significant uptake of the organism. The cilia on the surface of the gills may have aided the uptake. The digestive system remained unchanged in cell number, showing that this process of inoculation allowed only for the superficial contamination of the tissues. Similar results were seen during the depuration of inoculated and uninoculated oysters. Both the mantle and the adductor showed a decrease in counts. The nonciliated smooth epithelial surfaces of these tissues may have attributed to the reduction in Vibrio vulnificus from these tissues. The reduction in counts was greater in the tissues of the inoculated oysters than in the uninoculated oysters because the inoculation process involved only the external tissues. It appears that the inoculation and the depuration of oysters may be the interaction of Vibrio vulnificus with only the external tissues of the oysters since the cell counts in the digestive system remained relatively constant. The mucus and cilia on the oyster tissues may play a role in the uptake and removal of Vibrio vulnificus from oyster tissues.

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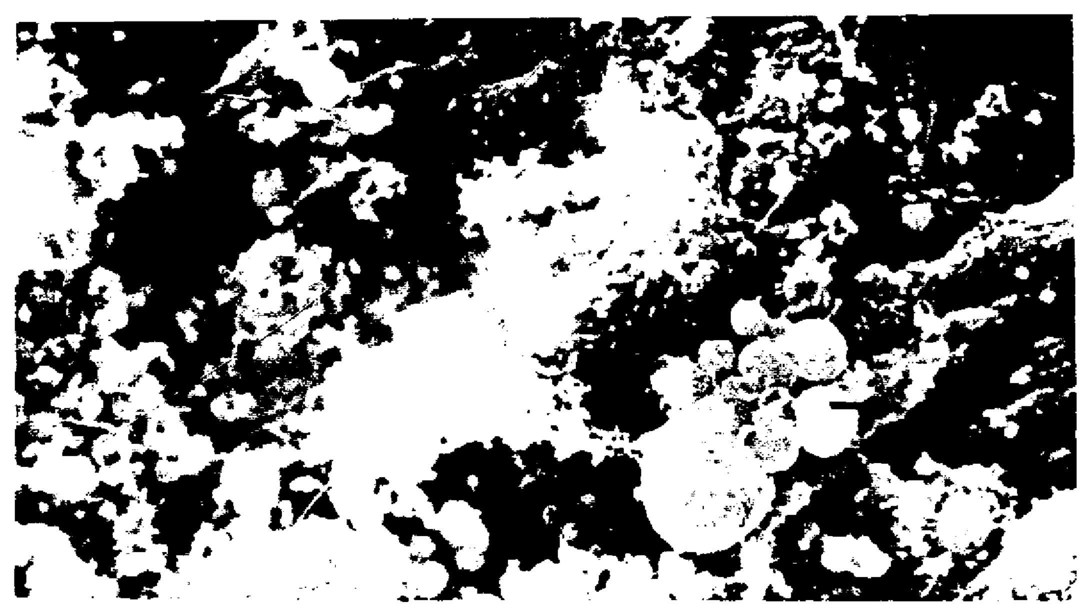

FIG 5. Scanning electron micrograph of the adductor tissue. Portions of the muscle fibers are covered with a sheet of mucus on the surface. Bar is 5 *u*m. Magnification is 1800 X.

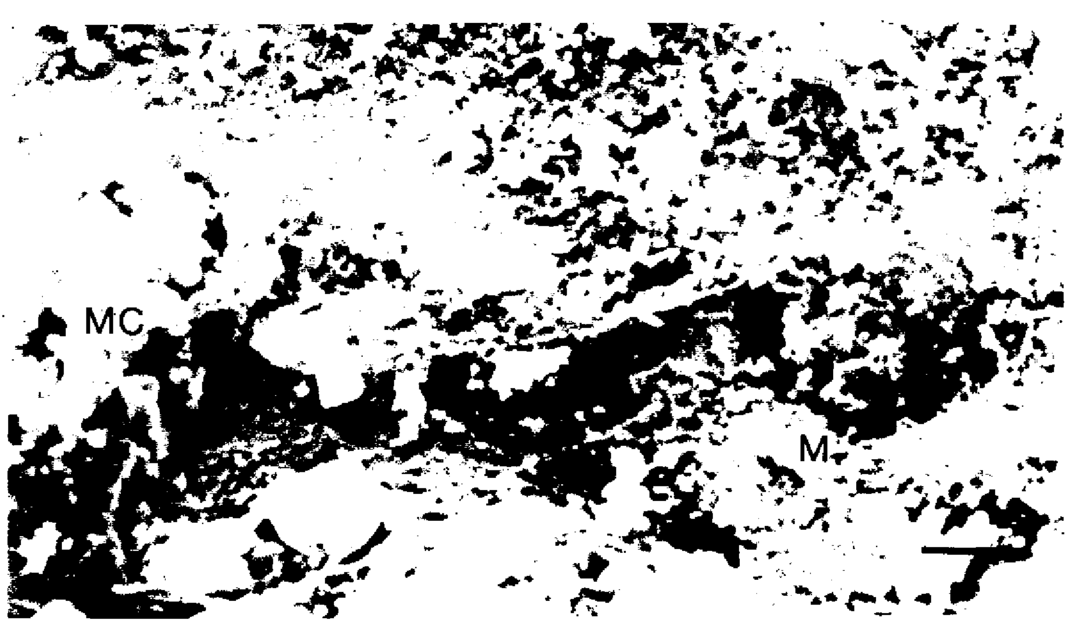

FIG 6. Scanning electron micrograph of the mantle tissue surface. This nonciliated surface is covered predominately with mucus (M) and mucocytes (MC). Bar is 5 *u*m. Magnification is 1800 X.

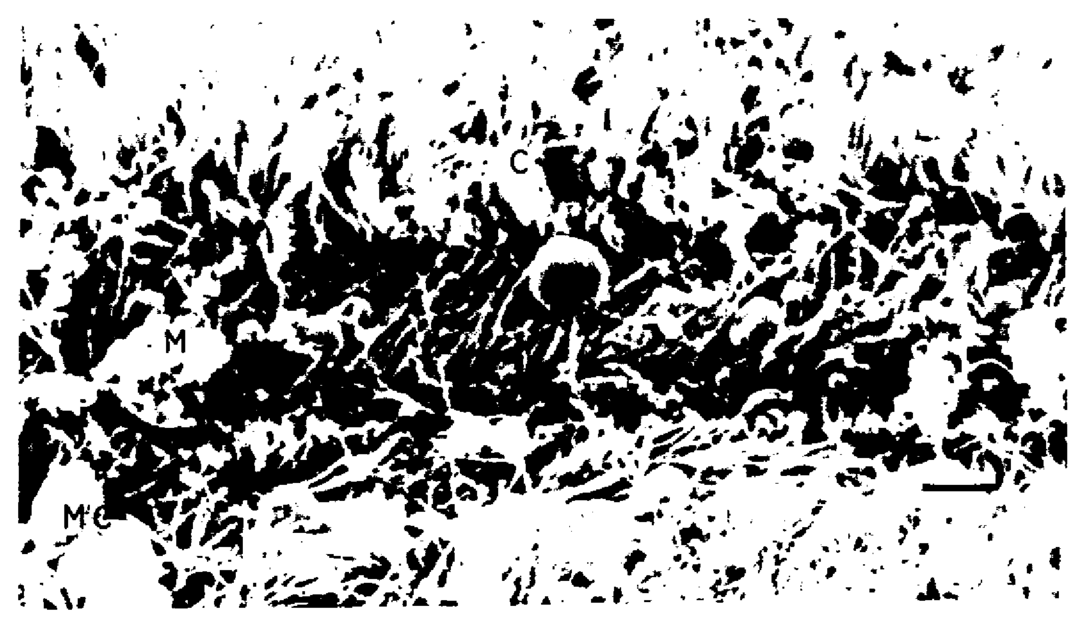

FIG 7. Scanning electron micrograph of the epithelial surface of gills. The surface is covered with cilia (C), mucus (M) and mucocytes (MC). Bar is 5 μm. Magnification is 1800 X.

FIG 8. Transmission electron micrograph of the transverse section of the gill tissue. Mucocytes (MC) are secreted through the ciliated surface. Bar is 0.5 μm. Magnification is 38,000 X.

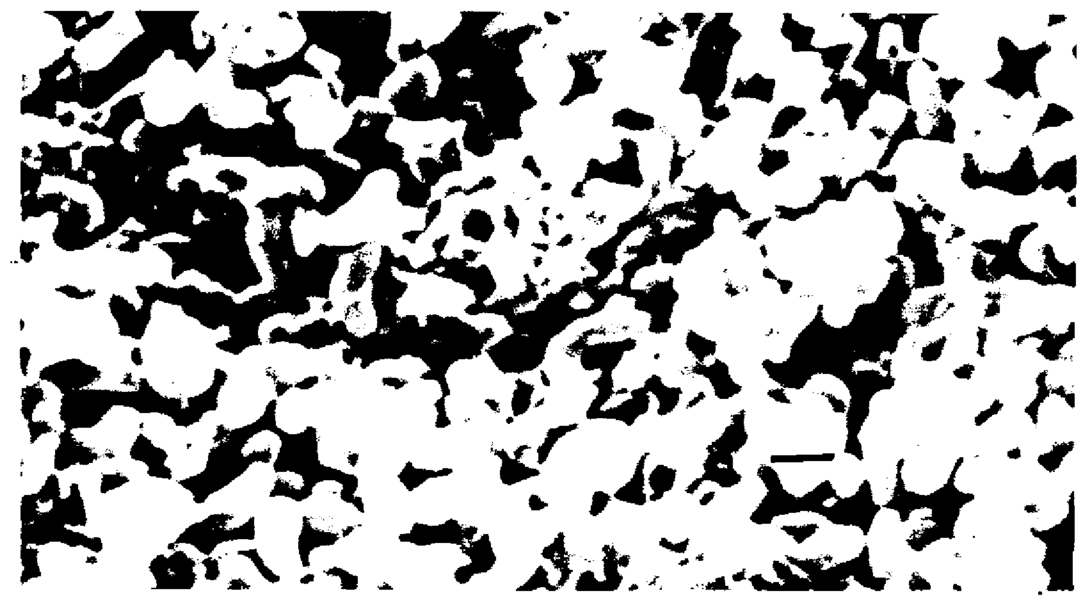

FIG. 9. Scanning electron micrograph of a colony of Vibrio vulnificus. The micrograph shows the vibrioid shape of this organism. Bar is 1 um. Magnification is 6,000 X.

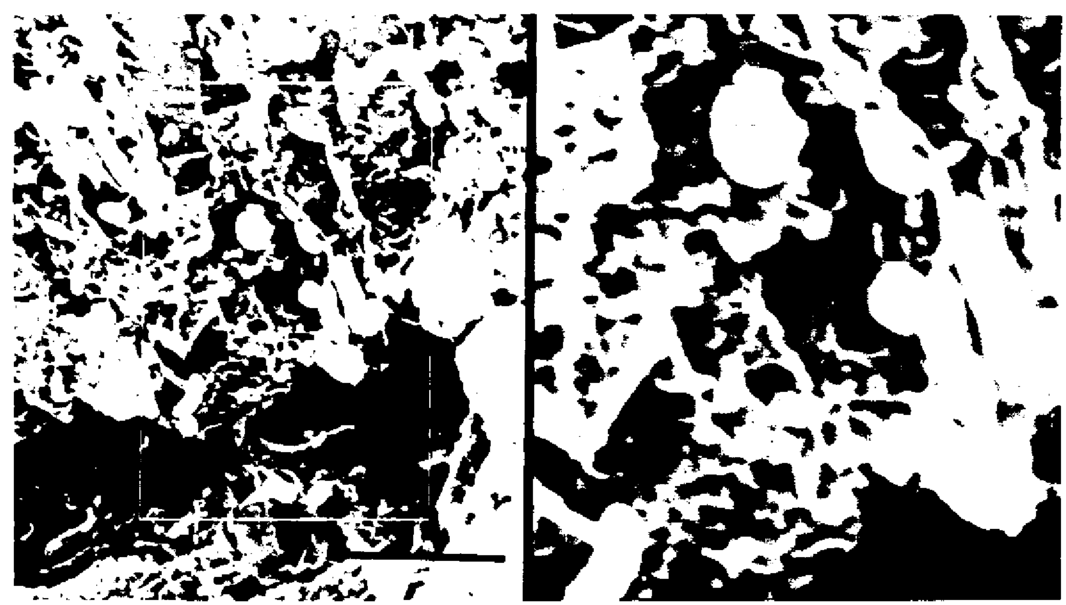

FIG 10. Scanning electron micrograph of the adductor tissue dissected from oysters inoculated with Vibrio vulnificus. Vibrioid organisms are associated with the mucus on the surface. The right micrograph is a further magnification of the vibrioid organisms. Bar is 5 um. The left micrograph is 2,500 magnification and the right micrograph is 6,000 magnification.

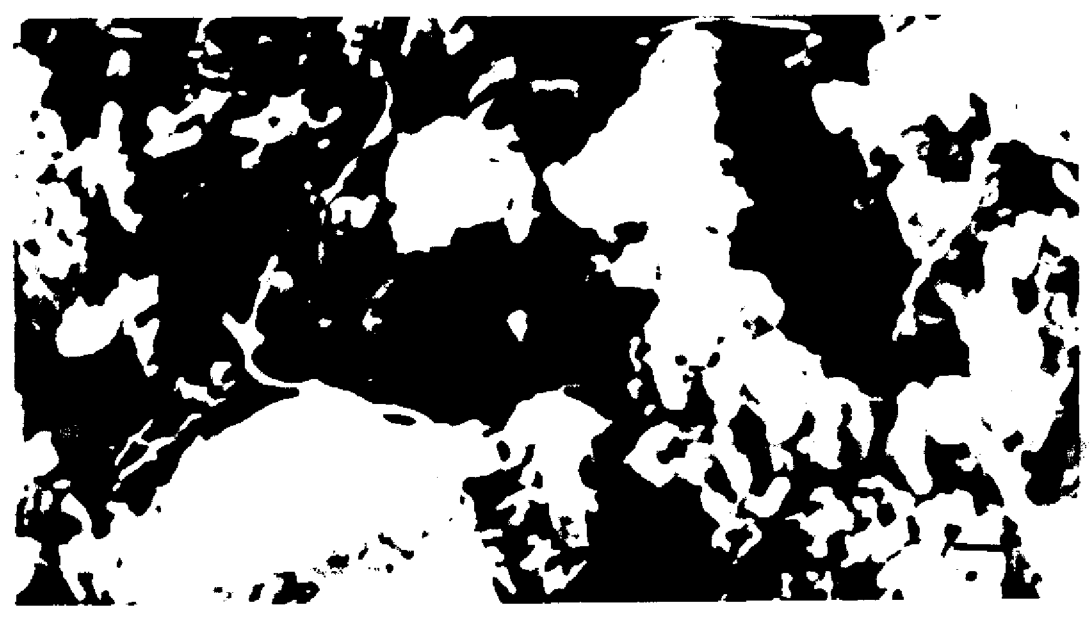

FIG 11. Scanning electron micrograph of the mantle tissue from oysters inoculated with Vibrio vulnificus. Clusters of vibrioid organisms are seen on the surface. Bar is 1 um. Magnification is 4,400 X.

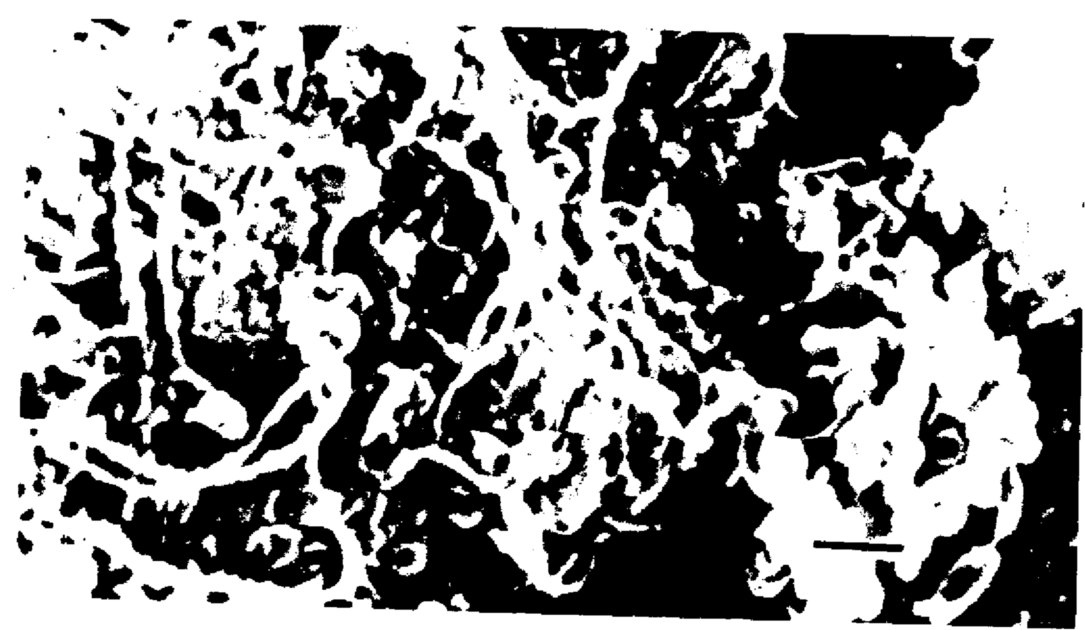

FIG 12. Scanning electron micrograph of the gill surface from inoculated oysters. Vibrioid organisms are present to the left of the cilia. Bar is 1 um. Magnification is 5,000 X.

LOW DOSE GAMMA IRRADIATION OF VIBRIO PARAHAEMOLYTICUS IN CRAYFISH TAIL MEAT

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In Louisiana, the crayfish (crawfish) industry is a significant part of the economy. Live crayfish are shipped and consumed locally, nationally, and world wide. Frozen crayfish tail meat is likewise shipped throughout the world. Quality assurance in the seafood industry has become a major focus of regulatory agencies, is being demanded by consumers, and requested by the industry itself. Several species of vibrios have been implicated as possible pathogens in warm water climates including Vibrio parahaemolyticus. Several vibrio species are natural inhabitants of warmer gulf coast waters. Vibrio parahaemolyticus is a known pathogen in blue crabs (2) and could easily be introduced into the processing plant and into crayfish during simultaneous processing of crab and crayfish.

Since the presence of this pathogen in crayfish tail meat may potentially cause serious health problems in individuals consuming contaminated crayfish, low dose gamma irradiation has been suggested as a possible processing method to inactivate this organism in various seafoods.

Therefore, the purpose of this study was to determine the effect of pasteurization levels of gamma irradiation on Vibrio parahaemolyticus in crayfish tail meat and the effect of refrigerated storage on the survival of the organism in the crayfish tail meat.

MATERIALS AND METHODS

Organism: Vibrio parahaemolyticus (Serotype 05:17) strain no. 116 was obtained from the Department of Microbiology, Louisiana State University. The organism was maintained at room temperature on tryptic soy agar slants and transferred to fresh media at weekly internals.

Preparation of Crawfish Homogenate: Fresh crayfish tail meat was purchased from a local seafood market. The nonsterile crayfish homogenate was prepared by blending one part tail meat with one part sterile saline (3% NaCl) in a Waring blendor to form a smooth paste. The sterile crayfish homogenate was prepared in the same manner, except that the crayfish was sterilized at 121 °C for 15 min before blending with saline.

Preparation of Inoculum: Growth phase cells of Vibrio parahaemolyticus were prepared by cultivating the organism through two transfers on tryptic soy agar slants with 3% NaCl incubated for 18 hrs at 37℃. A loopful (approximately 0.1 ml) of the organism was placed into 30ml of tryptic soy broth(TSB) with 3% NaCl, placed on a shaker at 125 rpm, and incubated for 2 hrs at 37℃. This incubated 30ml TSB served as the working culture.

After incubation, an appropriate quantity of the seeded broth was placed into another flask containing 50ml TSB until a reading of 0.1 absorbance (600nm) was obtained on the Bausch & Lomb Spectronic 70 (Bausch & Lomb, Inc., Rochester, NY). The seeded TSB was further incubated at 37℃ until a reading of 0.65 absorbance was observed, which was equal to approximately 1×10^8 CFU/ml. The Vibrio parahaemolyticus cell suspension was then centrifuged at 5000 rpm for 10 min in a Sorvall Superspeed RC5-C (Dupont Company, Newton, CT). The supernatant was discarded and the bacteria were resuspended in 20 ml of alkaline peptone water (1). This new cell suspension represented a population of 1×10^8 V. parahaemolyticus/ml. A known quantity of this prepared inoculum was added to the crayfish homogenate to produce a final concentration of 1×10^7 V. parahaemolyticus/g crayfish tail meat. The inoculated samples were then placed in 125 ml Nalgene bottles, packed in ice, and transferred to the Nuclear Science Center at Louisiana State University.

Irradiation of Samples: The Nalgene bottles containing the samples were paced in a water tight diving bell which was filled with ice and sealed. The diving bell was lowered into the pit to expose the samples to the cobalt-60 source which emitted 40 Gy/min. The samples were exposed to 0.10, 0.20, and 0.35 kGy. The nalgene bottles were then removed, packed in ice and transferred back to the Department of Food Science laboratory for analysis and storage. Control samples were treated in the same manner, except the irradiation treatments were omitted.

Enumeration of Vibrio parahaemolyticus: Samples were examined for the presence of V. parahaemolyticus at 0, 7, 14, and 21 days of storage at 4℃. Vibrio parahaemolyticus in the sterile and nonsterile homogenates was enumerated by pour plating appropriate dilutions on plate count agar (Difco) and thiosulfate-citrate-bile salts-sucrose plates (1). Pour plates were incubated at 37℃ for 48 hours prior to counting.

Irradiation of V. parahaemolyticus in sterile and nonsterile crayfish homogenates at 4℃ were performed in triplicate. The number of V. parahaemolyticus surviving was calculated by determining the average number of organisms recovered from replicate cultures of three samples subjected to the same irradiation treatment and refrigeration (Figure 1).

FIG 1: METHODOLOGY

BLEND

Crayfish tail meat and 3% NaCl

ADD

V parahaemolyticum (10^7 CFU/g)

IRRADIATE
(Cobalt-60 at 0.0, 0.1, 0.2, & 0.35 kGy)

STORE
(7, 14, 21 DAYS).

ENUMERATE
Microbial Survivors

RESULTS AND DISCUSSION

Effect of Radiation: In the sterile crayfish homogenate, the V. parahaemolyticus population was initially reduced from $\log_{10}7$ cfu/g to $\log_{10}4$ CFU/g with 0.10 kGy of gamma radiation (Figure 2). An additional 0.10 kGy of irradiation treatment immediately reduced the number of viable organisms to $\log_{10}2$ CFU/g crayfish tail meat. With an irradiation dose of 0.35 kGy the immediate reduction of viable microorganisms was even greater, less than 10 CFU/g recovered.

In the nonsterile crayfish tail meat homogenate, the V. parahaemolyticus population was initially reduced from $\log_{10}7$ CFU/g to $\log_{10}5$/CFU/g with 0.10 kGy radiation, to $\log_{10}3$ CFU/g with 0.20 kGy, and to less than 10 CFU/g with 0.35 kGy by day-7 and 0 by day 14 (Figure 3).

Note: Recent evaluation, in this laboratory, of the effect of irradiation on V. parahaemolyticus in nonsterile crayfish tail meat has demonstrated that 0.50 kGy dose of irradiation will reduce initial V. parahaemolyticus survival number to zero.

Effect of Time: In the non-irradiated sterile crayfish homogenate stored at 4℃, V. parahaemolyticus decreased from its initial population of $\log_{10}7$ CFU/g on day 0 to less than 10 CFU/g on day 21. The vibrio population in irradiated sterile samples treated with 0.10 and 0.20 kGy was also reduced to zero by Day 21. Microbial populations in sterile samples treated with 0.35 kGy were reduced immediately to 0 CFU/g on day-0 (Figure 2).

Survival in Sterile and Nonsterile Homogenates: Vibrio parahaemolyticus (10^7CFU/g of crayfish tail meat) survived at 4℃ for 21 days in both sterile and nonsterile crayfish tail meat (Figure 2 and 3). Also, as indicated in Figure 2 and 3, survival of V. parahaemolyticus was more pronounced in the nonsterile crayfish homogenate than in the sterile crayfish homogenate. From the data presented and from previous studies (3), it is evident that the nonsterile crayfish homogenate is more conducive to growth of the vibrios than are sterile homogenates even in non-irradiated control samples and after irradiation. It is possible that the microflora present in the nonsterile crayfish homogenate provide some degree of protection for the vibrios or that sterilization (121℃ for 15 min) renders the protein substrate less supportive of the growth of vibrios.

SUMMARY AND CONCLUSIONS

1. Vibrio parahaemolyticus (10^7CFU/g of crayfish tail meat) survived at 4℃ for 21 days in both sterile and nonsterile crayfish tail meat.

2. All doses of gamma irradiation (0.10, 0.20, and 0.35 kGy) were effective in immediately reducing the total number of viable V. parahaemolyticus.

3. Gamma irradiation at a dosage of 0.35 kGy was effective in immediately eliminating the v. parahaemolyticus from the initial inoculation of 10^7CFU/g on Day 0 in sterile crayfish tail meat.

4. Gamma irradiation at a dosage of 0.35 kGy was effective in immediately reducing viable V. parahaemolyticus in nonsterile crayfish tail meat by 6 log cycles and completely eliminating viable vibrio organisms in less than 14 days of refrigerated storage.

Low dose gamma irradiation is an effective method for insuring that fresh crayfish tail meat can be free of the environmental pathogen Vibrio parahaemolyticus.

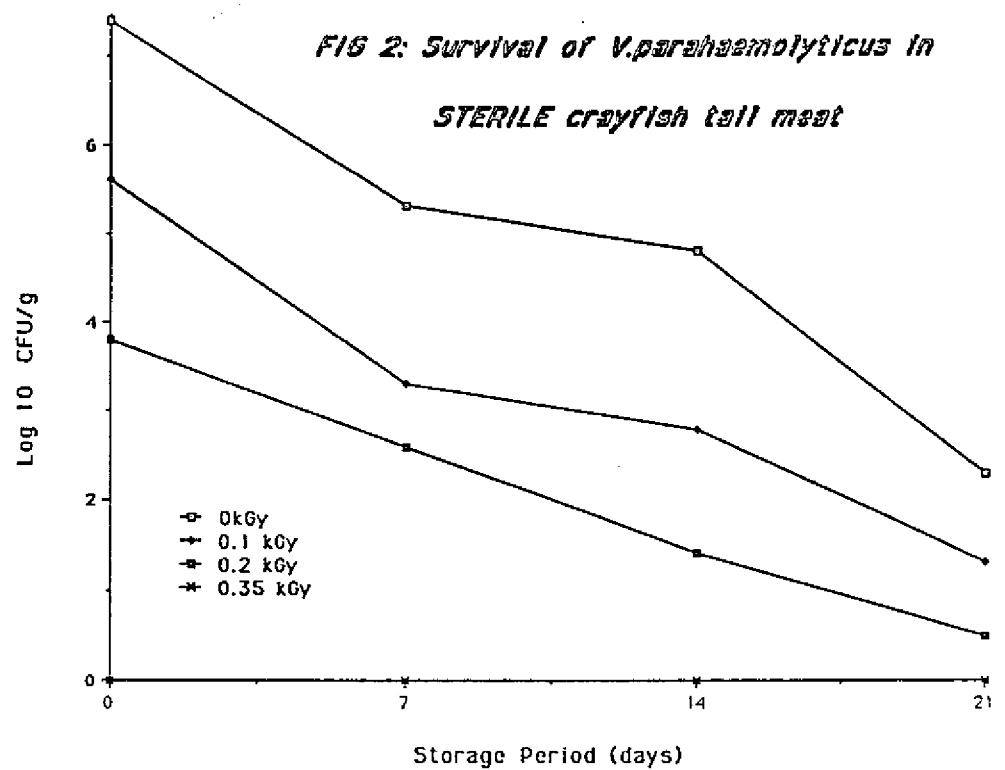
FIG 2: Survival of V.parahaemolyticus in
STERILE crayfish tail meat
Log 10 CFU/g
0
2
4
6
0
7
14
21
Storage Period (days)
0kGy
0.1 kGy
0.2 kGy
0.35 kGy

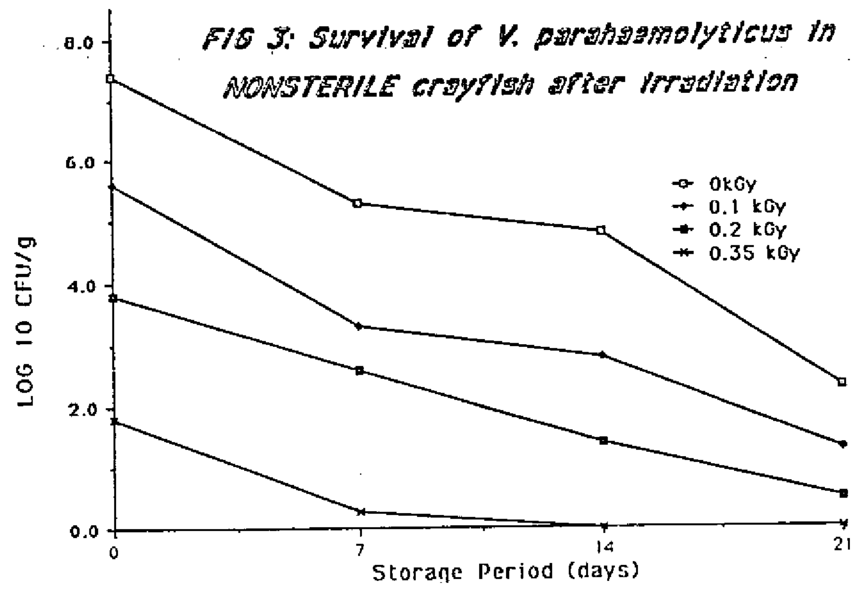
FIG 3: Survival of V. parahaemolyticus in
NONSTERILE crayfish after irradiation
LOG 10 CFU/g
0.0
2.0
4.0
6.0
8.0
0
7
14
21
Storage Period (days)
0kGy
0.1 kGy
0.2 kGy
0.35 kGy

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PERUVIAN CHOLERA EPIDEMIC: ROLE OF SEAFOOD

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Epidemic cholera, caused by toxigenic Vibrio cholerae O1, serotype Inaba, appeared in Peru in January 1991 after being absent from South America this century (1). An epidemic rapidly spread throughout Peru and into Ecuador, Colombia, Brazil, Chile, Mexico, Guatemala, Bolivia, Panama, and El Salvador. More than 300,000 cases and 3,000 deaths have been reported to the Pan American Health Organization (PAHO) (Cholera Update - September 16, 1991).

Although the source of cholera was unknown, there was initial speculation that seafoods, especially ceviche (raw fish marinated in lemon juice), were involved in the epidemic. The Peruvian Minister of Health advised the public not to consume raw seafood, and consumption of all domestic seafood (cooked or raw) plummeted. Seafood products are a major export of Peru. There was concern that the cholera epidemic might spread if food exports from Peru were contaminated with V. cholerae. In February 1991, the U.S. Food and Drug Administration (FDA) issued Import Alert #99-07, which called for sampling 100% of the seafood and water-processed produce from Peru for V. cholerae. Many other countries closed their borders to Peruvian foods.

FDA was asked to assist the Peruvian Certification Agency (CERPER) in assessing the control measures that were undertaken to prevent potential cholera contamination of fish products and produce exported to the United States. In March 1991, an FDA team (a microbiologist and an investigator) visited Peru and evaluated CERPER's inspection process and its ability to detect V. cholerae. A shrimp hatchery, a pond facility, and a number of food processing plants were inspected for sanitation and potential sources of cholera. Additionally, PAHO hired an investigator from the Brazilian Secretary of Health to conduct a survey of Peruvian street vendors' food for cholera contamination.

In this report we present cholera survey data from Peruvian street vendors' foods, Peruvian food exports, and food products imported into the United States from various Latin American countries. We also discuss the control measures used for reducing the risk of cholera contamination at Peruvian seafood processing plants and aquaculture facilities and report on the effectiveness of CERPER's certification program.

MATERIALS AND METHODS

The street vendor foods for the PAHO study were collected in Lima and Callao, Peru, in April 1991 by a team of investigators at the National Mayor University of San Marco in San Borj, Peru. The team coordinator was from the Office of the Brazilian Secretary of Health. The samples included raw seafood, ceviche, sweets, beverages, salads, dishwater, and swabs from food-contact surfaces.

Frozen seafood, fish meal, and water-processed produce for export were collected by CERPER inspectors from February through July 1991. These samples were analyzed at CERPER laboratories in Callao and Paita, Peru.

FDA inspectors collected samples from February through August 1991, as specified by Import Alert #99-07. Samples were analyzed by laboratories in Atlanta, Baltimore, Boston, Los Angeles, San Juan, Denver, and New York. Fresh and frozen seafood (ocean-harvested and pond-raised) from Peru, Ecuador, and Colombia were sampled by FDA during this period. Most of the samples were frozen whiting and shrimp. Water-processed produce (i.e., aspargus) from Peru was also sampled.

All samples for V. cholerae analysis were collected aseptically. In general, V. cholerae analyses were conducted by Bacteriological Analytical Manual (BAM) procedures (4). Samples were homogenized in blenders or stomachers and enriched in alkaline peptone broth at 35°C for 6-8 hr or overnight. A loopful of the enrichment culture was streaked onto thiosulfate citrate bile salts sucrose (TCBS) agar and incubated overnight at 35°C. Typical colonies were isolated for standard biochemical and serological evaluation.

FDA laboratories modified the BAM procedures to enhance recovery and increase the efficiency of the method; sometimes a Most Probable Number method was used for enumeration of V. cholerae. Samples usually consisted of multiple units of 5 (10 or 15 units per sample) which were composited before analysis. Cellobiose-polymyxin B-colistin (CPC) agar was used for isolation, in addition to TCBS. Cultures identified as V. cholerae were screened for the production of cholera enterotoxin by using the VET-RPLA (reversed-passive latex agglutination) kit (Oxoid, Columbia, MD).

Site visits to Peruvian seafood processing plants and CERPER were conducted by an FDA microbiologist and an investigator in March 1991. Six seafood processing plants in Paita and Tumbes were inspected for hygienic control of incoming raw product, plant sanitation, and chlorination of water. Employee health monitoring and quality control procedures, such as bacteriological testing, were also inspected. A hatchery and a pond facility in Tumbes were inspected for potential sources of cholera. CERPER was evaluated for the effectiveness of its inspection program and its ability to detect V. cholerae in foods.

RESULTS

V. cholerae O1, serotype Inaba was found in a variety of street vendors' food; the highest incidences were associated with seafood (Table 1).

Table 1. Vibrio cholerae Incidence in Street Vendors' Food in Lima and Callao, Peru

Sample Type	No.	% Positive
Raw seafood	5	100
Ceviche	37	35
Fish cutting board	6	33
Ceviche sauce	5	0
Chicken and rice	4	25
Pasta with sauce	12	17
Potato a la huancaina	10	10
Sorbet	10	10
Sauces	3	0
Green salads	8	0
Sweets	7	0
Fresh beverages	12	0
Dirty dishes	11	9
Dish water	15	7
Total	**153**	**18**

Pan American Health Organization study conducted in April 1991 by Instituto Adolfo Lutz, Secretary of Health, Sao Paulo, Brazil.

V. cholerae O1 was found in only 1 lot of seafood (<0.1%) for export and was not found in fish meal or produce analyzed by CERPER (Table 2).

Table 2. Vibrio cholerae in Peruvian Food Exports

Product	No.	No. Positive
Seafood	1011	1
Fish meal	194	0
Produce	873	0

Analyses conducted February-July 1991 by the Peruvian Certification Agency (CERPER).

The number and type of import samples analyzed by FDA for V. cholerae from each Latin American country are shown in Table 3. V. cholerae O1 was not found in any of the samples, but V. cholerae non-O1 was isolated from 9 seafood samples (primarily shrimp).

Table 3. U.S. Food and Drug Administration (FDA) Vibrio cholerae Analyses of Foods Imported from Latin America

Country	Product	No.
Peru	Seafood	174
Peru	Produce	25
Ecuador	Seafood	91
Colombia	Seafood	7

Analyses conducted February - August 30, 1991, by FDA and compiled by the Division of Field Science.

DISCUSSION

Shortly after the discovery of cholera in Peru, the Peruvian Ministry of Health recommended that raw seafood not be consumed. As a result, consumption of raw and cooked seafood plummeted. Subsequent epidemiological investigations from February 10 to March 12, 1991, were unable to obtain data on the risk of eating raw fish or shellfish because none of the people infected with cholera reported eating raw seafood (A. A. Reis, May 8, 1991, Memorandum; Foreign Trip Report-Peru, Centers for Disease Control, Atlanta, GA). Drinking unboiled water, consuming ready-to-eat foods and beverages from street vendors, and eating rice more than 3 hr after it was prepared without reheating it were common factors among people infected with cholera.

An investigation of water and sewage treatment in Peru in March 1991 found improper sewage treatment and inadequate chlorination of municipal water supplies (E. E. Geldreich and K. Fox, 1991, Investigation of the Microbial Quality of Water Supplies During the 1991 Cholera Outbreak in Peru, U.S. Environmental Protection Agency, Cincinnati, OH). Municipal water was frequently contaminated with fecal coliforms, and V. cholerae O1 was isolated in a few samples from several different locations.

Although unsanitary drinking water was implicated in the spread of cholera in Peru, the role of seafood was only speculative because epidemiological data on seafood consumption were unavaliable. The high incidence of V. cholerae O1 in seafoods sold by street vendors in Lima and Callao suggests that seafood may have been involved in the early transmission of cholera (before to the epidemiological studies). The highest frequency of cholera contamination (100%) was in raw finfish and molluscan shellfish. Ceviche (raw finfish and molluscan shellfish marinated in lemon juice) is popular in Peru and was contaminated with cholera more frequently than were other foods. Cooking may have inactivated V. cholerae in foods such as chicken, rice, and pasta. The occurrence of V. cholerae in a variety of street vendors' foods and in dish water is indicative of cross-contamination and maintenance at ambient temperatures for more than 3 hr.

Near-shore species, including molluscan shellfish, are the domestic seafoods primarily consumed in Peru. Cholera contamination is more likely in seafoods harvested ear-shore because of sewage out-falls and other shoreline pollution sources. Fresh water run-off also reduces salinity, and there is an inverse relationship between V. cholerae

abundance and increasing salinity (2, 5). Unsanitary processing conditions, inadequate refrigeration, and absence of regulatory control of domestic seafood processors and street vendors may also contribute to cholera contamination in ready-to-eat seafoods.

The low incidence of V. cholerae in Peruvian food exports may be attributed to the harvest of food from environments with little or no cholera contamination, good processing plant sanitation, and regulatory control. The FDA investigation assessed each of these factors; they are discussed separately below.

Peruvian seafood exports are primarily finfish and shrimp that are harvested in off-shore waters, well isolated from shoreline pollution sources. Pond-raised shrimp production is expanding rapidly in northern Peru, and most of the shrimp is exported. A hatchery and a shrimp farm were inspected for potential sources of cholera. The hatchery maintained post-larval shrimp for 18 days in concrete vats. The facility was modern and clean. Water in the vats was exchanged daily with well water that was filtered and treated with ultraviolet light. The shrimp ponds were of raised levee construction, which minimizes run-off into the ponds. The water source for most Peruvian shrimp farms was a lagoon that exchanged water with the Pacific Ocean during high tide. Pollution sources were not observed at this lagoon by either ground or aerial surveillance. Lagoon water was pumped through the pond system, and 10-20% was exchanged daily. Commercial feed pellets were the only feed source observed. Heat used in the pelleting process (80-90°C for 1-5 min; D. Harrad, 1991, Purina Mills, Montgomery, AL, personal communication) should inactivate any cholera present in the feed. Pond water analyzed for cholera by an independent laboratory was negative. In general, the practices at the hatchery and the ponds appeared to minimize the risk of cholera contamination.

The FDA inspection of seafood processing plants in the Tumbes and Paita areas indicated that most were properly controlled and the facilities, equipment, and hygienic practices of the personnel were adequate. The plants had raised concrete floors, sheet-metal walls, and high, thin sheet-metal ceilings. Generally, there was adequate space for the various operations. Tables, tools, and most equipment were of stainless steel, galvanized metal, or plastic construction. Sanitary controls, including monitoring the health status of workers, were effective. There was usually a health care provider on site or on retainer in addition to the health card system used by municipalities to monitor the health of workers. Hand-washing receptacles containing chlorinated water were available at key processing stages. Line workers wore uniforms and head coverings, and they usually wore rubber boots and gloves and sometimes face masks. In-plant chlorination was practiced, and acceptable chlorine levels were observed at all plants, but chlorine-monitoring records were not maintained.

CERPER, a government entity within the Ministry of Fishery, is responsible for the certification of agroindustrial and hydrobiologic products. CERPER performs inspections of food-processing facilities and product analyses. Certification by CERPER indicates that a product meets established standards of quality, hygiene, and sanitation and that it was processed or stored at establishments that meet prescribed standards. CODEX Alimentarius and nationally established technical standards are used as the basis for the CERPER sampling plan for frozen seafood products.

CERPER laboratories at Callao and Paita are responsible for all cholera analyses. These laboratories had not conducted cholera analyses before February 1991. The microbiologists in these laboratories were competent but inexperienced in cholera methodology. The equipment and materials required for cholera analyses were present but in short supply. The V. cholerae analysis was not equivalent to that used by FDA because of deviations from BAM V. cholerae procedures, inadequate records, and a

shortage of supplies, equipment, and personnel. The problems with methodology and records were addressed immediately but the intensity of analysis at CERPER continues to be far less than that at FDA. A regulatory sample for V. cholerae at FDA typically generates 24-36 suspect colonies but may yield up to 300 colonies for identification depending on the number of subsamples (usually 10-15), the type of product (oysters are enriched at various dilutions and temperatures), and the selection of optional enrichment broths or isolation agars [i.e., gelatin phosphate salt (GPS) broth, gelatin plates, and CPC plates). At CERPER, 1-2 suspect colonies per sample are typically selected for confirmation.

FDA and CERPER data agree that the seafood products exported to the United States from Peru and other Latin American countries are generally free of cholera contamination (toxigenic V. cholerae). The occasional isolation of V. cholerae non-O1 by FDA demonstrates that some strains are capable of surviving in frozen seafoods imported from Latin America. These data also suggest that commercial food shipments from Latin America are not responsible for the introduction of the Latin American V. cholerae strain along the U.S. Gulf Coast (DePaola et al., in preparation).

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SEAFOOD SAFETY AND INSPECTION - AN FDA PERSPECTIVE

by
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Food and Drug Administration

Good afternoon! During my 27 years with the National Marine Fisheries Service (NMFS), I worked with many of you. However, today, I wear a new hat and carry a new business card. I am now the Director of the Office of Seafood for the Food and Drug Administration, located within FDA's Center for Food Safety and Applied Nutrition.

This simply means that now - more than ever before - I am deeply involved with issues concerning safe and wholesome seafood.

Allow me to take this opportunity to tell you about the FDA's assessment of seafood safety in the U.S., and to talk a little about some of the new programs we are engaged in to better assure its safety, quality and proper labeling.

But before I get into the details, let me tell you a little about the driving forces that led to the implementation of many of our new seafood safety programs - including the establishment of my new office. After that, I will explain how the Office of Seafood is organized and how we are following a comprehensive seafood plan to make seafood safer.

Seafood has become much more important in the U.S., both in terms of its contribution to GNP and its role in the diet. In the United States alone, consumption has increased from 12 pounds to 16 pounds per capita in the last decade. The National Fisheries Institute estimates that it could reach 20 pounds by the year 2000. As a result, consumers, public health officials, the Congress, the media and even scientific organizations have been taking long looks at the efforts of both the seafood industry and the government with respect to assuring safety, wholesomeness and proper labeling.

Effective assurance of the safety of our seafood exports is important to our economy as well. As the world's largest exporter, we shipped $2.8 billion worth of our products throughout the world in 1990.

In the last two years, the U.S. Congress conducted a host of public hearings and introduced 10 legislative proposals to change or expand the U.S. regulation of both domestic and imported seafood. Instead, FDA was given two things: (1) a 62% or $15.5 million budget increase to expand and enhance its already existing seafood inspection program; and (2) a directive to develop and establish a new, voluntary, HACCP-based, fee-for-service inspection program in cooperation with the National Oceanic and Atmospheric Administration in the Department of Commerce. This was a welcome budget increase. In a few minutes, I will tell you some of the things we are doing with this money to enhance our existing program. Then I will describe our new voluntary program a little later.

Another driving force in the U.S. seafood safety arena was the publication in January of the U.S. National Academy of Sciences' report entitled "Seafood Safety". It essentially stated that seafood in the U.S. is safe. Of the illnesses that do occur, most are the result of eating raw shellfish. It recommended that control of domestic production should rest mostly with state and local authorities, with Federal oversight; but, that control of imports should lie with the Federal government. Overall, the report made about 70 recommendations for improving seafood safety, including recommending the

application to seafood of the Hazard Analysis Critical Control Points (HACCP) system that I mentioned earlier.

As a result of these major events - more money from the Congress and the NAS recommendations - the FDA began a series of seafood safety initiatives to augment those which were on-going.

In a vital first step, our new Commissioner, Dr. David Kessler, established in February of this year the new Office of Seafood, within FDA's Center for Food Safety and Applied Nutrition. This is the first time that an office has been created within the Center that is product specific. The office is charged with policy development, planning and coordination of all of FDA's seafood activities, including coordination of the FDA/NOAA joint program. It encompasses several laboratory facilities, as well as an expanded staff dedicated to addressing all seafood issues and problems. I was appointed its director in July 1991.

The Office of Seafood is divided into two divisions - the Division of Seafood Research and the Division of Seafood Programs. The Division of Seafood Research is made up of the Biological Hazards Branch and the Chemical Hazards Branch. This Division plans and conducts research on seafood, harvesting and processing, storage and distribution from both wild and farmed sources, as they may be affected by chemical and biological contamination. The Division of Seafood Programs is made up of three branches - the Policy Guidance Branch, the Program and Enforcement Branch and the Shellfish Sanitation Branch. This division is responsible for agency policies, planning and coordinating all seafood inspection and enforcement activities with regard to seafood, including molluscan shellfish.

When a new office such as ours is created, the seafood industry worldwide wants to know what it is we're going to do! In April 1991, the Office of Seafood published its FY 91 - 92 Seafood Plan. This essentially outlines the activities the Agency is undertaking to improve the safety, wholesomeness and labeling of seafood sold in the United States and exported to other countries. This plan follows the recommendations made by the National Academy of Sciences, and incorporates some new directions and priorities into FDA's on-going program as well as some totally new activities.

The Seafood plan is divided into five (5) areas - Water, Domestic Industry including Exports, Imports, Retail and Consumption or Consumers. It addresses the most serious problems presented by seafood consumption. I will give you a brief overview of what we have accomplished so far, and what we plan to accomplish in the coming year. Our activities center on inspection and enforcement, research, education and training.

As I mentioned before, the consumption of raw shellfish causes the most seafood-borne illnesses. According to data gathered by the Centers for Disease Control, 85% of seafood-borne illnesses in the U.S. over the past 10 years can be traced to consumption of raw shellfish. To get a better handle on this problem, we are conducting reviews of all 3,000 domestic molluscan shellfish harvesting areas classified as open for harvesting. We want to assure that those waters are truly safe. This involves 19 million acres of water in 24 states. Any misclassifications that we find will be corrected. In cooperation with NOAA, we are conducting undercover sting operations to stop "bootleggers". We believe that many shellfish illnesses can be traced to product bootlegged from closed waters.

To address the domestic industry, we have begun conducting inspections of all processors in the United States in order to get a better view of the industry. If problems are encountered, then the agency is taking whatever action is necessary to correct them. We have completed about half of these inspections and frankly, are pleased with what we're seeing so far. In our Southeast Region, for example, their entire inventory of over 1500 establishments has been visited. Only 1.5% of the inspections done were violative and required regulatory action. In addition, another 13% had problems which were able to be corrected right then and there by the firm. This is good news in that it confirms

both FDA and the NAS's contention that the vast majority of the processing sector is in compliance with existing regulations.

In the area of research, we are issuing "levels of concern" for nickel, cadmium, lead, chromium and arsenic in molluscan shellfish in the form of guidance documents for use by local authorities. These levels can be used by the individual states to stop the marketing of product that may present an unacceptable risk to consumers in their state. At the same time, we are considering whether to establish national tolerances.

This past July, we met with several key states regarding the type of information on toxicants that would be of greatest use to them. We asked whether a single national tolerance for a given contaminant would be best, or whether they want to use our toxicological evaluations to set regional standards that would be more applicable to their own consumption patterns and concerns. This approach was recommended by the NAS.

Similarly, we are working with the Environmental Protection Agency (EPA), National Oceanic and Atmospheric Administration (NOAA), the Fish and Wildlife Service (FWS) and the National Fisheries Institute (NFI) in compiling a new list of potential "regional contaminants" of concern in the U.S. This is based on a list of 45 substances put together by EPA, and will be added to as necessary. These substances could show up as contaminant residues and our expanded monitoring program will screen for them.

Through our research efforts, we have announced the availability of pure paralytic shellfish poison (PSP) reference standards, as well as domoic acid (ASP); and we have the capability to continuously provide these standards permanently. We are distributing these to appropriate state officials and researchers, upon request. This will help determine where and which type of saxitoxin might be present. Field tests are underway for genetic probes and a gene amplification system for shellfish waters and shellfish to determine if we can directly and more rapidly detect Norwalk agent and Hepatitis A virus.

Through our new study of Vibrios in the Gulf of Mexico, we are monitoring for the cholera bacteria that has become epidemic in Latin and Central America. To date we have detected its presence in only one bay in the Gulf of Mexico and all commercial harvesting has been curtailed from that area. We are coordinating efforts with the Centers for Disease Control. In order to better understand the relationship between water conditions and the numbers of various vibrio species in shellfish, we have joined forces with several Federal agencies to initiate a comprehensive study.

We began a surveillance of Federal waters outside 12 miles for natural toxins, covering clams, oysters and mussels, in the George's Bank area of New England.

We are testing a privately developed rapid, dip-stick system for detecting ciguatoxins and ciguatoxic reefs. If this works, we will distribute it for surveillance of both commercial and recreationally caught species from selected areas. We will be asking the states to help us in determining the location of known or suspected ciguatoxic reefs and get information out to properly warn the recreational fishing industry.

We are significantly increasing the number of wharf exams of imports and doubling the number of samples taken for analysis. This will be targeted to certain seafood to help us detect problems associated with these products. In this same area, we are connecting our FDA Headquarters and District Offices by computer with the U.S. Customs Service, in order to have a better handle on all entries. We also plan to expand the number of bilateral agreements with foreign countries in order to save our resources and to expedite the entry of products.

We are stepping up regulatory action against economic violations such as overglazing of shrimp and lobster tails, the excessive use of substances such as sodium tripolyphosphate with scallops and shrimp, the undeclared addition of water to falsely increase product weight, and species substitution. Economic adulteration is against the law and Commissioner Kessler has made this a priority enforcement area for both domestic and imported products.

Scombroid poisoning is one of the major causes of illness associated with finfish. It can be eradicated with rapid and proper refrigeration of products. We are working with the National Advisory Committee for Microbiological Criteria for Food to develop appropriate guidelines for time and temperature controls to be applied during harvesting, processing, shipping and marketing.

Training is an essential part of our program. We have been conducting training programs for state and local regulatory agencies, as well as for NOAA and our own inspectors. This will assure consistency among all those conducting inspections, sample collections and analyses and other FDA seafood related work. At the same time, we are working with national experts to develop training programs for industry in HACCP.

Something new on the horizon is Prime Connection - a new, user friendly computer system, accessible 24-hours a day, 7-days a week, toll free. This system in its final form, will make it possible to access important FDA information - the ISSC shippers list, retail food model codes and interpretations, the FDA Fish List, and the "Bug" Book - better known as "Foodborne Microorganisms and Natural Toxins". To date, there are over 850 registered users.

Last but not least, is consumer education. We feel strongly that a great deal of public concern has stemmed from misunderstanding and misconceptions about the safety of seafood. We have developed and distributed two videotape news releases for use by the television industry to explain both FDA's mandatory program and the FDA/NOAA joint program. Another one on shellfish safety is in the works. A new seafood safety hotline available in December will enable us to begin providing consumers with direct answers to questions about seafood. A new series of brochures aimed at high risk consumers will be published and distributed next year. On all of these materials, we are using this new logo and theme - "Get Hooked on Seafood Safety".

With respect to the new voluntary program, FDA and NOAA have all the essential talent, authority and ability to design and implement a new joint program which will serve as an adjunct to FDA's regular mandatory seafood inspection program and provide greater assurance of the safety, wholesomeness and labeling of seafood. It is based on the HACCP concept - Hazard Analysis Critical Control Points - which requires harvesters, manufacturers, importers and retailers to identify critical points in their operations, whereby a failure would result in an unacceptable public health, food hygiene or economic hazard. Then they apply controls at those critical points and monitor continuously to prevent hazards from developing. The HACCP concept has been in use by the low-acid canned food industry for 20 years and has been very successful.

The joint FDA/NOAA seafood inspection program, in its final form, will be voluntary and fee-for-service based, with a mark or seal signifying that the product has been produced under the program. We have just completed a pilot test for U.S. seafood processors, and intend to offer the formal program by the end of the year. We have begun a pilot for seafood sold at the retail level and hope to offer that inspection service later next year. We are also designing a pilot for the molluscan shellfish industry, which we hope will address products all the way from classification of harvesting waters to vessels, processing, transportation and retail sale.

In July, August and September of this year, we had meetings in Brussels, Mexico City and Kuala Lumpur to introduce the concept of our program to industry and government representatives. We will be proposing a pilot test for foreign processors in European, South and Central American and Asian countries. We were overwhelmed by the interest of the international community. This program

will require that either the foreign government be able to guarantee an inspection program equivalent to the new HACCP program in the U.S. for those plants wishing to participate, or we cooperate on an individual plant basis with the concurrence and cooperation of the government authorities. Initial and periodic verification inspections will be conducted by FDA and NOAA inspectors and the mark, signifying participation in the program, will be permitted on the product to be exported to the U.S.

Overall, we have taken on a tremendous task. But by working closely with other Federal and State agencies, and with industry, by increasing our research efforts, and by expanding and effectively targeting our inspectional efforts, we are providing a more efficient and effective regulatory network.

Thank you.

SPECTROPOLARIMETRIC PROPERTIES AND IMMUNOLOGICAL CHARACTERISTICS OF CRUSTACEAN POLYPHENOL OXIDASE

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INTRODUCTION

Polyphenol oxidase (PPO) (E.C. 1.14.18.1.), also known as tyrosinase, polyphenolase, phenolase, catechol oxidase, cresolase, and catecholase, is widely distributed in nature (Schwimmer, 1981). The unfavorable enzymatic browning caused by PPO on the surface of seafood products has been of great concern to food processors and scientists. Although melanin (blackening spot) formation does not affect the nutrient contents of food products, it is perceived as spoilage by consumers (Eskin et al., 1971). Economic loss resulting from this enzymatic action has always been significant and presents a problem to the food industry. Enzymatic browning of crustaceans (lobster, shrimp, and crab) due to PPO activity has been extensively studied (Chen et al., 1991a; Ferrer et al., 1989; Marshall et al., 1984; Simpson et al., 1988).

Recently Rolle et al. (1990) used circular dichroism (CD) spectro-polarimetry to demonstrate differences in the secondary structure between endogenously and trypsin activated forms of Florida spiny lobster PPO. Chen et al. (1991b) also showed that PPO's from different crustacean sources varied with respect to catalytic activity in the oxidation of DL-β-3,4-dihydroxyphenylalanine (DL-DOPA). They also showed variation in sensitivity to inhibition by kojic acid. Since the conformational structures among crustacean PPO's were not well documented, this study was undertaken to elucidate whether conformational difference exists among these PPO's using immunological techniques and circular dichroism spectropolarimetry.

MATERIALS AND METHODS

Fresh and non-sulfited white shrimp (*Penaeus setiferus*) and brown shrimp (*Penaeus aztecus*) were obtained from a local seafood store. Fresh Florida spiny lobster (*Panulirus argus*) tails were obtained from the Whitney Marine Laboratory (Marineland, FL) and transported in ice to the laboratory. Lobster cuticle and shrimp cephalothorax (head) were frozen in liquid nitrogen and ground into a fine powder using a Waring blender. The ground powder was stored at -20℃ until needed.

Extraction and Purification of PPO

PPO was extracted and purified according to the procedure of Chen et al. (1991a). Ground powder was added to 0.05 M sodium phosphate buffer (pH 7.2) containing 1 M NaCl and 0.2% Brij 35 at a ratio of 1/3 (w/v). The extract was stirred for 0.5 h at 4℃ and the suspension centrifuged at 8,000*g* (4℃) for 30 min. The supernatant was then dialyzed at 4℃ overnight against 3 changes of 4L 0.05 M sodium phosphate buffer (pH 6.5).

Crude PPO preparation was further purified using a nondenaturing preparative polyacrylamide gel electrophoresis (PAGE) system. Equipment utilized included a gel tube chamber (Model 175, Bio-Rad) and an EPS 500/400 power supply (Pharmacia). A one-mL aliquot of crude enzyme extract was applied to each of the eight gel tubes (1.4 cm I.D. x 12 cm length) containing 5% acrylamide gel and subjected to a constant current of 10 mA/tube in a buffer (pH 8.3) containing 5 mM Tris-HCl and 38 mM glycine (Sigma Chemical CO., 1984). PPO was visualized using a specific enzyme-substrate staining method; 10 mM DL-β-3,4-dihydroxyphenylalanine (DL-DOPA) in 0.05 M sodium phosphate buffer (pH 6.5) was used as substrate. After the R_f of PPO was determined using one of the eight gels, the remaining gels were sectioned at regions having the same R_f value. The sectioned gels containing PPO were homogenized in 0.05 M sodium phosphate buffer (pH 6.5) utilizing a tissue grinder. Following filtration, the filtrate was concentrated using an Amicon stirred cell (Model 8050).

Protein Quantitation and Enzyme Purity Determination

The protein contents of each PPO preparation was quantitated using the Bio-Rad Protein Assay kit with bovine serum albumin as standard. Enzyme purity was examined using a mini gel (7.5% acrylamide) system (Bio-Rad, 1985). PPO (20 μg protein/well) were loaded and electrophoresis was carried out at constant voltage (200 V) in an electrode buffer (pH 8.3) containing 25 mM Tris-HCl, 0.19 M glycine, and 3 mM sodium dodecyl sulfate (SDS) for 35 min. The purity of PPO preparations was examined by staining the gels with 10 mM DL-DOPA in 0.05 M sodium phosphate buffer (pH 6.5) and then with a Coomassie blue R-250 solution.

Enzyme Activity Assay

PPO activities were measured by adding 60 μL enzyme preparations to 840 μL 10 mM DL-DOPA in 0.05 M sodium phosphate buffer (pH 6.5) and monitored at 25℃ for 5 min. Maximal initial rate was determined as ΔA_{475nm}/min and one unit of PPO activity was defined as an increase in absorbance of 0.001/min at 25℃. Unless otherwise specified, experiments were carried out three times in duplicate. The enzyme activities of lobster, white shrimp, and brown shrimp PPO were determined as 4,500, 1,000, and 1,200 units/mg protein, respectively.

Antibody Production and Purification

One-mL purified lobster PPO (100 μg protein) was used as an antigen to inject into a hen biweekly. Antibody was isolated and purified from the egg yolk of immunized hen (Polson et al., 1985). One part egg yolk was added to 4 parts (v/v) 0.1 M sodium phosphate buffer (pH 7.6). The mixture was made up 3.5% (w/v) polyethylene-glycol (PEG) and stirred for 5 min. Following centrifugation at 5,000*g* (10℃) for 20 min, the supernatant collected was made up with 8.5% (w/v) PEG. The suspension was allowed to stand for 10 min followed by centrifugation at 5,000*g* (10℃) for 25 min. The pellet was dissolved in 2.5 volumes (v/v) of phosphate buffer (pH 7.6) and the suspension was made up with PEG to 12% (v/v). Again, the suspension was allowed to stand for 10 min and then centrifuged at 5,000*g* (10℃) for 25 min. The pellet was resuspended in 1/4 volume phosphate buffer and cooled to 0℃ before adding with an equivalent volume of 50% ethanol (-20℃). Following centrifugation at 10,000*g* (4℃) for 25 min, the precipitate was dissolved in 1/4 volume phosphate buffer and the suspension dialyzed overnight (4℃) against 4L 0.1 M sodium phosphate buffer (pH 7.6). The dialyzed antibody preparation was made up with NaN_3 to 0.1% and stored-refrigerated until needed.

Molecular Weight Determination of Antibody

The molecular weight of antibody preparation was determined using sodium dodecyl sulfate PAGE (SDS-PAGE). Mini slab gels (1.0 mm thickness) consisting of stacking gel (4% acrylamide) and separating gel (7.5% acrylamide) were used in this study. Prior to the addition of 20 µg aliquot to the sample well, antibody preparations were diluted with 4 volumes of sample buffer, and heated for 4 min at 95℃. An SDS-6H high Molecular Weight Protein Standards (Sigma) containing carbonic anhydrase (29 kD), egg albumin (45 kD), bovine albumin (66 kD), phosphorylase (97.4 kD), β-galactosidase (116 kD), and myosin (205 kD) was used. The methods of Weber and Osborn (1969) and Weber et al. (1972) were followed to estimate protein molecular wieghts.

IgY Titer Determination by Enzyme-linked Immunosorbent Assay (ELISA)

One hundred-µL lobster PPO containing 2.5 - 100 ng protein/well in 0.1 M $NaHCO_3$ and $NaCO_3$ (coating buffer, pH 8.6) was applied to an Immulon 2 microplate (Dynatech). Following overnight incubation at 4℃, the wells were aspirated and washed 4 times with PBS-Tween [0.01 M sodium phosphate, pH 7.4, containing 0.15 M NaCl and 0.2% (v/v) Tween 20]. After 100 µL primary antibody (0.01 - 10 µg) in PBS-Tween was added to each well, the plate was incubated at ambient temperature for 1 hr. Aspirations and washings were repeated as previously described before 0.1 mL secondary antibody (anti-chicken IgG-alkaline phosphatase conjugate) was added. Following another one-hour incubation, the microplate was aspirated and washed again with PBS-Tween. Following the addition of 0.1 mL *p*-nitrophenyl phosphate disodium (1.0 mg/mL) in assay buffer (0.05 M Na_2CO_3 0.05 M $NaHCO_3$ and 0.5 mM $MgCl_2$) to each well, the plate was further incubated at ambient temperature until color development. Absorbance at 405 nm was monitored using an ELISA reader. Coating buffer containing no antigen was used as the control.

Analysis of Antigenic Properties of PPO

The competitive ELISA adopted from Seymour et al. (1991) was employed to study whether PPO from white shrimp and brown shrimp possessed similar antigenic determinant as the Florida spiny lobster. After the microplate was coated with lobster PPO at 10 ng/well for one hour at room temperature, 100 µL aliquot of antibody-competitive PPO mixture was added. The antibody-PPO mixture was prepared by mixing the competitive PPO (lobster, white and brown shrimp; 0.2 - 2.0 µg/mL) with an equal volume of primary antibody solution (10 and 20 µg/mL of IgY) for 1 hr at ambient temperature. The assay procedures were conducted as previously described.

Immunoblotting

Purified lobster, white shrimp, and brown shrimp PPO and crude lobster PPO preparations were subjected to SDS-PAGE (mini gel system) and then electro-transferred to a nitrocellulose membrane. Fifty-ng aliquots of test samples were applied to each well and run with the SDS-6H protein standards.

Following electrophoresis, the gel was equilibrated in 100 mL of Towbin transfer buffer (25 mM Tris, 192 mM glycine, 0.1% SDS, and 20% methanol; pH 8.3) for 15 min. Electro-transfer was performed according to the Trans-Blot Electrophoretic Transfer Cell Instruction Manual (Bio-Rad, 1989) at a constant voltage of 50 V for 1.5 hr using Towbin buffer as the electrolytic buffer. The efficiency of protein transfer was examined by staining the gel and the nitrocellulose membrane with Coomassie blue solution.

Nitrocellulose membrane, following electro-transferring, was rinsed with phosphate-buffered saline (PBS) 3 times, and then incubated with Blotto/Tween blocking solution (5% w/v nonfat dry milk, 0.2% v/v Tween 20, and 0.02% w/v NaN_3 in PBS; Harlow and Lane, 1988) at ambient temperature with agitation for 1 hr. After washing twice for 5 min each in PBS, the membrane was incubated in the primary antibody (IgY) solution (10 µg/mL) at ambient temperature for 1.5 hr. Following washing with 4 changes of PBS for 5 min each, the membrane was treated for 1 hr with the secondary antibody (antichicken IgG-alkaline phosphatase conjugate, Sigma) at the dilution of 1/2000. The membrane was then washed again with PBS as previously described, and incubated with 100 mL alkaline phosphatase buffer (100 mM NaCl, 5 mM $MgCl_2$ and 100 mM Tris; pH 9.5) containing 0.016% bromochloroindolyl phosphate (BCIP) and 0.032% nitro blue tetrazolium (NBT) (Harlow and Lane, 1988) for 30 min. The reaction was stopped by rinsing the membrane with PBS containing 20 mM EDTA.

Spectropolarimetric Analysis of PPO

The circular dichroic spectra of PPO was scanned at the far UV (250 - 200 nm) range using a Jasco J-20 automatic recording spectropolarimeter (Japan Spectroscopic Co., 1985). A 1.0-cm Suprasil (Helma Cells) cuvette with a 1.0-cm light path was used. Four-mL of PPO (15 µg/mL) in 0.5 mM sodium phosphate buffer (pH 6.5) was analyzed at ambient temperature. Calculations of the secondary structures were carried out by computer analysis of the spectra using the SSE program (Japan Spectroscopic Co.) with myoglobin, cytochrome c, ribonuclease A, lysozyme, and papain as circular dichroic references.

RESULTS AND DISCUSSION

Antibody Production and Molecular Weight Determination

The production of lobster PPO specific antibody did not occur until after the second boosting; peak activity occurred on day 18 (Fig. 1). Antibody activity decreased gradually with time following the third and fourth boostings. The serum eventually lost antibody activity after day 35. The animals apparently had become tolerant to the antigen after the third boosting. Antibody produced on day 17 and 18 having a higher activity was pooled for molecular weight determination. Three distinct bands were observed on the SDS-PAGE gel (Fig. 2). The molecular weight for the lower band was estimated as 66 kD which was close to the value reported for the heavy chain of IgY (Jensenius et al., 1981).

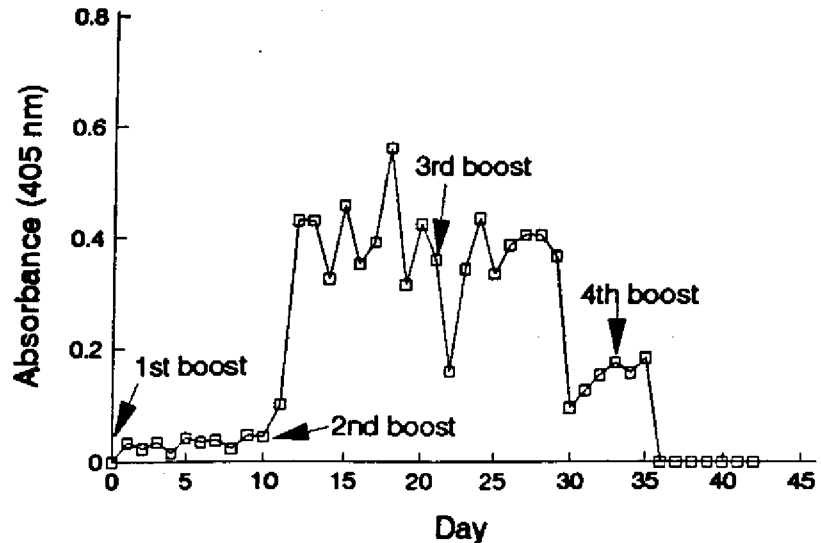

Figure 1. Production profile of anti-lobster PPO antibody.

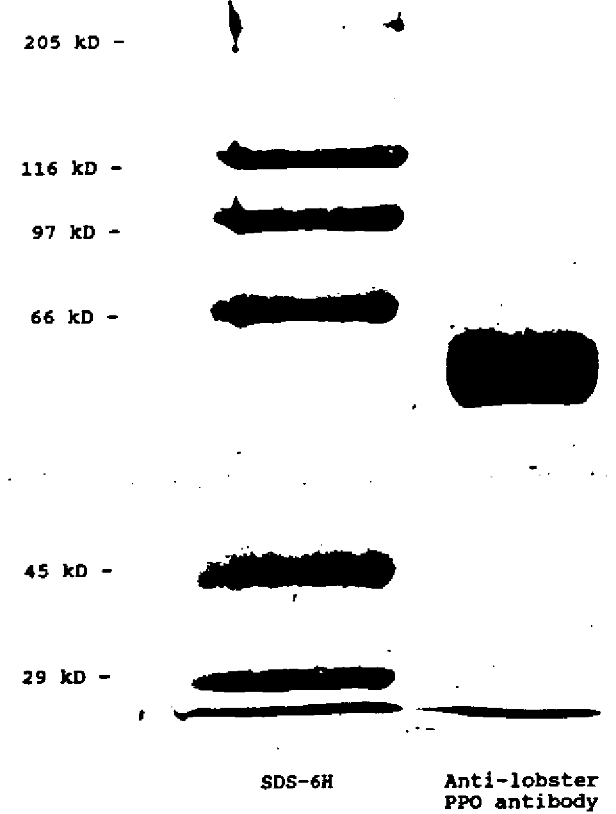

Figure 2. SDS-PAGE profile of anti-lobster PPO antibody. SDS-6H was used as protein standards.

Antibody (IgY) Titer Determination

The dose-related interactions of lobster, white shrimp, and brown shrimp PPO at 2.5 - 10 ng with lobster PPO-specific antibody at 2.5 μg are shown in Fig. 3. All test samples showed affinity for the antibody. Therefore, these antigens were partially cross-reactive and shared similar antigenic determinants. The appropriate dose of the various PPO to interact with antibody was determined to be 2.5 - 10 ng/well. When the PPO was added at more than 10 ng/well, the antigen-antibody reaction was saturated and the sensitivity of the ELISA became dull.

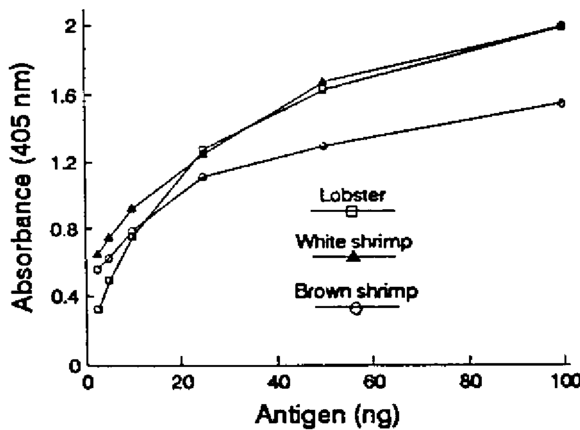

Figure 3. Titer determination of anti-lobster PPO antibody versus lobster (□), white shrimp (△), and brown shrimp (O) PPO.

Immunological Characteristics of PPO

The absorbance of the lobster PPO-antibody reaction mixture in the presence of various competitors (lobster, white shrimp, and brown shrimp PPO) at 405 nm as determined by ELISA is shown in Fig. 4. The color intensity was reduced by 18 - 79% of the control (Abs. = 0.967) when 12.5 ng of various competitors were added to the microplate containing 5 μg antibody/well. The increase of the competitor up to 100 ng/well slightly reduced the color intensity of the assay system. The results again indicated that these PPOs were partially cross-reactive and therefore shared similar structural components.

Complete transfer of PPO bands along with protein standards from acrylamide gel onto nitrocellulose membrane was achieved following electro-transferring; the staining of the treated gels with Coomassie blue revealed no protein bands (data not shown). The purified lobster, white shrimp, and brown shrimp PPO had only one protein band, while the crude lobster PPO preparation had 3 isozymes (Fig. 6a). The nitrocellulose membrane, following staining with lobster PPO-specific antibody, was found to contain dark spots corresponding to lobster, white shrimp, and brown shrimp PPO and crude lobster PPO preparation. Three dark spots were respectively observed for crude lobster PPO preparation, while only one dark spot for purified lobster, white shrimp, and brown shrimp PPO (Fig. 6b). Results from this study again verified our previous findings that these crustacean PPOs shared similar structural components.

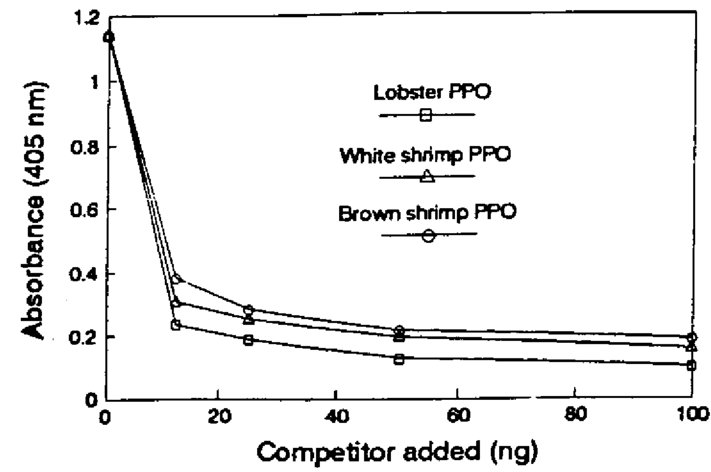

Figure 4. Analysis of antigenic properties of lobster (□), white shrimp (▵), and brown shrimp (O) PPO by competitive ELISA.

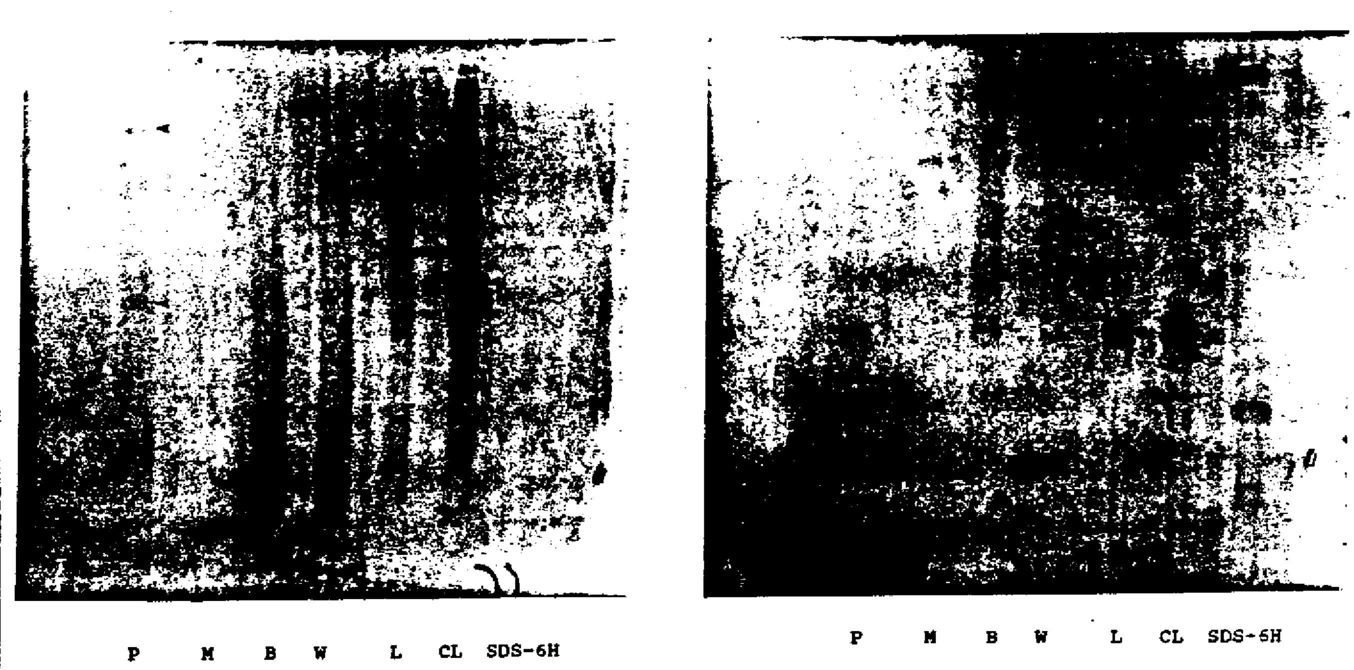

Figure 5. Protein profiles of electrotransferred standard SDS-6H, purified lobster (L), white shrimp (W), brown shrimp (B), and crude lobster PPO extract (CL) on nitrocellulose membrane (a). The nitrocellulose membrane was also stained with anti-lobster PPO antibody (b).

Spectropolarimetric Analysis of PPO

Lobster, white shrimp, and brown shrimp PPO showed similar circular dichroic spectra patterns (Figs. 6a, 6b, and 6c) but varied with respect to the secondary structures (Table 1). For example, the white shrimp PPO had a higher percentage of α-helix than brown shrimp PPO; they both showed the same broad negative ellipticity between 207 and 220 nm. The percentages of the secondary strucutures of these PPO estimated from the SSE program may not represent the absolute values. However, the results from this study showed that PPO from various sources possessed varied secondary structures.

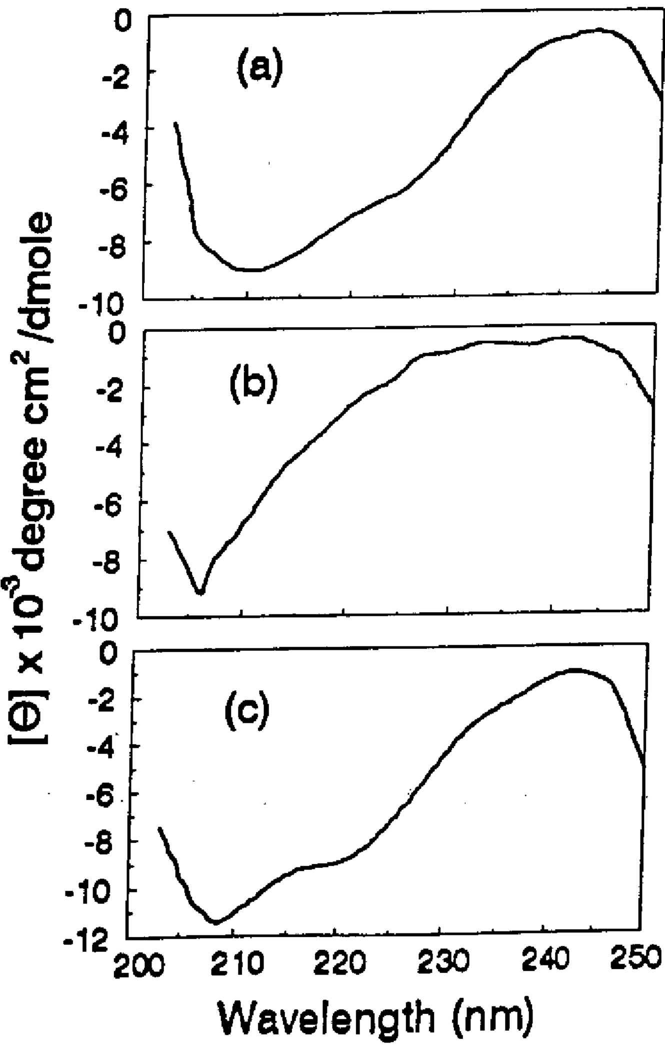

Figure 6. The far UV (250 - 200 nm) circular dichroic spectra of (a) lobster, (b) white shrimp, and (c) brown shrimp PPO.

Table 1. Secondary structure estimates of crustacean polyphenol oxidases (PPO) from far UV circular dichroic spectra

	% of Secondary structures			
PPO	α-helix	β-sheet	β-turn	random coil
Lobster	24.4	26.2	21.4	29.9
White shrimp	29.0	30.0	11.3	29.7
Brown shrimp	20.1	22.3	15.2	42.4

The circular dichroic spectra of PPO was scanned at the far UV (250 - 200 nm) range. Four-mL of PPO (10 - 20 μg/mL) in 0.5 mM sodium phosphate buffer (pH 6.5) was analyzed at ambient temperature.

CONCLUSION

Differences in the secondary structures (α-helix, β-sheet, β-turn, and random coil) among PPOs from Florida spiny lobster, white shrimp, and brown shrimp were verified by spectropolarimetric analysis. Using immunological assays, these PPO were shown to possess similar antigenic determinatants when they were tested against antibody (IgY) prepared from the hen immunized with lobster PPO.

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INACTIVATION OF CRUSTACEAN POLYPHENOL OXIDASE BY HIGH PRESSURE CARBON DIOXIDE

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INTRODUCTION

Undesirable enzymatic browning caused by polyphenol oxidase (E.C. 1.14.18.1; PPO) on the surface of seafood products has been of great concern to food processors because the formation of melanin reduces comsumer acceptability of these products. It thus has become a great challenge for food scientists to find solutions to inhibit and prevent undesirable browning. Several chemicals have been extensively studied for their effectiveness in inhibiting PPO activity (Madero and Finne, 1982; Ferrer et al., 1989; Chen et al., 1991b). However, problems related to off-flavor, off-odor, toxicity, and economic feasibility affect the application of these compounds (Eskin et al., 1971).

Supercritical (SC) carbon dioxide (CO_2) has been reported to inactivate peroxidase (Christianson et al., 1984), pectinesterase (Arreola, 1990), and apple PPO (Zemel, 1989). CO_2 is used as the SC fluid because it is non-toxic, non-flammable, inexpensive and readily available (Hardardottir and Kinsella, 1988). CO_2 has a relatively low critical temperature and pressure (Rizvi et al., 1986). Taniguchi et al. (1987) studied the retention of the activities of α-amylase, glucose oxidase, lipase, and catalase activity by SC-CO_2 Although SC-CO_2 has been shown to inactivate PPO, information regarding the inhibitory effect and inactivation kinetics of SC-CO_2 on purified PPO has not been elucidated. This study was thus undertaken to investigate the effect of SC-CO_2 on the inactivation of purified Florida spiny lobster and brown shrimp PPOs.

MATERIALS AND METHODS

Fresh Florida spiny lobster (*Panulirus argus*) tails were obtained from the Whitney Marine Laboratory (Marineland, FL) and transported in ice to the Food Science and Human Nutrition Department, University of Florida, Gainesville, FL and stored at -20℃. Non-sulfited fresh brown shrimp (*Penaeus aztecus*) were obtained from a local seafood store. Lobster cuticle and shrimp cephalothorax (head) were frozen in liquid nitrogen and ground into a fine powder using a Waring blender. The ground powder was stored at -20℃ until needed.

Extraction and Purification of PPO

PPO was extracted and purified following the procedures of Chen et al. (1991a). Ground powder was added to 0.05 M sodium phosphate buffer (pH 7.2) (1:3 w/v)

containing 1 M NaCl and 0.2% Brij 35, stirred for 0.5 h at 4℃, and then centrifuged at 8,000*g* (4℃) for 30 min. The supernatant was dialyzed at 4℃ overnight against 3 changes of 4L distilled water.

Crude PPO preparation was further purified using a nondenaturing preparative polyacrylamide gel electrophoresis system. Equipment utilized included a gel tube chamber (Model 175, Bio-Rad) and an EPS 500/400 power supply (Pharmacia LKB Biotechnology Inc.). A one-mL aliquot of crude PPO extract was applied to each of the eight gel tubes (1.4 cm I.D. x 12 cm length) containing 5% acrylamide gel and subjected to a constant current of 10 mA/tube in an electrode buffer (pH 8.3) containing 5 mM Tris-HCl and 38 mM glycine (Sigma Chemical Co., 1984). PPO was visualized using a specific enzyme-substrate staining method; 10 mM DL-β-3,4-dihydroxyphenylalanine (DL-DOPA) in 0.05 M sodium phosphate buffer (pH 6.5) was used as substrate. After the R_f was determined using one of the eight gels, the remaining gels were sectioned at the determined R_f. The enzyme was eluted from the gel by homogenization in distilled water utilizing a tissue grinder. The homogenates were filtered, pooled, and concentrated. The concentrated PPO was dialyzed overnight (4℃) against 2 changes of 4 L distilled water.

Protein Quantitation and Enzyme Purity Determination

The protein contents of PPO preparations were quantitated using the Bio-Rad Protein Assay kit with bovine serum albumin as standard. Enzyme purity was examined using a mini gel (7.5% acrylamide) system (Bio-Rad, 1985a). PPO (20 μg protein/well) was loaded and electrophoresis was carried out at constant voltage (200 V) in an electrode buffer (pH 8.3) containing 25 mM Tris-HCl and 0.19 M glycine for 35 min. The purity of enzyme preparations was determined by comparing gels stained with 10 mM DL-DOPA in 0.05 M sodium phosphate buffer (pH 6.5) and then with a Coomassie blue R-250 solution.

Enzyme Activity Assay

PPO activities were measured by adding 60 μL enzyme preparations to 840 μL 10 mM DL-DOPA in 0.05 M sodium phosphate buffer (pH 6.5) and monitored at 25℃ for 5 min. Maximal initial rate was determined as ΔA_{475nm}/min and one unit of PPO activity was defined as an increase in absorbance of 0.001/min at 25℃. Unless otherwise specified, experiments were replicated three times. For this study, the enzyme activities of lobster and brown shrimp PPO were determined as 3,750 and 740 units/mg protein, respectively.

Effect of High Pressure CO_2 on PPO Activity

Apparatus used to study the inactivation of PPO by high pressure CO_2 is illustrated in Fig. 1. Coleman grade CO_2 (99.99% pure, Liquid Air Co.) was connected to a high-pressure resistant stainless steel vessel (volume = 100 mL) equipped with valves through a metal hose. After the vessel was immersed into a water bath maintained at 43℃, a constant pressure of 58 atm (850 psi) inside the vessel chamber was achieved by adjusting the pressure-regulating valve. For each study, 80 mL lobster (0.15 mg protein/mL) or brown shrimp (0.27 mg protein/mL), PPO preparation was placed in the vessel. After treatment parameters were equilibrated to the desired conditions, the vessel was removed from the water bath and an 8 mL aliquot was removed for PPO activity determination. Each time after sampling, the vessel was immediately replaced into the bath. Sampling was done every minute during the first 5 min and 5 min thereafter.

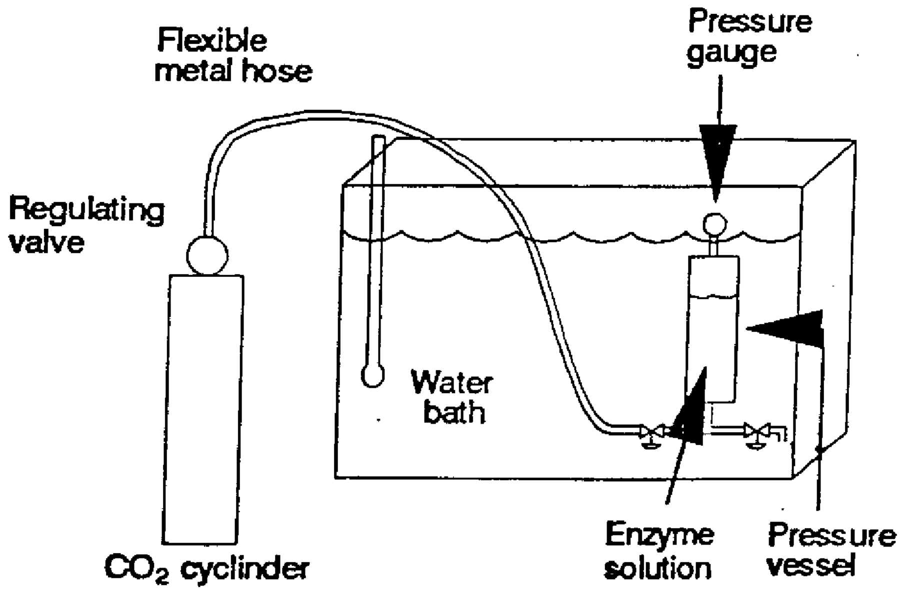

Figure 1. Apparatus for the study of polyphenol oxidase inactivation by high pressure CO_2

Following equilibration to ambient temperature, the PPO activity was determined as previously described. The activity for temperature controls was also determined. Percentage of relative activity was determined as $(E_t/E_o) \times 100$, where E_t were the PPO activities at time t and E_o the PPO activity without heat and CO_2 treatments. The pH change resulting from CO_2 treatment during the course of study was monitored using a pH meter.

Kinetics of PPO Inactivation

The reaction rate constant (k) for PPO inactivation in the presence or absence of CO_2 treatment was determined by measuring the maximal initial rate and plotting the logarithmic value of (V_t/V_o) versus time (t). V_t represents the activity of high pressure CO_2 treated PPO at time t and V_o the original activity of non-treated PPO (Segel, 1976). The negative slope of the equation equals to the reaction constant for PPO inactivation.

Mass Balance of CO_2 treated and Non-treated PPO

CO_2 treated PPO solution (1.5 mL) was centrifuged in an Eppendorf 5415 microcentrifuge at 13,000 rpm for 30 min. After the supernatant was removed, the pellet was redissolved in 0.5 mL 0.05 M. sodium phospahte buffer (pH 6.5), and the protein contents of both portions was quantitated. The combined protein contents from both portions were then correlated to that of an equal volume (1.5 mL) of non-treated PPO.

The protein patterns of CO_2-treated and non-treated PPO were also analyzed using mini sodium dodecyl sulfate (SDS) polyacrylamide gel of 7 x 8 cm (1 mm thickness). Twenty five-µL supernatant and pellet portions of CO_2-treated and non-treated PPO were separately loaded onto the sample well, and electrophoresis was carried out as previously described. Following electrophoresis, the gel was stained with the Bio-Rad silver stain kit. Molecular weights of PPO were determined by comparing the R_f values of proteins with those of standards (SDS-6H, Sigma) containing carbonic anhydrase (29 kD), egg albumin (45 kD), bovine albumin (66 kD), phosphorylase B (97.4 kD), β-galactosidase (116 kD), and myosin (205 kD).

Polyacrylamide Gel Isoelectric Focusing

A mixture containing 5% acrylamide, 5% glycerol, and 6.2% ampholyte (Pharmalyte 3-10, Pharmacia) was degassed for 5 min. After 5% (v/v) fresh ammonium persulfate and 0.1% (v/v) N,N,N',N'-tetramethylethylenediamine (TEMED) were added, the gel mixture was poured into 16 x 20 cm slab gel plates assembled with a 0.75 mm comb and allowed to polymerize for 1.5 hr (Bio-Rad Labs., 1985b). Following the removal of the comb, the buffer containing 0.2% (v/v) Pharmalyte 3-10 and 5% (v/v) glycerol was overlayed onto the polymerized gels and allowed to sit for 1 hr. Prefocusings at constant voltages of 200 V (15 min), 300 V (30 min), and 400 V (30 min) were alternately carried out after the overlaying buffer was changed. A 50-µL PPO preparation was loaded into the sample well and electrofocusing was performed at a constant voltage of 400 V for 17 hr. The gel was fixed with the fixative solution (sulfosalicylic acid/trichloroacetic acid/ methanol, 4:12.5:30, v/v) and then stained with Coomassie Blue R-250. The isoelectric point (pI) of PPO was determined by comparing the R_f value of the sample with those of protein standards (Broad pI Kit, pH 3-10, Pharmacia) containing amyloglucosidase, pI 3.50; soybean trypsin inhibitor, pI 4.55; β-lactoglobulin, pI 5.20; bovine carbonic anhydrase, pI 5.85; human carbonic anhydrase B, pI 6.55; horse myoglobin-acidic band, pI 6.85; -basic band, pI 7.35; lentil lectin-acidic band, pI 8.15; -middle band, pI 8.45; -basic band, pI 8.65; and trypsinogen, pI 9.30.

Spectropolarimetric Analysis of PPO

The circular dichroic (CD) spectra of CO_2-treated and non-treated PPO were scanned at the far UV range (250 - 200 nm) using a Jasco J-20 automatic recording spectropolarimeter using a 1.0-cm Suprasil (Helma Cells) cuvette with 1.0-cm light path. Four-mL PPO (10 µg/mL) in 0.05 M sodium phosphate buffer (pH 6.5) was measured at ambient temperature. Secondary structure calculations were performed by computer analysis of the CD spectra using the SSE program (Japan Spectroscopic Co., 1985) with myoglobin, cytochrome c, ribonuclease A, lysozyme, and papain as CD references.

Study of Restoration of PPO Activity

To examine the reactivation ability of PPO following CO_2 treatment, a portion of CO_2-treated sample was stored at -20°C for 6 weeks in a microcentrifuge tube. After thawing at ambient temperature, the pH of the PPO solution was measured using a digital pH meter. The enzyme activity was determined as previously described and the assays were performed weekly. Percentage of relative activity was determined as $(ER_t/ER_o) \times 100$, where ER_t were the activities of CO_2-treated PPO stored at time t and ER_o the original activity of non-treated PPO.

RESULTS AND DISCUSSION

Effect of High Pressure CO_2 on PPO Activity

Heating of lobster and brown shrimp PPO at 43℃ for 30 min caused some loss of activity (Figure 2). The treatment of these two PPO with high pressure (58 atm) CO_2 at 43℃, however, caused a dramatic loss of enzyme activity (Figure 2). Lobster and brown shrimp PPO after treatment for 1 min respectively retained only 2% (ΔA_{475nm}/min = 0.001 vs. 0.083) and 22% (0.010 vs. 0.046) of the original activity, respectively. Extended treatment of lobster and brown shrimp PPO for more than one min caused a complete loss of the activity. In these two PPO, the treatment for 10 and 15 min respectively caused protein precipitation. CO_2 treatment thus caused PPO denaturation and the loss of lobster and brown shrimp activity. The results also showed that brown shrimp PPO was slightly more resistant than lobster PPO to high pressure CO_2 treatment at 43℃. In comparison with the studies of Christianson et al. (1984) and Taniguchi et al. (1987), it appears that lobster and brown shrimp PPO were more vulnerable to high pressure CO_2 than corn germ peroxidase, α-amylase, glucoamylase, β-galactosidase, glucose oxidase, glucose isomerase, lipase, thermolysin, alcohol dehydrogenase, and catalase.

High pressure CO_2 treatment at 43℃ for 1 min caused a drop in pH from 9.1 to 5.4 for lobster PPO and 6.5 to 4.8 for brown shrimp PPO (Figure 2). The treated lobster and brown shrimp PPO was then constant at pH 5.3 and 4.5, respectively, throughout the experiment.

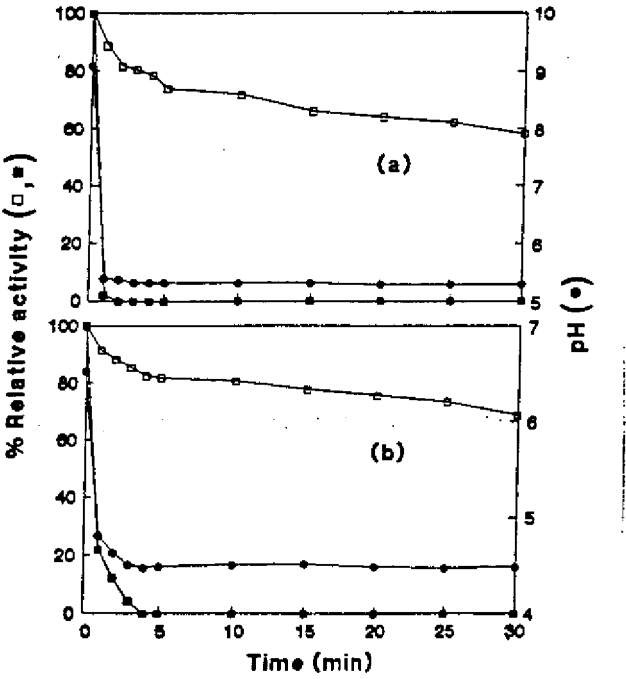

Figure 2. Effect of high pressure (58 atm) CO_2 treatment at 43℃ on changes in pH (●) and enzyme activity (■) of (a) Florida spiny lobster and (b) brown shrimp PPO. The treatment at 43℃ on PPO activity in the absence of CO_2 (□) was also conducted.

Kinetics of PPO Inactivation

The reaction constants (k) for lobster and brown shrimp PPO inactivation at 43℃ were determined to be 1.6×10^{-2} and 9.4×10^{-3}, respectively. Under the same heating condition in the presence of CO_2 (58 atm), the k value for brown shrimp PPO was changed to 0.98 min^{-1}. The k value for lobster PPO was expected to be greater than that of brown shrimp PPO since no enzyme activity was detected after lobster PPO was exposed to CO_2 at 43℃ for 1 min. Brown shrimp PPO was thus more resistant than lobster PPO to high pressure CO_2 at 43℃. The results also showed that the combined treatment of heat and high pressure CO_2 enhanced the inactivation of brown shrimp PPO by 104 (0.98 min^{-1} / $9.4 \times 10^{-3} min^{-1}$) fold than by heat alone.

Mass Balance of CO_2-treated and Non-treated PPO

Protein precipitation occurred after lobster and shrimp PPO were subjected to high pressure CO_2 treatment. The combined protein contents in the supernatant and pellet portions of the treated samples were close to that of their respective non-treated controls (Table 1). The combined protein patterns of the supernatant and the pellet portions of CO_2-treated lobster PPO matched those of the non-treated control (Figure 3). The results thus indirectly suggest that high pressure CO_2 treatment could denature enzyme molecules and cause loss of catalytic function.

Table 1. Mass balance of protein contents of CO_2-treated and non-treated PPO

	Protein content (µg)		
		CO_2 treatment	
PPO	Control*	Supernatant	Pellet
Lobster	464±4[@]	132±7	332±5
Brown shrimp	507±5	148±3	359±6

* The total protein contents in 1.5 mL PPO solution was quantitated using the Bio-Rad Protein Assay kit.

@ Mean value ± standard deviation

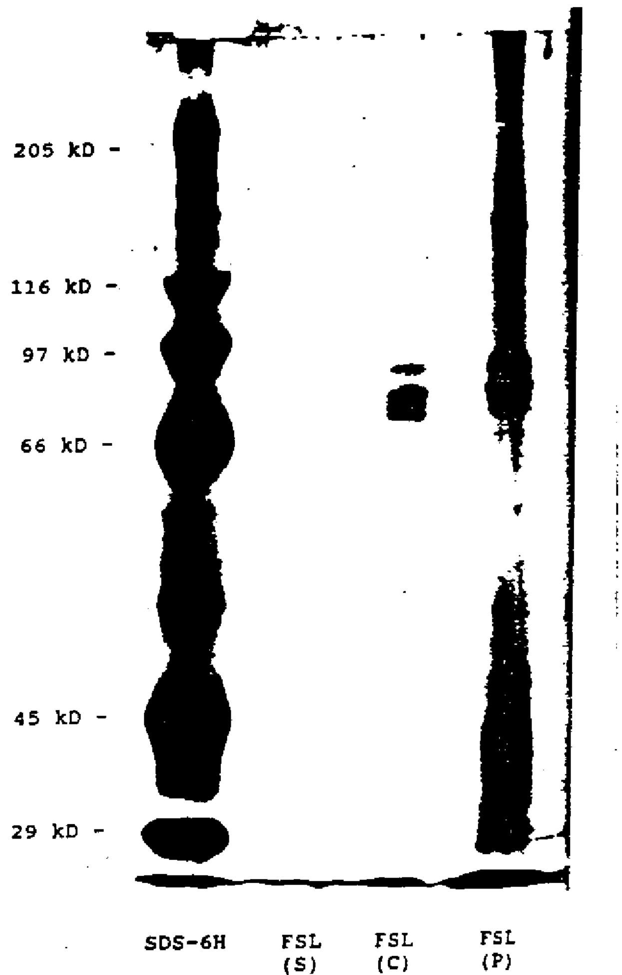

Figure 3. SDS-PAGE profiles of Florida spiny lobster (FSL) PPO. FSL (C) is the non-treated PPO, while FSL (S) and FSL (P) respectively represent the supernatant and pellet portions of high pressure (58 atm) CO_2 treated PPO following centrifugation.

Polyacrylamide Gel Isoelectric Focusing

Non-treated lobster and brown shrimp PPO on IEF gels only showed one protein band with an isoelectric point (pl) of 6.0. These PPO's upon high pressure CO_2 treatment, however, all showed more than one protein bands including the one with the pl of 6.2 (data not shown). Such treatment thus disintegrated PPO molecules.

Spectropolarimetric Analysis of PPO

The CD spectra at far UV range of non-treated and high pressure CO_2-treated lobster and brown shrimp PPO are given in Figure 4. The negative ellipticity between 207 and 220 nm of the untreated controls were quite different from those of CO_2-treated PPO. CO_2-treatment caused changes in the secondary structures (α-helix, β-sheet, β-turn, and random coil) (Table 2). Both lobster and brown shrimp PPO showed alterations in the composition of α-helix and random coil. Chen et al. (1991c) has demonstrated that the change of the pH of the assay system and the bubbling effect due to the use of CO_2 could not exclusively be responsible for the loss of PPO activity. This was in agreement with the previous findings of Miller et al. (1981) who proposed that the pressure-induced effect from SC-CO_2 treatment could cause changes in protein backbone structure and subunit disocciation and thus inactivate the enzyme.

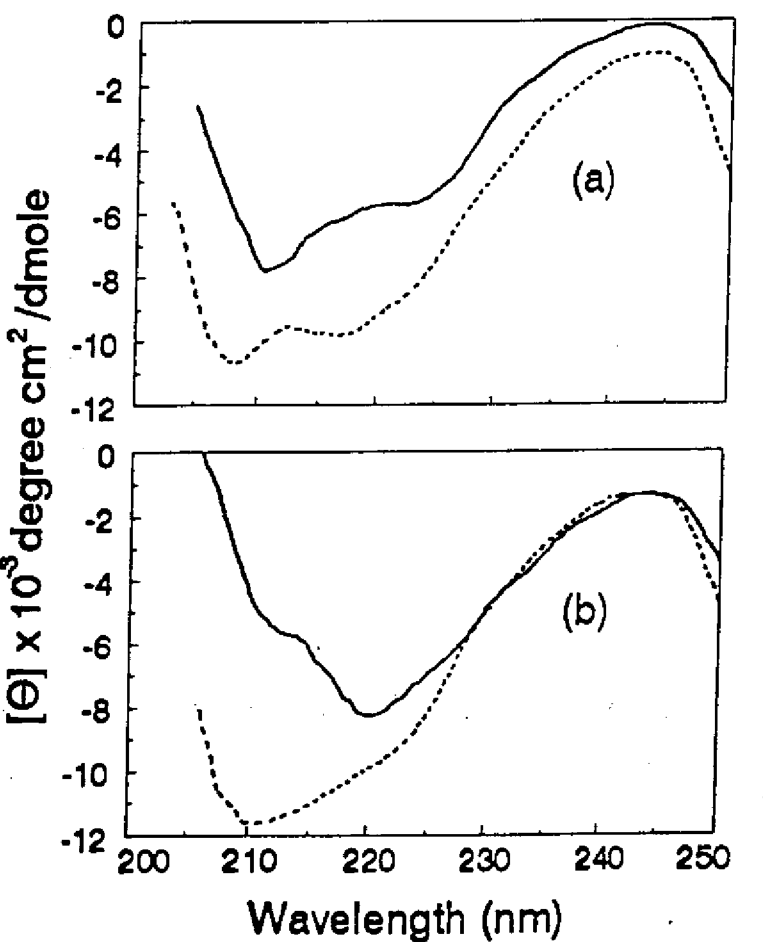

Figure 4. The circular dichroic spectra of (a) Florida spiny lobster and (b) brown shrimp PPO before (......) and after (—) high pressure CO_2 treatment.

Table 2. Secondary structure estimates of non-CO_2treated and high pressure CO_2 treated Florida spiny lobster and brown shrimp polyphenol oxidases (PPOs) from far UV circular dichroic spectra

		% of secondary structure			
PPO		α-helix	β-sheet	β-turn	random coil
Lobster	non-treated	24.4	26.2	21.4	29.9
	CO_2treated	19.7	25.9	15.2	39.3
Brown shrimp	non-treated	20.1	22.3	15.2	42.4
	CO_2treated	29.6	18.9	18.2	33.3

The circular dichroic spectra of PPO was scanned at the far UV (250 - 200 nm) range. Four-mL PPO (15 μg/mL) in 0.05 mM sodium phosphate buffer (pH 6.5) was analyzed at ambient temperature.

Restoration of Enzyme Activity of CO_2treated PPO

Lobster and brown shrimp PPO after losing their enzyme activity following high pressure CO_2treatment at 43°C did not restore the activity upon 6-week of frozen storage. However, the pH of these two systems came back from 5.3 to 9.1, and 4.5 to 6.5, respectively (Figures 5a and 5b).

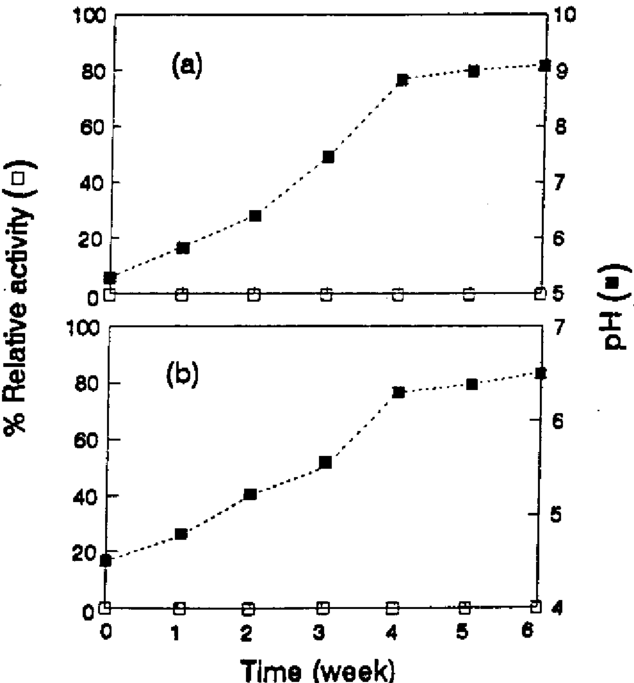

Figure 5. The restorative ability of high pressure CO_2treated (a) Florida spiny lobster and (b) brown shrimp PPO activity (□) and the pertinent environmental pH changes (■) during frozen storage.

CONCLUSION

Purified Florida spiny lobster and brown shrimp polyphenol oxidase (PPO) showed a time-related decrease in activity when subjected to high pressure CO_2(58 atm) treatment at 43℃. Lobster PPO was more vulnerable than brown shrimp PPO to high pressure CO_2 Studies using gel electrophoresis showed that there were differences in the isoelectric profiles and protein patterns between the CO_2treated and non-treated PPO. Spectropolarimetric analysis revealed that CO_2treatment caused changes in the secondary structures of the PPO.

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COMPARISON OF BLUE CRAB PROTEIN PATTERNS BY ISOELECTRIC FOCUSING

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INTRODUCTION

There has been a tremendous growth in seafood consumption due to changes in consumer attitudes about health, nutrition and other factors. In order to meet the growth in demand, the U.S. seafood market has become increasingly dependent on imports from other countries (19). This has generated concerns about availability, price, safety and quality. Willful or unintentional adulteration by substituting lower quality and/or lower priced seafood products for higher priced products has led to an increasing demand for identifying species of fish, crab or other seafood in the market place. There are at least three broad testing methods available for species identification: chemical, electrophoretic and immunological methods (13). Several official electrophoretic methods, such as starch gel-zone electrophoresis, thin layer polyacrylamide gel isoelectric focusing and cellulose acetate strip, have been approved as the official AOAC method (1984) to differentiate species of seafood or seafood products.

Isoelectric focusing (IEF) has been extensively used for seafood species identification because of its high resolution in separating proteins. IEF provides reliable and reproducible electrophoretic protein patterns for differentiating closely related species (15). IEF resolution is so great that differences among individuals of the same species are also apparent as in the case of monkfish (16). Identification of cooked or processed seafood has not been successfully done as yet using this methodology. The difficulties encountered are primarily due to alterations of protein molecules by heat treatment. Heat treatment reduces the amount of water-extractable proteins as demonstrated by comparing electrophoretic patterns of heated to non-heated samples on polyacrylamide gels (6,7,10,20). The extractibility of heat denatured proteins can be increased by the use of sodium dodecyl sulfate (SDS) or urea. Krzynowek and Wiggin (11,12) showed that the genus of cooked or frozen crab meat could be identified with 98% accuracy among 79 samples, when crab meat proteins were extracted with urea and analyzed by IEF. However this procedure was not sensitive enough to identify individual species. A similar result was shown by An et al. (1) using an 8M urea extract of boiled shrimp proteins for analysis with SDS-polyacrylamide gel electrophoresis (SDS-PAGE). They also used 1% SDS to extract boiled shrimp proteins and reported results comparable to urea extracts. Urea has been incorporated in electrophoretic gels to enhance protein separation and resolution (5,8,17,18). By utilizing 9.2M urea IEF gel for the first dimension, an *Escherichia coli* lysate was successfully separated into over 1000 components by two-dimensional gel electrophoresis (17). An et al. (3) have also achieved a good protein separation for shrimp, fish and surimi samples using 9.2M urea and 2% ampholyte in the IEF gel.

The objectives of this study were to compare the IEF protein profiles of the water extracts of raw, male and female, lump and claw crab meat and the water and urea extracts

of cooked male and female, lump and claw crab meat obtained from different places within the U.S. Samples obtained from Florida during different months of the year were also compared. This was a part of a wider study aimed at detection of adulteration or mislabelling of crab meat obtained from the foreign and domestic markets.

MATERIALS & METHODS

Crab samples

Live blue crabs were obtained from the local seafood store (Gainesville, Florida) during different months of the year, J. M. Clayton Co.(Cambridge, Maryland), the Motivatit Seafoods, Inc. (Houma, Louisiana), and the South Carolina Crab Company (McClellanville, South Carolina) for comparison of their protein profiles using isoelectric focusing.

Protein extraction and sample preparation for gel electrophoresis

Water and 8 M urea (electrophoresis purity, BioRad) were used to extract proteins from raw and cooked crab samples. Both solvents contained 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM EDTA and 0.01% sodium azide to inhibit proteases and microbial growth. The meat samples were chopped and each combined separately with the individual extractants in a ratio of 1:3 (w/v). The samples were then homogenized at room temperature for 1 min using a Polytron (Brinkmann Instruments) and the homogenates were centrifuged at 26,900 x g for 20 min at 5℃. The supernatants were collected and the protein concentrations determined using the Lowry method (14). The final protein concentration was adjusted to 5 mg/ml with water and 6% sucrose added. Each sample was then put into small vials in 200-300 μl aliquots and stored at -70℃.

Isoelectric focusing

A gel mixture containing 4% (w/v) acrylamide, 2% (w/v) Triton X-100 and 9.2 M urea was heated at 37℃ with shaking for 5 min to dissolve urea. Following the addition of ampholyte mixture at a final concentration of 2% (v/v) [Pharmalyte 6.2% v/v (Pharmacia), 20% pH 3-10 and 80% pH 4-6.5], the mixture was degassed for 3 min. Fresh ammonium persulfate solution and N,N,N',N'-tetramethyl-ethylene diamine (TEMED) at a final concentration of 0.02% (v/v) and 0.14% (v/v) respectively were added. This gel mixture was then poured into 16 X 20 cm slab gel plates (0.75 mm thick) assembled with a comb. The gel was allowed to polymerize for 1-2 hr. The comb was removed and the gel overlayered with lysis buffer containing 9.2 M urea, 2% Triton X-100, and 2% (v/v) Pharmalyte (20% pH 3-10 and 80% pH 4-6.5). The gel was allowed to sit for another hour.

After the lysis buffer was replaced with fresh one, prefocusing of the gel was done at 200 V for 15 min, 300 V for 30 min and 400 V for 30 min in a Protean II electrophoresis slab gel unit (BioRad) using 10 mM phosphoric acid as the anode solution and 20 mM sodium hydroxide as the cathode solution. The lysis buffer and cathode solution were removed after prefocusing.

To each well, 100 μg of the protein sample was applied and the sample overlayered with an aqueous solution containing 2% (w/v) Triton X-100 and 2% (v/v) pharmalyte. The gel plate was then reassembled in the electrophoresis unit, and fresh cathode solution added to the chamber. Proteins were focused at room temperature for 17 hrs at 400 V with circulating tap water as coolant. The focused gel were fixed with 12.5% trichloroacetic acid and 4% sulfosalicylic acid, stained with 0.04% Coomassie blue R-250 in 27% isopropanol, 10% acetic acid and 0.5% copper sulfate and destained using 12% propanol, 7% acetic acid and 0.5% copper sulfate (2).

Determination of apparent pI values of protein bands

Apparent pI values of the crab proteins were determined indirectly by comparing their R_f values on the gel with those of the protein standards (Broad pI kit, pH 3-10, Pharmacia) containing: trypsinogen, pI 9.30; lentil lectin-basic band, pI 8.65; -middle band, pI 8.45; -acidic band, pI 8.15; horse myoglobin-basic band pI 7.35, -acidic band, pI 6.85; human carbonic anhydrase B, pI 6.55; bovine carbonic anhydrase, pI 5.85; ß-lactoglobulin A, pI 5.20; soybean trypsin inhibitor, pI 4.55; and amyloglucosidase, pI 3.50. The pH profile of the whole gel was determined using a flat surface combination pH electrode (Corning Glass Works). A linear relationship of gel pH and the R_f values of protein standards enabled the determination of apparent pI values for the corresponding standards. From the apparent pI and R_f values of the protein standards and the R_f values of the crab meat proteins on the gel, the apparent pI values of crab meat proteins were determined (2).

Gel processing and storage

Positive image was developed from the stained gel using electrophoresis duplicating paper (Eastman Kodak Company). The gels were then dried using a BioRad slab gel drier and stored for future reference. Protein profiles of the dried IEF gel were scanned using the video densitometer (Model 620, BioRad).

RESULTS & DISCUSSION

Comparison of crab meat protein patterns of male and female, and of lump and claw

The protein profiles of the water extracts of raw, male and female, and of lump and claw crab meat, obtained from Florida were very similar (Fig. 1). More bands were observed on the cathodic side of the gel. The characteristic bands were with apparent pI values of 5.54, 6.19, 6.35, 6.89, and 7.15.

Heat treatment of crabs reduced the water extractibility of proteins (Table 1). Heat treated samples showed a different protein pattern where the number of bands was greatly reduced (Fig. 2) when compared to raw samples. Dowdie and Biede (7) also observed a reduction in band numbers. The major bands were clustered in the pH range of 5.4 to 6.0.

The extractibility of heat denatured proteins was increased using 8M urea (Table 1) and an increase in the number of bands was observed on the focused gel. The characteristic bands were with pI values of 5.54, 5.65, 6.19, 6.35, and 6.89 (Fig. 3). The water extracts of raw and cooked, and urea extracts of cooked samples had overall different protein banding patterns on the focused gel. However the protein band with pI value of 5.54 was predominantly present in these patterns.

Comparison of the protein patterns of Florida crab meat obtained in different months

Characteristic protein profiles of the water extracts of raw male lump and the water and urea extracts of cooked male lump, of crab meat obtained from Florida in July, August, 1990 and January, April, and June, 1991, were obtained on the focused gels (Figs. 4, 5, and 6). There was no detectable variation in the number of protein bands during the different months. However, in some cases there was a difference in the intensity of the bands.

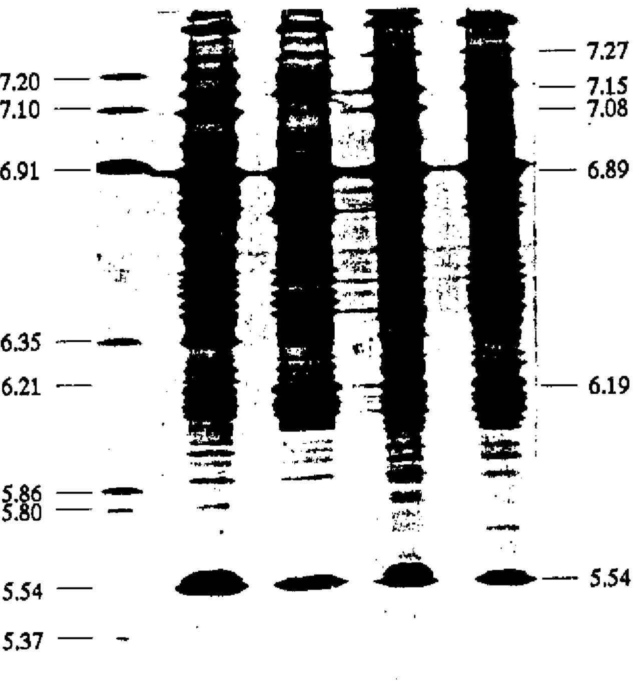
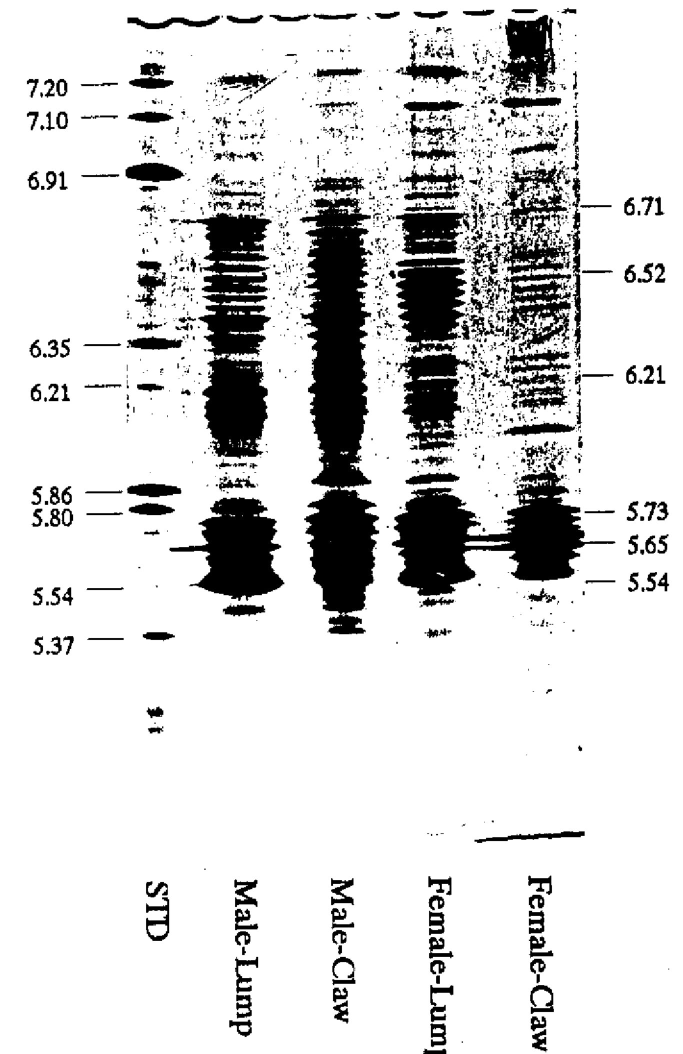


Fig. 1. IEF patterns of water extracts of raw blue crab meat, male-lump and claw and female-lump and claw (cathode on top). Protein standards (Std) are also included. The numerical designations indicate apparent pI values of the protein bands.



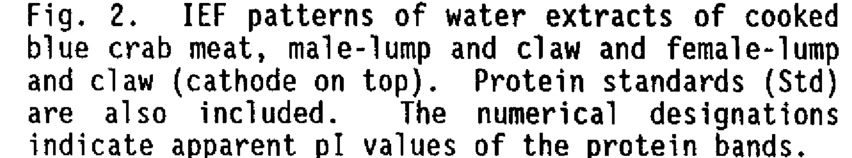


Fig. 2. IEF patterns of water extracts of cooked blue crab meat, male-lump and claw and female-lump and claw (cathode on top). Protein standards (Std) are also included. The numerical designations indicate apparent pI values of the protein bands.

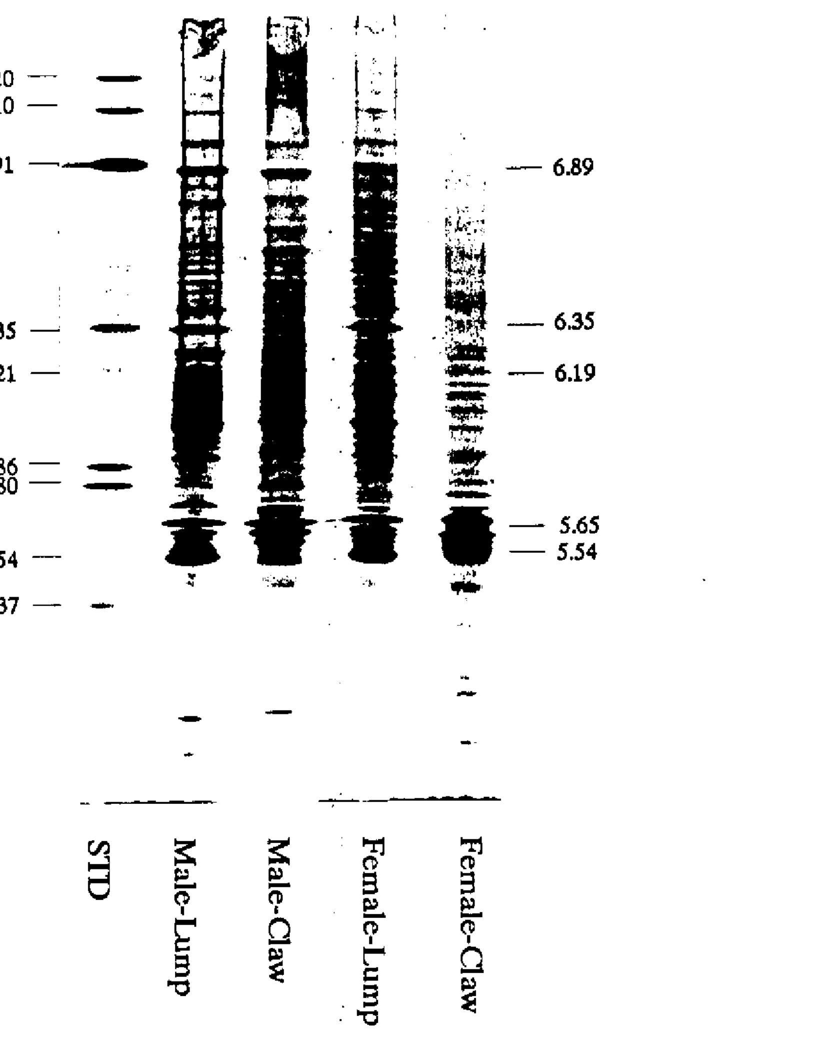


Fig. 3. IEF patterns of urea extracts of cooked blue crab meat, male-lump and claw and female-lump and claw (cathode on top). Protein standards (Std) are also included. The numerical designations indicate apparent pI values of the protein bands.

Fig. 4. IEF patterns of water extracts of raw, male-lump, Florida blue crab meat, obtained during the different months (cathode on top). Protein standards (Std) are also included. The numerical designations indicate apparent pI values of the protein bands.

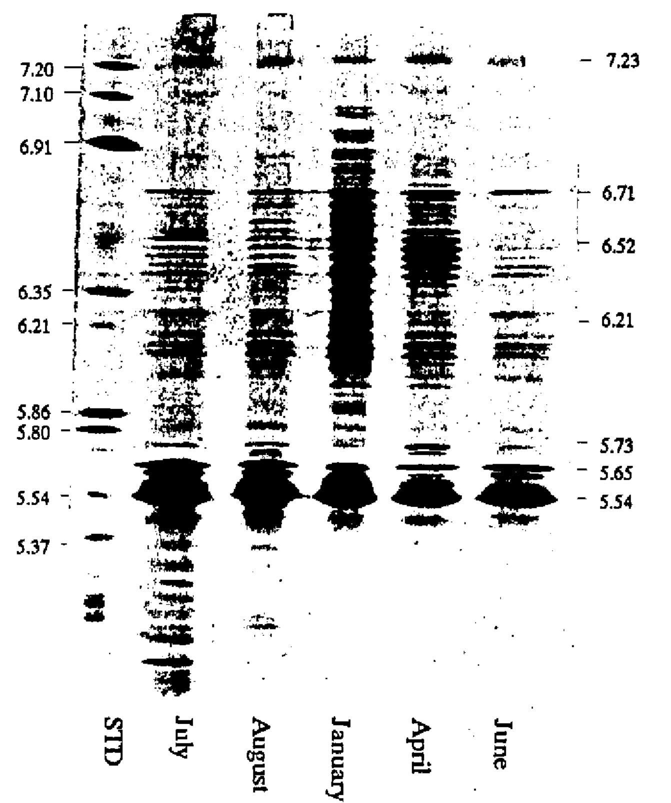
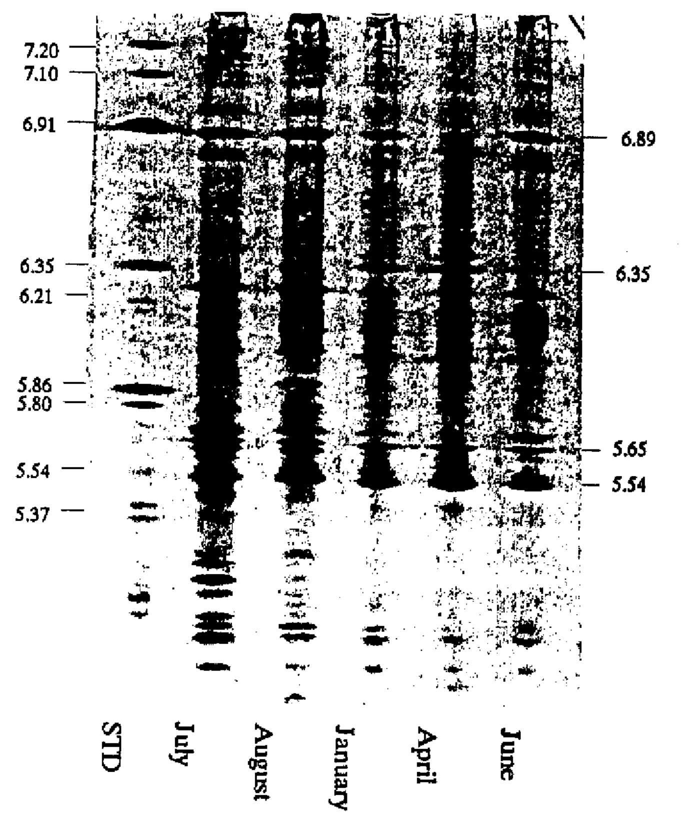


Fig. 5. IEF patterns of water extracts of cooked, male-lump, Florida blue crab meat, obtained during the different months (cathode on top). Protein standards (Std) are also included. The numerical designations indicate apparent pI values of the protein bands.



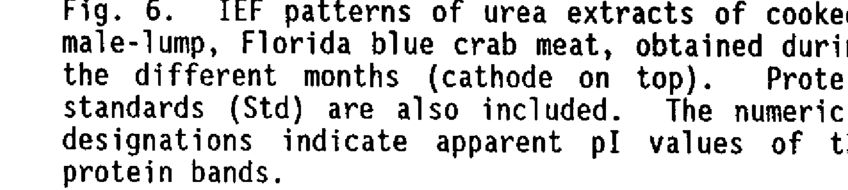


Fig. 6. IEF patterns of urea extracts of cooked, male-lump, Florida blue crab meat, obtained during the different months (cathode on top). Protein standards (Std) are also included. The numerical designations indicate apparent pI values of the protein bands.

Table 1: Protein concentration in crab meat extracts from various domestic origins (mg/ml)

	RAW		COOKED			
	Water		Water		Urea	
	Lump	Claw	Lump	Claw	Lump	Claw
Florida						
Male	6.60	6.70	2.23	2.51	11.83	820
Female	8.88	9.36	2.62	2.47	11.90	128
Maryland						
Male	9.91	7.61	4.23	4.39	15.33	109
Female	10.76	10.96	1.75	2.31	9.84	625
Louisiana						
Male	5.20	5.60	2.08	2.35	10.87	124
Female	6.88	7.04	2.05	2.42	14.46	893
South Carolina						
Male	6.89	7.04	3.00	2.86	6.96	682
Female	4.90	5.30	1.43	2.46	6.65	660

Comparison of the protein patterns of crab meat from various domestic origins

Table 1 gives the protein concentrations of the water extracts of raw, male and female, lump and claw and the water and urea extracts of cooked, male and female, lump and claw crab meat obtained from Florida, Maryland, Louisiana, and South Carolina. Characteristic protein patterns of all the samples were obtained on the focused gel. The protein banding patterns of the water extracts of raw male lump and the water and urea extracts of the cooked male lump, of samples from the four places within the U.S. is in general, very similar, however there is a difference in band intensities (Figs. 7, 8, and 9). The similarity of the protein banding pattern and the difference in intensity was evident in the densitometric scanning profiles (Fig. 10).

CONCLUSION

IEF is an effective method for comparing the protein profiles of the blue crabs. There is no difference in protein banding patterns within the blue crab species with respect to sex, body part chosen for sampling, and different months of sampling. The protein banding patterns of the samples from Florida, Maryland, Louisiana and South Carolina are also very similar, although there are some differences in band intensities. Heat-treatment of the crab meat caused a loss of water extractable proteins, and the number of protein bands was reduced. Urea greatly increased protein extractibility of the heat-treated samples and the number of bands was increased.

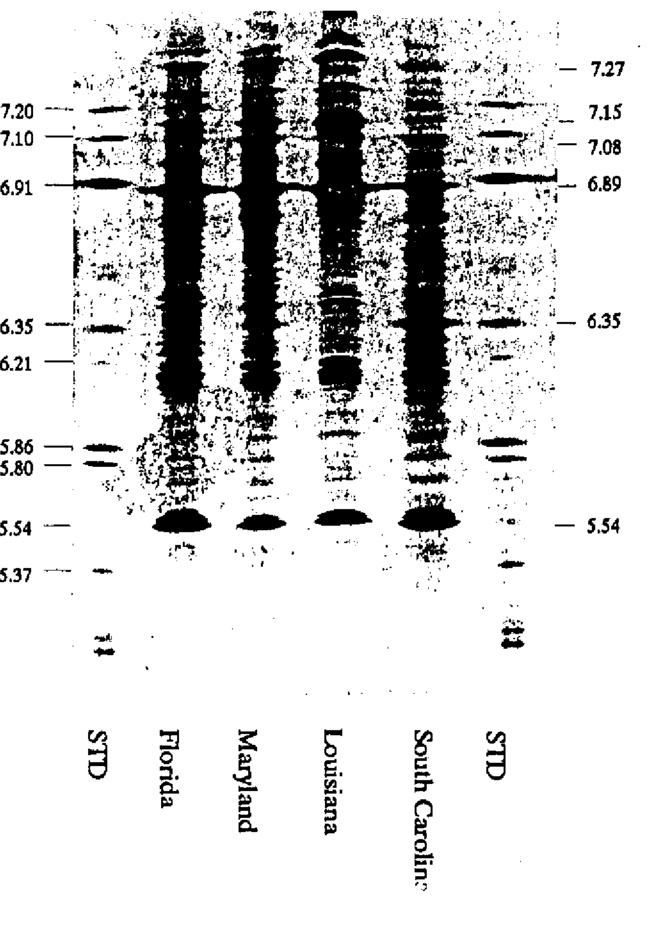
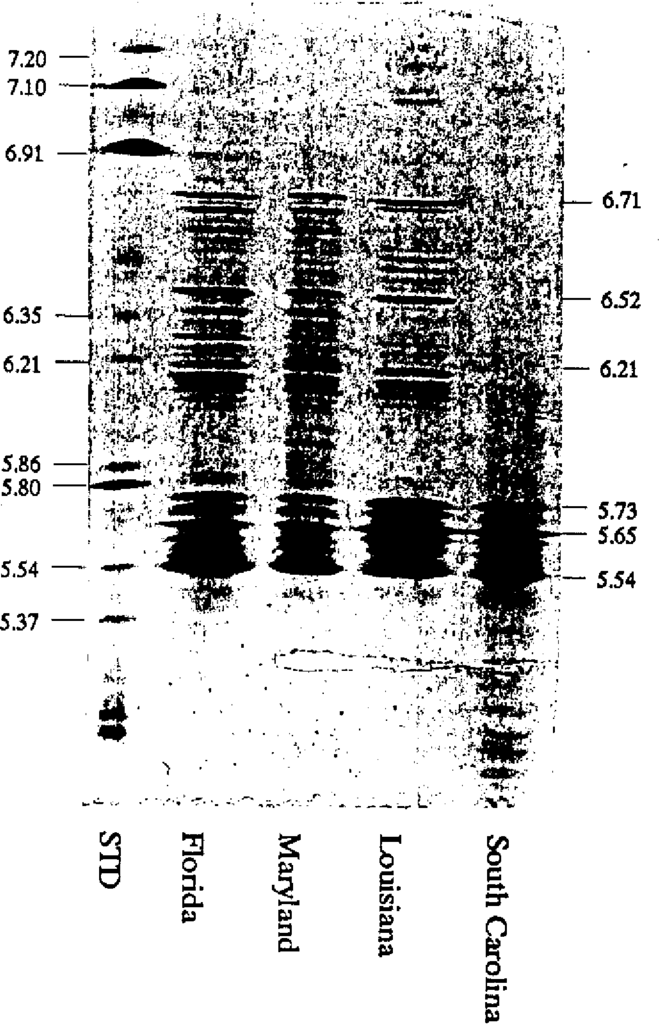


Fig. 7. IEF patterns of water extracts of raw, male-lump blue crab meat, obtained from various regions (cathode on top). Protein standards (Std) are also included. The numerical designations indicate apparent pI values of the protein bands.



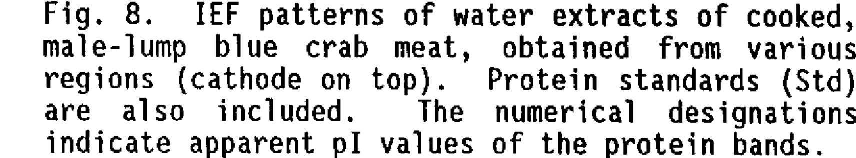
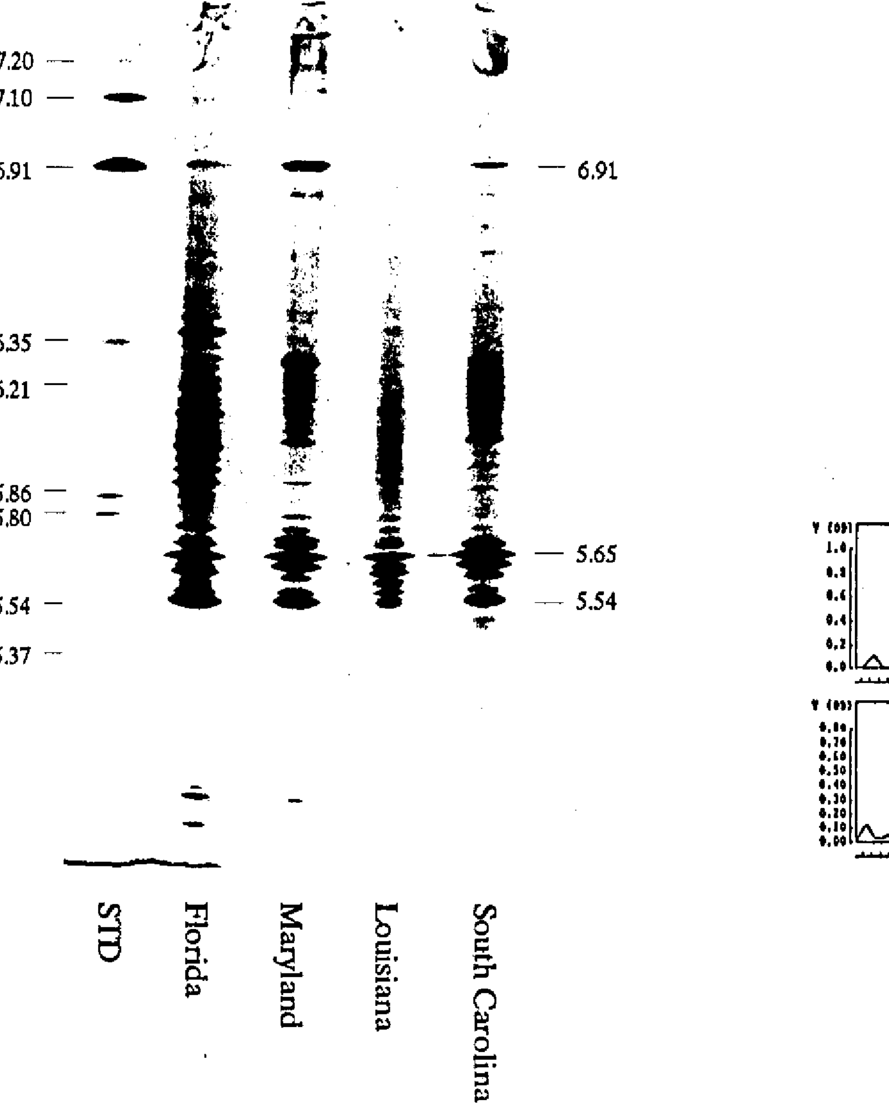


Fig. 8. IEF patterns of water extracts of cooked, male-lump blue crab meat, obtained from various regions (cathode on top). Protein standards (Std) are also included. The numerical designations indicate apparent pI values of the protein bands.



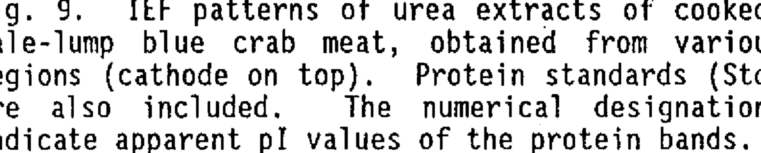


Fig. 9. IEF patterns of urea extracts of cooked, male-lump blue crab meat, obtained from various regions (cathode on top). Protein standards (Std) are also included. The numerical designations indicate apparent pI values of the protein bands.

Florida

Maryland

5 6 7 8 9 10 11 12 13

Distance from the cathode (cm)

Fig. 10. Densitometric profiles of water extracts of cooked, male-lump blue crab meat from Florida and Maryland.

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PURIFICATION AND CHARACTERIZATION OF A TRYPSIN-LIKE ENZYME FROM THE HEPATOPANCREAS OF CRAYFISH (PROCAMBARUS CLARKII)

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INTRODUCTION

Proteolytic enzymes have been used in a number of industrial applications including laundry detergents, feed, cheese making, meat tenderizing, fermented sauces, and the production of pharmaceuticals (18). Proteolytic enzymes obtained from livestock offal do not adequately meet the demand on a world basis and the future availability of traditional enzyme sources is sometimes dependent on the political and agricultural policies (10). Moreover, traditional animal enzyme sources have been restricted to relatively few species, namely, bovine and porcine offal.

Recent interest has developed concerning the proteases found in stomachless marine organisms. Stomachless marine organisms are deprived of the acid denaturation which takes place in the stomach, making the protein in the feed more amenable to subsequent degradation in the intestine by trypsin, chymotrypsin and other proteases or peptidases. Indeed, trypsin and chymotrypsin from higher vertebrates do not hydrolyze native proteins or do so at a very low rate compared to denatured proteins (15, 19). Additional evidence showed that a protease from crayfish hydrolyzed native ribonuclease and inactivated native lactate dehydrogenase while proteases from species having a stomach were not effective (22).

This paper describes the purification and characterization of a trypsin-like enzyme from the hepatopancreas of Louisiana swamp crayfish. The properties of this crayfish enzyme are also compared to those of bovine trypsin and trypsins from other marine organisms.

MATERIALS & METHODS

Preparation of crayfish extract

Louisiana swamp crayfish (*Procambarus clarkii*) purchased live from a local seafood store were decapitated, and the hepatopancreas removed, rapidly frozen in liquid nitrogen and ground to a powder using a Waring blender. The powdered hepatopancreas was stored at
-70℃.

Twenty-five grams of the powdered hepatopancreas was homogenized for 1 min in 125 mL of ice-cold distilled water and 30 mL of tetrachloromethane using a Waring blender. The homogenate was centrifuged at 27,000 x g for 20 min at 4℃ and the supernatant was collected for enzyme purification.

Purification of protease

Unless otherwise specified all steps were carried out at 4℃. The defatted crude extract was subjected to ammonium sulfate fractionation and the precipitate from the 30-70% saturation range was collected and resuspended in 25 mL of 10 mM Tris-HCl buffer (pH 7.0) containing 0.7 M $(NH_4)_2SO_4$ This sample was applied slowly (0.2 mL/min) to a Phenyl Sepharose CL-6B column (1.6 x 40 cm, Pharmacia). Elution was accomplished with a 0.7-0 M linear gradient of $(NH_4)_2SO_4$ in 10 mM Tris-HCl (pH 7.0). Material passing through the column was collected in fractions of 4 mL/tube using a Bio-Rad fraction collector (model 2110). Absorbance at 280 nm was recorded using a spectrophotometer. Fractions absorbing light at 280 nm were assayed for esterase activity according to the method of Hummel (14).

Fractions eluting between 120 and 160 mL were pooled and concentrated to 3 mL via ultrafiltration utilizing a 50K omegacell filter (Filtron) and applied to a 2.5 x 50 cm Sephadex G-100 column (Pharmacia) which was equilibrated and eluted with 50 mM Tris-HCl buffer (pH 7.0) at 0.2 mL/min. Material passing through the column was collected in 4 mL/tube fractions and checked for absorbance at 280 nm and esterase activity. Fractions eluting between 128 and 160 mL were pooled and applied to a 1.6 x 40 cm column packed with DEAE cellulose which had been equilibrated with 50 mM Tris-HCl (pH 7.0). Elution was accomplished using a 0-1 M linear gradient of NaCl in 50 mM Tris buffer at 0.2 mL/min. Material passing through the column was collected in 4 mL/tube fractions, and checked for absorbance at 280 nm and esterase activity. Fractions eluting between 120 and 168 mL were pooled and stored at -20℃ for further studies.

Activity measurement

Esterase activity of the protease was assayed using tosylarginine methyl ester (TAME) as substrate. Tosyl arginine released at 25℃ from TAME was measured by an increase in absorbance at 247 nm in a spectrophotometer under the assay conditions in which 100 μL enzyme preparation was mixed with 0.3 mL 0.01 M TAME, and 2.6 mM Tris-HCl (pH 8.1) containing 11.5 mM $CaCl_2$ (14). One TAME unit of activity was defined as ΔA_{247nm}/min x 1000 x 3 divided by 540; where 540 is the extinction coefficient of tosyl arginine (1).

Amidase activity was determined according to the method of Erlanger et al. (5) using N-α-benzoylarginine-*p*-nitroanilide (BAPA) as substrate. A 200 μL aliquot of the isolate was added to 2.8 mL of 1 mM BAPA in 0.05 M Tris-HCl (pH 8.2) containing 0.02 M $CaCl_2$ and the release of *p*-nitroanilide was measured at 410 nm at 25℃. One BAPA unit of activity was defined as ΔA_{410nm}/min x 1000 x 3 divided by 8800; where 8800 is the extinction coefficient of *p*-nitoanilide (5).

For assays with *p*-toluenesulfonyl-L-lysine methyl ester (TLME) and benzoyl-tyrosine ethyl ester (BTEE), conditions were the same as for TAME except for the substitution of the indicator substrate at a final concentration of 1 mM. A unit of TLME and BTEE activity is defined as the increase in absorbency of 0.001/min at 25℃ (14).

The influence of pH on protease activity and stability

Protease activity as influenced by pH was determined using TAME as substrate. The pH optimum for TAME hydrolysis was determined by preparing the substrate in the various buffer solutions: 0.1 M citrate-HCl, pH 2.0; 0.1 M citrate-NaOH, pH 3.0; 0.1 M citrate-NaOH, pH 4.0; 0.1 M citrate-NaOH, pH 5.0; 0.1 M citrate-NaOH, pH 6.0; 0.1 M Tris-HCl, pH 7.0; 0.1 M Tris-HCl, pH 7.5; 0.1 M Tris-HCl, pH 8.0; 0.1 M Tris-HCl, pH 9.0; and 0.1 M

glycine-NaOH, pH 10.0, and allowing hydrolysis by the enzyme to proceed as above. The kinetic constants (K_m' and V_{max}) were then estimated for each pH by Lineweaver-Burk plots at various concentrations of TAME (0.3 - 1.0 mM). Data were weighted using "EZ-FIT" software as described by Perrella (21).

Stability of protease at various pH's was determined by preincubating 25 µL of the enzyme in 275 µL of the various buffer solutions in an ice bath for 5 min prior to assaying for esterase activity.

Temperature stability

Thermostability of the protease was determined by equilibrating the enzyme at various temperatures for 30 min, cooling rapidly in an ice bath for 5 min and then adding substrate at 25℃.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Electrophoresis was performed under reducing conditions (17) utilizing a 5% stacking gel and a 12% separating gel. Proteins were detected by staining with 0.1% Coomassie brilliant blue R-250 dissolved in a solution containing 40% methanol and 10% acetic acid in water. Molecular weights were estimated according to the method of Weber and Osborn (27) using a Sigma low MW standard kit containing bovine serum albumin (66 KD), egg albumin (45 KD), glyceraldehyde-3-phosphate dehydrogenase (rabbit muscle, 36 KD), carbonic anhydrase (bovine erythrocytes, 29 KD), trypsin (24 KD), soybean trypsin inhibitor (20.1 KD), and α-lactalbumin (14.2 KD).

Isoelectric Focusing

Precast agarose isoelectric focusing gels (IsoGel), pH 3-10, 0.6 mm thick, were purchased from FMC Bioproducts. Purified protease (5µL) and isoelectric focusing standards (Broad pI kit, pH 3-7, Pharmacia) were loaded onto strips of blotting paper (Schleicher and Schuell Inc.) placed in the center of the gel and run for 2 h at 8 W (500 V limiting) on a Hoefer Isobox flatbed system using 10 mM phosphoric acid as the anode solution and 20 mM sodium hydroxide as the cathode solution. Gels were stained in 0.1% solution of Coomassie brilliant blue R-250 in 25% ethanol and 9% glacial acetic acid.

Protein Determination

Protein determinations were based on the dye binding assay as described in the Bio-Rad protein standard assay bulletin (Manual 82-0275-1282).

Inhibition Study

Enzyme inhibitors including phenylmethyl sulfonyl fluoride (PMSF), tosyl lysine chloromethyl ketone (TLCK), tosyl phenylalanine chlomethyl ketone (TPCK), aprotinin, soybean trypsin inhibitor (SBTI), benzamidine, 1,10-phenanthroline, and 8-hydroxyquinoline on the activity of crayfish protease (CP) were separately determined by incubating each inhibitor with the enzyme at 25℃ for 30 min before application of TAME and measurement of the initial rate of enzyme activity.

Activation study

The influence of the bivalent ions Ca^{+2}, Mg^{+2}, Zn^{+2}, Cu^{+2}, Hg^{+2} and Ag^{+1} on the CP activity was separately determined by incubating each ion with the enzyme at 25°C for 30 min before application of TAME and measurement of enzyme activity.

Antibody preparation

Immunological cross reactivity between proteases was tested by using purified protease from crayfish as an antigen to induce antibody production in hens. Animals were injected with 100 µg of the enzyme, and boosted every 2 weeks with an additional 100 µg. Antibodies were purified from egg yolk by the method of Polson et al. (23). Yolk was separated from the white, diluted with 4 volumes of 0.1 M sodium phosphate buffer (pH 7.6), and mixed with 3.5% (w/v) polyethelene glycol (PEG) until a homogeneous mixture was obtained. The suspension was centrifuged at 5,000 x g for 20 min. To the supernatant was added 8.5% (w/v) PEG, and mixed. After standing for 10 min, the mixture was centrifuged at 5,000 x g for 25 min. The precipitate obtained was dissolved in the phosphate buffer at a volume equal to 2.5 times that of the egg yolk. The mixture was made to 12% PEG (w/v) and let to stand for 10 min before centrifugation at 5,000 x g for 25 min. The pellet was dissolved in a phosphate buffer at a volume equal to 0.25 times that of the egg yolk, cooled to 0°C and then a same volume of 50% ethanol (v/v) precooled at -20°C was added. After centrifugation at 10,000 x g for 25 min, the pellet obtained was dissolved in phosphate buffer at a volume equal to 0.25 times that of the egg yolk. The antibody solution was dialyzed with stirring at 4°C against 50 volumes of phosphate buffer for 24 hr, made to 0.1% sodium azide, and stored at 4°C.

ELISA procedures

Diluted antigen (1 µg/mL in 0.1 M sodium carbonate buffer, pH 9.6) at 100 µL was added to each well of an Immulon plate (Fisher Scientific Co.). After incubation for 2 h at room temperature, the plate was washed four times with PBS-Tween buffer. Following the addition of diluted antibody solution (1/1000, 1/10,000 and 1/100,000 with PBS-Tween) at 100 µL per well and incubation for 2 h at room temperature, the plate was washed four times with PBS-Tween. Each well was then added with 75 µL anti-chicken alkaline phosphatase conjugate diluted 1/500 with PBS-Tween and incubated for an additional 2 h at room temperature. Following washing the plate four times with PBS-Tween, alkaline phosphatase substrate (*p*-nitrophenol acetate) dissolved in substrate buffer (0.1 M sodium carbonate and 0.5 mM magnesium chloride, pH 9.4) at 1 mg/mL was added (75 µL per well). Following incubation for 15 min, absorbance at 405 nm was read with an ELISA reader. The blank controls contained no antigen or antibody; the higher color intensity of the two was taken as the blank determination.

In competitive ELISA experiments, various amounts of competitors were mixed with the primary antibody solution prior to allowing them to interact with the bound antigen. Anti-CP antibody was used at 1/1000 dilution and 100 ng of CP was plated as antigen. CP, trypsin, chymotrypsin and pepsin at 0-100 ng were added as competitors to the anti-CP antibody. To exclude the possibility of antibody being digested by the proteases, either PMSF (5 mM) or benzamidine (10 mM) was added prior to incubation with antibody.

RESULTS & DISCUSSION

Purification of crayfish protease

A summary of CP purification is presented in Table 1. The specific activity of the purified CP (corresponding to 7% yield) was about 40-fold greater than that of crude extract. The yield of CP was low even though the extraction conditions (eg. pH and temperature) were in the stability range for the enzyme. The presence of other serine proteases that have high esterase activity toward TAME in the extract may have contributed to the low yield. Another possibility for the low yield could be attributed to the low content of the protease.

Table 1. Purification of crayfish protease from *Procambarus clarkii* hepatopancreas.

Step	Total protein (mg)	Total activity (units*)	Specific activity (units*/mg)	Yield (%)	Purification fold
Defatted crude extract	258.50	900	3.5	100	1.0
30-70% $(NH_4)_2SO_4$	75.20	825	10.9	92	3.1
HIC	8.90	696	78.2	77	22.3
G-100	1.41	144	102.0	16	29.1
DEAE-cellulose	0.40	63	157.0	7	44.9

*One unit catalyzes the hydrolysis of one μmol TAME/min at 25℃, pH 8.2.

Enzyme specificity

Purified CP only hydrolyzed TAME and TLME but not BAPA (Table 2). CP thus cleaves at sites next to arginine and lysine residues when coupled with methyl or ethyl esters, but not at sites next to arginine when coupled with *p*-nitoanilide (BAPA). Similar observations were reported with crustacean and bovine trypsins. Kimoto et al. (16) and Dendinger and O'Connor (3) reported a lack of activity when benzol-arginine naphthylamide (BANA) was used with trypsin-like enzymes from *Euphausia superba* and *Callinectes sapidus*, respectively. CP was also shown to have more than twice the activity with TAME than with TLME. It had no chymotryptic activity against BTEE.

Electrophoresis

The purified CP following ion exchange chromatography had only one single band with a molecular weight of about 33.6 KD on the SDS-PAGE gel (data not shown). This crayfish protease has a higher molecular weight than most of the other crustacean trypsin-like enzymes: crayfish, 24 KD (4); shrimp, 24 and 25 KD (8, 9); crabs, 16.7 and 20.5 KD (2); and spiny lobster, 24 KD (7). Trypsins with higher molecular weights were also isolated from krill (28-30 KD) (16) and Atlantic blue crab (33.5 KD) (3).

Crayfish protease also appeared as a single band on isoelectrically focussed gels (pH 3-10) (data not shown). It had a pI value of around 3.0 indicating that this CP is high in acidic amino acids. Zwilling et al. (29) found the trypsin-like enzyme of crayfish had a pI of 3.8, while Kimoto et al. (16) reported a value of 2.6 for Arctic krill trypsin-like enzyme. Trypsin-like enzymes from some crustacean species were reported to have more than twice as many acidic amino acids as the bovine form (26).

Table 2. Hydrolysis of some synthetic substrates by crayfish protease. Activity measured at 25℃ and pH 8.2.

Substrate	Activity*
TAME	157.1
LME	71.4
BAPA	0.0
BTEE	0.0

*Specific activity (units/mg protein) as described in Materials and Methods.

pH optima and stability

CP was found to have a narrow pH optimum with an estimated maximum at about pH 8.0 (Fig. 1A). Serine proteases, particularly
the trypsin-like enzymes, from invertebrates (9, 12, 13, 15, 24) and vertebrates (5, 24) were reported to have similar pH optima values.

CP exhibited optimum stability over a pH range of 7.5-9.0 (Fig. 1B). This result is similar to that of most proteases thus far characterized for marine organisms. Many of these proteases are highly unstable under acidic but very stable at neutral to slightly alkaline conditions (12, 13, 15, 20, 28).

Temperature stability

This CP had a similar temperature stability as enzymes from other fish species (13, 24). Approximately 50% of the initial activity was retained after incubation at 55℃ for 30 min, but only 20% remained after 30 min at 65℃ (Fig. 2B). Proteases from fish thus far characterized tend to inactivate at 40-50℃ (13, 22, 24).

The influence of various inhibitors on crayfish protease

PMSF has been described as a serine protease inhibitor; it reacts with the essential serine residue at the active center of proteinases (6, 11). The inhibition of CP by PMSF but not by 8-hydroxyquinoline and 1,10-phenanthroline (Table 3) suggests that CP is a serine protease rather than a metallo- or sulfhydryl-enzyme. Jany (15) demonstrated that trypsin from a stomachless bonefish was inhibited by PMSF while Hjelmeland and Raa (13) similarly observed inhibition of capelin trypsins by PMSF. The inhibition of CP by TLCK, SBTI, aprotinin and benzamidine, but not by TPCK also indicates that the enzyme has an action mechanism similar to that of trypsin-like enzymes (Table 3).

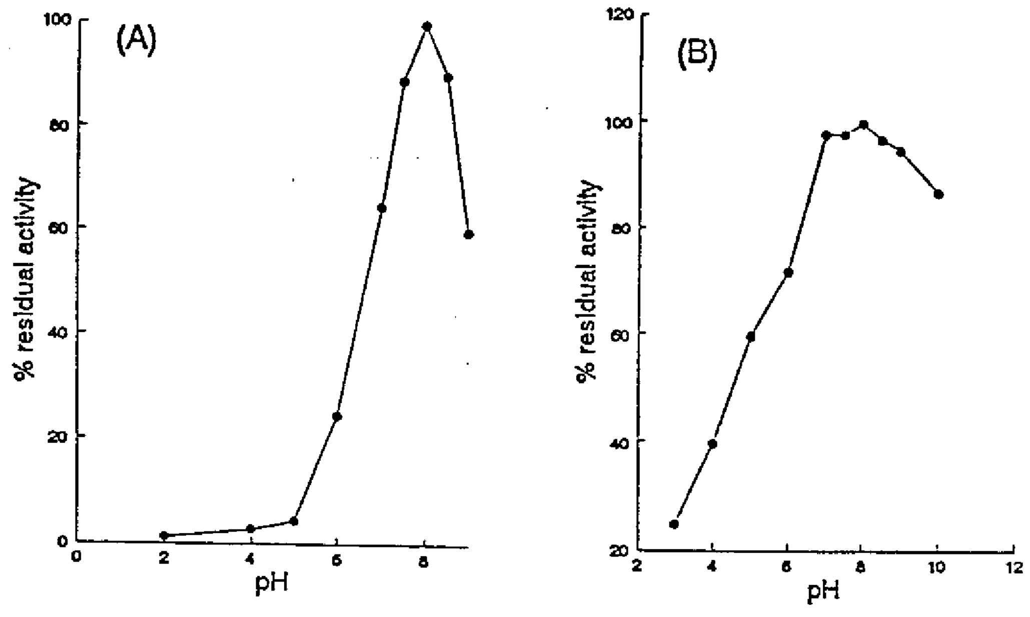

Fig. 1. pH optimum (A) and pH stability (B) of crayfish protease. Activity was measured using TAME as substrate.

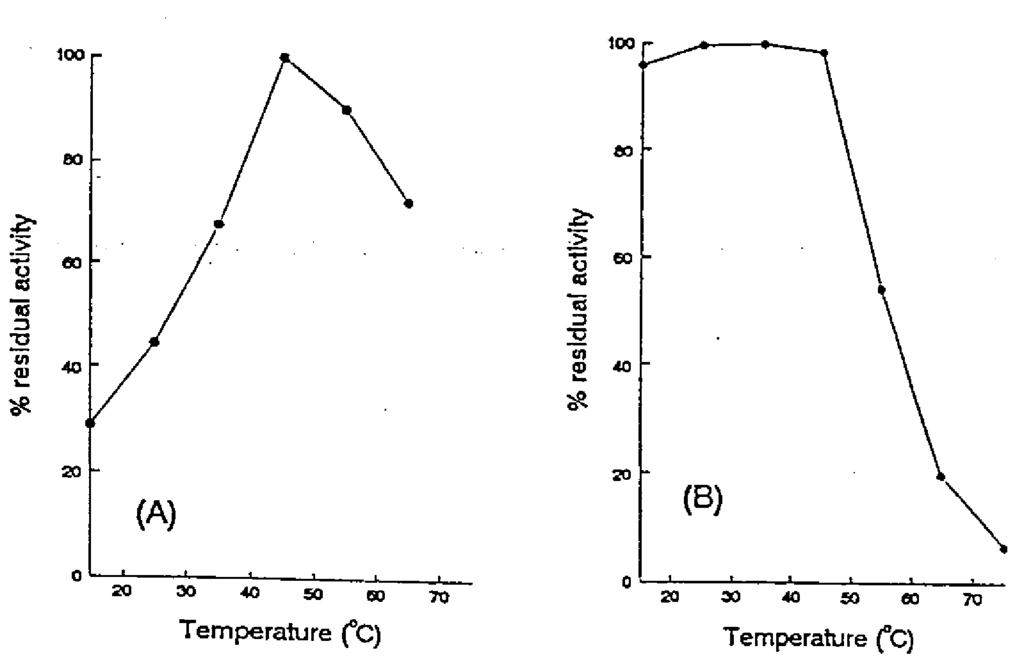

Fig. 2. Temperature optimum (A) and thermostability (B) of crayfish protease. Activity was assayed using TAME as substrate.

Table 3. Effect of protease inhibitors on the esterase activity of crayfish protease.

Inhibitor	Concentration	%inhibition
No inhibitor	-	0.0
PMSF	2 mM	50.6
	5 mM	89.4
SBTI	25 mM	98.5
	50 mM	99.1
TPCK	1 mg/mL	0.0
TLCK	2 mM	100.0
Aprotinin	0.25 TIU*	98.8
	0.50 TIU*	100.0
Benzamidine	1.3 mg/mL	46.0
1,10-Phenanthroline	1 mM	0.0
	5 mM	0.0
8-Hydroxyquinoline	1 mM	0.0
	5 mM	0.0

*TIU stands for trypsin inhibitor units.

Inhibitors were incubated separately with equal volumes of enzyme for 30 min at 25 ℃ before assay for residual esterase activity.

Effect of metal ions

The addition of heavy metals including Zn^{2+}, Hg^{2+} and Cu^{2+} caused a substantial inhibition of CP activity (Table 4). However, Ca^{+2} and Mg^{+2} had nearly no effect on CP activity. A similar trait has also been noted for the tryptic activities of shrimp (8, 9) and spiny lobster (7). In contrast, the inhibition of the enzyme activity by calcium ions has been found in crayfish (29) and blue crab (3).

Kinetic constants

The kinetic parameters were determined from a double reciprocal plot of the initial reaction rates at various concentrations of TAME. The K_m and V_{max} for TAME were 0.133 ± 0.004 mM and 168.34 ± 0.70 TAME units/mg, respectively. The enzyme appeared not to act on BAPA, thus attempts to determine K_m value for BAPA was not possible.

Table 4. Effect of some bivalent ions on the esterase activity of crayfish protease.

Ion	Concentration (mM)	Relative activity (%)
No ion	-	100
Ca^{2+}	5	99
Mg^{2+}	5	94
Zn^{2+}	1	64
Hg^{2+}	1	41
Cu^{2+}	1	40
Ag^{+1}	1	100
	5	100

Metal ions were incubated separately with equal volumes of enzyme for 30 min at 25°C before assaying for residual esterase activity.

Antibody preparation and immunological analysis

Four antibody samples with the highest titer as determined by ELISA were pooled and used to analyze cross-reactivity between CP and some of the well characterized proteases including bovine trypsin, bovine chymotrypsin, and porcine pepsin. As can be seen from Figure 3, color intensity was reduced greatly when 20 ng of either CP or bovine trypsin was added, and to a lesser extent when bovine chymotrypsin was added. The color intensity was further reduced in a nearly linear fashion over the range 20-100 ng. When pepsin was used as competitor, no reduction in color intensity was observed. CP and bovine trypsin are thus partially cross-reactive and have shared structural components.

CONCLUSION

The 33,600 mol. wt trypsin-like protease prepared from crayfish hepatopancreas has similar properties as those trypsin-like enzymes isolated from other crustacean. It shares structural components with bovine trypsin as determined by immunological study.

ACKNOWLEDGEMENTS

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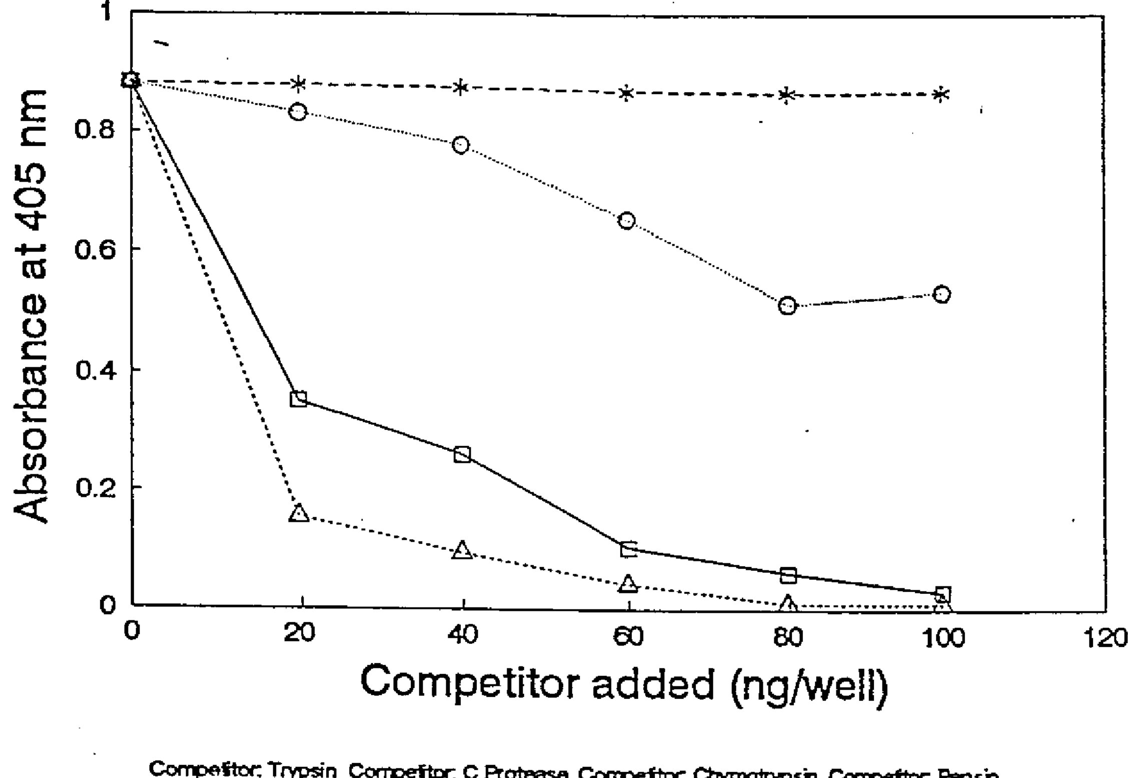

Fig. 3. Analysis of antigenic properties of crayfish protease by competitive ELISA. Crayfish protease (100 ng) was plated and reacted with anti-CP antibody plus 0-100 ng/well of various competitors.

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MEASUREMENT OF LACTATE RESIDUALS ON TREATED SHRIMP USING A YSI LACTATE ANALYZER

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Lactic acid is commonly used in the meat industry and is being used more and more in the seafood industry. Its primary function is to increase the shelflife of the product. Lactic acid is produced naturally and has GRAS status, generally recognized as safe. The most commonly used neutral salt form of lactic acid is sodium lactate. It has been noted that concentrations of sodium lactate equal to that of lactic acid have less negative taste consequences. Sodium lactate was used throughout this study.

Objective

Yellow Springs Instrument Incorporated (YSI) has developed a compact instrument in which to accurately measure lactate levels. The objectives of this study were to evaluate the use of the YSI lactate analyzer for the detection of lactate treated shrimp.

Treatment

Shrimp were untreated for the controls and soaked for various periods of time in various concentrations of sodium lactate for the three individual studies. In the first study, shrimp were treated in several concentrations of sodium lactate (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0) for 1 minuted and 5 minutes. Lactate residuals were then measured on both raw and cooked samples with the YSI lactate analyzer. Secondly, shrimp were treated for 1 minute in, 1.5% sodium lactate. The treated samples and controls (untreated samples) were then held on ice in refrigerated storage (4°C) for 10 days. The lactate residuals were analyzed with the YSI lactate analyzer every two or three days.

Extraction

Key West pink shrimp, Penaeus duodorum, were used for this study. The shrimp in the treated or untreated samples were chopped and mixed. Ten gram subsamples, in triplicate, were homogenized in 25ml of 10% TCA. The samples were then centrifuged at 4000 rpm for 20 minutes. The supernatant was filtered and 25ul injected into the lactate analyzer.

Reaction Mechanism

The YSI lactate analyzer consists of a polarographic probe over which a three layer membrane is mounted, Figure 1. The sample is injected into the reaction chamber and mixed with the instrument buffer. It then passes through the polycarbonate membrane, which allows only substances with molecular weights less than 300,000 pass through. The sample then comes into contact with immiobilized lactate oxidase. The lactate present is oxidized to pyruvate and hydrogen peroxide. The hydrogen peroxide passes through the cellular membrane and is oxidized at the surface of the anode, which is polarized at +0.7 volts verses Ag/AgCl, producing electrons, Figure 2. The steady state hydrogen peroxide concentration or current is linearly proportional to the concentration of lactate present in the sample.

Results

When the shrimp were treated for 1 & 5 minutes in increasing concentrations of sodium lactate, the residual levels of lactates on the product also increased, Fig 3. Also, the lactate residual on the samples soaked for one minute in the lactate solutions were less than the residuals on the samples treated for 5 minutes. The samples were also cooked and the cooked samples had less residual lactate than the raw samples which were treated in the same manner. In the storage study, the lactate residuals of the treated shrimp increased over the 10 day storage period as well as that of the controls, Figure 4.

Instrument Evaluation

The YSI lactate analyzer has many unique features. It is compact, water resistant and has a rechargeable 12 volt DC battery for field use and an AC adapter. It is controlled by a microprocessor, has an alpha-numeric liquid crystal display and menu driven interface. It is easy to use, has automatic calibration and sample result recall. A small sample size (25ul) is required. The sampling speed is approximately 90 seconds per sample. The detection range is 0 to 30 mmoles per liter and the resolution is 0.01 mmoles per liter. Linearity depends on the response of the membrane and as the membrane ages, the linearity decreses slightly. The linearity may be checked throughout an analysis by simply injecting a linearity check standard. The average standard deviation for all analyses was +/- 0.01.

Conclusions

The YSI lactate analyser is a very useful instrument in which to measure lactate residuals on treated shrimp. It is compact, easy to use, very reproducible and very little sample preparation is required. It is necessary to measure lactate residuals on treated shrimp in order to determine was concentrations of lactate to use and to evaluate the proper means of application of the lactate. With a 1 minute dip in a 3% sodium lactate solution, the residual lactate was measured to be 200 mg/100g shrimp and this amount was detected and disliked by half of the panelist.

Fig. 1 *SENSOR PROBE AND ENZYME MEMBRANE*

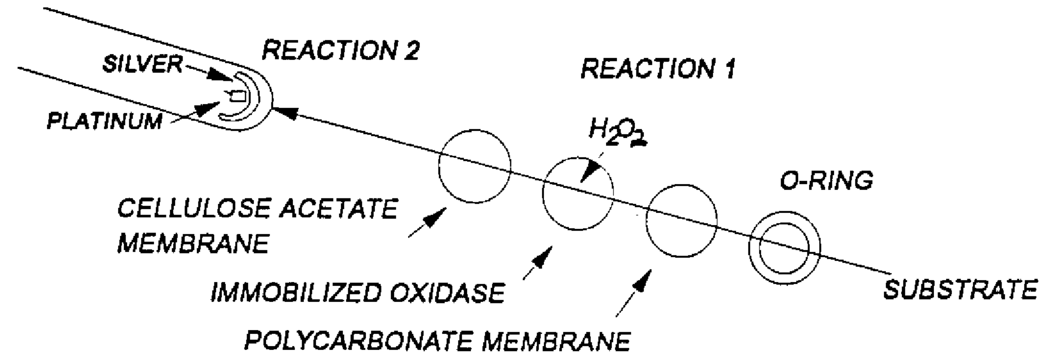

Fig. 2 LACTATE SENSOR

Electron flow is linearly proportional to the steady state H_2O_2 concentration and therefore proportional to the concentration of lactate.

RX 1 L - Lactate + O_2 $\longrightarrow$ Pyruvate + H_2O_2

RX 2 $H_2O_2 \longrightarrow 2H^+ + O_2 + 2e^-$

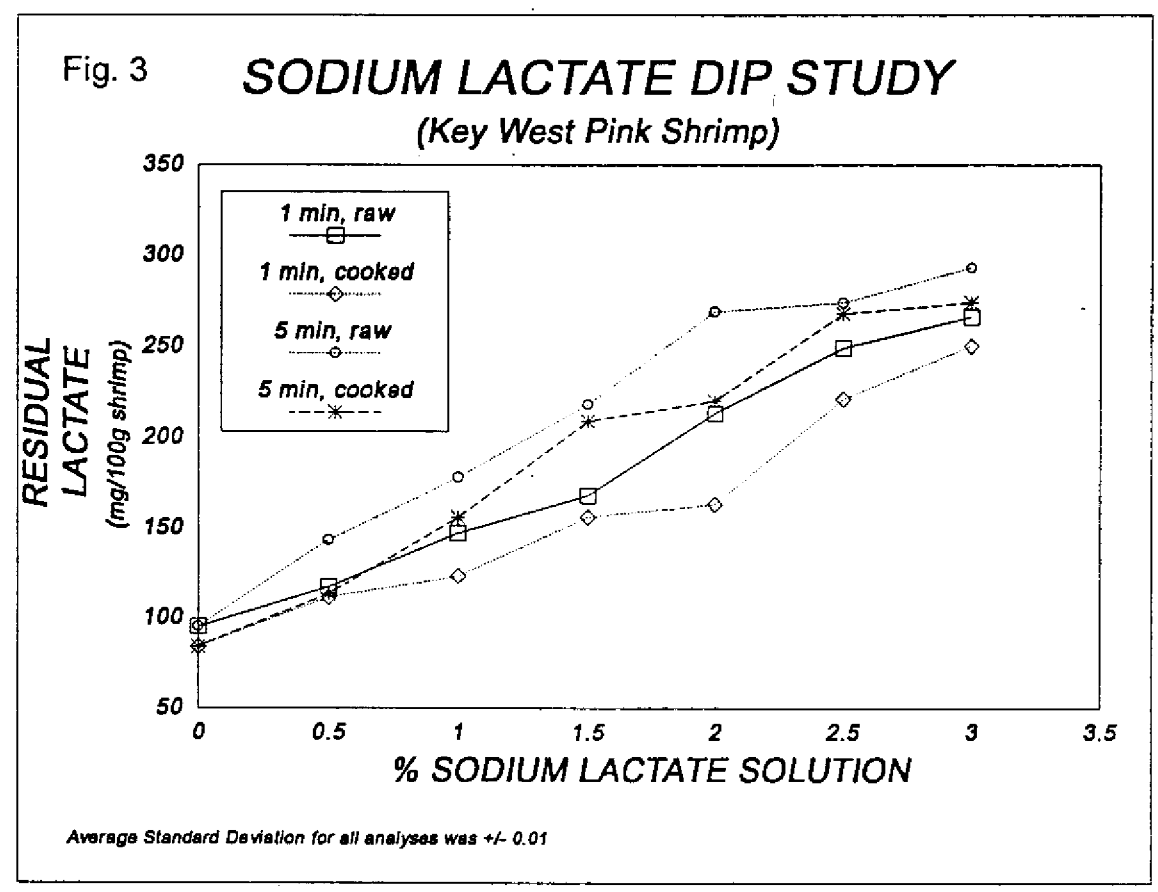
Fig. 3
SODIUM LACTATE DIP STUDY
(Key West Pink Shrimp)
1 min, raw
1 min, cooked
5 min, raw
5 min, cooked
RESIDUAL LACTATE
(mg/100g shrimp)
350
300
250
200
150
100
50
0
0.5
1
1.5
2
2.5
3
3.5
% SODIUM LACTATE SOLUTION
Average Standard Deviation for all analyses was +/- 0.01

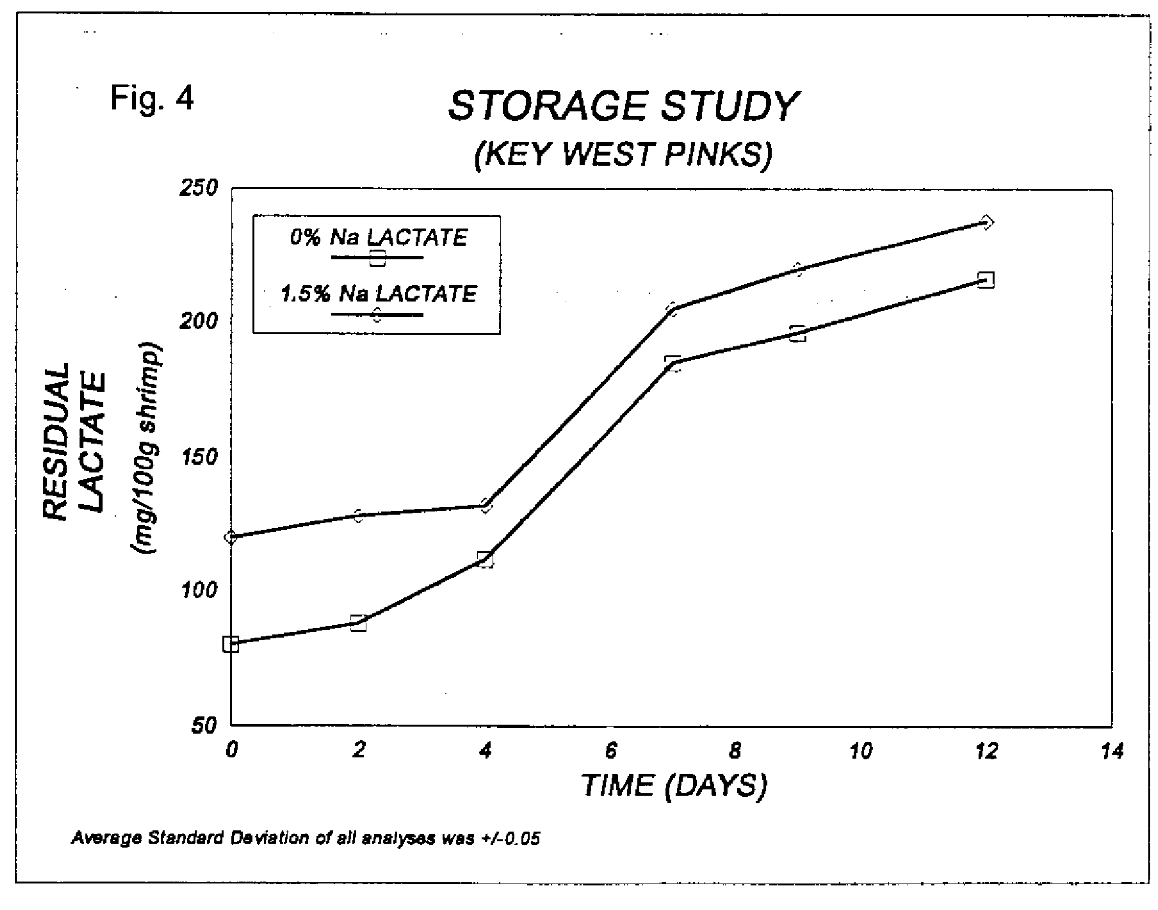
Fig. 4
STORAGE STUDY
(KEY WEST PINKS)
0% Na LACTATE
1.5% Na LACTATE
RESIDUAL LACTATE
(mg/100g shrimp)
250
200
150
100
50
0
2
4
6
8
10
12
14
TIME (DAYS)
Average Standard Deviation of all analyses was +/-0.05

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DETERMINATION OF FISH QUALITY USING A MICROFRESH BIOSENSOR

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Many physical and chemical methods have been used, other than sensory methods, to determine the freshness of fish. Ammonia, amines, volatile acids, catalase activity, and trimethylamine have been proposed (Gruger, 1972). Recently, nucleotide degradation in fish muscle has been found to be a reliable indicator of the freshness of raw fish (Scott et al.,1986). The K-value is derived by dividing the concentrations of hypoxanthine and inosine in the fish flesh by the concentrations of inosine monophosphate, inosine and hypoxanthine present.

The MICROFRESH biosensor, developed by Pegasus Biotechnology, is a quantitative device for measuring the K-value in fish tissues. The MICROFRESH biosensor is designed for both routine applications and research. The objective of this study was to evaluate the use of the Microfresh Biosensor form Pegasus Biotechnology for possible use in the seafood industry as a monitor for fish freshness.

Principle of Measurement

Immediately after the death of the fish, ATP in fish muscle degrades to uric acid through the following autolytic pathway:

ATP --- ADP --- AMP --- IMP --- HxR --- Hx --- X --- U where

ATP, ADP, AMP = adenosine triphosphate, diphosphate and monophosphate

IMP, HxR = inosine monophosphate, inosine

Hx, X, U = hypoxanthine, xanthine, uric acid

In many fish species, ATP and ADP disappear within 24 hours following the death of the fish. Concentrations of AMP are also negligible after this time period. Concentrations of IMP increase initially following death and then decreases corresponding to an increase in HxR and Hx. The-K value is derived from the following equation:

$$K = [HxR] + [Hx] / [IMP] + [HxR] + [Hx]$$

Generally, fish having K-values of less than 0.2 are excellent and these with less than 0.4 are good. These values are hard to achieve commercially so values up to 0.65 are considered good quality for raw fish.

Reaction Mechanism

The MICROFRESH Biosensor consists of a polarographic probe which is polarized +0.7 volts with respect to a silver cathode and platinum anode. Hydrogen peroxide is oxidized at such a polarized potential. Uric acid is also electroactive and provides a limiting current at the same potential where hydrogen peroxide is oxidized.
The oxidation of hydrogen peroxide produces electrons. The steady state concentration of electrons produced is linearly proportional to the concentration of inosine and hypozanthine present in the sample.

$$H_2O_2 \text{---- } 2H^+ + O_2 + 2e^-$$

$$\text{Uric Acid} + O_2 + 3H_2O \text{ ---- Allantoin} + H_2O_2 + HCO_3^=$$

Nucleoside phosphorylase (NP) and xanthine oxidase are immobilized on a membrane and attached to the sensing area of the polarographic probe. The probe is mounted in a sample measurement chamber. For the determination of [HxR] + [Hx], the sample is injected directly into the detection chamber. The sample comes into contact with the immobilized enzymes on the membrane. For the determination of [Hx] + [HxR] + [IMP], the sample is first transferred to the test tube containing immobilized nucleotidase. The IMP in the sample is converted to HxR. This is then injected into the sample compartment and reacts with the membrane.

MATERIALS AND METHODS

Extraction

Three grams of fish flesh were homogenized in ten ml of 10% trichloroacetic acid (TCA) using a glass mortar and pestle. After the solids settle, three ml of the liquid was filtered, placed in a test tube, diluted 2 fold with buffer and neutralized using 2M NaOH. A small portion (50 ul) of the neutralized sample was injected into the sample compartment. From the same sample tube, 500 ml was placed into the reaction tube containing immobilized enzyme and vortexed for 2 minutes. A small portion (50 ul) of the enzyme reacted sample was then injected into the instrument. The K value was digitally displayed on the front of the biosensor.

Methodology

Three different storage studies are presented in this paper. In the first study, 5 species of fish were obtained fresh and placed on ice in plastic containers with holes drilled in the bottom for drainage. The samples were stored at 4 degrees C for two weeks with the lids of each container open during the day and closed at night. The five species included catfish (Ictalurus punctatus), hybrid striped bass (a genetic cross between striped bass Morone saxatilis and white bass Morone chrysops, tilapia (Tilapia aurea), grouper (Mysteroperca bonaci) and salmon (Salmo salar). An initial (day 0) K-value was determined for each and then subsequent K value measurements were made every few days. The raw fillets were also organoleptically evaluated at each sample time. A second trial with grouper fillets involved storage as in the first trial. Again, K-values were obtained on day 0 and every few days following. Organoleptic evaluations were also made at each sampling time. The final trial with catfish fillets involved similar storage on ice in refrigeration for 2 weeks. Samples were taken daily and evaluated organoleptically when K-values were also measured.

Sensory Evaluations

Raw fillets in each of the three studies were evaluated on the same days that K-values were measured. The fish samples were rated on appearance, texture and odor by 5-10 panelist who were experienced in descriptive evaluation of fish and shrimp. The panelist were presented the raw fillets in the storage container and a rating scale of 1 to 10 was used where 1 was excellent and 10 unacceptable. The score of 5 was considered marginal. To determine the texture, the panelist used a fork to separate the meat and were allowed to handle a small portion of the fillets. Average scores for each fillet were calculated.

RESULTS

In the first study, initially all five species of fish were of exceptional quality, with K values less than 0.2 (Figure 1). On day 5, tilapia and salmon remained fresh (K-values<0.2, organoleptic rating<2) while the catfish, hybrid striped bass and grouper were beginning to show organoleptic signs of degradation. The edges of the fillets were drying and the overall raw muscle surface color yellow (K-values approximately 0.4). At day 7, tilapia and hybrid striped bass showed more severe signs of degradation having organoleptic ratings of 10 which corresponded to K-values slightly greater than 0.4, while the catfish, grouper and salmon were organoleptically rated 2-4. After 10 days, all fish species were showing organoleptic signs of spoilage and tilapia and hybrid striped bass scored higher than the other species (K-values 0.68-0.95). In most cases, the muscle fibers were gaping, the edges of the fillets were dry, the overall color of the fillets was dark yellow to brown and an ammonia, sour odor was detected (organoleptic ratings 6-10). Organoleptically, the catfish, grouper and salmon were rated from 4-7 which was slightly below marginal to slightly above marginal. The K values corresponded well with the organoleptic ratings (Figure 2). At the day 5 rating both the tilapia and the hybrid striped bass were above grade 5 on the sensory scale. On day 10 all the species were unacceptable except the salmon and the grouper. A similar trend was seen for the grouper storage study. The K value increased significantly between day 2 and 4 but remained the same until day 7 and then increased. As seen in Figure 3, the sensory ratings also increased steadily over time and the grouper became unacceptable at day 12 when the K values were very close to 1.0. In the third study, the catfish had very low K values until day 7 and then steadily increased (Figure 4). This also corresponded well with the sensory ratings. On day 7 the sensory evaluations were higher than the K values and the catfish was rated unacceptable on day 15 while the K values were still in the acceptable range.

CONCLUSIONS

The K-values obtained with the MICROFRESH Biosensor appear to correlate well with work found in the literature. This instrument provided an easy and reproducible means in which to measure K values for several species of fish. The K values obtained using the Biosensor from Pegasus Biotechnology also compare well with sensory ratings. Further analytical work involving varible storage temperature, larger sample sizes and more fish species is necessary to confirm these results.

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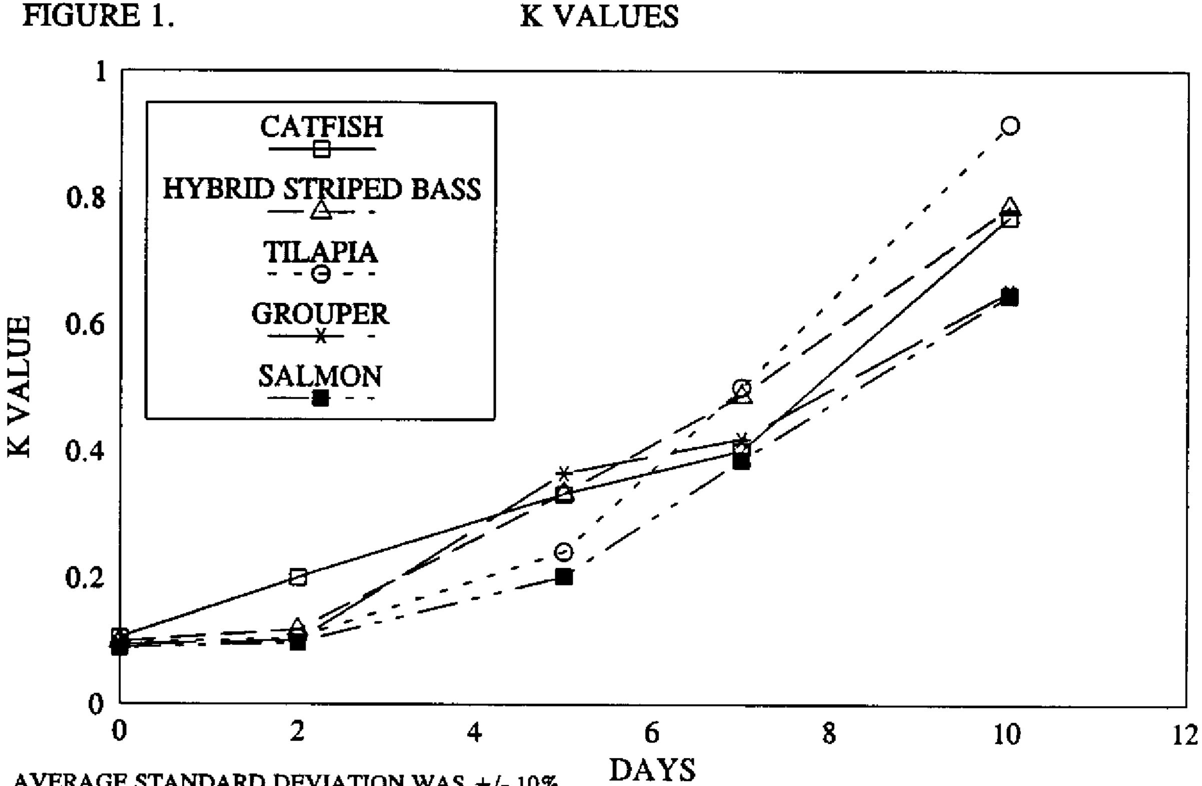
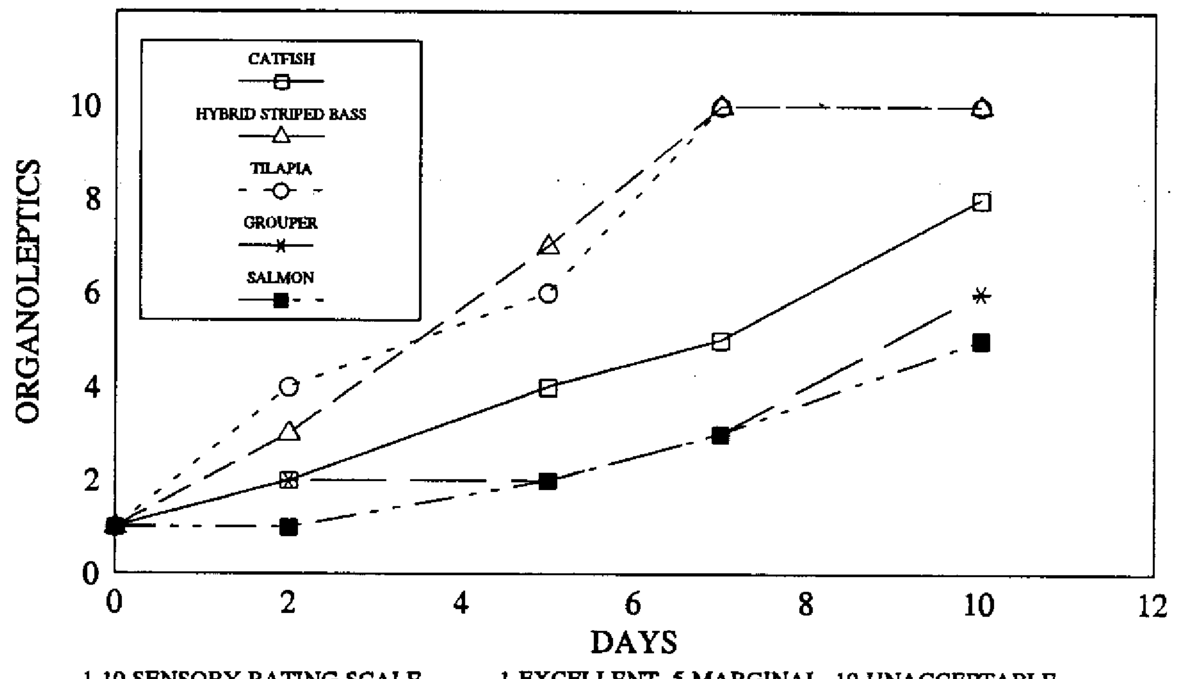
FIGURE 1. K VALUES



AVERAGE STANDARD DEVIATION WAS +/- 10%.

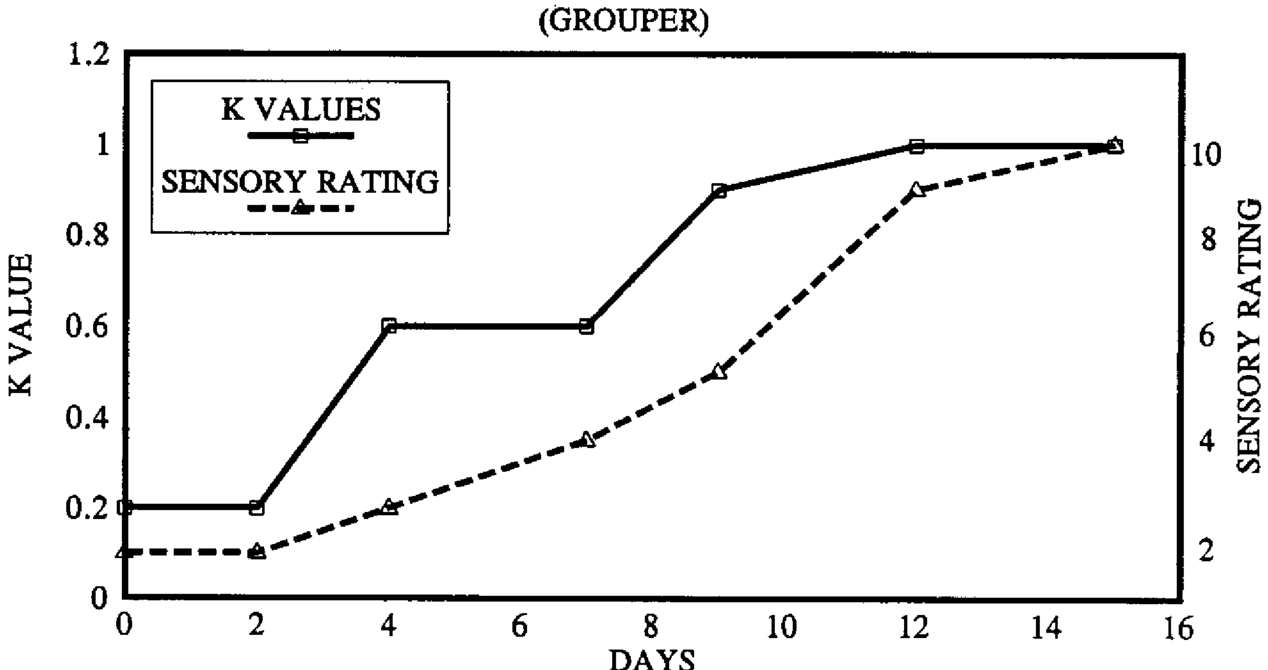
FIGURE 2. ORGANOLEPTIC EVALUATION



1-10 SENSORY RATING SCALE 1-EXCELLENT 5-MARGINAL 10-UNACCEPTABLE

FIGURE 3. STORAGE STUDY

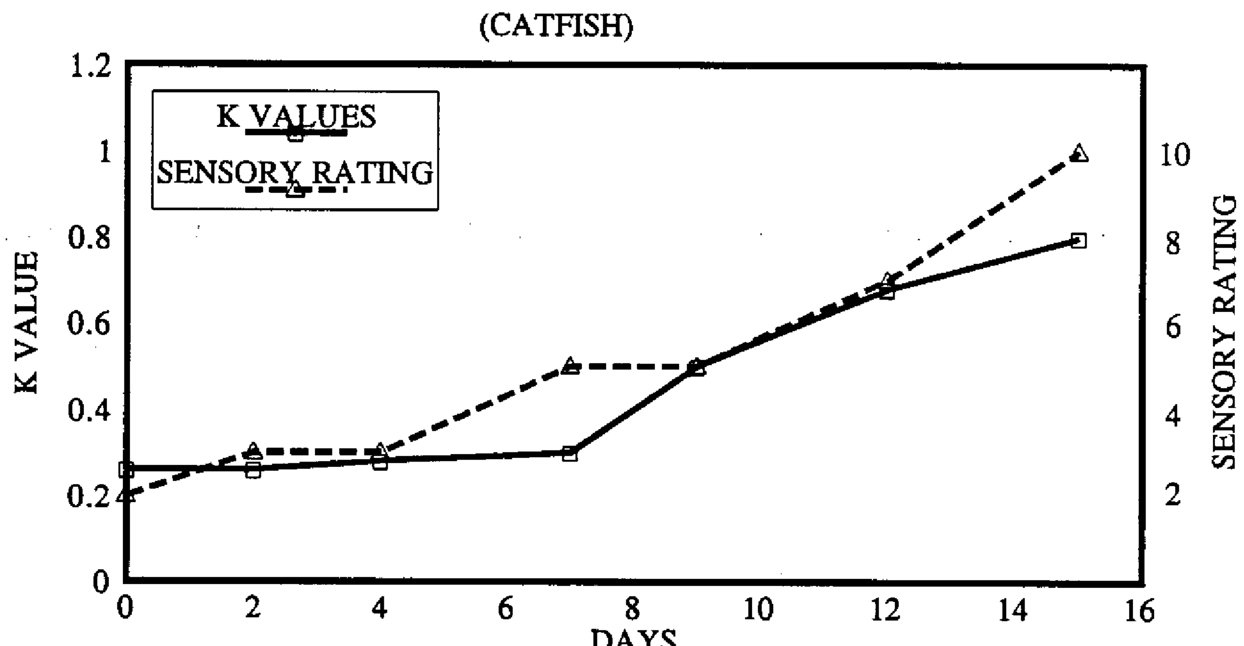
(GROUPER)



AVERAGE STANDARD DEVIATION FOR ALL ANALYSES WAS +/- 0.05
SENSORY SCALE 1-10: 1-EXCELLENT 5-MARGINAL 10-UNACCEPTABLE

FIGURE 4. STORAGE STUDY

(CATFISH)



AVERAGE STANDARD DEVIATION FOR ALL ANALYSES WAS +/- 0.05
SENSORY SCALE 1-10: 1-EXCELLENT 5-MARGINAL 10-UNACCEPTABLE

SEASONAL VARIATIONS IN FATTY ACIDS OF 34 SPECIES OF FINFISH

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Consumers are becoming more aware of the importance of nutrition to human health, and with this interest the popularity of seafood has surged. Seafood contains n-3 or omega-3 fatty acids which may play a role in preventing and lessening the impact of certain diseases. The increased interest in seafood brings with it concern for consistency of seafood products. Any wild caught product is subject to a broad spectrum of variability factors, not the least of which is the variation in nutrient level imposed by seasonal influences.

As early as 1973, Stansby (11) observed strong seasonal variations in lipid content in fish. He concluded that fish that stored triglycerides in their muscle tissue showed maximum fat storage in the summer. However, Kinsella (5) in a review of seasonal variations observed that for some fishes the fat storage peaked in colder months. Other studies (2,3,4,8,9) also showed that there were significant seasonal fluctuations in lipid composition and that these variations were not consistent among species.

In a 3-year study of fatty acids and other nutritional components in Gulf of Mexico finfishes, an effort was made to collect 42 targeted species in all seasons. These collections were examined to define both the mean seasonal values of lipid and fatty acid concentrations and the ranges these values might encompass due to seasonal variability. In addition, these collections allowed assessment of seasonal availability of Gulf fishes as well as comparison of fatty acid levels and distributions to those in cold water and tropical fishes.

MATERIALS AND METHODS

Sample preparation

Immediately after delivery of fresh fish to the laboratory, fish were measured, weighed and state of health recorded. Fish were filleted and given identification number, placed in plastic containers, flushed with N_2 to remove O_2 and then frozen at -40°C. At the end of each season, the entire collection of each species of individual fishes were grouped into a minimum of three pools. Identical quantities of tissue from each of the fish in the subgroups were used in the composites so that no bias was placed upon size of fish. Tissues were taken from the entire longitudinal cross section of fillet so that the sample was representative of all of the edible tissue of each fish.

Analytical procedure

All solvents used in analysis were HPLC grade or analytical reagent grade. Standards were purchased from NuCheck Prep, Inc. (Elysian, MN). Fillets were homogenized using a Waring blender and 0.5 g aliquots weighed into screw-capped (Teflon-lined) centrifuge

tubes (30 ml) and saponified at ambient temperature with ethanolic KOH under N_2 using a magnetic stirrer for one hour. Care was exercised in the volumes of saponifying mixtures used to keep the water level, derived from tissue, sufficiently high to prevent trans-esterification. Solvent ratios were those suggested by Nelson (7). After dilution with distilled water, the neutral fraction was extracted with hexane. The remaining alkaline solution was acidified with 6N HCl, and free fatty acids were extracted with benzene. Benzene aliquots were combined and concentrated using a rotary evaporator. All evaporations were closely monitored to ensure that distillation temperatures did not exceed 25°C. Fatty acids were converted to methyl esters using 7% BF_3MeOH by the method of Metcalfe et al. (6) modified to use ambient temperatures and a one-hour reaction period.

Identification of fatty acid methyl esters (FAME) was obtained by capillary gas chromatography (GC) using a Perkin-Elmer model Sigma 2000 gas chromatograph equipped with flame ionization detector and fitted with a 30 m x 0.25 mm i.d. fused silica capillary column coated with a 0.25 µm film thickness of Dura Bond WAX (J & W Scientific) and operated with a split ratio of 100:1. The carrier gas was He, maintained at 20 psi. Oven temperature was programmed at 90-250°C at a linear rate of 4°/min. Data was processed using a Perkin-Elmer Sigma 10 data system with quantification of all compounds based on individual peak area response by GC compared to the internal standard methyl tricosanoate. Quantitative data were corrected for differences in detector responses that were determined through analysis of authentic standards of each reported fatty acid. FAME were tentatively identified by comparison with retention times with those of authentic standards. Verification of identification on select samples was accomplished through gas chromatography mass spectrometry analysis conducted by Charleston Laboratory, National Marine Fisheries Service.

Sample protection

Several precautions were taken to ensure that no degradation or other alteration of lipids occurred during extraction and saponification. All analytical steps were conducted at ambient temperatures, and samples were constantly flushed with N_2 to prevent oxidation. Further, as many steps as possible were conducted in a single extraction tube to reduce loss and degradation that occurs with sample transfer. All solvents were flushed with N_2 immediately before use to remove dissolved O_2 and to prevent oxidative degradation. Likewise, samples requiring storage were placed in sample bags which were then flushed with N_2 before being placed in freezers. In addition, the antioxidant BHT was added in a concentration of 0.005% (w/v) to extraction solvents to prevent oxidative degradation of unsaturated lipids.

Data analysis

One way analysis of variance (ANOVA) with post facto 95% confidence level range test (12) was used to compare fatty acids as well as certain parameters derived from fatty acid data. Those parameters that indicated statistical difference within comparison groups were identified ($p<0.05$).

RESULTS AND DISCUSSION

Seasonal availability

Of 42 species of fish included in this study, 34 were collected in two or more seasons. Collections are listed in Table 1. Though many of these species are not typical of those found in seafood markets, all have been identified as having good market potential.

Table 1. Seasonal Collections of Gulf of Mexico Finfishes*

Atlantic croaker		Su		Wi	Pigfish	Sp	Su	Fa	
At. sharpnose	Sp	Su			Pinfish	Sp	Su		
Atlantic stingray	Sp	Su			Red drum		Su	Fa	Wi
Bay anchovy	Sp	Su	Fa		Red porgy	Sp		Fa	Wi
Black drum	Sp	Su	Fa	Wi	Red snapper	Sp		Fa	
Blackfin tuna	Sp	Su		Wi	Round herring	Sp			
Blackchin tilapia			Fa		Rough scad	Sp			
Blacktip shark	Sp				Sand seatrout	Sp	Su	Fa	
Blue runner	Sp	Su			Scaled sardine	Sp	Su	Fa	
Bluefish	Sp	Su	Fa		Sheepshead	Sp	Su		Wi
Cobia	Sp	Su			Snowy grouper	Sp			
Dolphin	Sp	Su		Wi	Southern flounder	Sp	Su		
Gafftopsail catfish	Sp	Su			Southern kingfish	Sp	Su		
Gulf butterfish	Sp	Su	Fa	Wi	Spanish mackerel		Su	Fa	
Hardhead catfish	Sp	Su	Fa	Wi	Spot	Sp	Su	Fa	Wi
Harvestfish	Sp				Spotted seatrout	Sp	Su	Fa	Wi
Jack crevalle		Su			Striped bass,			Fa	
King mackerel		Su			Striped anchovy		Su	Fa	
Ladyfish	Sp	Su			Striped mullet				Wi
Lane snapper	Sp				Tilefish	Sp	Su		
Little tunny	Sp	Su			Vermillion snapper	Sp	Su		Wi

*Collections within each season indicated by: Sp-spring, Su-summer, Fa-fall and Wi-winter; collections only listed for which analyses have been completed as of this report.

Total lipids and fatty acids

Much of the interest in seafood as a consumer product lies in the fact that fish is low in fat and cholesterol and is a good low-fat alternative to other protein sources. A fat level of <5% is generally considered to be "low fat". In this regard Gulf of Mexico fishes generally fall in the low fat category. Lipid and total fatty acid concentrations are displayed graphically in Figure 1 for those fishes collected and analyzed in three or four seasons. Both variables displayed similar seasonal trends, and using these trends the fishes fell into the two categories previously described by Shul'man (10). One group which he called "heat loving" deposited fat in the summer and fall with sexual maturation occurring in spring and spawning in summer. The "cold loving" group deposited fat in the spring and summer, and sexual

maturation occurred in the fall and spawning in winter. Bluefish, pigfish and spot fit the "heat-loving" description having greater fat in summer and fall while hardhead catfish, sheepshead and spotted seatrout fit the "cold-loving" description having fat storage in winter and spring.

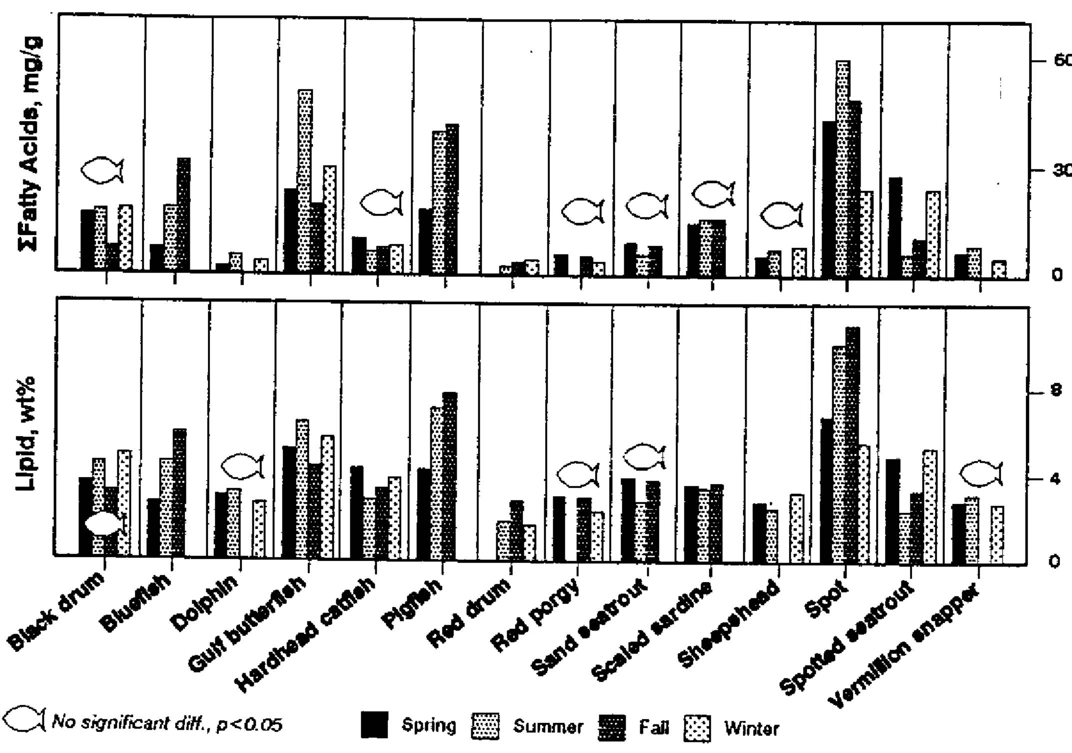

Figure 1. Mean lipid and total fatty acids in Gulf of Mexico fishes.

N-3 fatty acids

The quantity of n-3 fatty acids in seafood is of particular interest to the astute seafood consumer because of the purported benefits associated with replacing some of the excessive quantities of n-6 fatty acids derived from beef, pork and poultry in the American diet with n-3 fatty acids from seafood. In Figure 2, n-3 fatty acid concentrations are shown both as absolute amounts of mg/g in fish tissue and as relative percent of total fatty acids. All fishes, regardless of season, showed appreciable levels of n-3 in edible tissue (1-14 mg/g) but with two contrasting seasonal variation patterns. In one pattern, bluefish, dolphin, hardhead catfish and red porgy displayed inverse trends in absolute and relative amounts of n-3 i.e. seasonal increases in absolute quantities of n-3 were accompanied by decreases in the relative percent of total fatty acids. These fish apparently accumulate n-3 fatty acids less readily than other fatty acids during periods of high fat deposition. Another group of fish, gulf butterfish, red drum, scaled sardine and sheepshead showed similar trends for both absolute and relative concentrations of n-3. This indicated that seasonal increases in absolute n-3 fatty acids were accompanied by increases in relative percent of total fatty acids. These observations most likely reflect particular dietary patterns of the various Gulf finfishes, with diet serving as the major influence on the n-3 fatty acid levels in fish tissue.

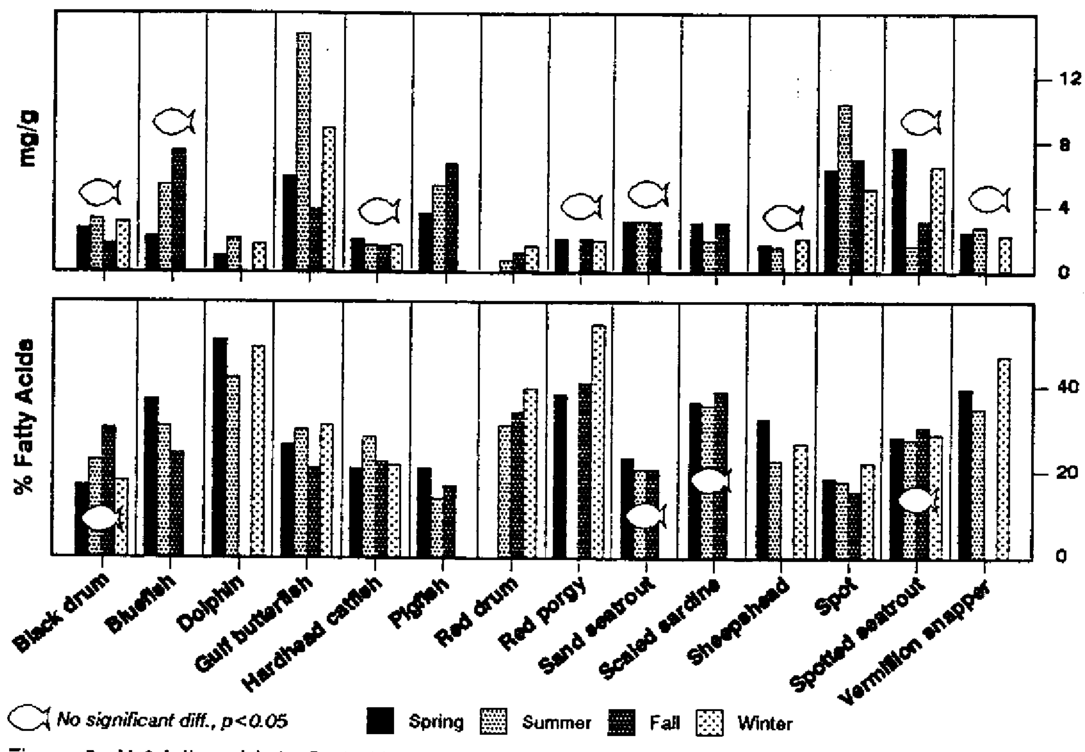

Figure 2. N-3 fatty acids in Gulf of Mexico fishes.

Specific n-3 and n-6 fatty acids

Attention has been focused on three polyunsaturated fatty acid components, the n-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and the n-6 fatty acid, arachidonic acid (AA). Excessive quantities of arachidonic acid and its precursors in the diet can lead to formation of certain eisosanoids that may produce undesirable health effects. These effects can be countered by addition of n-3, primarily EPA and DHA, to the diet. Figure 3 displays seasonal variations of the ratio of EPA+DHA to AA. The ratio was >1 regardless of species or season. Several of the fishes have maxima in this ratio in the winter or spring season duplicating seasonal trends seen in overall n-3 to n-6 ratios shown in Figure 4 and discussed below.

EPA is the primary n-3 component found in cold water fish, and for that reason has received considerable attention in research concerning the role of n-3 fatty acids in the diet. DHA has been reported as the predominant n-3 fatty acid in tropical fish (1) and has received much less attention in medical research. It is interesting that Evans (1) noted an increase in DHA/EPA ratios in fish from more tropical waters suggesting that DHA predominance is linked to geographical temperature zones. Our study showed DHA/EPA ratios >1 in Gulf fish with some interesting seasonal trends. Patterns of DHA/EPA seen in Figure 3 closely resembled those of n-3 (% fatty acids) shown in Figure 2. This relationship suggests that when fatty acids in Gulf fishes were most enriched in n-3 fatty acids that DHA also reached its maximum values. It is hypothesized that EPA may be the conservative component of the n-3 fatty acids with fluctuations in these n-3 fatty acids primarily attributable to variations in the levels of DHA.

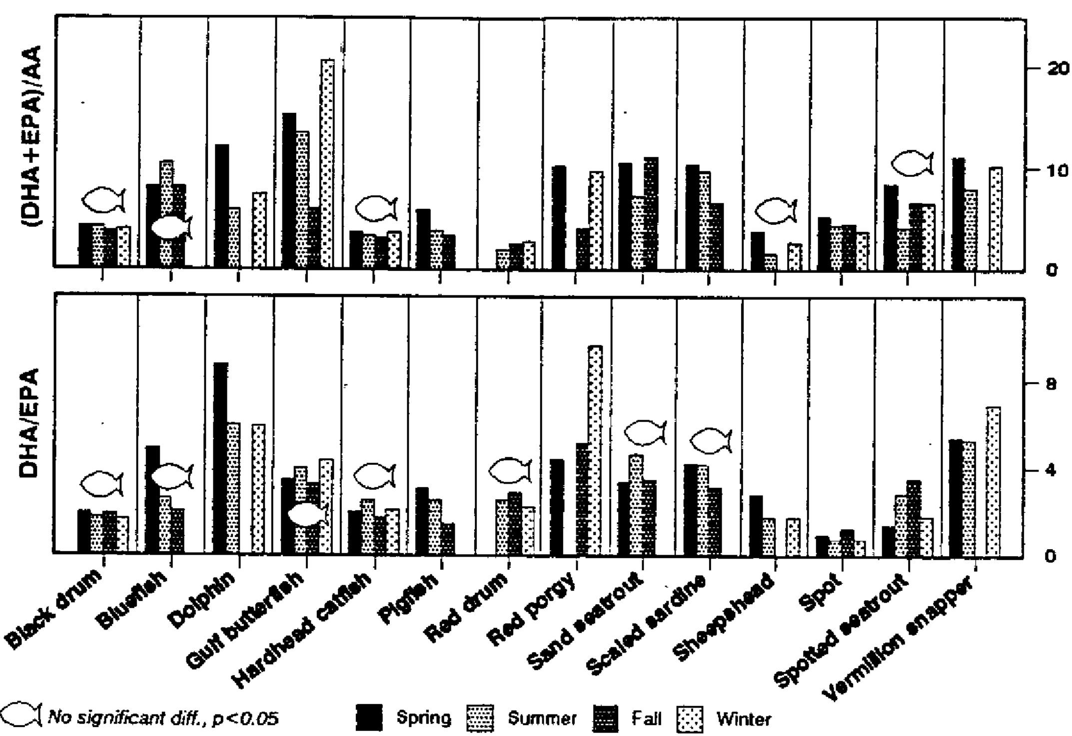

Figure 3. Eicosapentaenoic (EPA), docosahexaenoic (DHA) and arachidonic acids (AA) in Gulf of Mexico fishes.

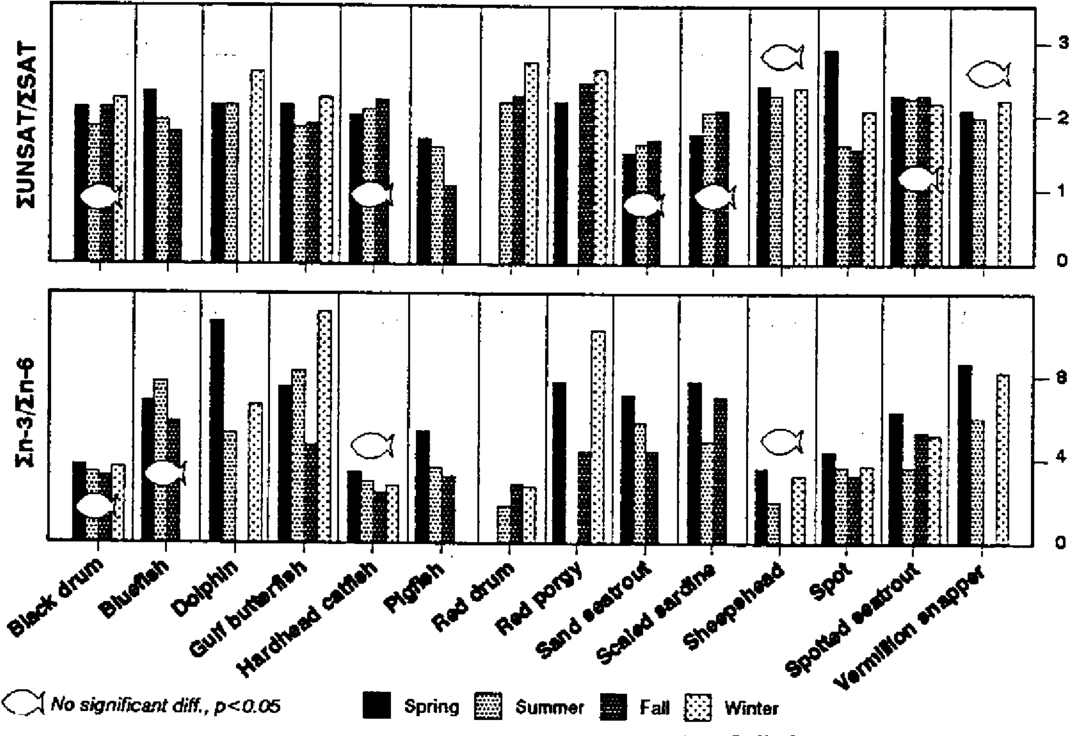

Figure 4. Ratios of n-3/n-6 and unsaturated/saturated fatty acids in Gulf of Mexico fishes.

Ratios of n-3/n-6 and unsaturated/saturated

In Figure 4 the ratios of total unsaturated fatty acids to saturated fatty acids are remarkable for their consistency not only across season but across species. Though some seasonal trends indicate statistically significant differences, absolute differences from a consumer perspective appear to be inconsequential. This ratio may be regarded as a conservative property of Gulf seafood regardless of season or species. The levels of total n-3 to total n-6, like the (EPA+DHA)/AA ratios, tended to maximize in winter and spring for many of the fishes. Using the criterion of enhanced n-3/n-6 or enhanced (EPA+DHA)/AA as a desirable trait of seafood, there may be some slight seasonal advantage to Gulf fish caught in either the winter or spring months.

Interseasonal trends

Another way of examining seasonal fatty acid data for multiple species is to examine one or two variables within one season for all fish species and see how trends among fishes differ between seasons. Such a treatment is shown in Figures 5-8 which are seasonal plots of n-3 and n-6 as % of fatty acids vs lipid as wt % of tissue. Individual data points represent mean values for one species during a season. Correlation coefficients (R) for all plots except the spring and fall n-6 fatty acids were significant at the $p<0.05$ level and indicated significant relationships between these pairs of variables. With leaner fish represented by points to the left and fattier fish to the right, it can be seen that n-6 levels did not differ appreciably between fattier and leaner fish during any season, and in the spring showed no distinction whatever. The negative slopes in all the n-3 plots indicated that as one progresses towards the leaner fish, one encounters fish with fatty acids progressively enriched in the n-3 fatty acids, an observation also noted previously (1). The slopes of -4.1 and -4.8 for winter and spring collections (vs -2.1 and -2.4 for summer and fall) indicated greater enrichment in n-3 fatty acids in leaner fish (compared to fattier fish) in the winter and spring months. This observation supports the earlier contention that Gulf fishes, particularly the leaner fishes, have some advantage in both quantities and balance of the n-3 fatty acids in the winter and spring months.

Warm water vs cold water fishes

Published literature extols the benefit of seafood consumption and often suggests that one consume cold water fish. Many cold water fishes have greater absolute quantities of n-3 in edible tissue than warm water fishes, but at the expense generally of higher total fats. This offers little if any advantage in terms of n-3/n-6 balance in the diet. Enhancement of

n-3/n-6 in the diet is best accomplished by lowering the fat intake and enhancing the n-3/n-6 ratio rather than offsetting excessive n-6 fatty acid consumption with large quantities of additional fat, that is enriched in n-3 fatty acids. A low fat diet is compatible with the leaner Gulf of Mexico warm water fish, which coupled with other low fat foods, can effectively raise the n-3/n-6 ratio in the diet to more desirable levels.

Conclusion

Gulf of Mexico fishes are low in fat but both lipid and fatty acids have considerable variation during the year. The n-3 fatty acids are predominant over n-6 fats with somewhat higher ratios observed in the winter and spring seasons. Seasonal variations are the result of food availability as well as feeding and migratory patterns; this accounts for a large part of the diversity in seasonal patterns observed with warm water fishes. The choice of seafood need not be a choice of cold or warm water species or a particular species or one caught during a certain season but should be a choice of a variety of whatever fresh fish is available in the region.

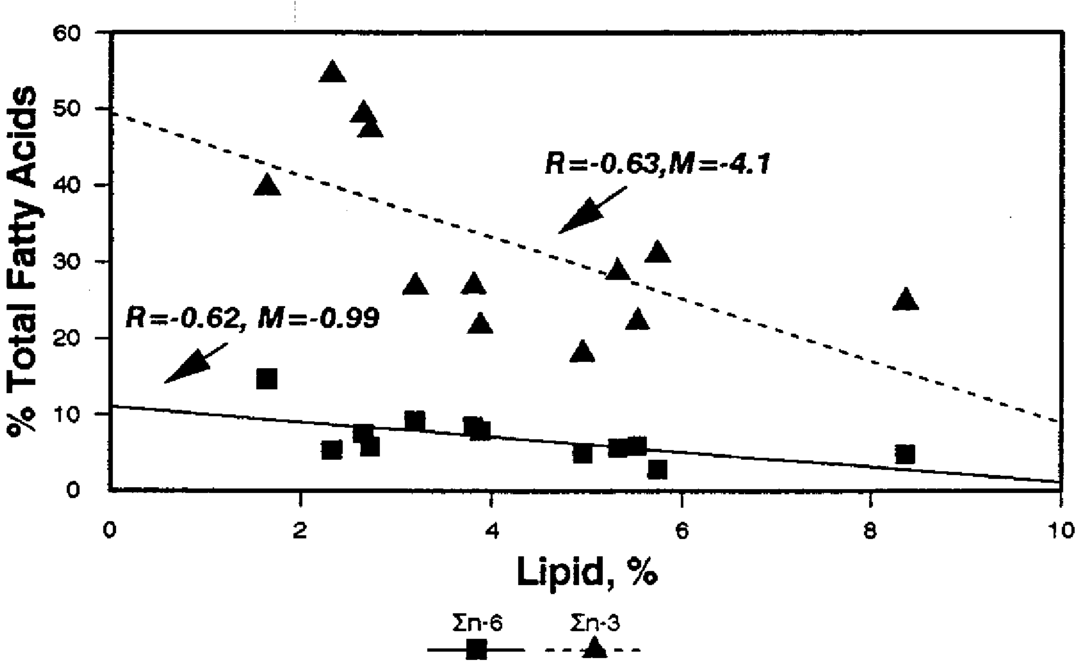

Figure 5. N-3 and n-6 fatty acids vs fats in winter collections. Each point represents mean for one fish species.

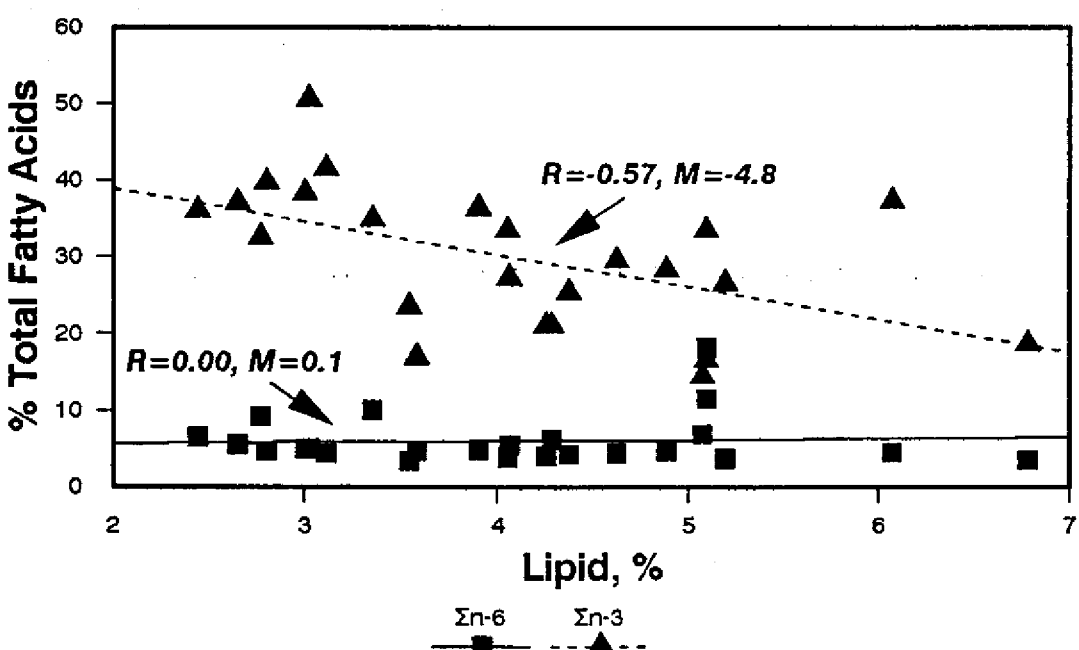

Figure 6. N-3 and n-6 fatty acids vs fats in spring collections. Each point represents mean for one fish species.

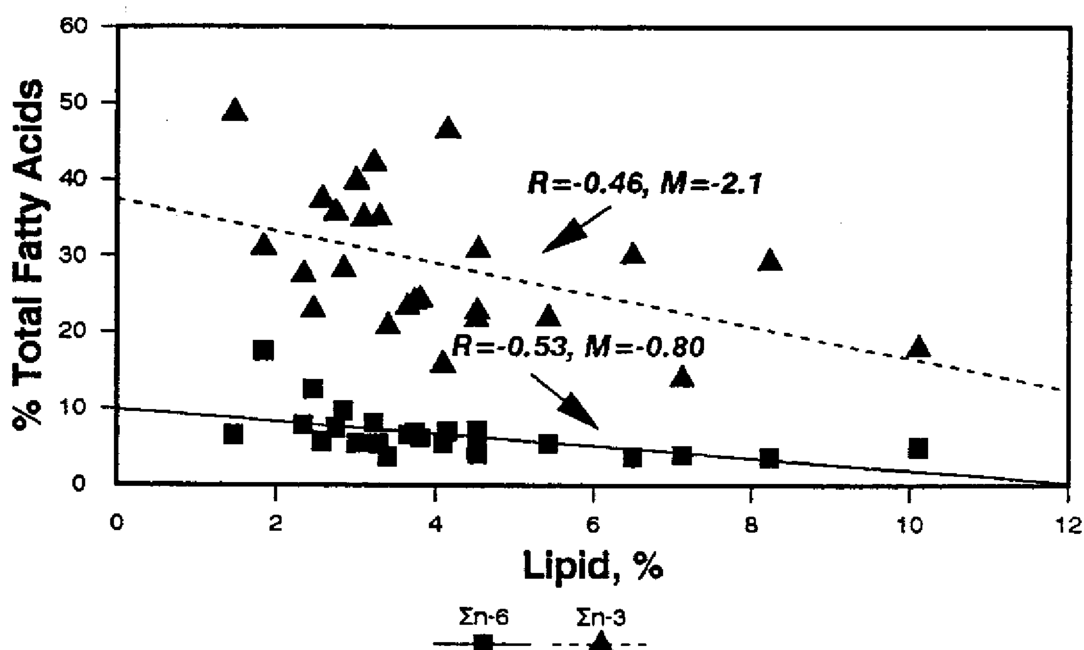

Figure 7. N-3 and n-6 fatty acids vs fats in summer collections. Each point represents mean for one fish species.

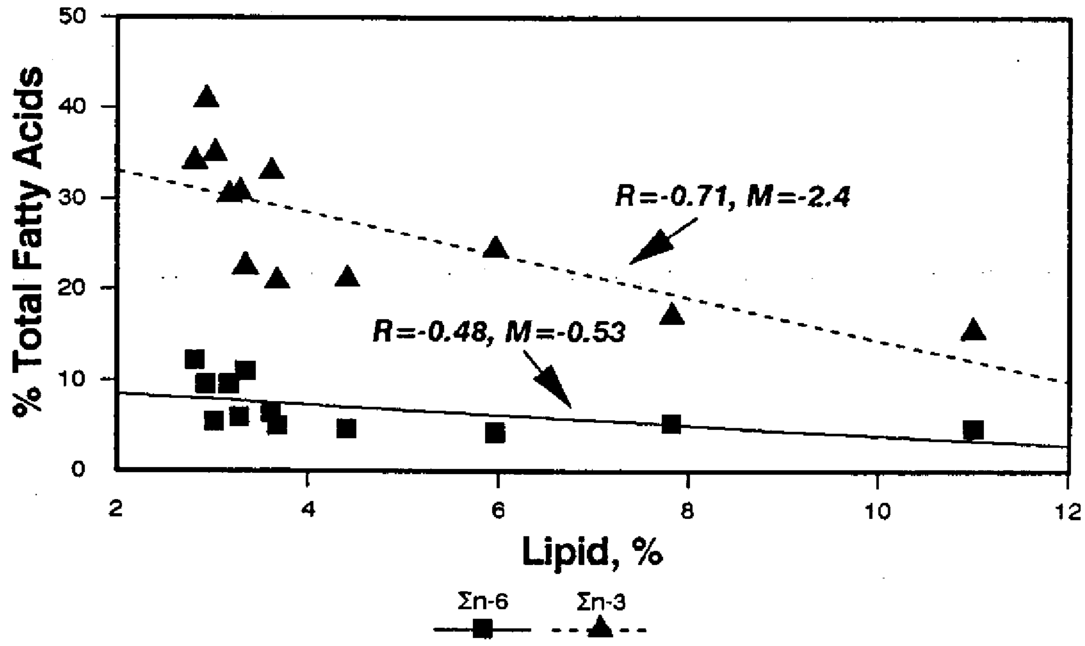

Figure 8. N-3 and n-6 fatty acids vs fats in fall collections. Each point represents mean for one fish species.

ACKNOWLEDGMENTS

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FATTY ACID ALTERATIONS DUE TO BIOCHEMICAL CHANGES DURING THE LIFE CYCLE OF GULF OF MEXICO FINFISH

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Recent studies in our laboratory have shown that fatty acid concentrations and distributions in muscle tissue of Gulf finfishes vary not only with species but with size, sex, sexual maturation and age (6,7). In some species, the variations are significant, while in others, the changes are very small. Fatty acid modifications which occur as a result of biological change may be due to one or more of the following: changes in diet, changes in hormone levels, changes in sensitivity to hormone actions with age, changes in food availability, changes in health and changes in environment (8).

Nutritional information for fatty acids as well as the saturated, monounsaturated and polyunsaturated fats cannot be adequately evaluated without knowing the extent of these variations. Our laboratory has examined extensively 45 species of warm water finfish species from the Gulf of Mexico to determine the extent of these variables on fatty acid concentration and distribution of muscle tissue.

Eleven species of Gulf finfishes will be presented to illustrate fatty acid changes with observed biological change.

MATERIALS AND METHODS

Sample design

A collection scheme was devised that would permit a measure of the fatty acid variability associated with biological changes in fish species (1,2,3,4,5,9,10). Each species was collected during four seasons in sufficient quantities to generate information on seasonal variability of fatty acid concentration and distribution for each of the species as well as biological variations such as age, size, sex and sexual maturity.

Sample preparation

Immediately after delivery of fresh fish to the laboratory, the fish were measured, weighed, sexed and state of health recorded. The fish were filleted and given a unique identification number, placed in plastic containers, flushed with N_2 to remove O_2 and then frozen at -40°C. At the end of each season, the entire collection of each species of individual fishes were grouped into a minimum of three pools of unique characteristics (e.g. size, sex, sexual maturity, age). Identical quantities of tissue from each of the fish in the subgroups was used in the composites so that no bias was placed upon size of fish. Tissues were taken from the entire longitudinal cross section of fillet so that the sample was representative of all of the edible tissue of each fish. All tissue from individual fish not used for compositing was archived.

Analytical procedure

All solvents used in analysis were HPLC grade or analytical reagent grade. Standards were purchased from NuCheck Prep, Inc. (Elysian, MN). Fillets were homogenized using a Waring blender and 0.5 g aliquots weighed into screw-capped (Teflon-lined) centrifuge tubes (30 ml) and saponified at ambient temperature with ethanolic KOH under N_2 using a magnetic stirrer for one hour. Care was exercised in the volumes of saponifying mixtures used to keep the water level, derived from tissue, sufficiently high to prevent trans-esterification. Solvent ratios were those suggested by Nelson (7). After dilution with distilled water, the neutral fraction was extracted with hexane. The remaining alkaline solution was acidified with 6N HCl, and free fatty acids were extracted with benzene. Benzene aliquots were combined and concentrated using a rotary evaporator. All evaporations were closely monitored to ensure that distillation temperatures did not exceed 25°C. Fatty acids were converted to methyl esters using 7% BF_3MeOH by the method of Metcalfe et al. (6) modified to use ambient temperatures and a one-hour reaction period.

Identification of fatty acid methyl esters (FAME) was obtained by capillary gas chromatography (GC) using a Perkin-Elmer model Sigma 2000 gas chromatograph equipped with flame ionization detector and fitted with a 30 m x 0.25 mm i.d. fused silica capillary column coated with a 0.25 µm film thickness of Dura Bond WAX (J & W Scientific) and operated with a split ratio of 100:1. The carrier gas was He, maintained at 20 psi. Oven temperature was programmed at 90-250°C at a linear rate of 4°/min. Data was processed using a Perkin-Elmer Sigma 10 data system with quantification of all compounds based on individual peak area response by GC compared to the internal standard methyl tricosanoate. Quantitative data were corrected for differences in detector responses that were determined through analysis of authentic standards of each reported fatty acid. FAME were tentatively identified by comparison with retention times with those of authentic standards. Verification of identification on select samples was accomplished through gas chromatography mass spectrometry analysis conducted by Charleston Laboratory, National Marine Fisheries Service.

Sample protection

Several precautions were taken to ensure that no degradation or other alteration of lipids occurred during extraction and saponification. All analytical steps were conducted at ambient temperatures, and samples were constantly flushed with N_2 to prevent oxidation. Further, as many steps as possible were conducted in a single extraction tube to reduce loss and degradation that occurs with sample transfer. All solvents were flushed with N_2 immediately before use to remove dissolved O_2 and to prevent oxidative degradation. Likewise, samples requiring storage were placed in sample bags which were then flushed with N_2 before being placed in freezers. In addition, the antioxidant BHT was added in a concentration of 0.005% (w/v) to extraction solvents to prevent oxidative degradation of unsaturated lipids.

Data analysis

One way analysis of variance (ANOVA) with post facto 95% confidence level range test (11) was used to compare fatty acids as well as certain parameters derived from fatty acid data. Those parameters that indicated statistical difference within comparison groups were identified ($p<0.05$).

RESULTS AND DISCUSSION

Male vs Female Fatty acid and lipid differences were observed in males and females of the same species of similar size. These differences, however, were significant only during certain seasons of the year. Male and female spot (*Leiostomus xanthurus*) caught in January show little variation in relative or absolute concentrations of classes of fatty acids or lipids. Figure 1 is representative of the format used for all figures. The top division provides concentrations expressed as relative percent of the total fatty acids; the middle division, concentrations are expressed as absolute amounts in µg/g; the lower division affords results of measured parameter ratios of nutritional significance including percent lipid. As shown in Figure 1, there is no significant difference between male and female spot caught in January from various near shore areas along the Mississippi Coast. Figure 2 depicts fatty acid in male and female spot caught in October from the same areas. In these samples, fatty acids found in males and females are significantly different whether expressed as relative or absolute concentrations. The exception is absolute concentrations of total fatty acids. Even though there are large differences between mean values for males and females, the individual variations were so large as to show no significance when statistics were applied to this parameter.
Male and female harvest fish (*Peprilus burti*) caught in spring show very little significant fatty acid variations (Figure 3). Again, the mean percent fat in females is higher than males though statistically not significant. Male and female pigfish (*Orthopristis chrysoptera*) exhibit significant differences in percent lipid and absolute amounts of n-3 fatty acids (Figure 4). When comparing differences between other male and female species, there is a trend for males to have less fat than females and for males to have a higher relative amounts of omega-3 (denoted by n-3) fatty acids than females.

Age Cobia (*Rachycentron canadum*) was the only species in which ages were ascertained. Ages were determined by Mr. Jim Franks, Gulf Coast Research Laboratory (GCRL) fisheries biologist, by counting the number of rings in their otoliths using the method of image analysis. Figure 5 represents female cobia caught in late summer of 1988. The older 4-7 year females in these fish are lower in fat with higher relative n-3 fatty acid concentrations as compared to the younger 2-3 year females. On the other hand, the older 4-6 year females caught in May 1988 (Figure 6) are higher in fat and lower in relative n-3 concentrations indicating that cobia may exhibit greater variation among themselves than other species. Relative differences in cobia of different ages are small or insignificant while absolute differences are much greater.

Size Red snapper (*Lutjanus campechanus*) caught in the fall of 1989 were pooled into three size groups ranging from 800 to 1500 g. All were caught from one geographical area and each group contained both males and females. No significant differences in fatty acid parameters are observed for the three sizes as indicated in Figure 7, however, the range in the three weight groups was small. Blackfin tuna (*Thunnus atlanticus*) caught in June of 1990 ranged in weights from 13 to 23.5 lbs. and were pooled into three size groups (Figure 8). There is wider variation in fatty acids among the three size groups with relative concentrations of saturates, n-6 and n-3 showing no significant differences.

Juvenile and adult maturation Atlantic croaker (*Micropogonias undulatus*) have a much wider fatty acid range of variations than the majority of species examined which may be due in part to their feeding habits. Even though the range is wide, there are no significant differences in relative concentrations nor for measured ratios of fatty acid parameters. Saturates, polyunsaturates, n-3 and n-6 are significantly different when measured as absolute concentrations (Figure 9). Southern kingfish (*Menticirrhus americanus*) (Figure 10) and hybrid striped bass (*Morone saxatilis x Morone chrysops*) show similar trends

Saturates
Monounsat
PUFA
n-6
n-3
% Total Fatty Acids
0 10 20 30 40
ΣFatty Acids
Saturates
Monounsat
PUFA
n-6
n-3
μg/g (1000's)
0 5 10 15 20 25 30
Σunsat/Σsat
Σn-3/Σn-6
Lipid, %
0 1 2 3 4 5 6 7
Male
Female
No sign. diff., $p<0.05$

Figure 1. Fatty acids in male and female spot (winter).

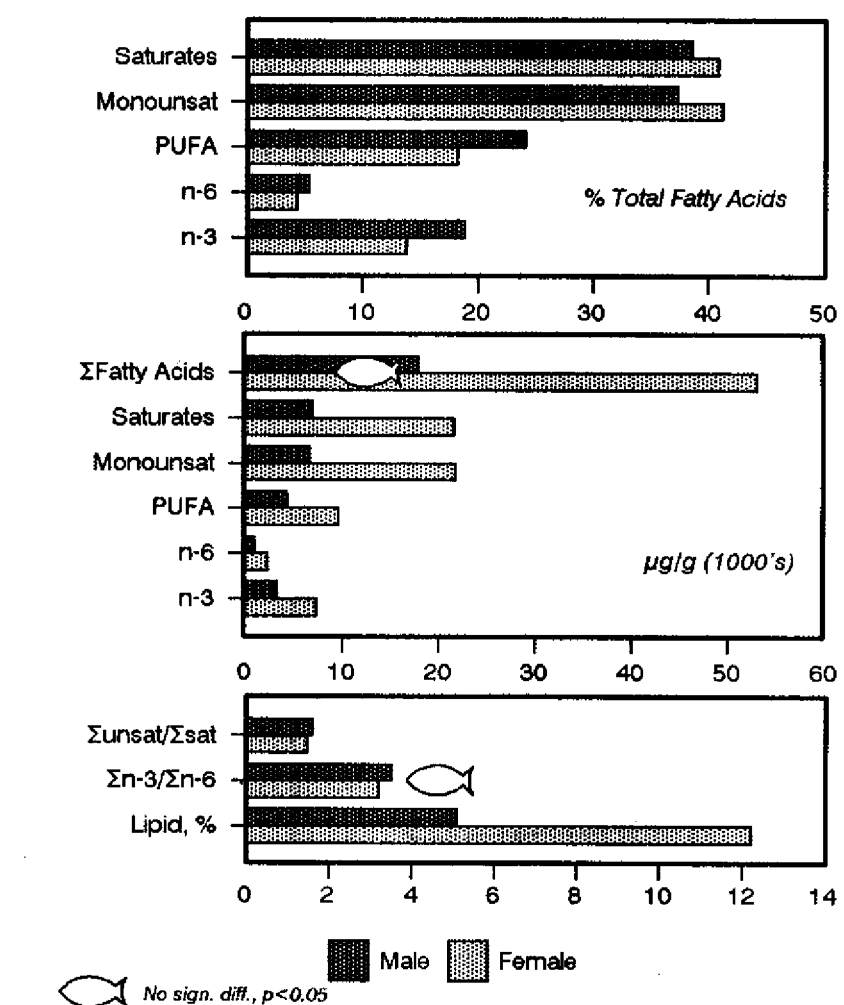

Figure 2. Fatty acids in male and female spot (fall).

Figure 3. Fatty acids in male and female harvestfish.

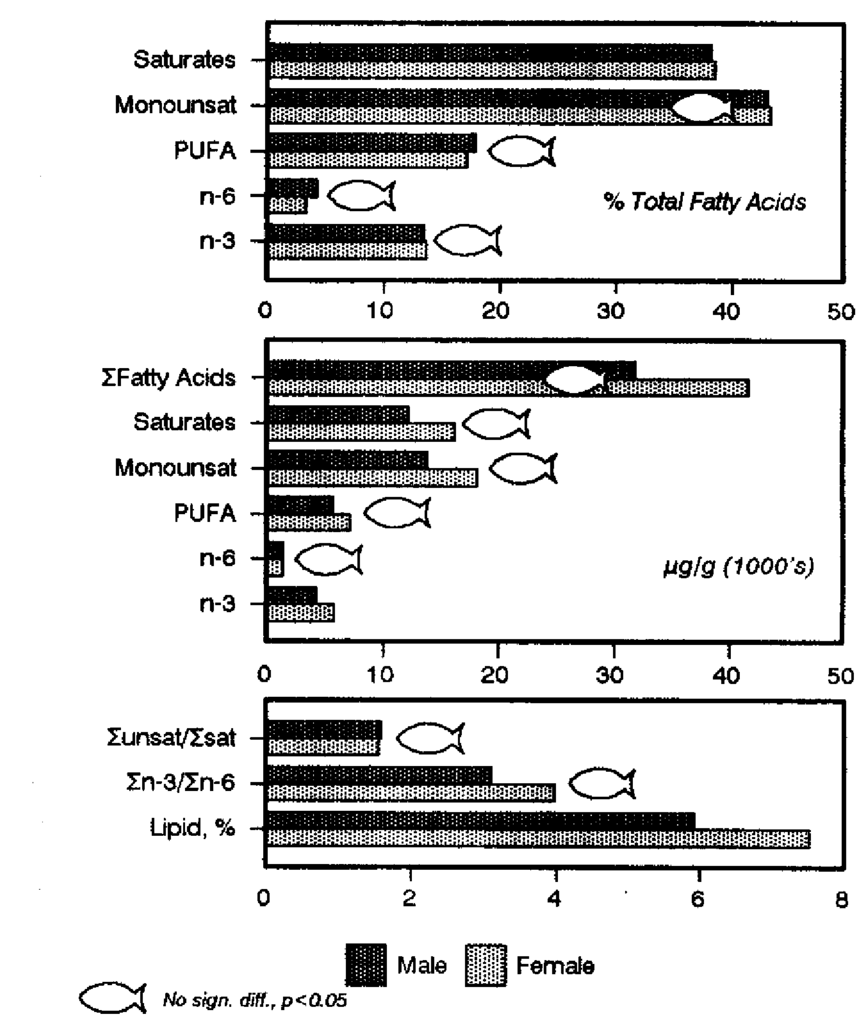

Figure 4. Fatty acids in male and female pigfish.

Figure 5. Fatty acids in female cobia of various ages (summer).

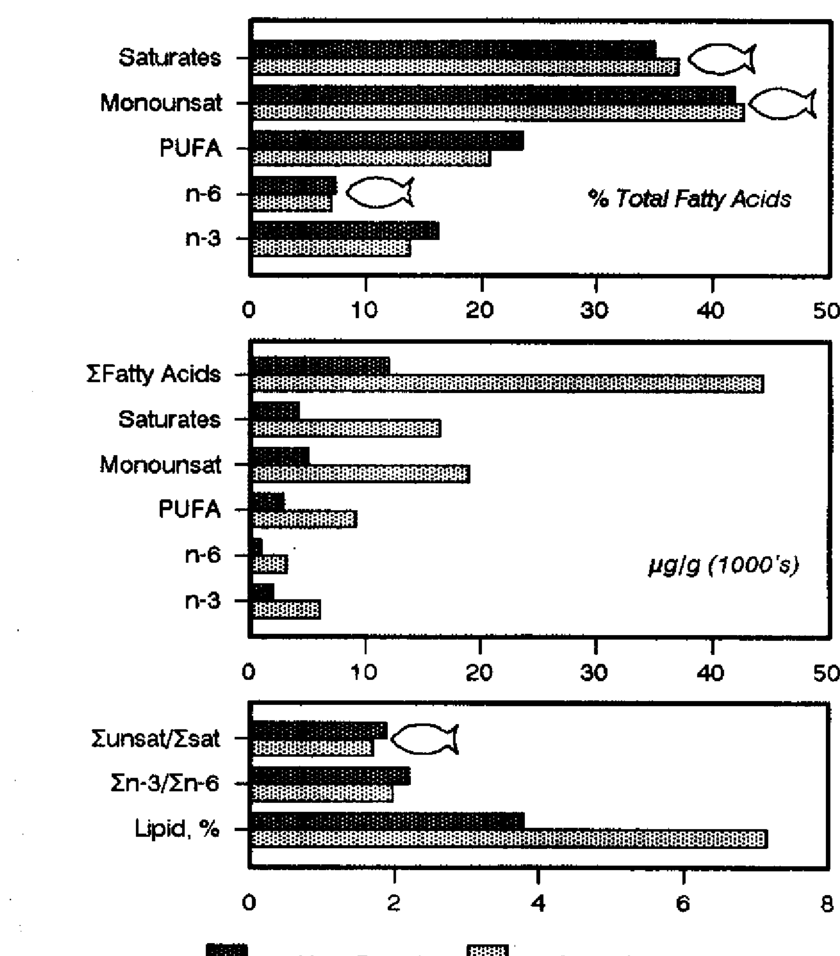

Figure 6. Fatty acids in female cobia of various ages (spring).

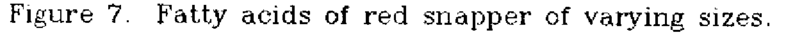

Figure 7. Fatty acids of red snapper of varying sizes.

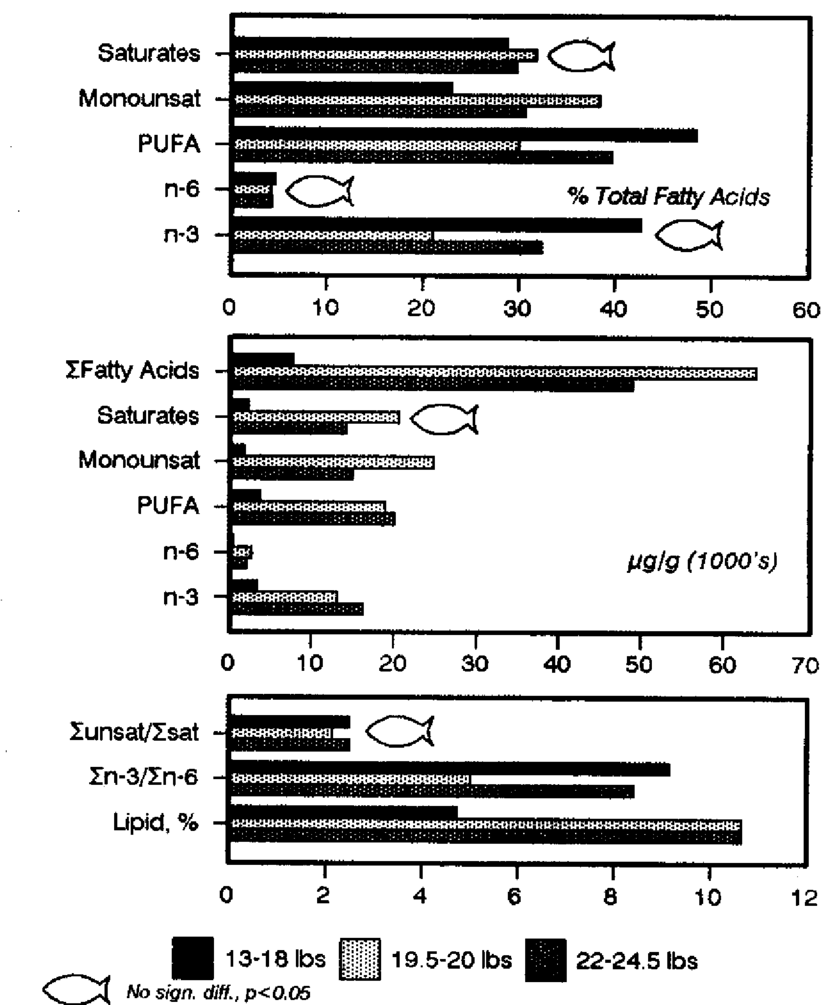

Figure 8. Fatty acids of blackfin tuna of varying sizes.

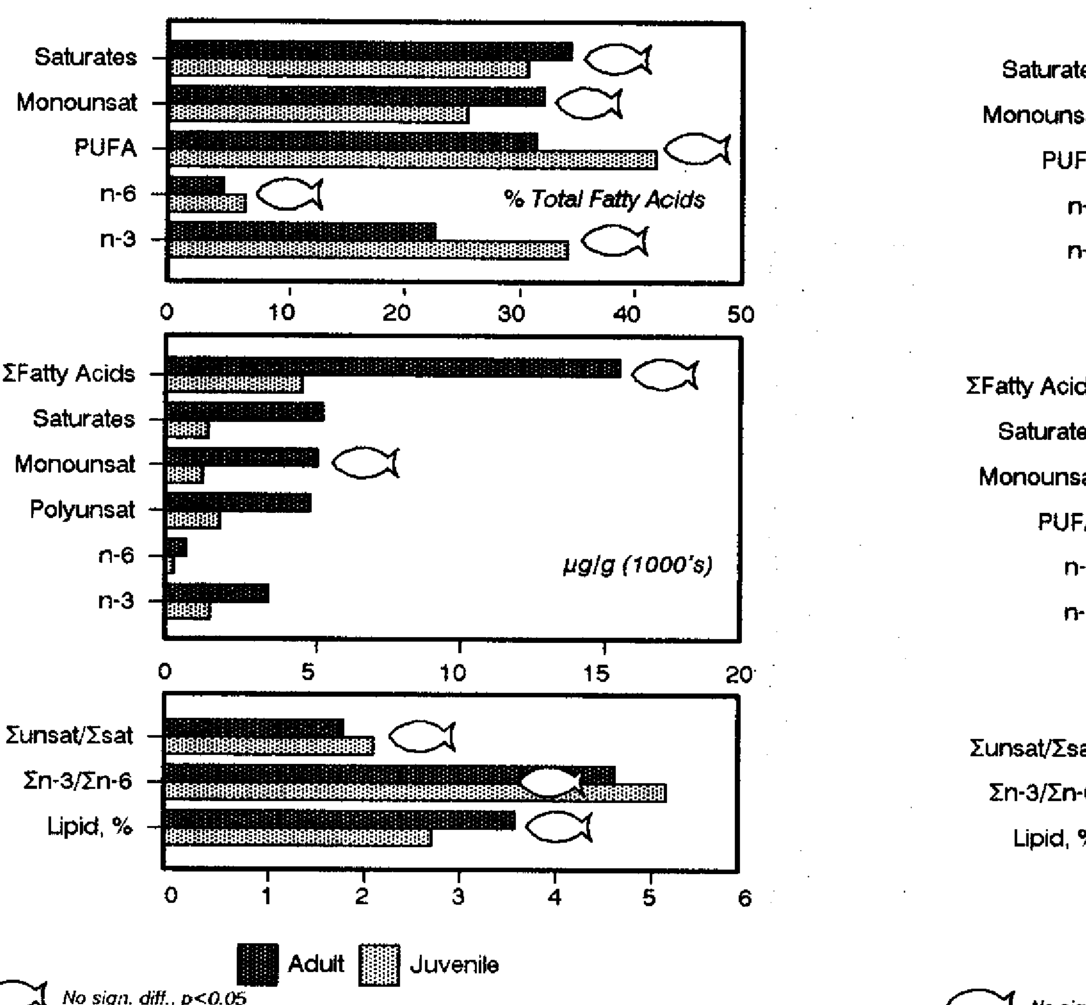

Figure 9. Fatty acids of adult and juvenile Atlantic croaker.

Figure 10. Fatty acids of adult and juvenile southern kingfish.

between juvenile and adult species (Figure 11). That is, juveniles have less fat but higher relative amounts of n-3 fatty acids. Juveniles of all species have higher total n-3/total n-6 ratio. Some fatty acid differences between adult and juvenile hybrid striped bass may also be due to differences in diet. Juvenile hybrid striped bass were reared in 500 gallon indoor tanks fed trout grower 1/8" by Zeigler from week 15 until sampled at approximately 5 months. Adult fish were reared in outdoor ponds for 6 months before sampling where they most likely fed on the epifauna and flora of the pond in addition to the same commercial feeds that fish reared in indoor tanks were fed.

Maturation development Red porgy (*Pagrus pagrus*) males caught in March, 1990 from the same geographical area but in developmental stages 2 and 4 show no significant differences except relative differences in total fatty acids (Figure 12). On the other hand, female ladyfish (*Elops saurus*) caught during the summer months from the Mississippi Sound in early developmental stage 1 and in gravid development, show significant differences whether expressed as relative concentrations or absolute concentrations (Figure 13). Only lipid and the ratio unsaturated/saturated are not significantly different. Greatest differences in fatty acids are seen when comparisons are made on species having the greatest differences in developmental stages. Southern kingfish (*Menticirrhus americanus*), for example, caught on June 20, 1989 from the Mississippi Sound were separated into developing females stage 3 and gravid females stage 5, and results indicated no significant differences (Figure 14).

CONCLUSIONS

Variations in fatty acids are observed in males and females during certain seasons of the year. These differences are most likely due to hormonal changes during maturation/spawning which may also change eating habits. Males are usually leaner and enriched in n-3 compared to females. Likewise, variations in size of species produce variations in the fatty acid patterns. Most likely, these changes are due to wide differences in fat content. Younger fish of all species examined are enriched in relative percent n-3 fatty acids compared to older fish. Juvenile species are enriched in n-3 and have higher n-3/n-6 ratios than do adult species. Species within families (pinfish and red porgy, little tunny and blackfin tuna) show similar trends. However, diet most likely is the over-riding factor in biological variability.

ACKNOWLEDGMENTS

This research was supported by NOAA, National Marine Fisheries Service, U.S. Department of Commerce, Grant Number NA88AA-H-SK018 and by the Gulf Coast Research Laboratory (GCRL) and the State of Mississippi. We acknowledge Constancia Ramos, Lynda O. Baker and Faye Mallette for their technical assistance and Gloria Seaborn for performing mass spectral analyses on a portion of the samples reported here. We are grateful for the fish supplied by Clark Seafood, Pascagoula, MS; Jim Franks, fisheries biologist, GCRL; the Parasitology Section, GCRL, Ocean Springs, MS; and Mike Murphy, fisheries biologist, Mississippi State University Aquaculture Station, Gulfport, MS.

Figure 11. Fatty acids of adult and juvenile hybrid striped bass.

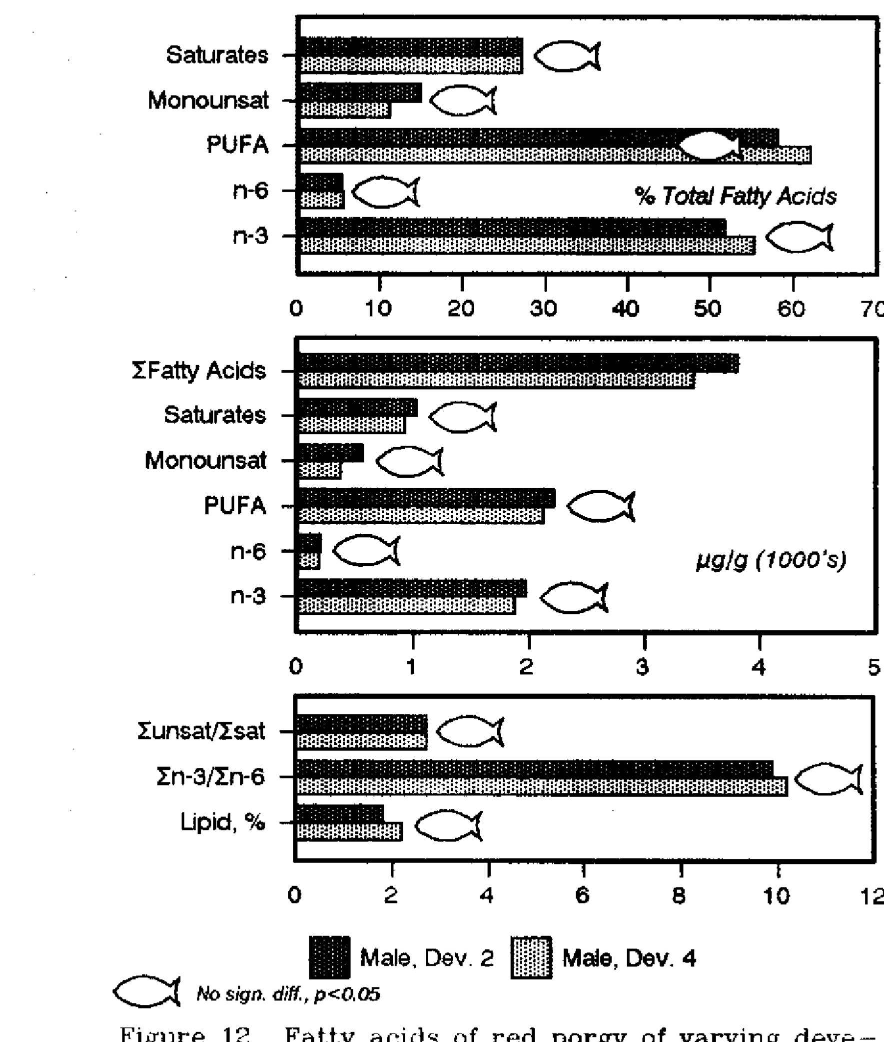

Figure 12. Fatty acids of red porgy of varying development state.

Figure 13. Fatty acids of ladyfish of varying development state.

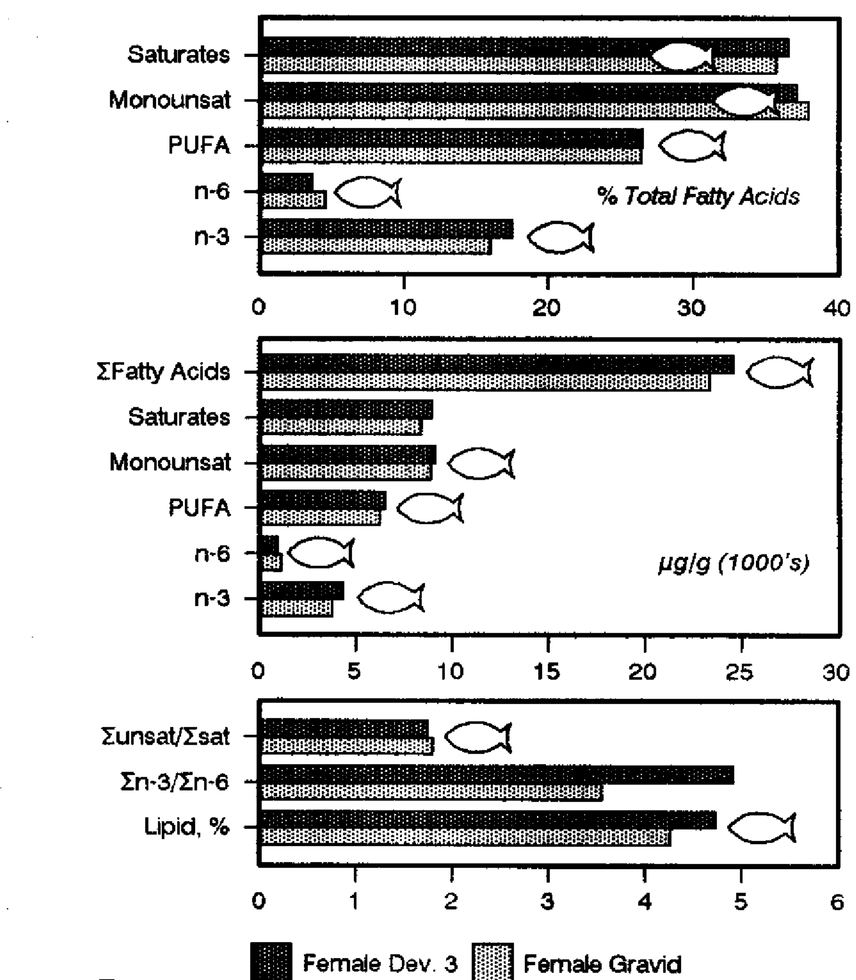

Figure 14. Fatty acids of southern kingfish of varying development state.

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FLAVOR AND TEXTURE COMPARISON OF HYBRID STRIPED BASS (Morone saxatilis/chrysops) AND RED SNAPPER (Lutjanus campechanus)

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The increasing consumption of seafood in the United States (1) has encouraged the development of new seafood products by the aquaculture industry. One of the newer aquaculture species, hybrid striped bass, is the object of much attention by aquaculture promoters (5). Information regarding consumer acceptance of hybrid striped bass could be beneficial when developing marketing strategies for this new aquaculture species.

Hybrid striped bass is a cross between striped bass and white bass. This new species has gained much attention recently and most sea grant universities have it under study with production occurring predominantly in the Middle Atlantic region of the United States. "At least two farms, one in California and one in North Carolina, are now harvesting product" (5). Hybrid striped bass is fairly cold tolerant and has many of the characteristics of saltwater finfish.

Market acceptability of new aquaculture species depends on the acceptability of flavor and texture attributes associated with what consumers consider good fish flavors and tenderness. Obviously, consumer acceptability of new products has a direct impact on the economic outcome of any food industry. The primary objective of this study was to establish flavor and texture descriptors for the hybrid striped bass, and secondly, to compare these descriptors to those of another popular saltwater finfish, red snapper.

MATERIALS AND METHODS

Volunteer sensory panelists were selected from undergraduate students, graduate students, faculty and staff in the College of Agriculture, Louisiana State University. Eight panel members were selected from potential panelists screened for their ability to identify and distinguish intensities of various control flavors and textures and for their frequent finfish consumption. Previous sensory and/or consumer panel studies involving seafood were reviewed (2)(4)(6) to provide input into planning and conducting training sessions for the eight-member panel and to establish the evaluation criteria for the actual evaluation sessions. Following established procedures, the panel was trained using red snapper as the control fish. Procedures outlined by Meilgaard, et al. (3) were followed, during three training sessions, to assist the panel members in identifying the kinds and intensities of flavors and textures present in the red snapper. Training in recognizing good fish flavor and texture in the red snapper was used in rating the overall acceptability of the unknown samples.

Three formal sensory panel sessions were held, with each panel member evaluating unidentified duplicate samples of both hybrid striped bass and red snapper finfish using a randomized complete block design. The sensory room was lighted with red light to reduce the influence of flesh color on the flavor evaluation. Each panel booth was equipped with evaluation forms, cooked samples, expectorant cups, water and scissors (for opening plastic bags). The identities of all the fish samples were unknown to the panelists.

The hybrid striped bass used in the evaluation was pond reared by the Fisheries Department at the Louisiana State University. Red snapper was purchased from a local seafood specialty store. All products were filleted, wrapped, frozen and stored in a -20℃ freezer until use. Just prior to testing, the fillets were removed from the freezer (cut while in frozen state) into approximately 3 cm squares and sealed individually in 4" x 4" polyethylene bags.

All fish fillet samples (sealed in polyethylene bags to retain moisture and flavor) were cooked in a boiling water bath (100℃) for 8-9min, optimum cooking times for texture was determined for both species prior to actual panel sessions. Cooking times longer than 8-9 min for these two species resulted in a drier and tougher meat protein structure. Each of the samples was clearly labeled with random numbers and presented in random order to the panelists. Each panelist rated all samples for flavor, juiciness, texture, and overall acceptability using a 0-9 hedonic scale. The hedonic acceptability scale ranged from 0 - highly unacceptable to 9 - highly acceptable. In addition, the panelists were asked to record specific flavor and texture attributes identified in each sample. The panel results were used to determine mean values for the panel as a whole and also with the panel divided into two groups based on their preference and consumption of fresh or salt water finfish.

Freshly thawed hybrid striped bass filets and red snapper were also breaded in a "Cajun" style fish fry breading and pan fried in pure vegetable oil (175℃) for 3-5 min until golden brown. Samples of breaded and fried hybrid striped bass and red snapper were rated for overall acceptability by the panel following the final session of evaluating boil-in-bag samples.

RESULTS AND DISCUSSION

Flavor descriptors identified by the panel for the red snapper and hybrid striped bass are presented in Table 1. All of the flavor and texture descriptors identified by the panel during the training sessions for the red snapper were also identified in the blind samples of red snapper and hybrid striped bass with the addition of pleasant, grainy, and buttery/oily identified in the hybrid striped bass only. The majority of the descriptors (mild fishy, sweet, moist, firm, etc.) were considered "good fish flavors" expected from a marine finfish such as red snapper. A few variations among panelists occurred in determining the degree of desirable fishy flavor in the boiled fish fillets. The "fishy" flavors described by the panel members, particularly those who consumed marine finfish on a regular basis, were considered good and expected flavors for fresh saltwater fish.

Overall acceptability ratings of the boiled fish samples are presented in Table 2. The overall mean acceptability scores for red snapper and the hybrid striped bass were very similar with ratings of 3.85(acceptable) and 3.18 (somewhat acceptable) respectively. When the panel scores were split into two groups

based on the panelists normal consumption of fresh or salt water fish, the acceptability of both fish species approximately doubled to around 7.00 (very acceptable)
for the salt water fish group specifically.

Table 1. Flavor and texture descriptors identifie in red snapper and hybrid striped bass.

Flavor related	Texture related	Miscellaneous
weak fishy	flaky	nutty
good fishy	moist	creamed corn
mild fishy	firm	cardboard
bland		
strong fishy		
buttery/oily*		
sweet light	grainy*	
typical fishy		
pleasant*		

* Descriptors identified in hybrid striped bass only.

Table 2. Acceptability* Rating.

	Whole Panel	Ocean Fish Panel	Whole Panel
	Unseasoned/boiled	Unseasoned/boiled	Seasoned/Fried
HSBass	3.18	7.12	7.89
Snapper	3.85	6.93	7.15

*Acceptability scores were measured on a 9 point hedonic rating scale. (9 = highly acceptable; 0 = highly unacceptable).

Acceptability ratings, by the entire panel, for the red snapper and hybrid striped bass prepared in a seasoned batter and fried were markedly higher than unseasoned samples with scores of 7.15 for red snapper and 7.89 for hybrid striped bass (Table 2).

CONCLUSION

Flavor and texture descriptors identified with hybrid striped bass were very similar to those described for red snapper. The overall flavor and texture acceptability scores rated by this sensory panel was similar for the two species. Consequently, it would appear that the farm raised hybrid striped bass could favorably compete with a popular marine species such as red snapper. The investigators recognize the limited scope of this experiment and recommend test marketing of hybrid striped bass also be conducted in other regions of the United States.

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QUALITY CHANGES OF AQUACULTURED HYBRID STRIPED BASS FILLET MEAT RESULTING FROM POST-HARVEST COOLING OR CO_2 TREATMENTS

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Aquacultured finfish and shellfish comprise an increasing share of all edible fishery products which is now approximately 12% of the worldwide supply. Additionally, aquaculture production increased 290% between 1980 and 1989 in the United States (3).

The hybrid striped bass has become an increasingly popular aquacultured food fish in the United States along with catfish, trout, salmon and tilapia. This fish is suitable for intensive culture because of good biological traits, rapid growth, schooling behavior, hardiness and high market value (10).

The primary objective of this study was to examine ways to increase the quality or shelf life of hybrid striped bass fillets from fish raised in an indoor recirculating aquaculture system. In this study, the post-harvest metabolism of hybrid striped bass was reduced with either a carbon dioxide gas treatment or a cooled water treatment.

Stress and high metabolic activity associated with harvesting and transportation of live fish may affect final product quality and shelf life. Of all the flesh foods, fish are the most susceptible to autolysis, oxidation and hydrolysis of fats and microbial spoilage (2). Rigor mortis is especially important in fish preservation because it can retard post-mortem autolysis and bacterial decomposition. Rigor mortis is hastened by struggling, lack of oxygen and warm temperatures and delayed by low pH and cooling (2,6). Shelf life can be prolonged by procedures to lengthen rigor mortis which include lowering muscle activity, careful handling and lowering of holding temperatures.

Another study objective was to develop quality indices for this species which have been lacking. Many previous studies that have reported biochemical, microbiological and sensory indices of quality and shelf life in other fish have widely varying or contradictory results. Since the hybrid striped bass used in this project were raised under controlled conditions, many of the variables (i.e. diet variations or seasonal water temperatures and photoperiods) that can affect quality indices were not factors.

MATERIALS AND METHODS

Source of Fish

The reciprocal cross hybrid striped bass used in this study were a cross between the female white bass Morone chrysops and the male striped bass Morone saxatilis. This cross is readily available from commercial sources and is the most common cultured hybrid striped bass. The accepted common name of this cross is the Sunshine Bass (4). All fish were obtained from the Virginia Polytechnic Institute & State University Aquaculture Research Facility (ARF). Fingerlings were purchased from Keo Fish Farms (Lonoake, Arkansas) and stocked at 1800 per culture tank (approximately 4 fish per cubic ft.).

Recirculating Aquaculture System

The Aquaculture Research Facility contains nine, indoor independent recirculating aquaculture systems. Each system consists of five major components: an 8,330 liter rectangular culture tank , a 1,970 liter sump tank with a multi-tube clarifier for the removal of suspended solids, a 1/4 hp pump (50 gpm), a 1,990 liter biofilter tank housing a three stage rotating biological contact filter, and a U-tube aeration system incorporating pure oxygen injection. Rotating biological contactors aid to maintain appropriate ammonia, nitrate and nitrite levels. Oxygen concentration is maintained by surface aerators, injection of liquid oxygen and the use of U-tube aerators.

Air and water temperatures in the facility are controlled by four propane heaters suspended in each corner of the building. Lighting in the building is kept to a minimum to reduce fish stress and algal growth. Lighting simulated an approximately 14 hour light and 10 hour dark cycle.

Water quality parameters were consistently measured. These parameters included ammonia, nitrate, nitrite, dissolved oxygen, pH, alkalinity, hardness, and temperature. Culture tank water temperatures at time of fish harvest were 24.4 - 25.1 °C.

The fish were fed a high protein floating diet (Biosponge Aquaculture Products, Sheridan, Wyoming) formulated for hybrid striped bass. The diet was composed of 44% crude protein, 8% fat, 3% crude fiber and 13% moisture. Feed was administered once or twice daily depending on water quality and feeding activity. Fish were not fed within 15 hours of harvest time.

Treatments

For each treatment or control group, 25-40 fish were harvested from a single tank by grading and dip net. Fish were transported to the Department of Food Science & Technology (FST) for filleting.

Fish were sacrificed on day zero. All analyses were started the following day (day one). Four test groups were used as follows in each of three experiments.

Control group fish were harvested, placed in waxed cardboard boxes, transported to FST and filleted.

Stressed Control group fish were harvested, and placed in a truck mounted holding tank for transport to FST. Transport tank water was obtained from the fish culture tank. After arrival at FST the fish were transferred by dip net to another holding tank. Approximately 200 gallons of water for the second holding tank was obtained from

municipal supply and aerated by a surface agitator. Sodium bicarbonate ($NaHCO_3$ 200 g) and calcium chloride ($CaCl_2 \cdot 2H_2O$, 215 g) were added to increase alkalinity and hardness. Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$, 20 g) was added to inactivate chlorine.

Fish were held in this tank for approximately three hours and subjected to periodic agitation. Also, these fish were additionally stressed since they were transferred between culture tank, transport tank and holding tank and experienced a change in water quality in the holding tank. After three hours, the fish were removed by dip net, weighed and filleted within three hours.

The cool water treatment group (CW) was harvested and placed in a holding tank at ARF for approximately two hours. Water for the holding tank was obtained from the culture tank and aerated with a surface agitator. Crushed ice was periodically added to the water to lower the temperature to 10-12 ℃ at the end of two hours. Sodium thiosulfate (10 g) was added to approximately 100 gallons of water in the tank to inactivate chlorine from the melting ice. At the end of the holding period the fish were removed by dip net, placed in waxed cardboard boxes with crushed ice and transported to FST for filleting.

The carbon dioxide treatment group (CO_2) was harvested and placed in a holding tank at ARF for approximately 30 minutes. Carbon dioxide (CO_2) was injected at 10-20 cfh until most fish remained on the bottom of the tank. After five to ten minutes, fish were gasping at the water surface. Water (approx. 200 gallons) for the holding tank was obtained from the culture tank and aerated only prior to addition of fish. Dissolved oxygen level dropped from approximately 6.7 ppm to 4 ppm over 30 minutes as measured with a YSI model 58 dissolved oxygen meter (Yellow Springs Instrument Co., Yellow Springs, OH). At the end of the holding period the fish were removed by dip net, placed in waxed cardboard boxes and transported to FST for filleting.

Sampling of fillet meat

Skinless, boneless fillets were removed from all fish within 30 minutes after arrival at FST, except filleting occurred after the three hour holding period for the stressed control group. Fillets were stored in ice covered plastic boxes in a refrigerator maintained at 1-4 ℃.

For each experiment, 15 fillets from the control group were vacuum packaged in plastic bags. These fillets were stored at -20 ℃ and used as a reference for the sensory evaluation panel.

Analyses

Aerobic Plate Count

Microbial levels of thawed fillets were determined with an aerobic plate count. For each group, a composite sample of 20-25g from three fillets was tested. The composite was mixed with 0.1% peptone (Difco Laboratories, Detroit, MI) to acheive a 10^{-1} dilution. The dilution was blended in a Stomacher Model 400 (Tekmar Co., Cincinnati, OH) for two minutes. Further dilutions in 0.1% peptone were prepared and plated in duplicate on Plate Count Agar (Difco Laboratories) using a pour plate technique. Plates were incubated at 20 ℃ for four days.

pH

The pH level of a fillet meat composite was determined on the same test days as the aerobic plate count. A composite sample of approximately five grams (from three fillets) was mixed with an equivalent weight of distilled water with a glass rod in a beaker. Determinations of pH level were performed with a Corning Model 240 pH meter (Corning Glass Works, Corning, NY) meter with a combination electrode (Corning #476530).

Texture measurement

Measurements of the texture of raw and cooked fillets were performed with an Instron Model 1011 Universal Testing Instrument (Instron Corp., Canton, MA) equipped with a L.E.E. - Kramer Shear/Compression Cell (1). Approximately 15 g pieces of raw (experiment 2) and cooked (experiments 1 and 2 only) fillets were brought to room temperature prior to testing. Cooked pieces were oven baked at 350°F for eight minutes. Two pieces per fillet, and three fillets per group were tested each test day. Measurements of peak force in units of kilograms force (kgf) to compress the sample to 30% of its original thickness were recorded.

Sensory evaluation panel

An experienced taste panel of 15 graduate students or employees of the Department of Food Science & Technology were used to evaluate the taste, aroma, appearance and texture of cooked fillets. Fillets presented to the panel were prepared by cutting 10-15 gram portions, wrapping in aluminum foil, labelling and baking for eight minutes in a 350°F convection oven. Each panelist was presented five cooked portions of fish and asked to rate the samples with a nine-point scale (1 = inedible, 5 = borderline, 9 = excellent) for appearance, odor, texture and taste (8). Each panelist received one piece each of fish representing each of the four test groups. A fifth fish portion for each panelist was a portion from the vacuum packaged and previously frozen at -20°C fillets, and was coded as "A". Panelists were directed to compare all attributes of the other fillet portions to the reference "A" portion.

Statistical analysis

Analysis of variance was performed with the General Linear Models procecure (Version 6.06, SAS Institute, Inc., Cary, NC). Additional comparisons were made with Duncan's Multiple Range Test, Tukey's Studentized Range Test and Dunnett's T test. The results of three experiments are combined and reported.

RESULTS AND DISCUSSION

Hybrid striped bass measurements

The average weight of the 369 fish filleted was 337 g (11.9 oz.). The average fillet weight obtained from each fish was 98.8 g (49.4 g per fillet). The fillet operation yielded an average of 29.2% fillets per fish. All fillets were skinless, boneless, J-cut and without belly flap. Fillets were studied because they are a popular market form and are less susceptible to autolytic spoilage by digestive tract enzymes than either whole or headed and gutted fish (9). The average flesh temperatures of the fish groups prior to filleting were 9.6°C for the CW fish group and approximately 23°C for the other three groups.

Aerobic plate count

The aerobic plate count of all test groups exceeded finfish spoilage levels of 10^7 cfu/gm (5) by day 11 (Figure 1). The cool water treatment fillets reached this level by day 9 and the two control groups near day 10. The fillets from the carbon dioxide treated fish did not achieve log 7 growth of microorganisms until eleven days of storage. Also, log phase growth in the CO_2 treatment fillets did not occur until between day 7 and 8 (Figure 1). A one log increase in counts occurred at least one day earlier for the other three test groups of fillets. Differences in levels of aerobic organisms were not significant ($p \leq 0.05$) between test groups.

pH

The pH of the refrigerated fillets in all groups increased from an average of approximately 6.24 initially to 6.54 by day 14 and 6.86 by day 18. Throughout the test period the pH of the cool water treatment fillets were highest overall and were significantly higher ($p<0.05$) than that of the control fillets. On day 1 the pH of the CO_2 fillets (6.13) was lowest. A significant correlation ($p<0.001$, $r = 0.57$) existed between pH level and aerobic plate count (log cfu/gm) over time for all test groups combined.

Texture measurement

Treatment differences and time differences due to aging of fillets were highly significant ($p<0.001$) for cooked fillet pieces for peak force energy measurements. Initial peak force measurements on cooked fillets (Figure 2) ranged from an average of 40.1 kilograms force (kgf) for the CW fillets to 60.2 kgf for the control group fillets. Cool water fillet peak force measurements were significantly lower ($p<0.05$) throughout the test period than the control and CO_2 fillets.

Differences in peak force energy measurements on raw fish portions (one experiment only) were not significant over time (18 days) or between test groups. Average initial (day 1) peak force was 26.2 kgf for raw fish portions and 49.4 kgf for cooked portions. Average final peak force was 22.7 kgf for raw fish portions and 46.8 kgf for cooked portions.

Sensory evaluation panel

Significant differences ($p<0.05$) over time (day 1 - 14) exist for all four sensory attributes. The average sensory attribute scores across all test groups declined over 14 days as follows: appearance score from 7.51 to 6.58, taste score from 7.06 to 6.00, odor score from 7.44 to 6.43, and texture score from 7.08 to 6.40. Texture scores and Instron measurements of fillet texture were not correlated. Texture scores and microbial levels (log cfu/g) were not correlated ($r=-0.31$, $p=0.053$).

The only significant ($p<0.05$) treatment difference for a sensory attribute was that the control group fillets were rated lower in appearance than the other three groups throughout the test period. In Figure 3 the sensory scores at each time have been combined and averaged to show differences in sensory qualities of fillets of each test group by attribute. Overall, the two treatments were rated higher in sensory quality than both control groups when all times are combined.

In Figure 4 the four attribute scores have been combined and averaged at each time to show the changes in sensory qualities of each test group over time. The control group fillets were rated lowest initially (day 1-3), and the CO_2 group fillets were rated highest late in the test period (days 10-14).

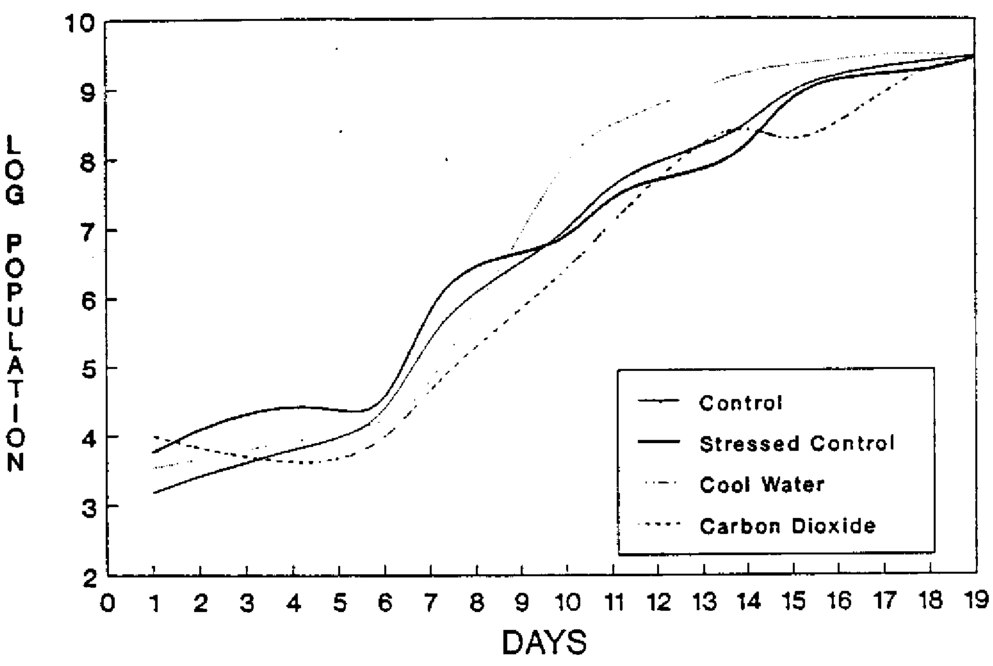

Figure 1. Mean aerobic plate count of fillets stored at 1-4℃.

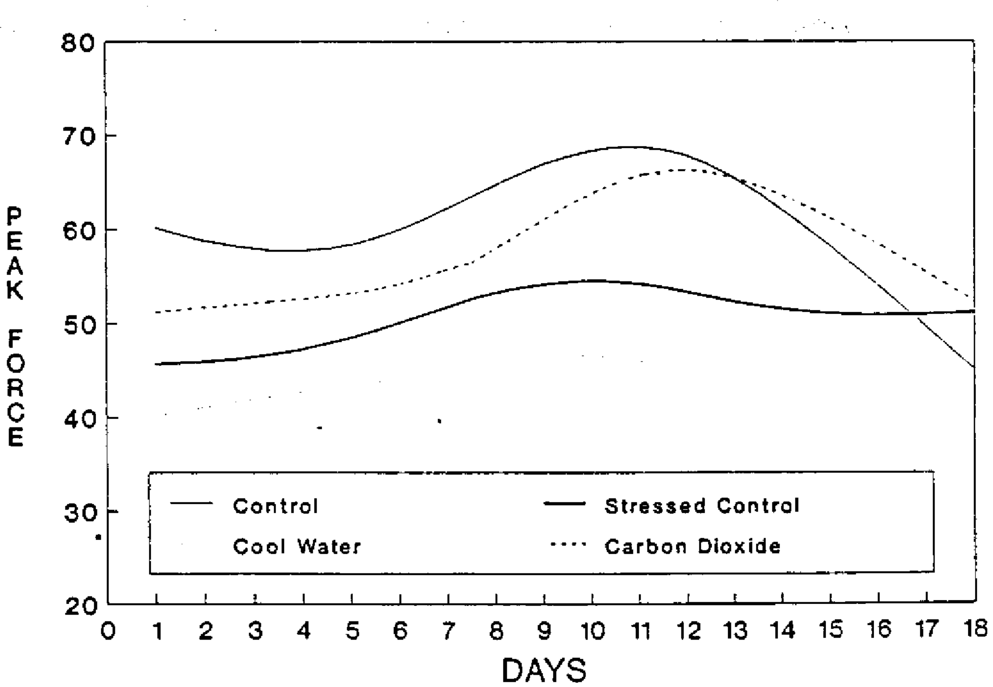

Figure 2. Mean peak force energy (kgf) for compression of cooked fish portions.

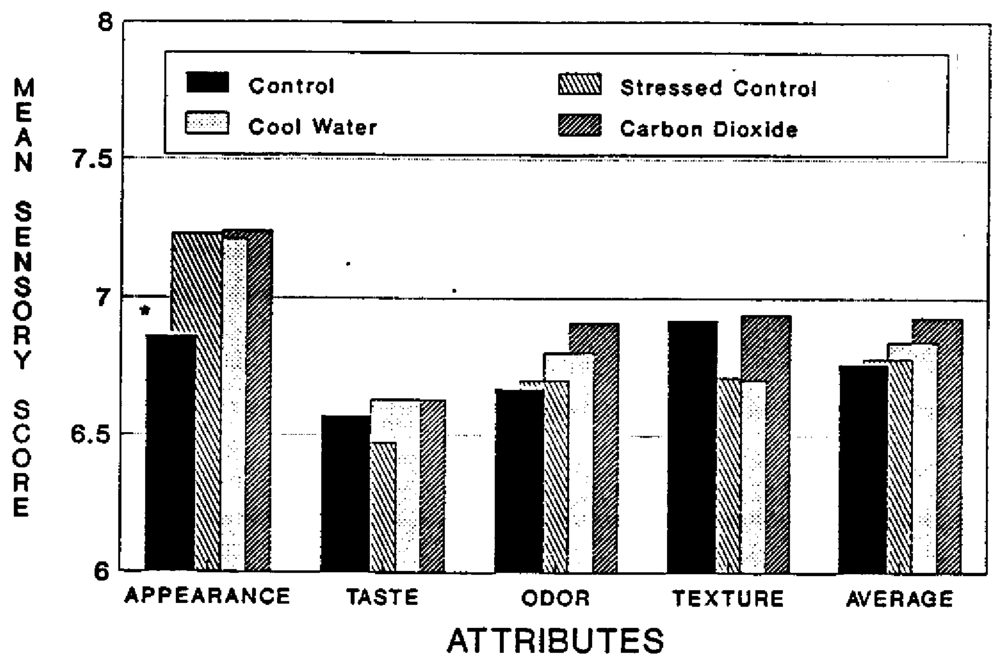

Figure 3. Mean sensory evaluation panel ratings for each test group per attribute. * = significance, $p<0.05$.

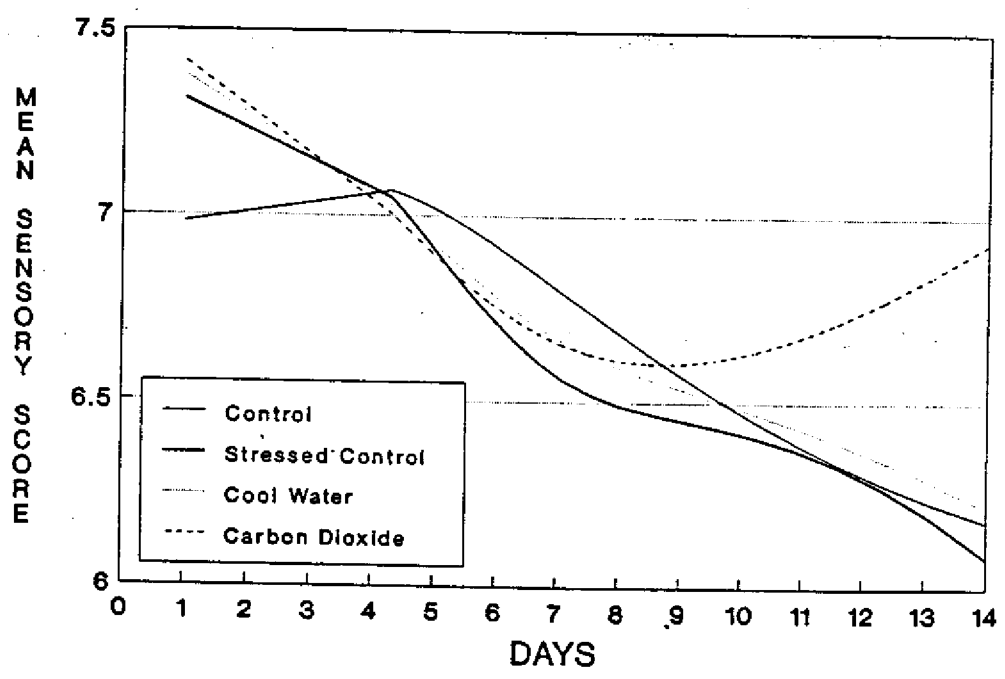

Figure 4. Mean sensory evaluation panel ratings for each test group over time. Appearance, odor, texture and taste scores are combined.

CONCLUSION

The CO_2 treatment increased the shelf life of fillets by at least one day over the other treatments used since log phase growth and the time to reach a seven log microbial level was delayed. In addition, sensory attributes of fillets from the carbon dioxide treated hybrid striped bass were judged superior during extended storage of fillets. Also, the firmness of cooked fish portions as measured by a Kramer Shear Compression Cell (peak force) was significantly higher for these fillets than the cool water treatment fillets.

Procedures to reduce fish stress during handling and processing are critical to increased productivity of intensive aquaculture (7). The use of a post-harvest CO_2 treatment to increase the quality and shelf life of fish obtained from a recirculating aquaculture facility can lead to several economic benefits. First, an increase in consumer confidence in the high quality and consistency of hybrid striped bass will lead to a greater market demand and increases in price or sales. Increased shelf life can lead to distribution over greater distances and a product less sensitive to storage temperature abuse. Also, processing plants can operate year-round since a recirculating aquaculture system can produce a predictable supply of raw material leading to planned production schedules (9). Finally, post-harvest use of carbon dioxide gas on fish awaiting processing is less costly than the use of ice or refrigeration to slow the metabolism of live fish.

The carbon dioxide treatment described here may be useful for extensively aquacultured fish after harvest and during or after transport to a processing or packaging facility. The results of this research may be applicable to other food fish species that are raised in recirculating aquaculture systems.

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STORAGE STABILITY OF TILAPIA IN RELATION TO LIPID AND TOCOPHEROL COMPOSITION

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Displaying an efficient conversion of low protein diets, an ability to breed easily, a resistance to handling stress and disease, and a high palatability, tilapia are considered to be one of the most important aquacultured food fish in the world. In the United States, culture of tilapia has been restricted to the warmer states. Despite this limitation, production of these fish within this country could increase dramatically necessitating in turn that a greater percentage of the product be marketed as frozen. The storage stability of two varieties of tilapia was therefore examined to explore the potential of tilapia to be marketed in this form. The lipid and tocopherol composition of these tilapia was also examined in an attempt to understand the degree to which these components dictate the tilapia's susceptibility to oxidation.

MATERIALS AND METHODS

Sample preparation

Two varieties of tilapia were obtained from the Owens and Williams Fish Farm in Hawkinsville, Georgia. One variety, *Tilapia nilotica*, exhibited the traditional black/blue coloration of skin and was designated as "Blue" tilapia (BT). The other variety designated "Red" tilapia (RT) was a hybrid, having inherited a red body coloration from a cross of *Tilapia nilotica* and *Tilapia mossambica* 10 generations earlier. Both fish had been spawned in April, stocked the following month in a 0.38 acre pond at 12,000 fish/acre, and harvested in October of the same year. Transported on ice to Griffin, seven fish from each variety were weighed, filleted, pooled to form a homogeneous sample, and minced.

Storage

The samples, divided into 4 portions, were subjected to varying periods (0, 3, 6 and 9 months) of fluctuating frozen storage (-6 to -18°C), then stored at -100°C until the various analyses could be performed on the samples. At the time of analysis, samples were removed from the -100°C freezer and thawed in cold running water.

Moisture Analyses

Moisture content was determined on 2.0 g minced tissue samples by microwave drying. Samples were dried at 100% power using an Automatic Volatility Computer, Model AVC-80 (CEM Corporation; Indian Trail, NC) until constant weight was achieved.

Lipid Extraction and Characterization

Chloroform:methanol (2:1) was used to extract lipid from 1.00 $\pm$ 0.02 g muscle tissue as described by Erickson (3). Polar (phospholipids) and nonpolar lipid (triacylglycerol and cholesterol) fractions were obtained using silica Sep-Pak cartridges (5).

Fatty acid composition of the phospholipid and total lipid fractions was monitored by esterification using 4% H_2SO_4 in methanol and subsequent gas chromatography of the fatty acid methyl esters.

Triacylglycerols were measured by saponifying an aliquot of the nonpolar lipid fraction and quantitating the glycerol moiety (6).

Following saponification of another aliquot of the nonpolar lipid fraction, quantitation of total cholesterol was based on the formation of a chromogen with $FeCl_3$ (11).

Tocopherol Analyses

After saponification in the presence of ascorbic acid and ethanol, tocopherols were extracted from 1.0 g minced muscle tissue with 10% ethyl acetate in hexane (3). The extract evaporated to dryness under nitrogen was reconstituted in 1.0 ml methanol:water (98:2). Reverse-phase high performance liquid chromatographic separation of tocopherols was conducted as described by Vatassery and Smith (7). The chromatographic system consisted of a Micromeritics 752 Gradient Programmer, Micromeritics 750 Solvent Delivery System, and a Brinkmann 656 Electrochemical Detector.

Oxidative Measurements

Two five ml aliquots of lipid extract (25 ml total) were dispensed into separate clean tubes and each washed with 1 ml of 0.88% KCl. After removing the upper layer by aspiration, one of the tubes was analyzed for hydroperoxides according to Buege and Aust (1), while the other tube was analyzed for conjugated dienes by the spectrophotometric procedure (A_{232}).

Fluorescent pigments were determined on 10.0 ml of a lipid extract which had been washed with 2.50 ml of 0.88% KCl. Diluted samples of both the aqueous and organic layers were measured in a Turner fluorometer, Model 112, using a quinine sulfate standard (1×10^{-8}M) set equal to 100 fluorescence units.

Thiobarbituric acid reactive substances (TBA-RS) were quantified according to the extraction procedure of Vyncke (8). Results were expressed as nmol malonaldehyde/g dry weight.

Following heating of tissue (1.5 g) in a 5 ml conical vial for 15 minutes at 90°C, headspace (1.5 ml) was removed with a gas-tight syringe and subjected to chromatographic conditions described by Young (10). Peaks corresponding to propanal and hexanal were tentatively identified by comparison of their relative retention times to purified standards (Sigma, St. Louis, MO) subjected to the same heating and chromatographic conditions as the samples. Based on the response of the internal standard, 4-heptanone, quantities of propanal and hexanal were calculated.

RESULTS AND DISCUSSION

At harvest, BT's fish weights ranged from 375 to 508 g with an average of 414 g while RT's fish weights ranged from 309 to 508 g with an average of 363 g. As shown in Table I, BT was found to have larger quantities of both lipid and moisture than found in RT. By accounting for the moisture differences between the two tilapia varieties, differences in fish weights were largely attributed to the larger quantities of triacylglycerol deposited in the BT. In addition, BT contained slightly higher quantities of phospholipid and cholesterol than RT on a dry weight basis.

Table I. Lipid composition of tilapia.

	RT	BT
Percent Lipid	1.7	1.9
Percent Moisture	79.4	80.2
mg Triacylglycerol/ g dry wt.	58.5	71.8
mg Phospholipid/ g dry wt.	22.0	23.2
mg Cholesterol/ g dry wt.	2.4	2.6

The two most abundant polyunsaturated fatty acids present in both tilapia's triacylglycerol fractions were linoleic acid (18:2) and docasahexaenoic acid (22:6). RT's triacylglycerol fraction, however, was characterized with a greater degree of unsaturation than BT's when expressed as a percentage of the total fatty acids in this fraction (Table II). When all fatty acid percentages and the varying susceptibilities of each fatty acid to oxidize are used to calculate a peroxidizability index (9), RT's triacylglycerol fraction is considered more unstable than BT's fraction. Alternatively, when the peroxidizability index is modified to take into account the quantity of each fatty acid that would be present in one g of tissue, BT's tissue would be considered more susceptible to oxidation than RT if triacylglycerols serve as the primary site of lipid oxidation.

While representing a much smaller percentage of the total lipid, the phospholipid fractions from each tilapia variety were also characterized as to their fatty acid composition (Table II). Again, 18:2 and 22:6 were the most dominant polyunsaturated fatty acids in both fractions and constituted a larger percentage of the total fatty acids and hence higher peroxidizability index in RT's phospholipid fraction than BT's fraction. The tissue peroxidizability index in turn indicated that RT's tissue could be more susceptible to oxidation than BT's tissue if phospholipids alone were the primary rate determining factor.

The tocopherol antioxidants, however, must also be factored into the susceptibility of phospholipids to oxidize. Residing in membranes, the tocopherols act as antioxidants by scavenging oxygen- or carbon-centered fatty acyl radicals via donation of a hydrogen atom from the phenyl hydroxyl of the chromanol ring. In tilapia, both the alpha and gamma isomers of tocopherol were present in greater abundance in BT than RT (Table III). Adjusting for the gamma isomer being approximately 31% as effective an antioxidant as the alpha isomer (2), BT's tocopherol protection was nearly 1.2 times greater than RT. Therefore, in accounting for both the phospholipid and tocopherol composition (peroxidizability index/nmol alpha-tocopherol equivalents), RT's minced muscle sample with a ratio of 2.2 could be expected to oxidize sooner than the BT sample with a ratio of 1.8 provided membrane phospholipids serve as the primary site of lipid oxidation.

Table II. Fatty acid composition and peroxidizability of triacylglycerol and phospholipid fractions.

	TRIACYLGLYCEROL		PHOSPHOLIPID	
	RT	BT	RT	BT
Percent 18:2	11.6	11.2	12.3	11.2
Percent 22:6	2.7	2.3	19.4	18.5
Peroxidizability Index[a]	54.2	50.6	243.8	231.0
μmol 18:2/g dry wt	23.0	27.2	7.0	6.7
μmol 22:6/g dry wt	5.3	5.6	11.0	11.1
Tissue Peroxidizability Index[b]	107.4	123.1	138.4	137.2

[a] Peroxidizability Index = (sum % monoenes * 0.025) + (sum % dienes * 1) + (sum % trienes * 2) + (sum % tetraenes * 4) + (sum % pentaenes * 6) + (sum % hexaenes * 8)

[b] Tissue Peroxidizability Index = (sum mg monoenes * 0.025) + (sum mg dienes * 1) + (sum mg trienes * 2) + (sum mg tetraenes * 4) + (sum mg pentaenes * 6) + (sum mg hexaenes * 8)

Table III. Tocopherol content of tilapia muscle tissue.

	NMOL TOCOPHEROL/G DRY WT	
	RT	BT
Alpha-Tocopherol	58.8	68.6
Gamma-Tocopherol	15.7	19.0
Alpha-Tocopherol Equivalents	63.7	74.5

During the first 3 months of frozen storage, no change was noted in the alpha-tocopherol equivalents for either BT or RT (Fig 1). Between 3 and 6 months, degradation of tocopherol had occurred in both tilapia varieties but at a similar rate. After 6 months, a dramatic drop in tocopherol levels was seen for both tilapia varieties increased rate of tocopherol degradation which occurred after 6 months implied that a critical concentration had been reached at which point the tocopherol was no longer able to compete effectively for the peroxyl radicals and prevent the propagation step of oxidation. Previously, this critical tocopherol concentration was found to be dependent on the effectiveness of the oxidative stress (4). Consequently, it may be surmised that the higher apparent critical concentration exhibited by BT arose from a greater oxidative stress in BT than RT muscle tissue.

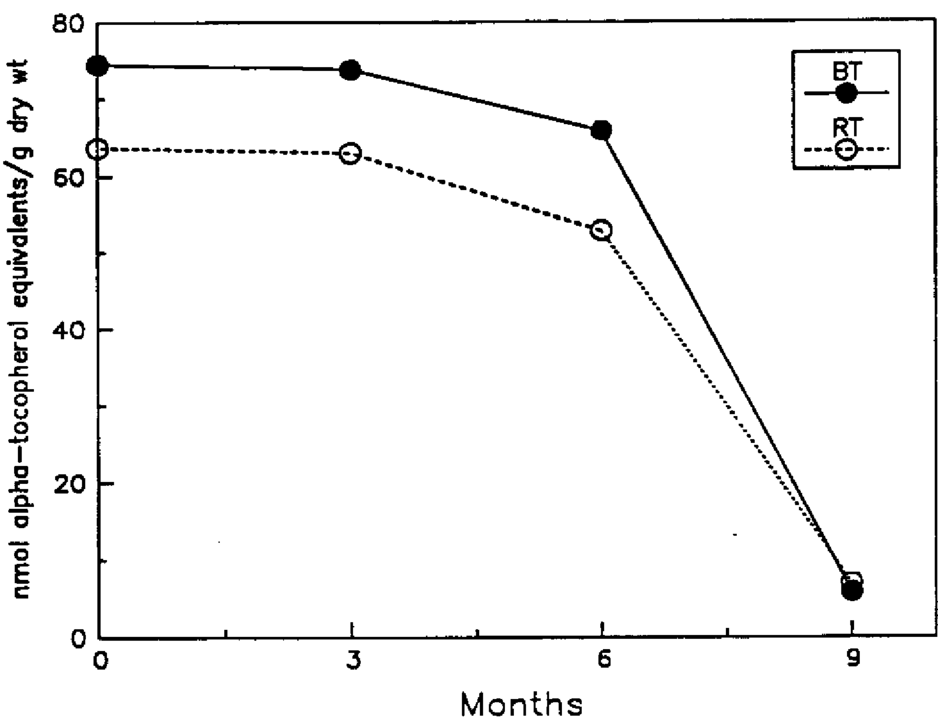

Fig. 1. Tocopherol content of tilapia muscle tissue following frozen storage.

Measurement of the oxidative products, hydroperoxides, also demonstrated the presence of a lag phase for both RT and BT (Fig. 2). In the case of this data, though, increased rates of formation occurred prior to 6 months for BT muscle. The larger hydroperoxide levels found in BT muscle compared to RT muscle supported the tocopherol data in indicating that BT was more susceptible to oxidation than RT. The conjugated diene data, however, did not follow the same trend as the hydroperoxide data (Fig. 2). Higher levels of conjugated dienes were found in BT at 0, 3 and 6 months, yet at 9 months, levels of conjugated dienes in RT surpassed those in BT.

For the TBA test which measures some of the breakdown products of hydroperoxides, the lag phase was evident for both RT and BT only during the first 3 months of storage (Fig. 3). Notable differences in rate of formation of these oxidative products between the two varieties was just evident during the last 3 months of storage. Similarly, dramatic differences for propanal and hexanal were only noted between varieties in muscle tissue which had been stored 9 months (Fig. 4). The larger production of propanal compared to hexanal which occurred is likely attributed to the relative susceptibility of the fatty acids from which they originate, propanal being an endproduct of n-3 fatty acid oxidation (i.e. 22:6) and hexanal being an endproduct of n-6 fatty acid oxidation (i.e. 18:2).

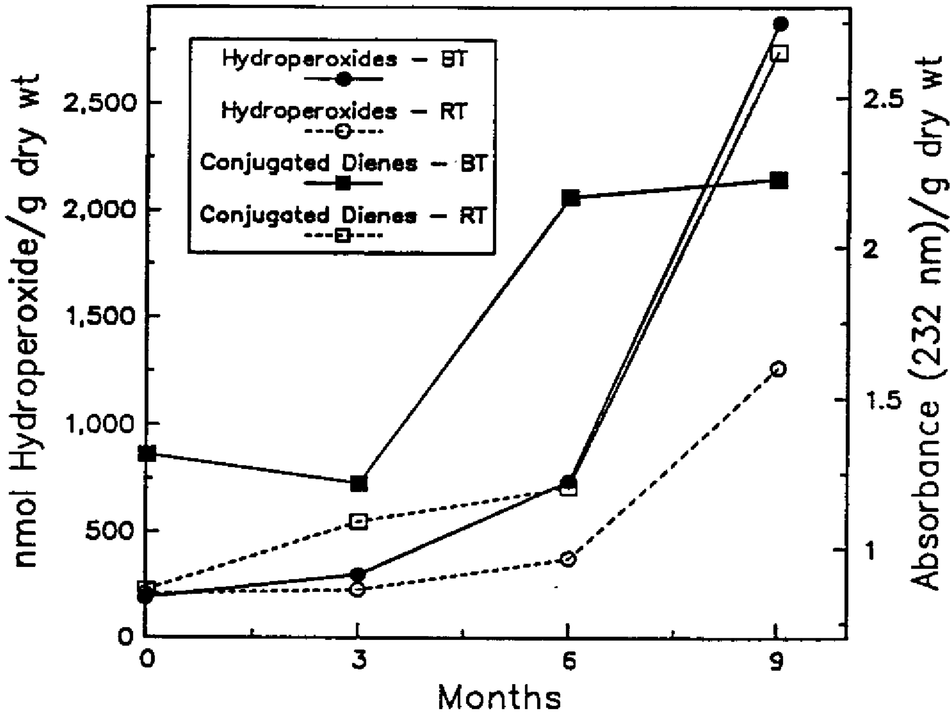

Fig. 2. Hydroperoxide and conjugated diene content of tilapia muscle tissue following frozen storage.

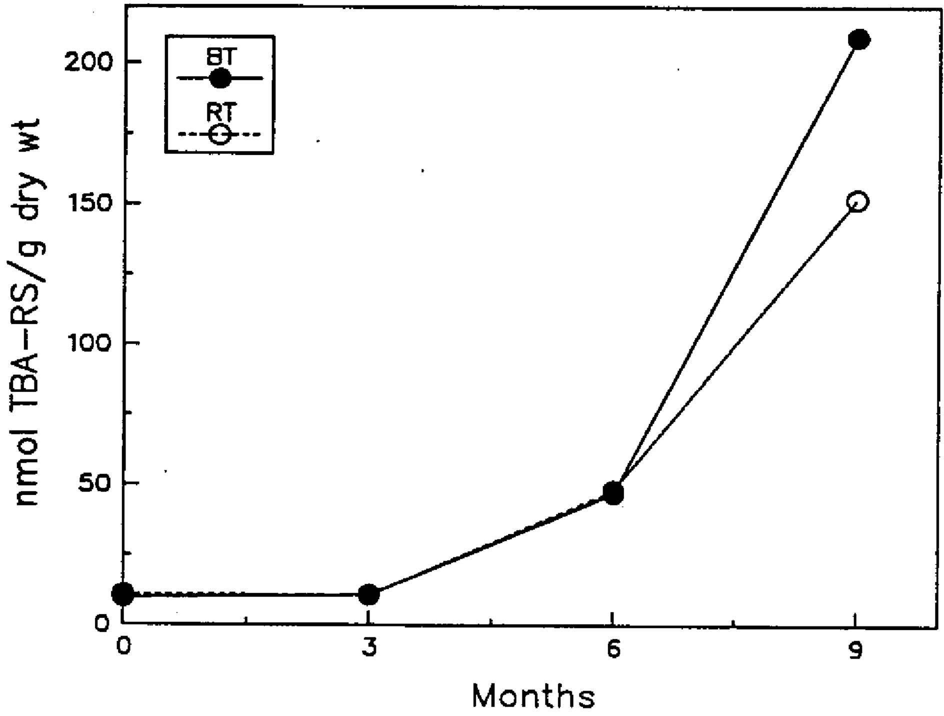

Fig. 3. TBA-RS content of tilapia muscle tissue following frozen storage.

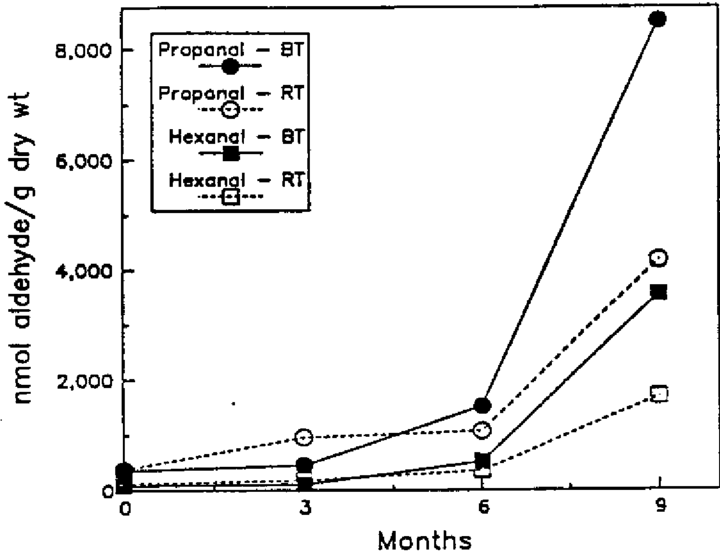

Fig. 4. Propanal and hexanal content of tilapia muscle tissue following frozen storage.

Fluorescent pigments, another type of secondary oxidative product, was also monitored on the stored samples (Fig. 5). Consisting of carbonyls that have formed a Schiff base to the amine group of a phospholipid, organic fluorescent pigments were produced at similar rates in the two varieties. On the other hand, aqueous fluorescent pigments, or those carbonyls that have formed Schiff bases to a primary amino acid or peptide, formed to a greater extent in the BT than RT muscle tissue. In contrast to the TBA data, the lag phase extended through 6 months of storage for both types of fluorescent pigments. Such a delay, however, is expected given that the fluorescent pigment precursor, carbonyls, must first be produced.

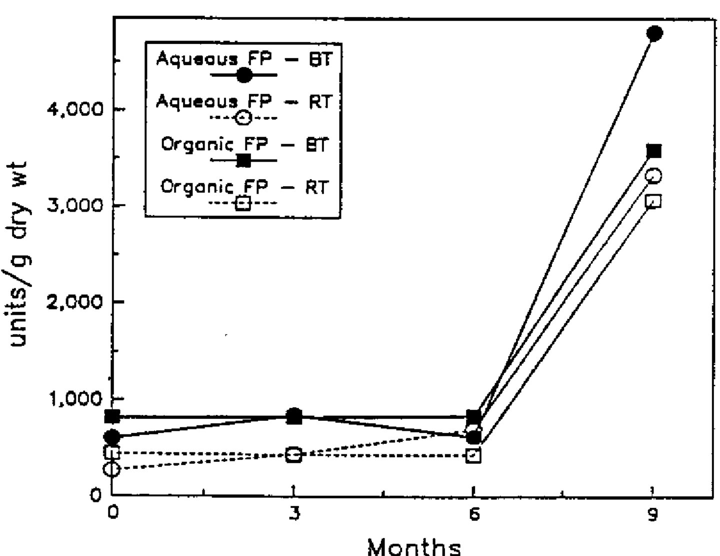

Fig. 5. Fluorescent pigment content of tilapia muscle tissue following frozen storage.

CONCLUSION

Based on the majority of oxidative measurements conducted in this study BT was considered less stable than RT. From the phospholipid and tocopherol composition data, however, the opposite scenario would have been predicted. Only when the absolute amount of triacylglycerol and the relative oxidative stabilities of this fraction's fatty acid composition were considered was BT predicted to be less stable than RT. These results suggest that the triacylglycerols would be serving as the primary site of lipid oxidation and not the phospholipids in these minced fish. It is further speculated that the triacylglycerol contribution to oxidation is via the accelerated action of lipases released on mincing. Since the contribution of lipases in an intact muscle tissue would be minimized, it is unknown whether similar oxidative susceptibilities would exist for these tilapia varieties with unminced muscle tissue. Thus, further studies are needed on both minced and unminced muscle tissue to provide us with additional information on the relative contributions of compositional factors to the oxidative stability of tilapia tissue.

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ANTIOXIDANT PROPERTIES OF PHOSPHOLIPIDS IN A SALMON OIL MODEL SYSTEM

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INTRODUCTION

During the processing of both seed oils and fish oils, the major content of phospholipids is removed during the washing and clean-up of crude lipid extracts. Since phospholipids (PL) are generally of higher unsaturation, a great deal of emphasis has been placed on their effective removal to assure enhanced stability of refined oils. Recent studies (12,5), however, have shown that the addition of small quantities of PL possessed antioxidant properties in enhancing the storage stability of processed vegetable oils and animal fats. Previous studies in the authors laboratory (7) have also shown that the addition of PL extracted from bluefish (Pomatomus salatrix) to a salmon oil model system had greater antioxidant properties than Neutral lipids in spite of the higher polyunsaturated fatty acid content of the PL. There, the objectives of this study were to measure the antioxidant properties of selected PL in a heated salmon oil model system and to examine the relationship between color intensity and antioxidant property.

METHODS

Commercial phospholipid standards of greater than 98% purity were obtained from Sigma Chemical Co (St. Louis, MO). These PL standards included Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidyl serine (PS), sphingomyelin (SPH), and phosphatidylinositol (PI). The PE, PC, SPH, and PG were obtained from egg yolk, whereas the PS and PI were obtained from bovine brain and liver, respectively. The salmon oil was obtained from Body Products Research, Inc. (Chatsworth, CA) without the addition of stabilizers or antioxidants. Prior to

use, the endogenous phosphopholipid contents of the oil and of the commercial PL were checked by high performance liquid chromatography (HPLC) (4) and phosphorus analyses (1).

Model oil systems: All PL standards were dissolved in chloroform-methanol (2:1, v/v) prior to their addition to 20 mg of salmon oil (SO), followed by vortexing and evaporation of solvent under a stream of nitrogen. All treatments were placed in screw-cap glass test tubes without caps and heated in a forced-draft oven at 180° C for periods of 0, 15, 30, 60, 120, and 180 min.

Experiment I: The first experiment consisted of four treatments that included (1) control salmon oil-no PL, (2) SO + 0.01% PC, (3) SO + 0.10% PC and (4) SO + 1.0% PC.

Experiment II: Treatments consisted of the following (1) control:SO-no PL (2) SO + 1% PG, (3) SO + 1% PI, (4) SO + 1% PS, (5) SO + 1% PE, (6) SO + 1% PC (7) SO + 1% LPC, and (8) SO + 1% SPH.

Test of Oxidative Stability: The oxidative stability of the model systems was determined by measuring the change in fatty acid composition (Polyene ratio) (11), and the formation of malonaldehyde as measure by the 2-thiobarbituric acid assay (TBA) (6). The polyene ratio or index was measured by gas chromatographic analysis (9) of fatty acids expressed as the ratio polyunsaturated fatty acids(PUFA) to saturated (SAT) fatty acids using the loss of docosahexaenoic acid (DHA) to palmitic acid (i.e. C22:6/C16:0) as the index of oxidation. A Hewlett Packard, Model 5890 (Avondale, PA) equipped with a flame ionization detector was used to analyze prepared methyl esters (8). As an indicator of the possible formation of browning reaction products, 20 mg aliquots from each treatment were removed for spectrophotometric measurement of color changes using a Shimadzu Recording Spectrophotometer UV-240 (Schimadzu Corp., Kyoto, Japan) set at 430 nm (3).

Statistical Analysis: A randomized complete block design containing two replication was used to analyze all data (2,10).

RESULTS AND DISCUSSION

The addition of PC at all three levels improved the overall stability of salmon oil subjected to thermal stress at 180° C

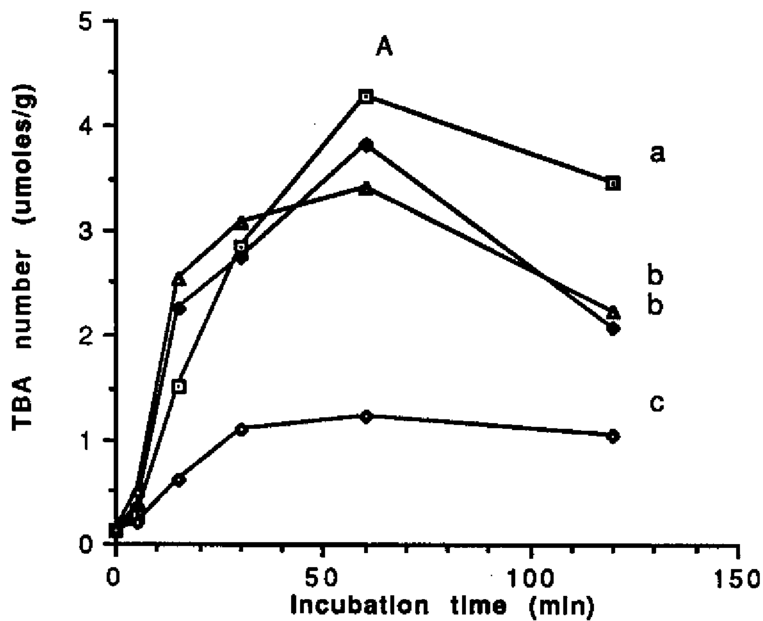
(Fig. 1A). Additions of PC at the 1% level appeared to be most effective in reducing TBA values, whereas additions at 0.01% and 0.10% did not differ significantly from each other. The polyene index showed a pattern similar to TBA values in that additions at the 1% and 0.10% levels were most effective in preserving PUFA. Additions of PC at the 0.01% did not differ significantly from the control containing no PC.

Examination of the change in color intensity measured at 490 nm (Fig. 2) indicate that increasing concentrations of PC were associated with increasing color intensity. Additions of PC at the 0.01% and 1.0% were significantly higher than control and 0.01%, which did not differ from each other. Comparisons of TBA numbers, polyene index, and color intensity indicate significant correlations between the three parameters as well as length of heating (Table 1). For example, TBA values were inversely correlated to PC levels at the 30 and 60 min heating periods, whereas the polyene index was strongly correlated to PC levels toward the end of the heating cycle reflecting the generally slower loss of PUFA over time.

The results of experiment II indicate that the PL differed in their ability to control oxidation of PUFA subjected to heated storage (Fig. 3). The nitrogen-containing PL including PE, PC, LPC, and SPH appeared to be the most effective in stabilizing the oil whereas PG and PI were least effective. Examination of TBA numbers over various periods of heating indicate that while PI, PS, and PG were effective in increasing the induction period, they were not as effective as the nitrogen-containing PL over the entire course of heating. The polyene index values showed a pattern similar to that observed TBA numbers in that the antioxidant properties of PE, PC, LPC, and SPH were not significantly different from each other whereas PS, PI, PG, and the control were ineffective in controlling the loss of PUFA.

The change in color intensity followed a pattern similar to that observed in experiment I in that the most effective PL possessed the highest color intensity whereas the least effective PL showed increases in color intensity similar to the control containing no PL. However, there were differences in the rate of increases that appeared to be influenced by the effectiveness of certain PL. For example, treatments

Fig. 1: Model system I-Effects of 0.01, 0.10, and 1.0% phosphatidylcholine (PC) on (A) 2-thiobarbituric acid (TBA) numbers and (B) polylene index of salmon oil heated to 180° C for 180 min.



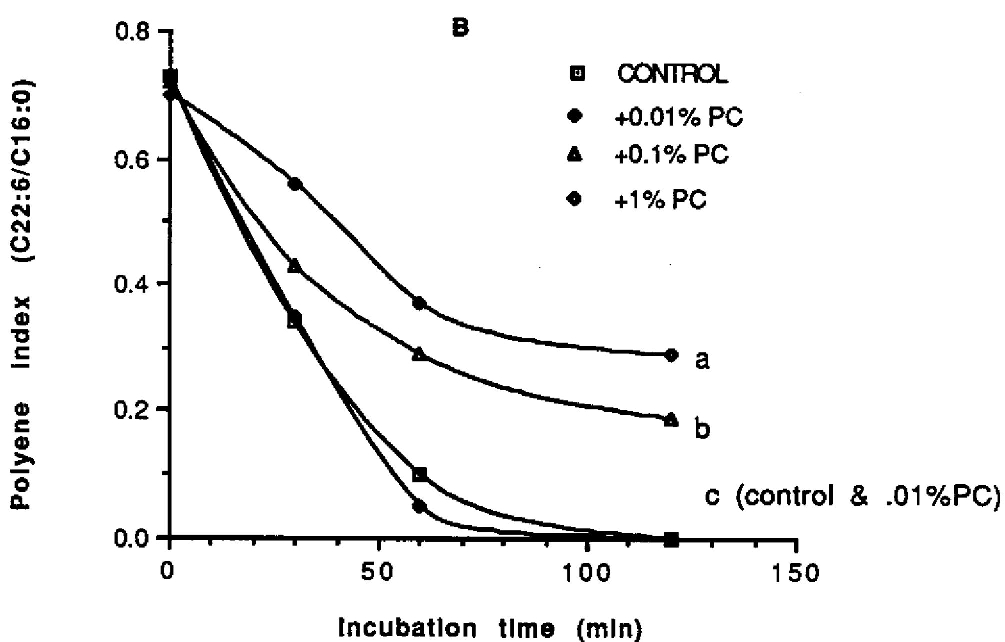


Fig. 2: Model system 1- Effects of 0.01, 1.0 and 1.0% phosphatidylcholine (PC) on absorbance values of salmon oil measured at 430 nm heated to 180° C for 180 min.

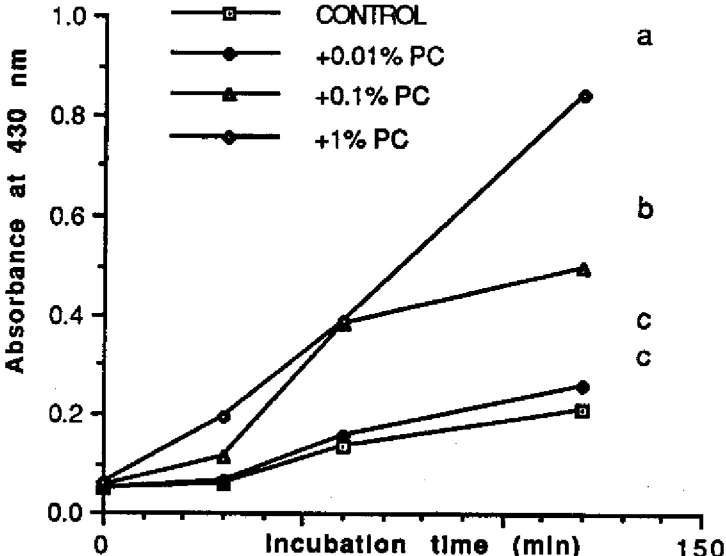
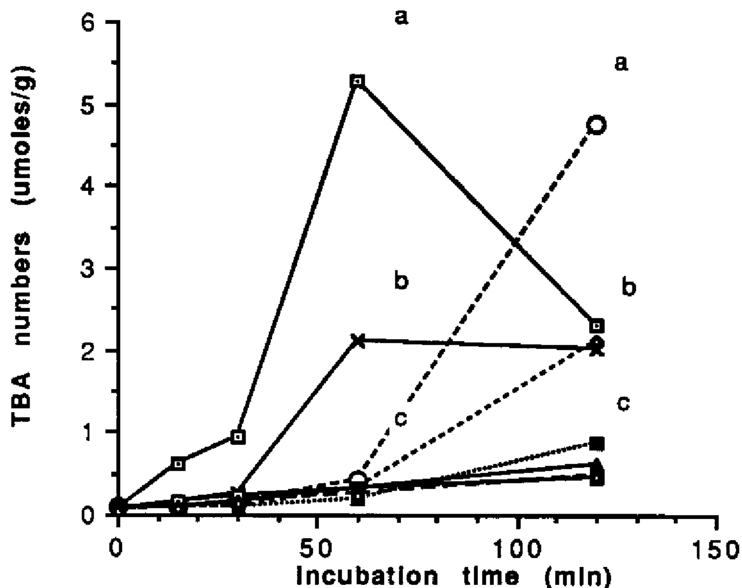
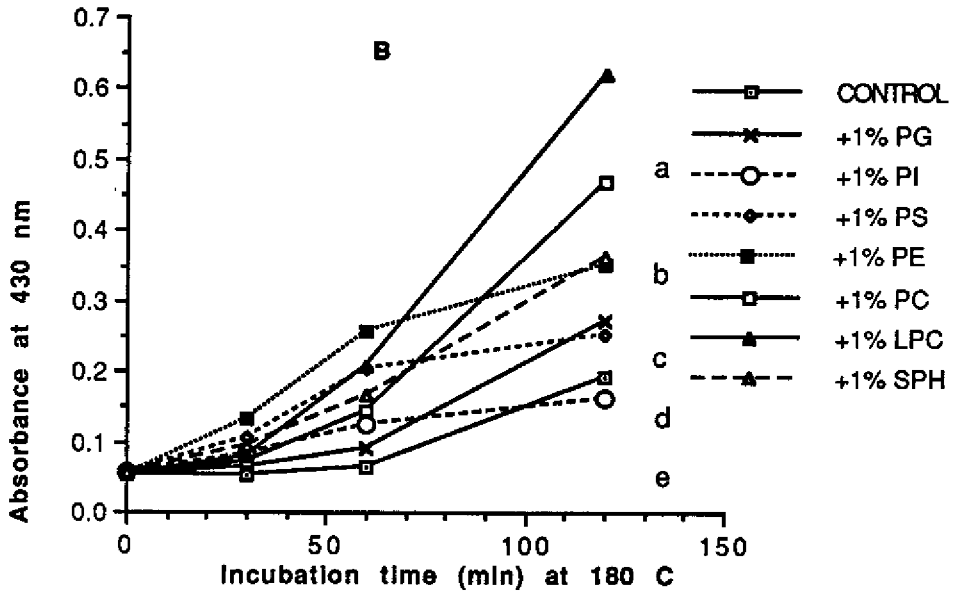


Fig. 3: Model system II-Effects of 1% additions of different classes of phospholipids on (A) thiobarbituric acid number (TBA) and (B) absorbance values of salmon oil heated to 180° C for 180 min*





*PG=phosphatidylglycerol; PI=phosphatidylinositol; PS=phosphatidylserine; PE=phosphatidylethanolamine; SPH=sphingomyelin; PC=phosphatidylcholine; LPC=lysophosphatidylcholine.

Table 1. Simple correlation coefficients between TBA numbers, polyene index, and browning color of salmon oil, and salmon oil + 0.01%, 0.1% and 1% Phosphatidylcholine over different heating times at 180° C.

Heating time (min)		Polyene ratio (22:6/16:0)	Browning	% of PC added
0	TBA numbers	-0.56	-0.52	0.45
	Polyene ratio		-0.44	-0.47
	Browning			0.84**
30	TBA numbers	-0.72	-0.82**	-0.93**
	Polyene ratio		0.94**	0.87**
	Browning			0.95**
60	TBA numbers	-0.78	-0.64	-0.95**
	Polyene ratio		0.83**	0.74*
	Browning			0.57
120	TBA numbers	-0.66	-0.69	-0.70
	Polyene ratio		0.90**	0.84**
	Browning			0.86**

* ($P < 0.05$).
** ($P < 0.01$).

containing PE and PS showed very rapid increases in color intensity within 1 hr of heating whereas treatments containing LPC, SPH, and PC showed more gradual increasing in color intensity.

The correlation patterns of Experiment II were the same as observed in Experiment I in that TBA values were negatively correlated with the formation of browning reaction products and the polyene index. However, color intensity was positively correlated with the polyene index, indicative of the preservation of PUFA by the addition of selected PL.

Table 2 shows the change in fatty acid concentration of the control salmon oil and the treatments containing PL prior to and following heating over the course of the 3 hr heating. As can be observed from the initial zero period of heating, the total PUFA content as well as the total n-3 PUFA content of the controls and of the treatments containing PL did not differ significantly from each other. However, following 3 hr of heating, the total PUFA content, n-3 PUFA as well as n-3/SAT ratio showed major differences in the ability of the PL to protect the stability of the heated oil. The SPH and LPC appeared to be equally most effective in stabilizing the lipid, followed by PC and PE. Treatments containing PG, PS, and PI were ineffective and did not differ from the control salmon oil containing no PL. The fatty acid composition of the individual PL also did not appear to have an impact on the antioxidant properties of the PL as PG and PC were almost identical in fatty acid composition yet differed significantly in their antioxidant properties.

CONCLUSION

The results of these studies demonstrated that the addition of selected PL enhanced the oxidative stability of salmon oil model systems subjected to heated storage. The nitrogen containing PL including PC, PE, SPH, LPC appeared to have the greatest antioxidant properties whereas PL containing glycerol (PG) and a reducing sugar (PI) were least effective. The addition of PC at different concentrations indicated a concentration gradient whereas the addition of different PL indicated that the antioxidant properties were not related to the fatty acid composition of the PL. The formation of

Table 2. Changes in fatty acid concentration of salmon oil and salmon oil plus 1% commercial PL during heating at 180° C for 3 hr*

Time (min)	Treat.	SAT. %	MONO. %	PUFA %	C18:2 %	n-3 %	Sln-3 %	n-3/SAT
0	OIL	35.2^{ab}	33.8^{a}	31.0^{a}	1.8^{b}	29.2^{a}	24.6^{a}	0.83^{ab}
	+PG	36.1^{ab}	33.9^{a}	30.0^{a}	2.3^{a}	27.7^{b}	24.0^{a}	0.77^{b}
	+PI	36.2^{ab}	33.2^{a}	30.6^{a}	1.9^{b}	28.7^{ab}	24.2^{a}	0.79^{ab}
	+PS	35.2^{ab}	33.3^{a}	31.5^{a}	1.8^{b}	29.8^{a}	25.0^{a}	0.85^{a}
	+PE	35.4^{ab}	33.3^{a}	31.3^{a}	2.2^{a}	29.1^{ab}	24.6^{a}	0.82^{ab}
	+PC	35.4^{ab}	33.5^{a}	31.1^{a}	2.3^{a}	28.9^{ab}	24.3^{a}	0.82^{ab}
	+LPC	36.7^{a}	32.9^{a}	30.5^{a}	1.8^{b}	28.6^{ab}	24.0^{a}	0.78^{ab}
	+SPH	34.7^{b}	33.5^{a}	31.7^{a}	1.8^{b}	29.9^{a}	25.1^{a}	0.86^{a}
60	OIL	43.5^{a}	38.6^{a}	17.9^{c}	1.5^{c}	16.4^{c}	13.7^{c}	0.39^{d}
	+PG	37.3^{bc}	36.4^{b}	26.3^{b}	2.3^{a}	24.1^{b}	20.1^{b}	0.65^{c}
	+PI	39.1^{b}	34.3^{cd}	26.7^{b}	1.8^{b}	24.8^{b}	20.5^{b}	0.64^{c}
	+PS	35.3^{c}	34.5^{bcd}	30.2^{ab}	1.8^{b}	28.4^{ab}	23.9^{ab}	0.81^{ab}
	+PE	35.4^{c}	32.7^{d}	31.9^{a}	2.1^{a}	29.7^{a}	24.8^{a}	0.84^{a}
	+PC	35.6^{c}	35.0^{bc}	29.4^{ab}	2.1^{a}	27.3^{ab}	22.8^{ab}	0.77^{abc}
	+LPC	38.0^{bc}	34.6^{bcd}	27.4^{ab}	1.8^{b}	25.7^{ab}	21.3^{ab}	0.68^{bc}
	+SPH	35.5^{c}	35.0^{bc}	29.6^{ab}	1.8^{b}	27.7^{ab}	23.0^{ab}	0.78^{abc}
120	OIL	51.2^{a}	43.0^{a}	5.9^{c}	0.9^{d}	4.9^{c}	4.1^{c}	0.10^{c}
	+PG	44.5^{b}	41.3^{a}	14.1^{b}	1.9^{abc}	12.2^{b}	10.6^{b}	0.28^{b}
	+PI	44.4^{b}	38.5^{b}	17.2^{b}	1.2^{cd}	16.0^{b}	13.3^{b}	0.38^{b}
	+PS	36.7^{c}	37.3^{bc}	26.0^{a}	1.7^{abc}	24.3^{a}	20.1^{a}	0.67^{a}
	+PE	38.3^{c}	34.4^{c}	27.3^{a}	2.2^{ab}	25.1^{a}	21.0^{a}	0.66^{a}
	+PC	38.5^{c}	36.3^{bc}	25.3^{a}	2.2^{a}	23.0^{a}	19.4^{a}	0.60^{a}
	+LPC	39.5^{c}	35.7^{bc}	24.9^{a}	1.4^{bcd}	23.5^{a}	19.5^{a}	0.60^{a}
	+SPH	39.1^{c}	37.6^{b}	23.3^{a}	1.8^{abc}	21.6^{a}	18.0^{a}	0.58^{a}
180	OIL	56.1^{a}	41.7^{a}	2.2^{d}	1.0^{ab}	1.2^{d}	1.2^{d}	0.02^{d}
	+PG	51.9^{a}	39.3^{a}	8.3^{c}	1.5^{ab}	6.7^{c}	3.3^{c}	0.13^{d}
	+PI	55.0^{a}	42.8^{a}	2.2^{d}	0.6^{b}	1.6^{d}	1.6^{d}	0.03^{d}
	+PS	52.0^{a}	40.5^{a}	7.5^{c}	0.7^{ab}	6.8^{c}	6.8^{c}	0.13^{d}
	+PE	45.2^{b}	38.8^{a}	16.1^{b}	1.7^{ab}	14.3^{b}	12.6^{b}	0.32^{c}
	+PC	42.6^{bc}	38.6^{a}	18.8^{ab}	1.9^{a}	16.9^{b}	14.4^{ab}	0.40^{bc}
	+LPC	41.9^{bc}	38.4^{a}	19.7^{ab}	1.6^{ab}	18.0^{ab}	15.3^{ab}	0.43^{ab}
	+SPH	39.8^{c}	37.5^{a}	22.8^{a}	1.9^{a}	20.9^{a}	17.3^{a}	0.53^{a}

*a, b, c Means with different letter superscripts within heating time and fatty acid category indicate a significant difference between treatments ($p<0.05$). Treat = treatment, SAT = saturated, mono = monounsaturated, PUFA = polyunsaturated fatty acids with two or more double bonds, n-3 = total of all fatty acids with n-3 double bonds, Sln-3 = combined C20:5 and C22:6, n-3/SAT = total n-3 fatty acids /saturated fatty acids, PG = phosphatidylglyerol, PS = phosphatidylserine, PL = Phospholipid, PE = Phosphatidylethanolamine, PC = phosphatidylcholine, LPC = Lysophosphatidylcholine, PI = phosphatidylinositol, SPH = sphingomyelin, C18:2 = linoleic acid.

browning reaction products and their effectiveness in stabilizing the salmon oil model system appears to be similar to the formation of Maillard reaction products which have been shown to have antioxidant properties in heated systems.

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EVALUATION OF PACKAGING ALTERNATIVES FOR FRESH AND PASTEURIZED CRAB MEAT

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Blue crab processors need packaging options for fresh and pasteurized crab meat that reduce processing and packaging costs, improve quality, safety, and shelf life, and accommodate smaller portion sizes. Traditional fresh and pasteurized crab meat containers are 16 oz polyethylene cups and 16 oz steel cans, respectively. Processors have been presented with a number of new commercial options for the packaging of both fresh and pasteurized crab meat. Little data is available to processors that would permit an objective evaluation of the effects of the new packaging materials on pasteurized or fresh picked crab meat stored on ice or at refrigerated temperatures. The following is a preliminary report of a continuing study to determine the effectiveness of commercial packaging materials on the quality, shelf life, and safety of fresh and pasteurized crab meat.

METHODS

Over a 10 month period lump meat pasteurized at 182°F was held between 32°F and 34°F in the following containers: (i) 16 oz steel cans (#401); (ii) 10 oz plastic cans with aluminum easy-open ends (# 307 co-polymer polyethylene cans holding 8 oz of crab meat, King Plastic Corporation, Orange, CA); (iii) 8 oz aluminum cans (#307 with easy-open ends); (iv) 8 oz non-barrier pouches (Cryovac P640 with nylon base and low density polyethylene sealant); and (v) 8 oz barrier pouches (Cryovac P640B with nylon base Saran[R] barrier and low density polyethylene sealant, Cryovac Corporation, Duncan, SC).

Webster et al. (14) determined that crab meat spoiled during refrigerated storage following pasteurization conditions that achieved a minimum F_{185}^{16} -value of 31 minutes, the process equivalent recommended by the National Blue Crab Industry Pasteurization Guidelines. Webster et al. (14) isolated two heat tolerant organisms that were identified as a *Bacillus* sp. and a *Clostridium* sp. All cans were processed to an F_{185}^{16} -value of approximately 40 minutes, in an effort to reduce the potential for bacterial spoilage in the investigated packaging materials. Cans were pasteurized in water at 182°F. Calculated F-values and cook times for each pasteurized can type can are shown in Table 1 (5,6).

Samples were collected from three containers of each can type and composited for: chemical, microbiological, and sensory analyses at zero time, 2, 4, 6, 8, and 10 months. Analyses were completed in duplicate. The study will be continued through 1 year of refrigerated storage.

TABLE 1. Cook times and calculated F_{185}^{16} -values for lump crab meat pasteurized at 182°F in the five investigated containers.

CAN TYPE	COOK TIME (MINUTES)	F-VALUE (MINUTES)
STEEL CAN	163	53.8
PLASTIC CAN	130	43.8
ALUMINUM CAN	120	39.7
BARRIER POUCH	70	42.8
NON-BARRIER POUCH	70	45.2

The following parameters were evaluated during 10 months of refrigerated storage. A Minolta Chroma Meter CR-200 (Minolta Corporation, Ramsey, NJ) was used to determine Hunter L, a, b color values of the meat (8). Whiteness index (WI) was calculated according to Stensby (12): WI = L - 3b + 3a. Stensby's whiteness index was adopted from the detergent industry where it has been used to determine the relative whiteness of fabrics. The index provides a quantitative evaluation of crab meat color. The index assigns 3 times as much weight to Hunter a, green levels, and Hunter b, blue levels, as it does to Hunter L or lightness levels. Components of blue and green create undesirable colors in blue crab meat. Stensby's whiteness index has not been correlated to the visual assessment of crab meat color. Aerobic and psychrotrophic plate counts were determined using standard dilution techniques (11). Ammonia levels (13) and pH (7) were measured. A five member trained panel developed sensory odor profiles on a continuous scale from 0 to 6 with 0 being none detected and 6 the strongest possible response for ammonia, sour, putrid, and crab odors (1,2,3,5,9). The panel's subjective like or dislike of meat color and appearance was rated on a continuous scale from 0 to 6.

Fresh special crab meat was held at 32°F or 39.2°F in the following: (i) 12 oz polyethylene cups; (ii) 8 oz polystyrene trays over-wrapped with Saran[R] (PVDC, polyvinylidene chloride film, Dow Chemical Co., Indianapolis, IN); (iii) 8 oz permeable pouches (Cryovac P640 with nylon base and low density polyethylene sealant, Cryovac Corporation, Duncan, SC) packaged using a Multivac AG 900 vacuum packaging machine (Multivac Sepp Haggenmüller KG, West Germany); and (iv) 8 oz polystyrene trays vacuum skin packaged by sealing film-to-tray with Trigon oxygen permeable Intact[R]Skin Packaging Film (ISPF) using a Trigon RM331 Mark III Mini Intact[R] machine (Trigon Packaging Corporation, Redmond, WA). Composite samples were collected at 0, 3, 7, 11, 14, 18, and 23 days of storage. Monitored parameters were the same as described previously for pasteurized crab meat.

Statistical analyses were performed on chemical, sensory, and microbiological data by means of PC SAS (10). SAS GLM and Duncan's multiple range test at the 0.05 level were used to determine any significant differences among days or months of storage and packaging materials.

RESULTS AND DISCUSSION

Crab meat pH values were significantly different ($p < 0.05$) for all treatments during the first six months in the following descending order: barrier pouch > aluminum can > non-barrier pouch > plastic can > steel can (Figure 1). Ammonia levels showed no consistent patterns with any packaging treatment during the first 10 months of the pasteurization study. Hunter L-values, an objective measurement of lightness and darkness, are shown in Figure 2. At zero time, following pasteurization, meats in plastic and aluminum cans and non-barrier pouches had higher L-values, indicating a lighter meat color, than product in steel cans ($p < 0.05$). At 4 months meat in plastic containers had higher L-values than meat stored in barrier pouches. Hunter a and b values and Stensby's WI index showed no consistent differences with time.

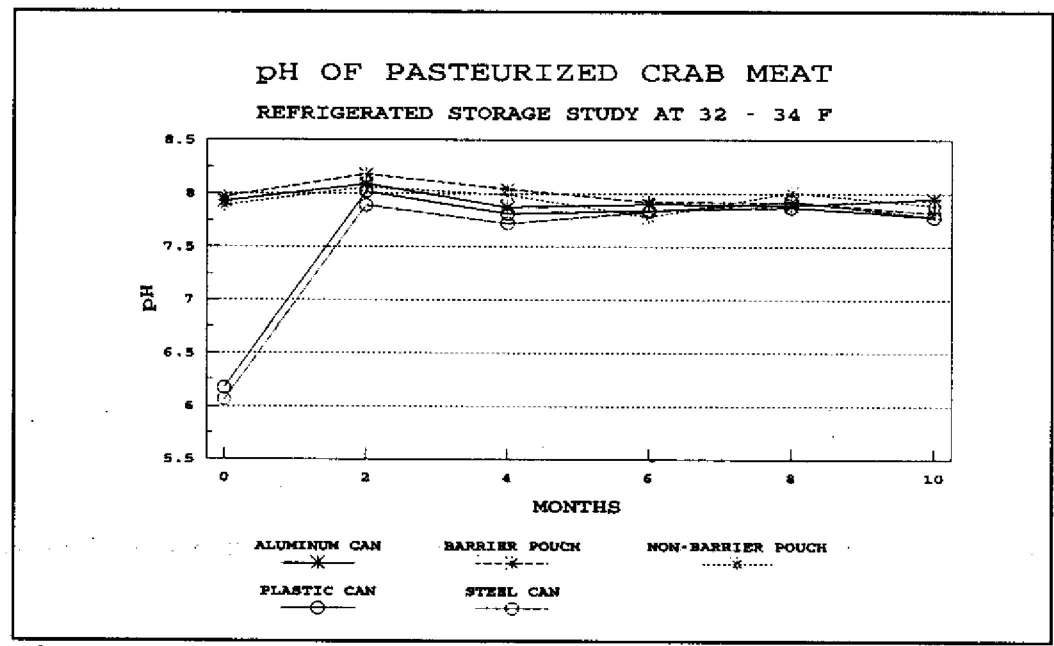

Figure 1. Pasteurized crab meat pH levels following refrigerated storage in the five monitored containers.

All treatments had low aerobic plate counts through 6 months of storage (Figure 3). Meat composited from plastic cans in the eighth month were microbiologically spoiled. However, plate counts from meat in steel cans, with the highest process F-value, were definitely less than the other treatments at the end of 8 months ($p < 0.05$). Meat collected from the plastic cans at 10 months had acceptable plate counts. The cause of the microbiological spoilage in meat sampled from plastic cans in the eighth month was not determined. Meat from barrier pouches had plate counts above 10^5 CFU/g at the end of 10 months. Plate counts determined for non-barrier pouches and steel cans were less than other treatments following 10 months of storage ($p < 0.05$) (Figure 3).

Psychrotrophic levels in plastic cans increased rapidly between zero time and 2 months, leveled off, and decreased between 8 and 10 months (Figure 4). Aluminum cans had the lowest psychrotrophic levels.

Sensory odor analysis that included ammonia, sour, putrid, and crab odors showed no consistent differences among the treatments over the storage period. At zero time meat from barrier bags and steel cans had the least acceptable color (Figure 5). Product from

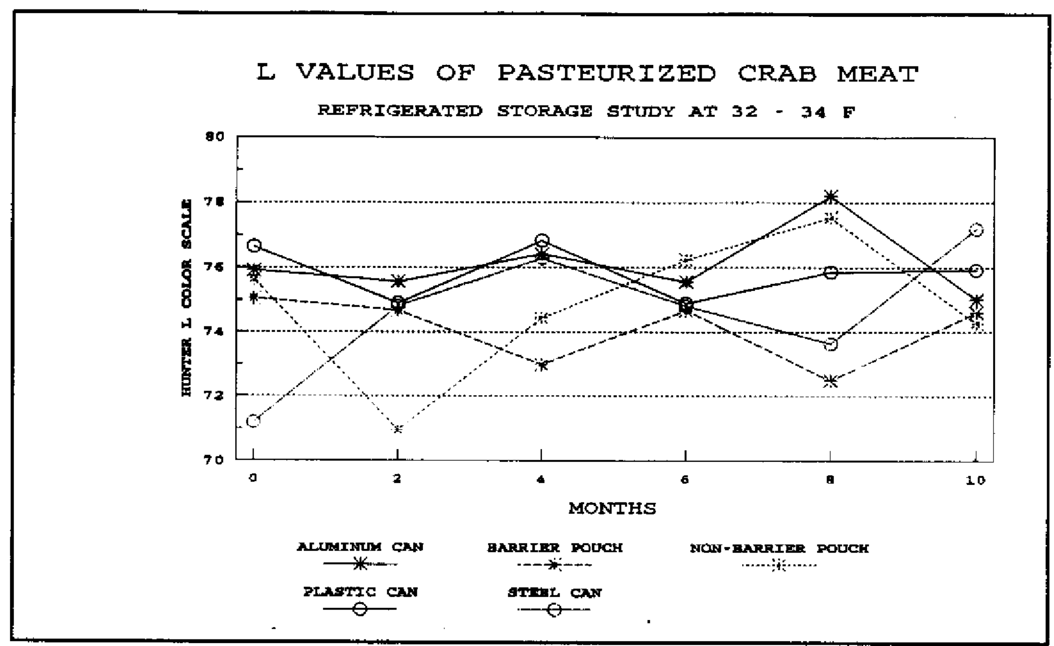

Figure 2. Hunter L-values of pasteurized crab meat following refrigerated storage in the five monitored containers.

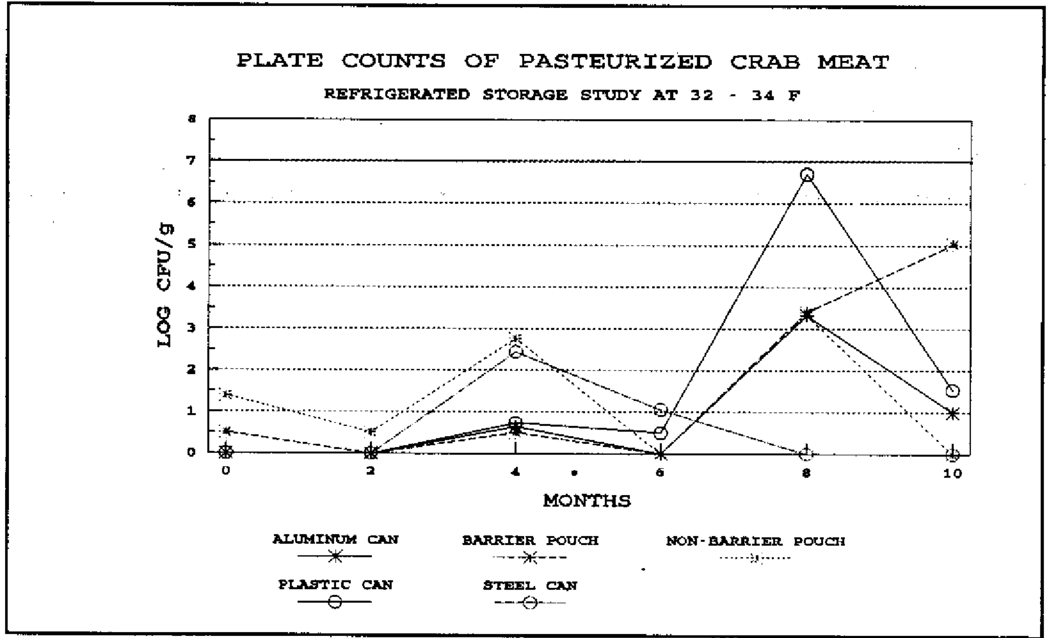

Figure 3. Log of plate counts from pasteurized crab meat following refrigerated storage in the five monitored containers.

barrier pouches received the lowest color acceptability ratings throughout the study. At 4 months meat from aluminum or plastic containers had better sensory color than meat from steel cans or barrier pouches ($p < 0.05$). Plastic and aluminum cans had the highest sensory color ratings at 6 months. The eighth month brought a change, with panel members indicating meat from steel and plastic containers had better color than product from aluminum cans and non-barrier pouches, in spite of definite microbiological spoilage

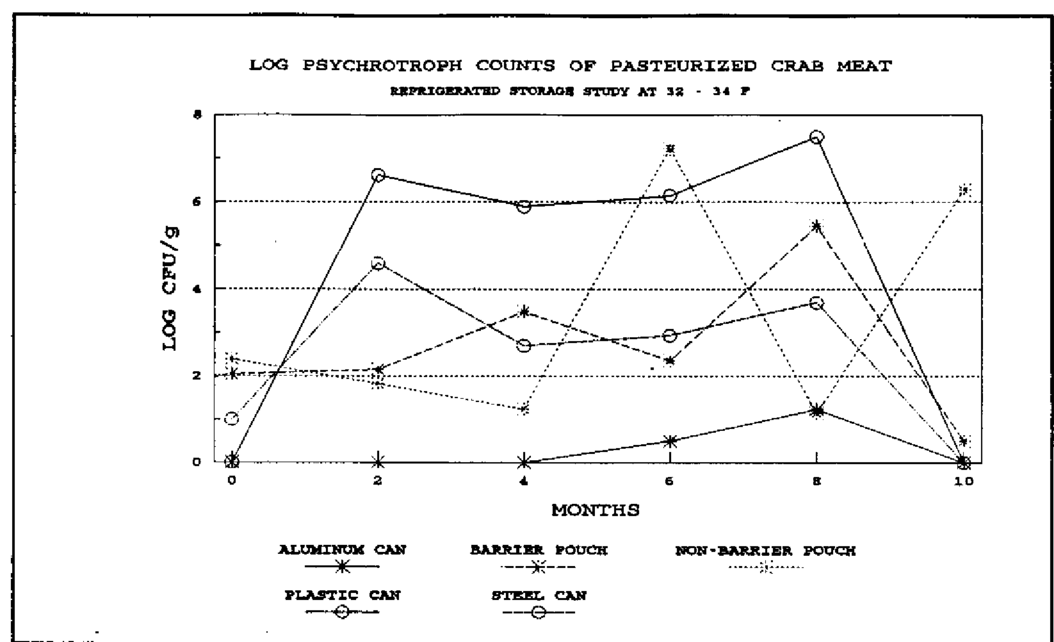

Figure 4. Log of psychrotrophic plate counts from pasteurized crab meat following refrigerated storage in the five monitored containers.

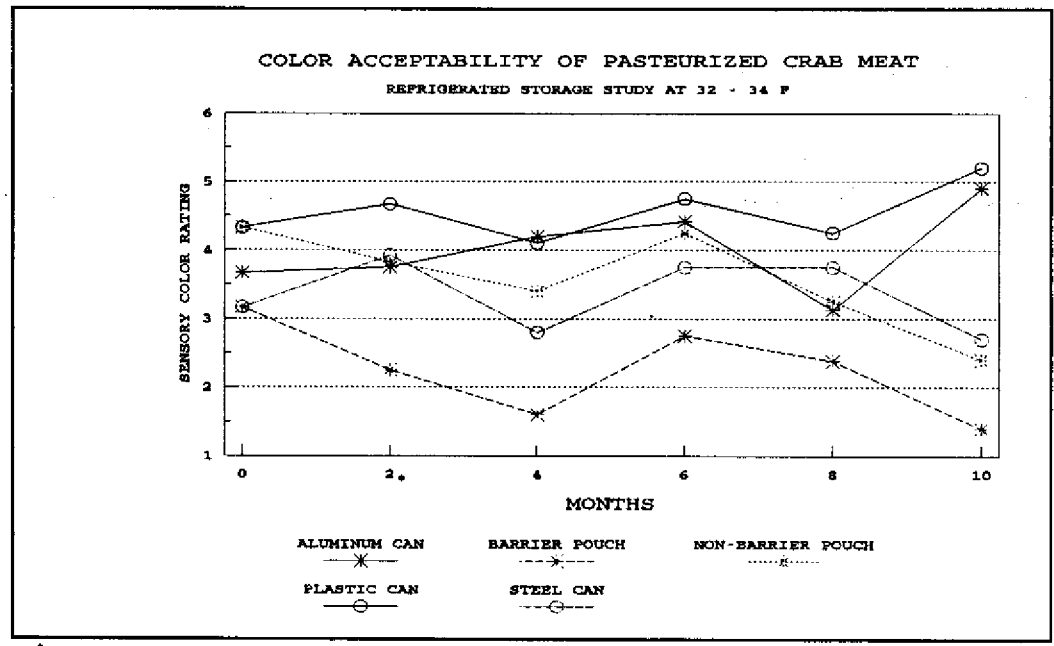

Figure 5. Sensory color acceptability of pasteurized crab meat following refrigerated storage in the five monitored containers.

determined for meat from the plastic containers. At 10 months meat from aluminum or plastic containers had better sensory color scores than the other samples ($p < 0.05$).

Plastic cans contained meat that rated the best sensory appearance at zero time ($p < 0.05$) (Figure 6). Meat from steel cans and non-barrier pouches had a better appearance rating than meat from aluminum cans or barrier pouches ($p < 0.05$). Meat

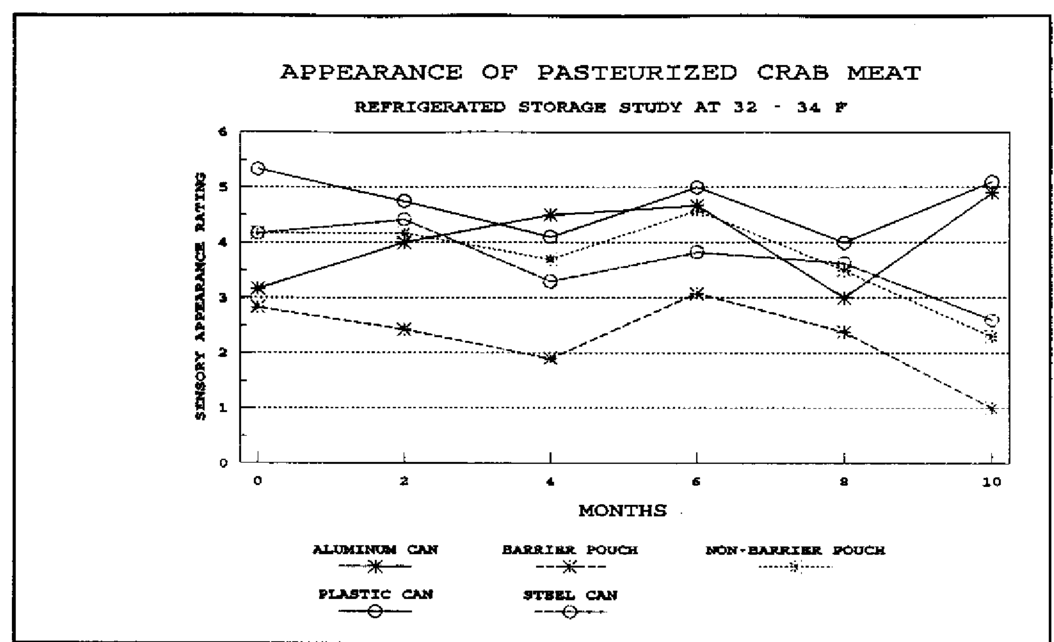

Figure 6. Sensory appearance acceptability of pasteurized crab meat following refrigerated storage in the five monitored containers.

from barrier pouches received the worst appearance ratings throughout the study ($p < 0.05$). Plastic cans were rated definitely better than steel cans or barrier pouches in the sixth month ($p < 0.05$). There were no significant differences in appearance by the eighth month. At 10 months meat from aluminum or plastic containers had better appearance scores than the other samples ($p < 0.05$).

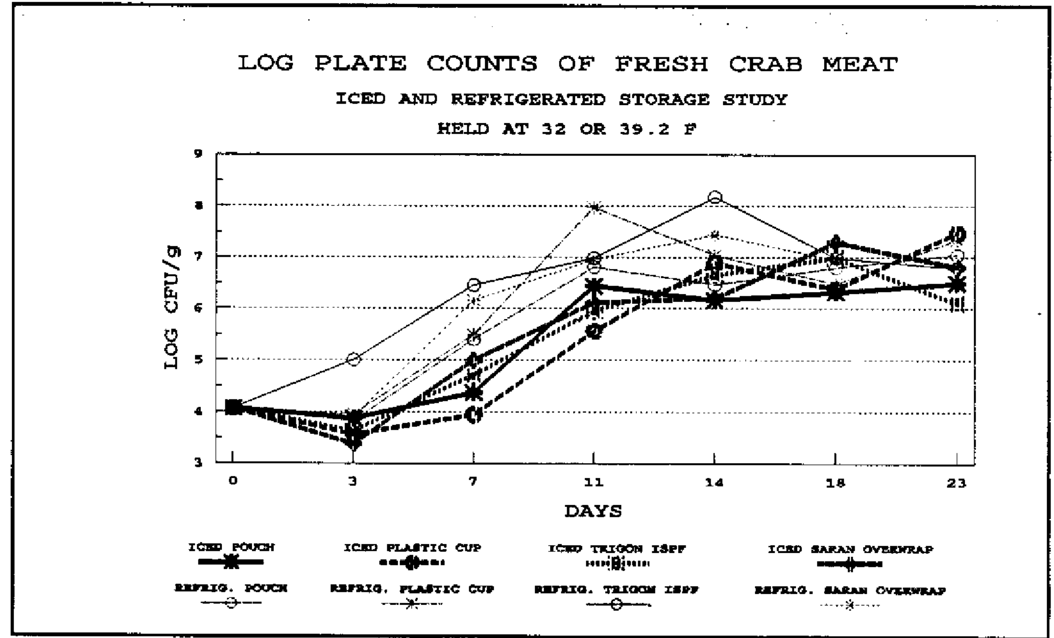

Figure 7. Log of plate counts from fresh crab meat stored in the four monitored containers at iced (32°F) or refrigerated (39.2°F) temperatures.

We determined microbiological quality of fresh picked meat by arbitrarily defining meat with plate counts under 100,000 CFU/g as good quality, meeting most state regulatory requirements for fresh picked crab meat (4).˙ Meat with plate counts between 100,000 and 1,000,000 CFU/g was considered poor quality, but typical of many products found at the retail level. Plate counts above 1,000,0000 CFU/g were considered to be microbiologically spoiled.

All iced samples maintained good microbiological quality through 7 days of storage (Figure 7). Saran[R] over-wrapped meat had higher plate counts than Trigon ISPF packaging, which had greater bacterial numbers than Cryovac pouches, which in turn had greater plate counts than those determined for plastic cups ($p < 0.05$). Meat in plastic cups and Trigon ISPF trays maintained market quality for 11 days of storage, with plate counts of meat packed in traditional plastic cups determined to be significantly less than all other treatments on days 7 and 11 ($p < 0.05$). All samples were microbiologically spoiled by day 14 (Figure 7).

All refrigerated samples retained good microbiological quality through 3 days of storage at 39.2°F. Trigon ISPF packages had greater plate counts than the other treatments at three days ($p < 0.05$). Refrigerated plastic cups and Cryovac pouches maintained minimum market quality for more than 7 days of refrigerated storage (Figure 7). Trigon ISPF trays had definitely higher plate counts than Saran[R] over-wrapped samples which had greater bacterial numbers than meat in plastic cups or Cryovac pouches at 7 days. All refrigerated samples were spoiled microbiologically by day 11 (Figure 7). Meat packaged in refrigerated Cryovac permeable pouches had lower psychrotrophic plate counts than all other treatments on days 3 through 14 ($p < 0.05$) (Figure 8).

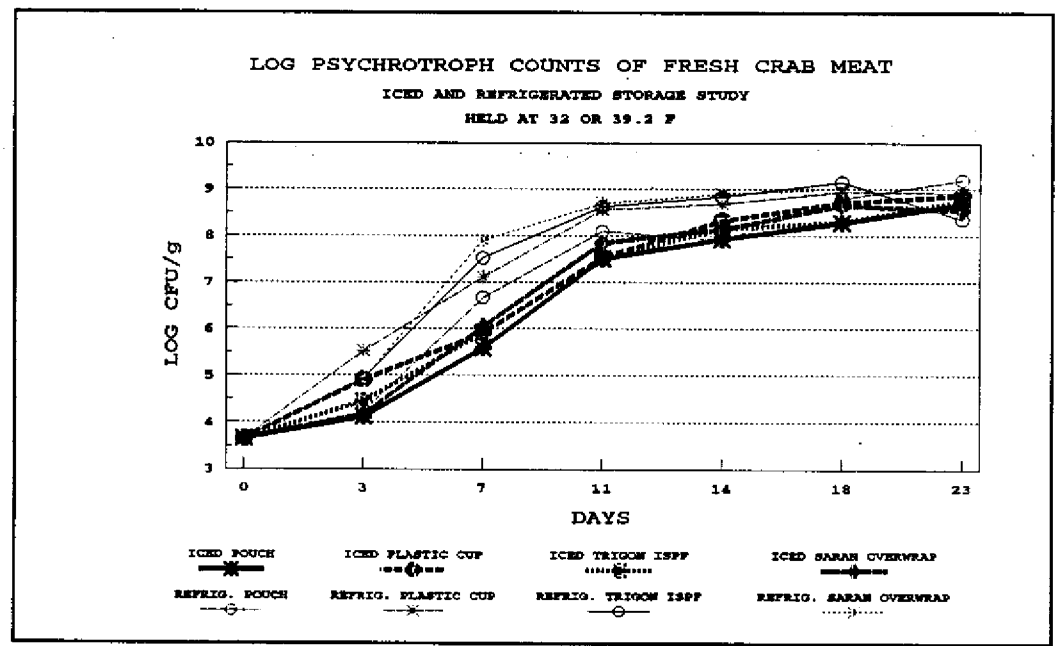

Figure 8. Log of psychrotrophic plate counts from fresh crab meat stored in the four monitored containers at iced (32°F) or refrigerated (39.2°F) temperatures.

Refrigerated crab meat held in trays overwrapped with Saran[R] had greater ($p < 0.05$) ammonia and pH levels than all other packaged meats on days 11 through 14 (Figures 9 and 10).

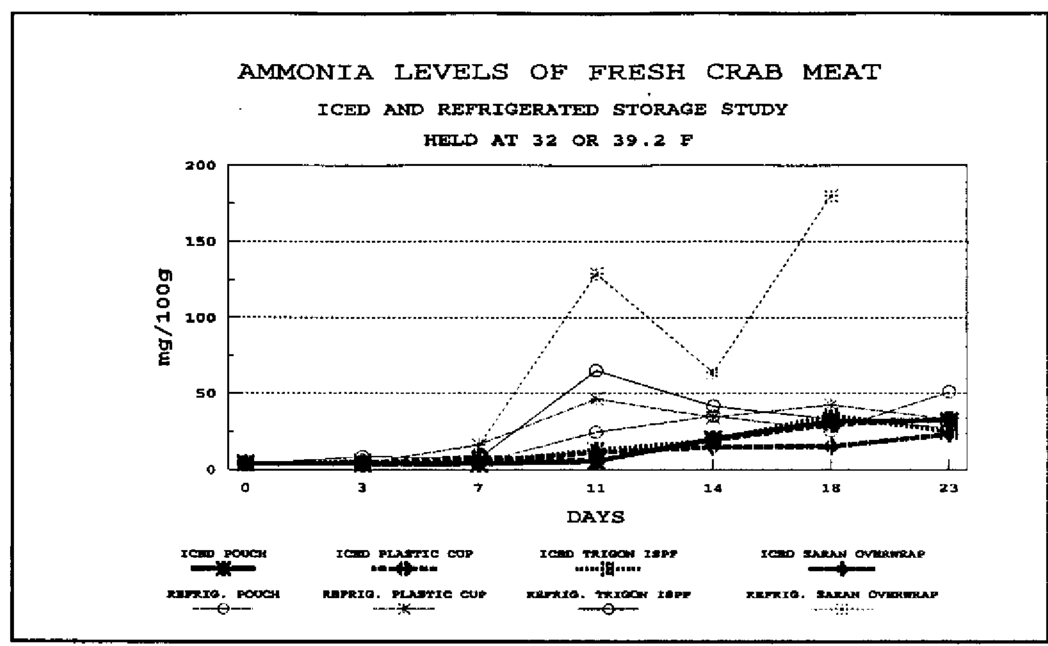

Figure 9. Ammonia concentrations determined from fresh crab meat stored in the four monitored containers at iced (32°F) or refrigerated (39.2°F) temperatures.

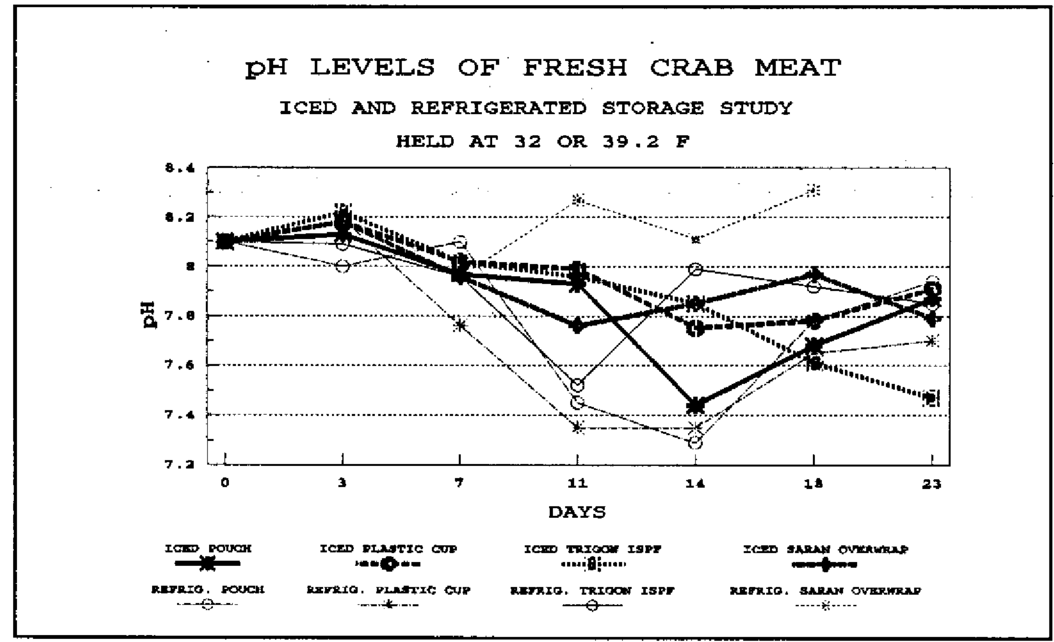

Figure 10. Levels of pH determined for fresh crab meat stored in the four monitored containers at iced (32°F) or refrigerated (39.2°F) temperatures.

Saran^R over-wrapped meat and meat stored in plastic cups were determined to have stronger sour odors than meat in Trigon ISPF trays on day 11 ($p < 0.05$) (Figure 11). Putrid odors were rated as greater in Saran^{R}over-wrapped trays than Cryovac pouches or Trigon ISPF trays at 11 days of iced storage (Figure 12). The panel found Trigon ISPF meat to have better color than meat held in plastic cups or Saran^{R}wrapped trays at 11 days of iced storage ($p < 0.05$) (Figure 13). Similar results were determined for appearance (Figure

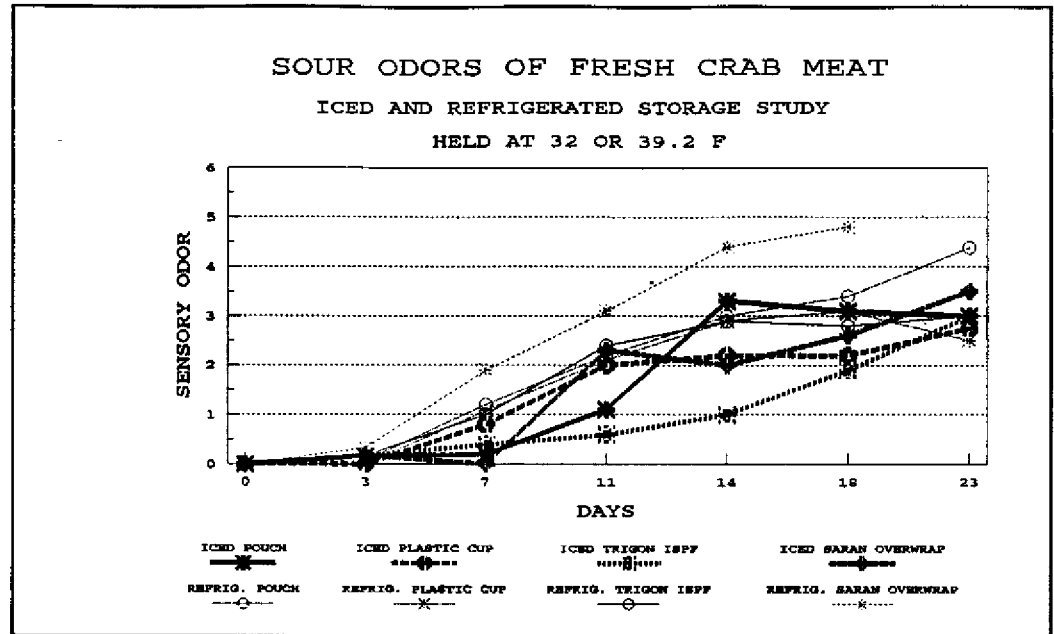

Figure 11. Sour odor of fresh crab meat stored in the four monitored containers at iced (32°F) and refrigerated (39.2°F) temperatures.

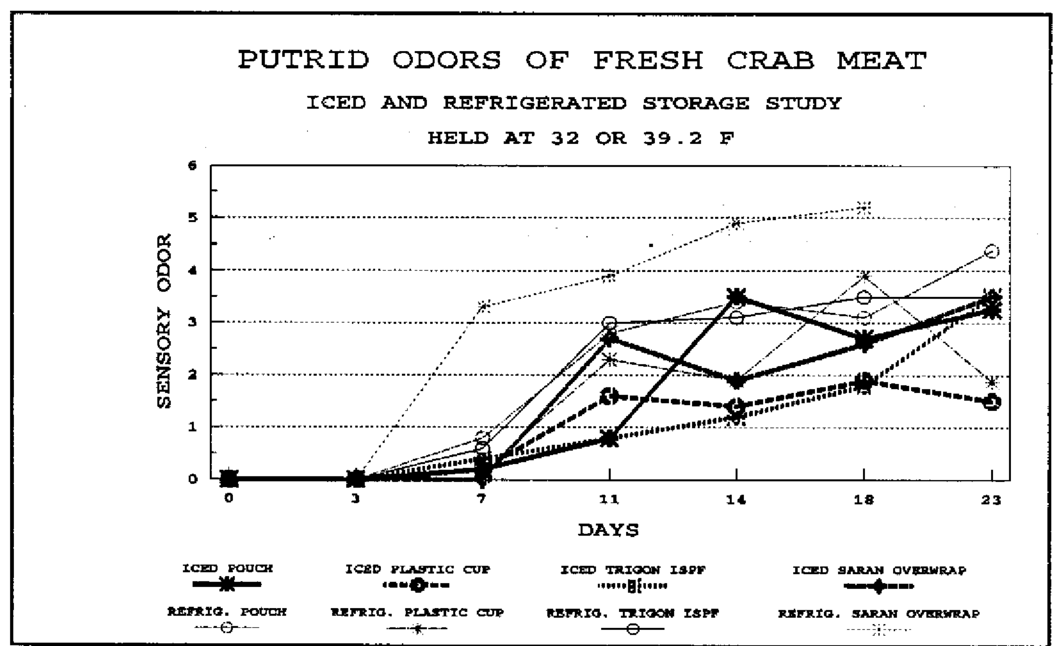

Figure 12. Putrid odor of fresh crab meat stored in the four monitored containers at iced (32°F) or refrigerated (39.2°F) temperatures.

iced storage ($p < 0.05$) (Figure 13). Similar results were determined for appearance (Figure 14). Refrigerated meat at 39.2°F did not show sensory differences similar to those determined for iced samples prior to microbiological spoilage.

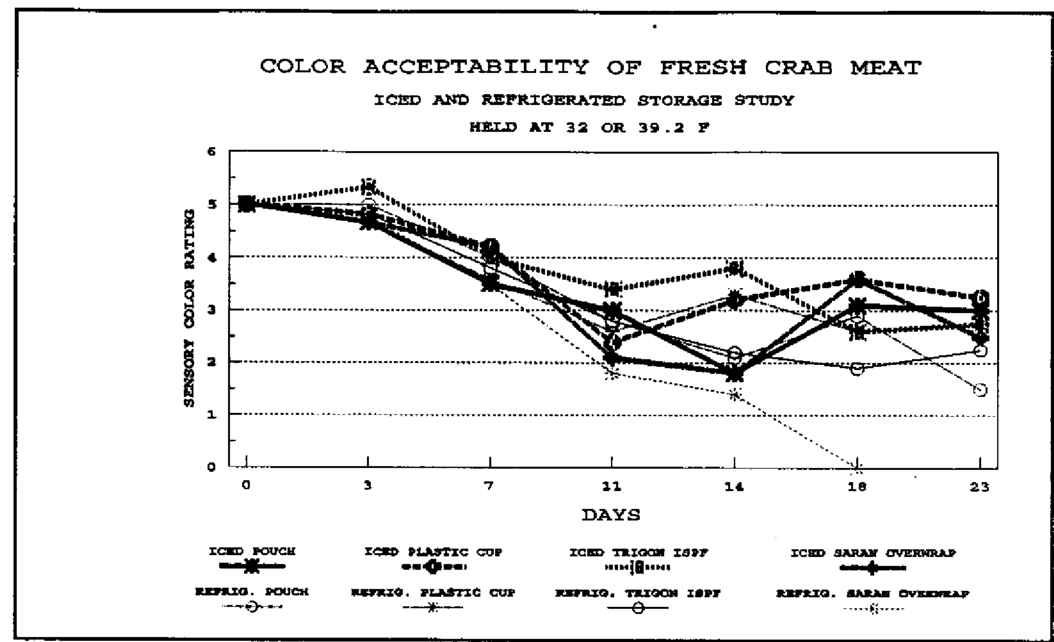

Figure 13. Sensory color acceptability of fresh crab meat stored in the four monitored containers at iced (32°F) or refrigerated (39.2°F) temperatures.

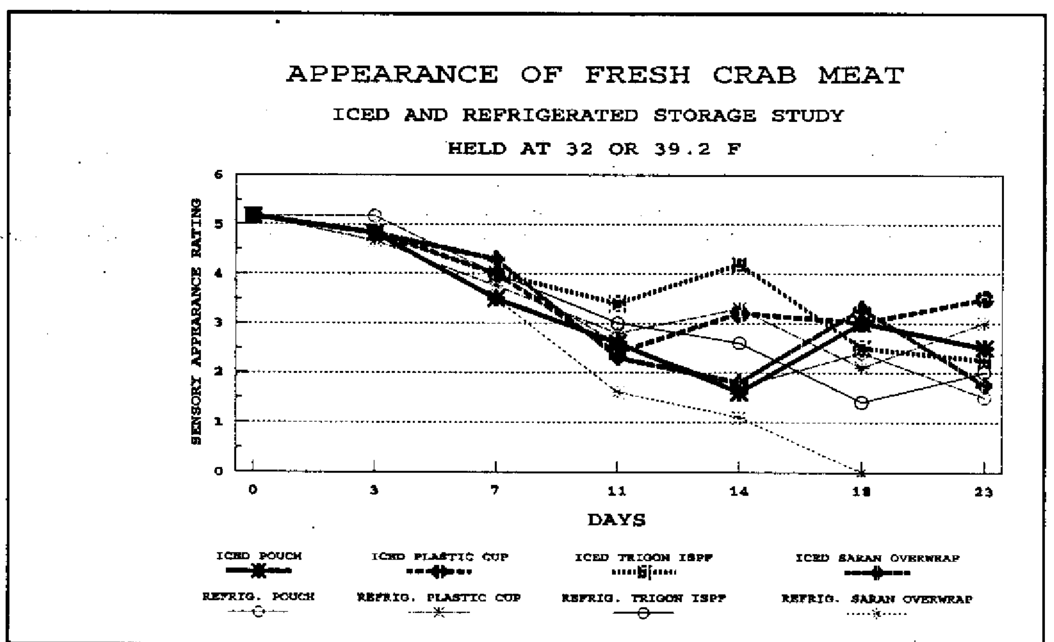

Figure 14. Sensory appearance acceptability of fresh crab meat stored in the four monitored containers at iced (32°F) or refrigerated (39.2°F) temperatures.

CONCLUSIONS

At zero time, following pasteurization, meats in plastic and aluminum cans and non-barrier pouches were lighter in color than product in steel cans as determined by significantly higher L-values. At 4 months meat in plastic containers had higher L-values than meat pasteurized in barrier pouches. At 10 months meat from aluminum or plastic containers had significantly better sensory color and appearance scores than pasteurized meat from steel cans or barrier and non-barrier pouches.

The longest microbiological shelf life for fresh special stored at 32°F was 11 days in both plastic cups and ISPF permeable packaging. Iced meat packaged in ISPF had better sensory characteristics than meat held in plastic cups at 7 and 11 days of storage. Refrigerated fresh special packaged in plastic cups or non-barrier pouches had the longest microbiological shelf life, 7 days at 39.2°F when compared to Trigon ISPF and Saran[R] overwrapped trays. Sensory quality of meat held at 39.2°F was not improved by any investigated packaging.

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APPLICATION AND EFFECTS OF LACTIC ACID AND CRYOPROTECTANTS ON THE STORAGE STABILITY OF FROZEN CRAB CORES AND CRAB MEAT

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INTRODUCTION

Fresh blue crab (Callinectes sapidus) meat is a highly perishable product with a limited shelf-life of up to 10 day. Since blue crabs are harvested seasonally with huge market gluts, industry has a continuing interest in expanding the marketing of crab meat beyond peak harvest. Such an extension of the processing and marketing of blue crab meat could lead to increased economic gains to all segments of the crab industry. Current methods of extending the storage of crabmeat involve pasteurization, sterilization, and freezing. All three methods, however, result in deteriorative changes in texture, appearance, flavor, and odor (1,3,10). Since fresh crabmeat currently represents the major method of marketing blue crabs while freezing results in the least deteriorative change in quality with storage, this study was designed to examine methods of extending the shelf-life of fresh and frozen blue crab meat and crab cores. The objectives were two-fold in that we wanted to determine the effects of lactic acid application on the storage stability of fresh crab cores and the effects of cryoprotectants on the storage stability of frozen crab meat.

METHODS

Sample Preparation: A local firm processed Atlantic Blue crabs (Callinectes sapidus) by steaming at 121° C (15 psi) for 12 min followed by cooling. Since crab cores could represent an intermediate product available for delayed picking during off-season or reduced harvest, cooked crabs were either fully picked to form processed crabmeat or debacked to create crab cores.

Part I: Application of lactic acid and cryoprotectants
Crab cores and picked crabmeat were vacuum infiltrated with a 3% aqueous solution of lactic acid for 10, 15, or 20 min or dipped for 10 or 20 min. Two cryoprotectants, sucrose and polydextrose were applied in aqueous solutions at concentrations of 10, 15, or 20% by vacuum infiltration and by dipping. The dipping procedures involved placing cores or picked crabmeat in solutions of lactic acid or cryoprotectants for 4 or 8 minutes, followed by draining for 2 min.

Vacuum Infiltration: Crab cores and picked meat were dipped into individual beakers containing solutions of lactic acid or cryoprotectants. The beakers were placed in the vacuum infiltrator (Nash Engineering Co., South Norwalk, CT) and the vacuum pressure allowed to reach 25 psi. The vacuum was immediately released and samples allowed to remain in the solutions for an additional 20 minutes prior to draining for 2 min. The effectiveness of the lactic acid and cryoprotectants in reaching the surface of the meat was measured by pH changes and sugar concentration, respectively. The pH was measured with a Fisher Scientific electrode (Fisher Accumet, Model 292) standardized against a pH 7.0 phosphate buffer. The concentration of cryoprotectants was measured by the phenol-sulfuric acid method for total carbohydrates (4) by measuring the absorbance at 490 nm against a standard curve using polydextrose or sucrose.

Part II: Effects of cryoprotectants on the storage stability of frozen crabmeat.
The second study was designed to compare the effectiveness of cryoprotectants, processing techniques, and storage methods on the chemical, physical, and sensory properties of crabmeat stored for up to eight months. Following the commercial processing of whole blue crabs, 1 lb aliquots of picked crabmeat were divided into the following treatments: (1) pasteurized; (2) untreated reference; (3) water; (4) polydextrose, 15% (wt/wt); (5) sucrose (7.5% wt/wt) + sorbitol (7.5% wt/wt) + tripolyphosphate, 0.5% (wt/wt). Treatments 3-5 were applied by dipping the crabmeat into their respective pre-chilled solutions (4° C) for 4 min and draining 10 min within a

refrigerated cooler. Following application of the treatments, samples were vacuum packaged as 1 lb units in low oxygen permeable Type B™ bags (W.R. Grace and Co., Duncan, SC), cryogenically frozen with liquid nitrogen (-23° C), and stored at -29° C until analyzed. The untreated reference sample was packaged similarly and stored at -65° C and used as a point of reference for chemical, physical, and sensory analyses. Samples designated as the pasteurized treatment were processed in 1 lb cans to an internal temperature of 85° C, cooled, and stored under refrigeration at 1.1° C until evaluated. Frozen samples were thawed under refrigeration (4° C) prior to evaluation at 0, 6, 12, 18, 24, 28, and 32 weeks of storage.

Chemical and Physical Measurements: Chemical indices of loss of quality consisted of measuring the 2-thiobarbituric acid number (8) for malonaldehyde formation and change in fatty acid composition as described by Sampugna, et al (9). Physical measurements consisted of texture, expressible moisture, and and color. An Instron Universal Testing Machine (Model CS-1; Food Technology Corp, Rockville, MD) was used to measure shear force. Drip loss and expressible moisture were determined as described by Jaregui et al. (2) while color changes were measured with a Spectrogard Colorsystem (Model, 96, Pacific Scientific, Silver Spring, MD) using the Hunter L a b scale.

Sensory Analyses: Sensory evaluations were conducted by a trained panel. The trained panel consisted of 12 members using quantitative descriptive analysis (6) to characterize changes appearance, aroma, flavor, and texture occurring during storage of crabmeat samples.

Statistical Analyses: All data were analyzed using a general linear model procedure (10) using a randomized complete block design with the entire study replicated two times.

RESULTS

The absorption of a 3% solution of lactic acid into crab cores and picked meat, via dipping or vacuum infiltration, is shown in figure 1. Significant differences ($P<0.05$) in pH were observed between crabmeat samples which were dipped and vacuum infiltrated. A comparison of the pH of crab cores and of picked

Fig. 1:Change in pH following vacuum infiltration (VI) and dipping of crab cores and crabmeat in lactic acid

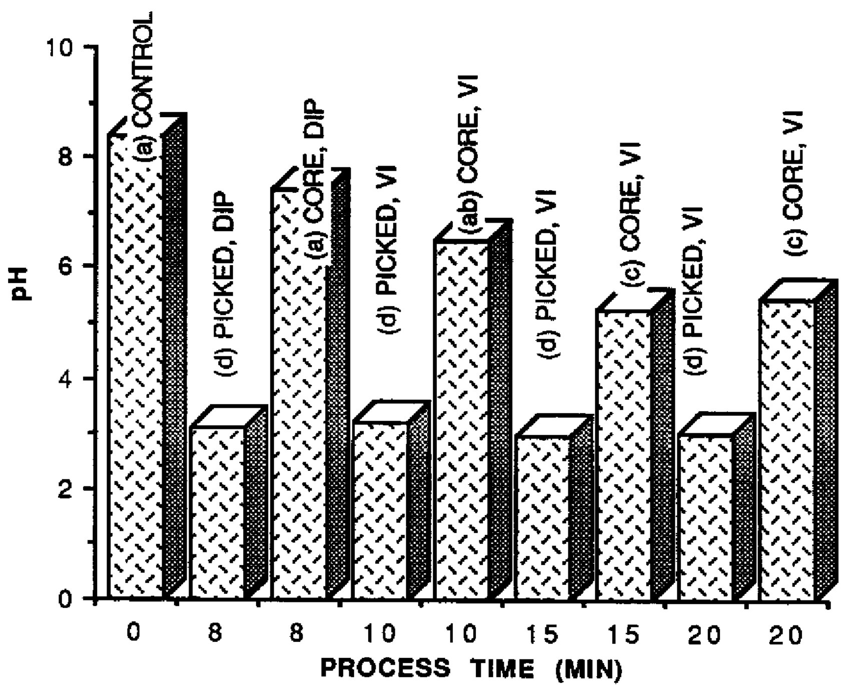
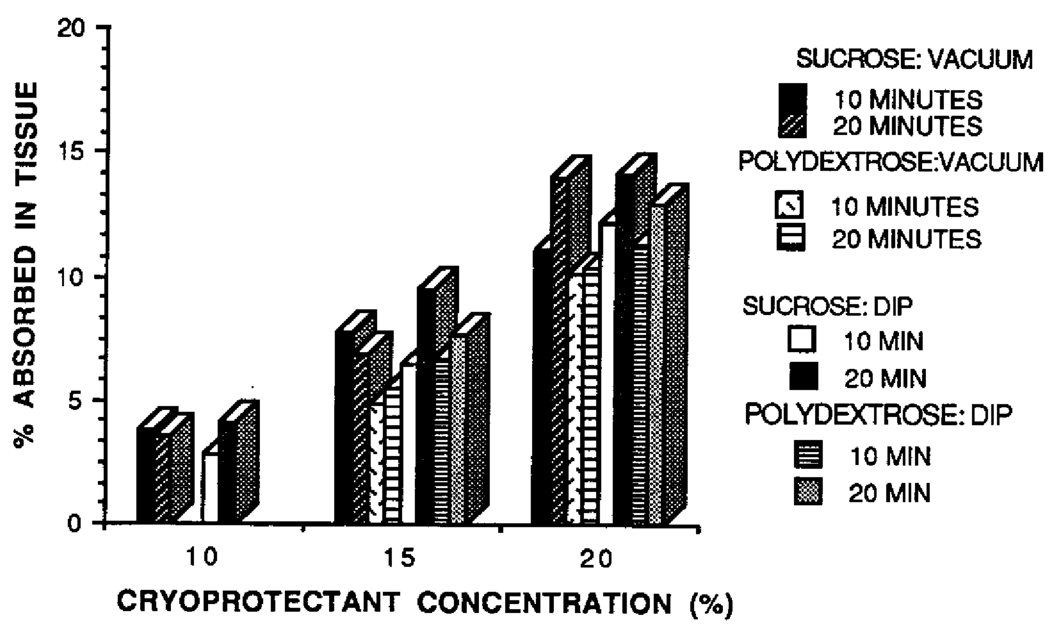


Fig. 2:Absorption of cryoprotectants following vacuum infiltration and dipping



meat dipped or vacuum infiltrated for 8 minutes found that the dipping of cores did not allow for effective penetration of lactic acid into the crabmeat. The pH of picked meat was approximately 2.4 times lower than cores, whereas no significant differences were observed in pH values between picked meat which had been dipped or vacuum infiltrated for 8, 10, 15, or 20 minutes. The absorption of polydextrose and sucrose followed a similar pattern to that observed for lactic acid (Fig 2). Significant differences in the concentration of cryoprotectants were observed between samples which had been treated with different concentrations of cryoprotectants. However, there were no differences in cryoprotectant concentration as a function of the processing times of 10 and 20 min of either picked meat or vacuum infiltrated cores.

The relative effectiveness of the two methods in applying the lactic acid or the cryoprotectants supports the findings of other investigators (3,7) in demonstrating that without vacuum injection or direct injection techniques, many applied additives may only have a surface-coating effect with very little of the chemical penetrating the interior tissues. Thus, to determine the effects of these additives in enhancing the storage stability of fresh crab cores or frozen crab meat, one has to be certain that the chemical is reaching the target tissue at a given level to be effective.

The effects of the treatments on the physical and chemical properties of crabmeat are shown in Table 1 with results representing an average over all sampling periods. Both drip loss and expressible moisture values were significantly higher for water treatments than the other treatments at all sampling periods. The mean drip loss of the sucrose/sorbitol/phosphate and polydextrose treated samples did not differ significantly from the untreated reference stored at a much lower temperature (-65° C). When drip loss and expressible moisture patterns were examined over the 32 week storage period, all samples showed increases in drip loss and expressible moisture values at week 12. The untreated reference, however, showed the greatest increase in expressible moisture at week 24.

Mean shear force values indicated that the water treatment had the highest mean shear force value, whereas no significant differences were observed between samples treated with

Table 1. The effects of the treatments on the mean physical and chemical characteristics of crabmeat[1].

Analyses	Untreated Reference	Pasteurized	Water	Polydextrose	†Sucrose
Physical Tests					
Drip Loss (%)**	26.49^{b}	23.50^{c}	29.05^{a}	26.72^{b}	25.28^{bc}
Exp. Moisture (%) **	48.41^{b}	45.39^{c}	52.60^{a}	51.11^{ab}	49.93^{ab}
Shear Force (Kg/g)**	2.80^{bc}	2.54^{c}	3.32^{a}	3.03^{b}	2.91^{b}
Total Energy(cm)	0.97	0.99	0.88	0.94	0.93
Total Energy/Shear Force*	0.41^{a}	0.40^{ab}	0.27^{c}	0.32^{bc}	0.34^{abc}
Hunter "L"**	72.44^{a}	71.37^{b}	72.86^{a}	72.58^{a}	73.20^{a}
Hunter "a"**	-0.03^{a}	-0.04^{a}	-0.33^{ab}	-0.57^{b}	-0.62^{b}
Hunter "b"**	12.24^{a}	11.48^{b}	11.67^{b}	11.90^{ab}	11.48^{b}
Chemical Tests					
Moisture (%)**	76.45^{b}	75.77^{bc}	78.42^{a}	76.26^{b}	74.86^{c}
Protein (%)*	19.72^{a}	19.03^{ab}	17.19^{bc}	15.99^{c}	17.67^{bc}
Fat (%)	1.55	1.50	1.34	1.25	1.28
TBA (mg/kg) **	0.30^{a}	0.28^{ab}	0.21^{c}	0.06^{d}	0.25^{bc}

[1] Means followed by the different letter in any given row were significantly different at .05 level (*) or Highly significant at .01 level (**). †Sucrose/sorbitol/phosphate

Table 2. Trained panel summary: The effects of the treatments on the mean sensory characteristics of crabmeat[1].

Sensory Attribute	Untreated Reference	Pasteurized	Water	Polydextrose	†Sucrose
Appearance					
Sheen**	5.00^{c}	5.85^{b}	5.58^{bc}	6.64^{a}	7.00^{a}
Grey/Blue**	3.51^{b}	5.62^{a}	3.49^{b}	3.08^{bc}	2.78^{bc}
Yellow/Green*	5.14	4.62^{ab}	4.61^{ab}	4.72^{a}	4.08^{b}
Aroma					
Fresh Crab**	4.64^{bc}	3.56^{d}	4.49^{c}	5.15^{ab}	5.46^{a}
Ammonia *	3.60^{abc}	4.08^{a}	3.83^{ab}	3.34^{bc}	3.11^{c}
Sour**	2.95^{b}	4.62^{a}	2.89^{b}	2.69^{b}	2.57^{b}
Flavor					
Sweet **	4.99^{c}	4.16^{d}	4.15^{d}	5.50^{b}	9.44^{a}
Fresh Crab**	4.61bc	3.08^{c}	4.08^{b}	5.16^{a}	5.08^{a}
Sour**	3.71b	5.54^{a}	3.26^{bc}	2.70^{cd}	2.18^{d}
Rancid**	3.12b	4.98^{a}	2.93^{bc}	2.34^{cd}	2.16^{d}
Texture					
Hardness	5.52	5.99	6.39	6.07	5.76
Toughness*	4.86^{a}	5.29^{b}	6.03^{a}	5.09^{b}	4.84^{b}
Moisture Persistence**	6.18^{a}	5.40^{b}	6.42^{ac}	7.17^{d}	7.14^{cd}
Total Intensity of Attributes**	5.11^{b}	3.27^{c}	4.73^{b}	6.40^{a}	6.57^{a}

[1] Means followed by different letters in any given row were significantly different at .05 level (*) or highly significant at .01 level; †Sucrose/sorbitol/phosphate.

cryoprotectants and the untreated reference. Examination of the total energy to shear force also revealed that the water treatment was more brittle (less deformable) than all the other treatments. By contrast, the pasteurized treatment was noted to be more rubbery and more deformable than all other treatments. The additional heat treatment resulting in additional protein denaturation may have been responsible for texture profile observed in the pasteurized sample.

The treatments appeared to have a significant impact on Hunter L, a and b values for white, red, and yellow colors. The pasteurized treatment had the darkest color (L values) whereas the polydextrose and sucrose/sorbitol/phosphate retained less red notes than the untreated reference, pasteurized, or water treatment. The pasteurized sample also had more blue color notes (b values) than all other treatments. All treatments became lighter at week 24 confirming moisture and drip loss data that moisture was being loss by all samples.

Examination of chemical data revealed that the treatments had a significant effect on TBA numbers. The polydextrose treatment had the lowest TBA values, whereas the untreated reference had the highest value with no significant differences observed between pasteurized, water, and sucrose/sorbitol/phosphate treatments. The treatments had no effect on the fatty acid composition in that the relative ratio of polyunsaturated fatty acids to saturated fatty acids did not differ over the course of the study.

Sensory Panel Evaluation: Table 2 shows the summary of the effects of the treatment on mean sensory attributes of crabmeat. Samples treated with polydextrose and sucrose/sorbitol/phosphate were rated highest in sheen, whereas the untreated reference sample was lowest. The pasteurized treatment showed highest values for grey/blue discolorations. Examination of the aroma profiles of treatments revealed that the polydextrose and sucrose-/sorbitol/phosphate samples retained more fresh crab aroma and less ammonia, whereas the untreated and water treatments contained more ammonia and less fresh crab aroma. The pasteurized treatment was observed to have more sour and ammonia aromas than all other treatments.

Treatments containing cryoprotectants were observed to have significantly higher scores for sweetness and fresh crab flavor than the untreated reference sample, pasteurized, or water treatment. The pasteurized treatment was observed to have the highest scores for sour and rancid notes followed by the untreated reference and water. Treatments containing the polydextrose and sucrose were equally and most effective in suppressing sour and rancid notes.
Examination of texture profiles of the treatments revealed greatest moisture persistence in samples treated with the cryoprotectants with the pasteurized sample showing least. Though no significant differences in hardness were found between treatments, the water treatment had the toughest texture with no differences observed between all other treatments.
Examination of the total intensity of attributes (TIA) most closely associated with fresh crab flavor and aroma indicated that treatments containing the polydextrose and sucrose/sorbitol-/phosphate were rated highest and equally effective in maintaining sensory attributes closest to fresh crabmeat. The pasteurized treatment was rated lowest followed by the water and untreated reference control.

Comparison of the data from trained panel evaluation of sensory attributes to chemical and physical measurements revealed several areas of agreement. Panel assessment of sheen and grey/blue discoloration agreed with Hunter L and b values showing that cryoprotectants had a positive effect on color. Expressible moisture, moisture persistence, and shear force values all indicate that as samples begin to lose moisture, the water treatment had significantly higher water losses and that this may have contributed to higher shear force values and toughness scores found by Instron measurements and trained panel scores, respectively.

CONCLUSIONS

The results from these studies indicate that the addition of the cryoprotectant, polydextrose and sucrose/sorbitol/phosphate significantly improved the sensory attributes of fresh crabmeat over an eight month period of frozen storage. Comparison of sensory data to chemical and physical measurements indicate improvements in moisture retention, texture, and possibly

decreased oxidation may be attributed to the addition of the cryoprotectants. Preliminary assessment of techniques to imbibe tissues and crab cores with the addition of lactic acid or cryoprotectants indicate that vacuum infiltration can be used to assure effective penetration of crab cores whereas either dipping or vacuum infiltration can be used to assure penetration of solutions into picked fresh crabment.

ACKNOWLEDGMENTS

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USE OF COMPOSITIONAL RATIOS TO DETERMINE PHOSPHATE-TREATED SHRIMP

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INTRODUCTION

Phosphates are commonly used during shrimp processing to reduce freezer/thaw drip losses, freezer burns and moisture loss during cooking. The phosphates are also thought to impart textural quality and reduce oxidative rancidity and other off-flavor development by sequestrating multivalent cations (Ellinger, 1972). The common food grade phosphates are generally recognized as safe (GRAS) substances (FDA-21CFR 182.184), and previous regulations have been proposed to set residual phosphate limits in seafoods destine for frozen storage (FDA-Federal Register 44(244)74845,Dec 18,1979).

Routine monitoring of phosphate residuals as a measure of previous phosphate treatments is complicated by variation in the indigenous phosphorus content in shrimp muscle. Previous nutritional summaries by Sidwell (1981) and Sullivan and Otwell (1992) report phosphate content in penaeid shrimp can vary from 39 to 397 mg/100g. Some customary phosphate treatments can result in phosphate residuals within this reported range. Likewise, Sturno and Marshall, 1987, used sensitive ion chromatographic analysis to demonstrate the rapid breakdown of added tripolyphosphate residuals to diphosphates and orthophosphates forms within 4 days at refrigeration temperature while samples in frozen storage had approximately 35% of the initial tripolyphosphates after 8 weeks. Confirmation of phosphate treated shrimp or the amount of added phosphate would require knowledge of the product handling and comparisons with untreated samples from the same original lot. These requirements are impractical for regulatory compliance or monitoring.

Since the primary regulatory concern is product adulteration with excessive water which could be considered economic fraud, this work introduces an alternative to monitor the use of phosphate treatments for shrimp. The method measures the primary adulterant of concern, water.

METHOD

Frozen and untreated shrimp of different penaeid species were sent to our lab from different locations around the world. All shrimp samples had no previous phosphate treatment or other exposure to chemicals. The shrimp arrived frozen in customary five pound boxed units. They were thawed in refrigeration without water contact, deheaded, peeled and deveined. Samples were treated with phosphate solutions to increase moisture content. The test treatments were 0% (control), 2% and 4% sodium tripolyphosphate. The tumble apparatus was as described by Barton and Otwell, 1989. From each lot per species, samples (100 g) were combined with 200 g of the phosphate solution and tumbled for 20 minutes. Following the application, the treated samples were drained for 2 minutes and weighed. The treated samples were frozen (-20 ^{o}F) for at least a week and then thawed to measure weight change and composition post-thaw and after cooking. Prior work had determined a routine immersion in boiling water assured an internal temperature above 160 ^{o}F. Cooking consisted of adding the shrimp to boiling water (1:20 W/W, shrimp:water ratio), returning to boil and boiling the shrimp for 1 minute. The cooked samples were drained and prepared for analysis. The controls were not treated with phosphates but followed the same procedure as the treated samples. These procedures provided raw and cooked samples to which protein, moisture, phosphorus and sodium percentages were determined following standard AOAC methodology (AOAC, 1990).

RESULTS AND DISCUSSION

Compositional ratios were determined for the various analyzed components per species. The percent moisture to percent protein (M/P) ratio increased as phosphate treatment increased (Table 1). For the control, non-phosphated samples, the M/P ratios ranged between 4.00-4.70. The 2% STP treated samples ranged from 5.21-6.24 while the samples treated with 4% STP ranged from 5.80-6.44. The M/P ratios for the cooked shrimp were also determined (Table 2), with a similar pattern of results. For the control, the M/P ratios ranged from 2.58-3.63. STP (2%) treated samples ranged from 3.71-5.07 and the 4% STP treated samples ranged from 4.39-5.93. Raw and cooked M/P ratios did not overlap between controls and treated samples (2% and 4% STP). However, the M/P ratios for the 2% STP and the 4% STP did overlap for the raw and cooked samples. M/P ratios appear to indicate whether or not the sample was treated with phosphate. However, this ratio alone did not predict the amount of phosphate used to treat the samples.

The most revealing pattern of results was for the ratio of % moisture to % phosphorus (M/Ph) (Table 3). These ratios decreased as phosphate treatments increased. The M/Ph ratio for the control ranged between 340-548. For the 2% STP treated samples, the M/Ph ratios ranged from 258-308 while 4% STP treated samples were between 164-200. The M/Ph ratios were also calculated for the cooked samples (Table 4). The controls ranged between 257-390 while the 2% and 4% STP treated samples ranged between 228-310 and 149-214, respectively. There was no overlap among the raw M/Ph ratios, yet some overlap resulted in the cooked M/Ph ratios. M/Ph ratios were more meaningful in identifying the degree of phosphate treatment in shrimp, specifically in raw shrimp. This has relevant importance to commercial shrimp processing since most shrimp is sold as raw product. Simple reliance on phosphorus content alone would not identify phosphate treated samples because of the minor differences among phosphorus residual levels between samples (control, 2% and 4% STP treated samples).

CONCLUSIONS

M/P ratio would indicate water addition to shrimp samples. However, it can not establish the actual treatment level. M/Ph ratio, at least in the raw product, appears to show a direct relationship with phosphate treatment. This is of more importance to the raw shrimp, since the majority of the shrimp sold in the USA is raw product.

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TABLE # 1

COMPOSITIONAL RATIO:

% moisture / % protein
(Raw Shrimp)

Specie	Control	2% STP	4% STP
Texas Browns (P. aztecus)	4.7	5.88	6.36
India Whites (P. indicus)	4.22	5.73	5.92
Colombia Whites (P. occidentalis)	4.35	6.24	6.24
Ecuador Whites (P. vannamei)	4.25	5.48	5.81
India Tigers (P. monodon)	4.64	5.50	5.90
Key West Pinks (P. duorarum)	4.00	5.21	5.57
East Coast Whites (P. setiferus)	4.28	5.64	6.44

All treatment brines included 1% NaCl

TABLE # 2

COMPOSITIONAL RATIO:

% moisture / % protein
(Cooked Shrimp)

Specie	Control	2% STP	4% STP
Texas Browns (P. aztecus)	3.55	5.07	5.21
India Whites (P. indicus)	3.14	4.33	4.74
Colombia Whites (P. occidentalis)	2.88	4.29	4.92
Ecuador Whites (P. vannamei)	3.07	4.44	4.87
India Tigers (P. monodon)	2.58	3.71	4.84
Key West Pinks (P. duorarum)	3.63	5.00	5.93
East Coast Whites (P. setiferus)	3.47	4.07	4.39

All treatment brines included 1% NaCl

TABLE # 3

COMPOSITIONAL RATIO:

% moisture / % phosphorus
(Raw Shrimp)

Specie	Control	2% STP	4% STP
Texas Browns (P. aztecus)	435.5	285.6	189.1
India Whites (P. indicus)	343.4	266.1	172.2
Colombia Whites (P. occidentalis)	432.2	261.1	167.3
Ecuador Whites (P. vannamei)	383.2	271.1	170.2
India Tigers (P. monodon)	548.1	308.6	200.4
Key West Pinks (P. duorarum)	340.4	258.4	167.2
East Coast Whites (P. setiferus)	369.9	266.5	164.0

All treatment brines included 1% NaCl

TABLE # 4

COMPOSITIONAL RATIO:

% moisture / % phosphorus
(Cooked Shrimp)

Specie	Control	2% STP	4% STP
Texas Browns (P. aztecus)	390.8	307.5	206.3
India Whites (P. indicus)	372.1	310.0	214.0
Colombia Whites (P. occidentalis)	320.4	290.4	208.4
Ecuador Whites (P. vannamei)	291.5	228.2	149.5
India Tigers (P. monodon)	280.9	252.3	184.6
Key West Pinks (P. duorarum)	257.7	241.0	157.6
East Coast Whites (P. setiferus)	268.3	252.1	177.7

All treatment brines included 1% NaCl

PRODUCT DEVELOPMENT: COWNOSE RAY (*Rhinoptera bonasus*)

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Large schools of cownose ray (Rhinoptera bonasus) reside in the Chesapeake Bay from early May to early October during which time young are born and mating occurs (3). During this residency period the rays feed extensively on commercially valuable shellfish species and destroy eel grass beds which reduces the biological productivity of shoal areas (4). Commercial fishermen of the Chesapeake Bay consider the cownose ray a nuisance, and for many years have advocated either eradication or utilization.

There have been numerous attempts to develop a market for cownose ray in the past. These efforts concentrated on exporting whole frozen ray wings to Europe with the intent to penetrate the existing market for skate wings. The dark color associated with ray meat, however, hampered marketing attempts. From those efforts valuable ray fishery, marketing, and product quality information was generated. Otwell and Lanier (5) demonstrated that: cownose rays can be effectively harvested utilizing conventional gear; that ray meat is a high protein, low fat meat source; and ammonia nitrogen levels are relatively low in comparison to other elasmobranchs. Additional research (2) was used to evaluated cownose ray meat stability during frozen storage, and it was concluded that its frozen storage shelf life would be acceptable for commercial distribution.

This study concentrated on the development of an effective, quality minded method of efficiently processing cownose rays with minimal equipment and labor costs, while providing marketable ray meat product forms for both export and domestic marketing efforts. Ray steaks and fillet market forms were considered by the investigators to provide the best chance for domestic and export market acceptance. Added value products, as ground ray meat and chunk meat, were also considered to maximize total yield of usable meat.

MATERIALS AND METHODS

Processing

Cownose rays used in this study were commercially harvested by pound nets deployed at the mouth of the York River and landed in Perrin, VA. Rays were purchased directly from the boat upon return to the dock, transported on ice to a processing facility, and processed within 8 hours post-harvesting. Processing was conducted by a labor force provided by local industry which was inexperienced in ray processing. Four processing trials were made on separate days, using 30-50 rays per trial (June 1991).

Preliminary ray processing efforts using hand knives quickly demonstrated the need for alternative, more efficient means of meat recovery. A Hobart Model 5214 meat cutting band saw was chosen for initial cutting procedures, and a Steen model 171 table top skinning machine was used for removing the skin. Both pieces of equipment were borrowed for this study, however, second hand pieces were readily available and inexpensive. Fillets were cut by hand using conventional fillet knives. A 4% (w/v) brine rinsing/soaking solution chilled with ice was used at various steps during processing. Ray meat was introduced to the brine solution at three points during processing. Ray meat was placed in Cryovac vacuum heat-sealed barrier bags with 1-1.5 pounds of meat per bag and vacuum packed in a Smith Supervac Model 6K-183 vacuum packaging machine. Vacuum packed meat was then either delivered fresh on ice to test markets, or placed in shallow (5 x 10 x 29 inch) corrugated shipping boxes (25 pound capacity), commercially blast frozen, and held at -30℃ (-22℉)in cold storage.

Product flow is diagrammed in Figure 1 for all ray meat product forms investigated. Prior to initial cutting with the band saw, ray tails were removed at the base of stingers to protect saw operator from barbed spines. Each whole ray was handled individually on the band saw table. The natural mucous associated with the ray skin surface provided easy maneuvering of rays on the table, therefore the slidable saw table remained stationary. This further decreased risk of injury to the saw operator. Figure 2 illustrates the cuts performed on the whole ray. The first cut on each side of the ray removed the wing tips, which were non-usable for our marketing effort but could possibly be used as crab or eel bait. The second cut trimmed off the trailing edge of each wing. This cut provided an exposed skin edge for which the skinning machine could grab and efficiently skin the product. The next series of cuts were determined by the product form desired. For filet product, the wings were cut free-hand into 3-4 inch wide strips. For steak product, the attached saw table fence was used,resulting in uniform cuts of 3/4 or 1 inch wide strips. Fillet and steak cuts were made on each side until the straight cuts contacted the body gill chamber. Once this point was reached, and both wings of a given ray were cut, a large U-shaped cut was made into both sides of the remaining ray body to detach usable body flesh. The remaining bodies and trimmed wing waste were refrigerated then transported to a local processor for evaluation as a possible pet food ingredient.

Product cut by the band saw was placed into the first iced brine solution. Four, 15 gallon totes containing the brine solution were used to accommodate back-logged product coming from the band saw station. Skin-on steaks were kept separated from skinned product by placing them in their own brine containers. Product to be skinned was removed from brine solution, skinned, and then placed into the second brine solution station. Skin-on steaks were allowed to remain in initial brine solution for periods equating that of an average dwell time of skinned product, then were transferred to the second brine solution station. Skinned product was then removed from second brine solution station and filleted. Filleted product was placed in respective containers of brine solution according to product market form (thick, medium, or thin fillets, and body flesh) to facilitate further processing.

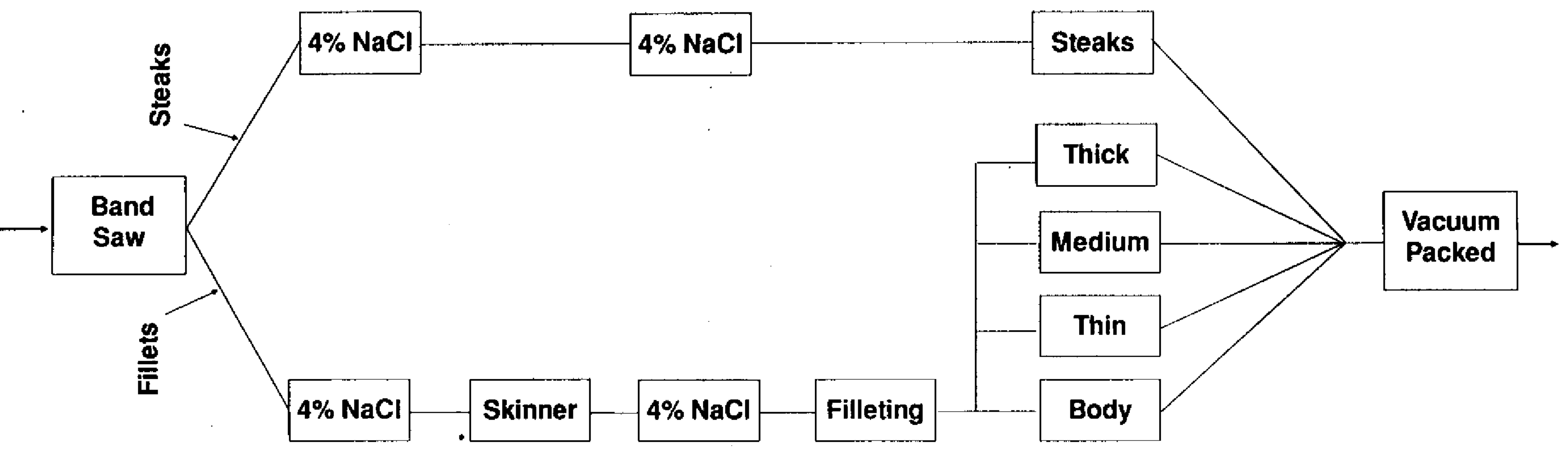

Figure 1. Cownose Ray processing flow chart. Ray meat is separated into product forms after filleting, and placed into the third of three chilled brine solutions prior to packaging.

Steaks were again transferred to the third brine solution station according to the skinned product flow. Brine solutions were discarded and remade according to excessive discoloration from bloody meat and/or elevated temperatures. From the third brine solution station, product was drained, bagged and vacuumed packed in a Smith Supervac Model 6K-183 vacuum packaging machine. Product to be frozen was boxed, weighed, blast frozen, and held at -30°C (-22°F) in commercial cold storage. Fresh product was weighed, iced, and distributed to various restaurants and a fresh seafood market.

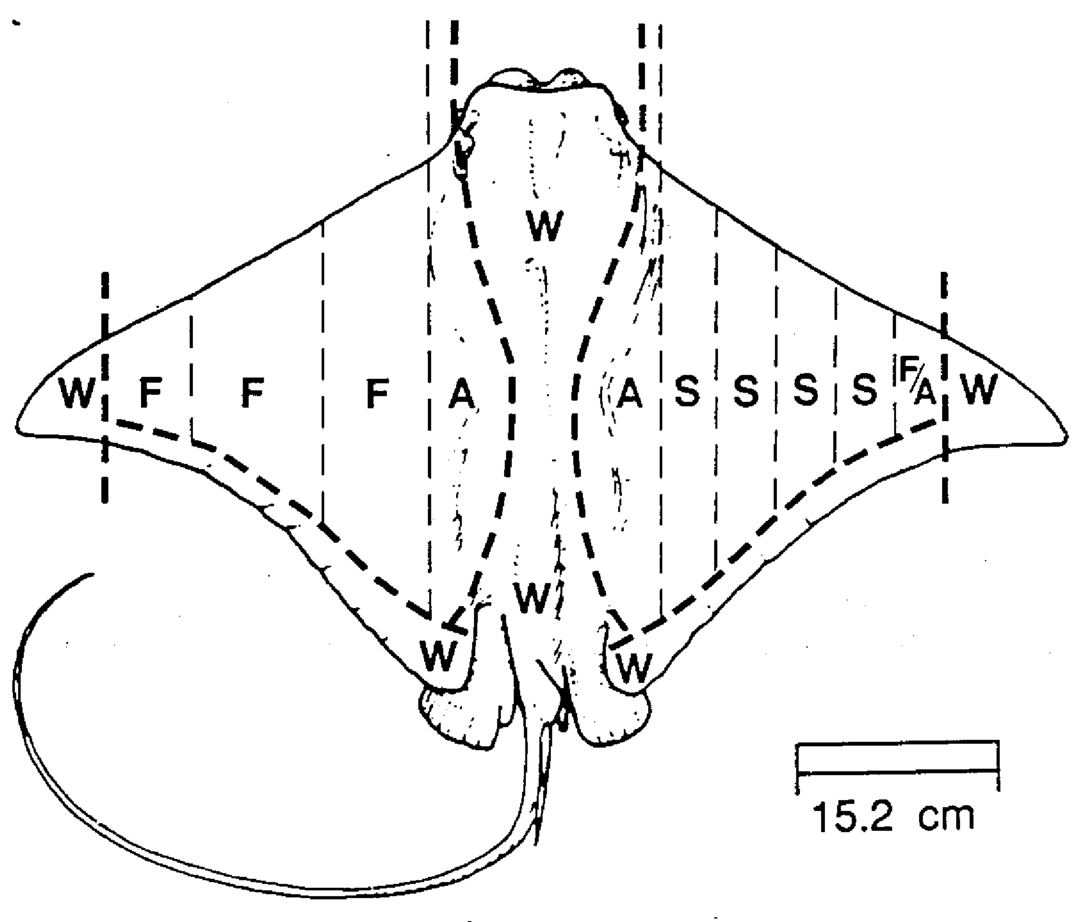

Figure 2. Bandsaw cuts made in cutting the cownose ray. Heavy dashed lines are cuts made that separate usable ray meat from waste product. F = Fillet cut; S = Steak cut; A = Added value cut; F/A = Fillet or added-value cut.

Economics

A three day production economics audit provided the basis for the cost analysis of cownose ray processing. For each day, a three man team measured (i) the amount of time per task, repetitively (via stopwatch); (ii) the amount or number of all inputs; and (iii) the number of employees involved in each task.

This data was then analyzed by work station and the input was used to derive a basic cost for each item, including labor. All equipment prices are 1991 used market equivalents. All inputs are at 1991 market prices. The representative costs were then applied to the parameters of the processing run on June 25, 1991.

Assumptions were made in order to facilitate general analysis. Assumptions include:

- All equipment is purchased used.
- The facility, equipment and crew is considered non-dedicated.
- The water discharge permit fee is a renewal.
- Labor is non-unionized and may switch among work stations.
- Labor costing is on a real-time (active time).

Marketing

The marketing analysis was conducted via three basic methods: (i) restaurant sampling followed with questionnaire; (ii) public/semi-public sensory analysis; and (iii) culinary institute evaluation. Other analysis, including baseline laboratory analysis and in-house evaluations contributed to the marketing conclusion.

Restaurants and retailers were provided with either fresh or vacuum packaged frozen products, per request and availability. Information from the culinary consultant was made available to the restaurants at the time of product transfer.

RESULTS AND DISCUSSION

Processing

Processing cownose rays with a band saw greatly facilitated processing. Cutting whole rays for fillets and added value product forms took an average time of 2.29 minutes per ray (n=8) and 1.05 minutes per ray (n=6) for steak cutting. This compares to preliminary hand butchering efforts which took an average of 12 minutes per ray to cut wings into strips for fillets and remove body meat. As processing continued, the inexperienced saw operator became more efficient indicating a learning curve for this operation. Therefore, cutting time at this station is likely to decrease with gained experience. The band saw also allowed easy trimming of ray wings and the removal of body meat. Previous investigators concentrated on ray wings, and discarded the ray bodies. Figure 3 shows dorsally and ventrally oriented body meat remaining after wings are removed. This meat was easily recovered by the large U-shaped cuts made on both sides of the ray body (Figure 4), therefore maximizing yield while reducing waste. Since ray wings are thickest at the point of body attachment and become thinner toward the wing tip; steaks become larger and fillets become thicker as cutting progresses toward the ray body. This results in fillets of varying thickness.

Figure 3. *Cownhose ray with wings detached exposing usable body meat not utilized in previous wing only marketing efforts.*

Figure 4. *Cownose ray carcass after removal of usable body meat.*

Exposing ray meat to a series of chilled brine solutions during processing produced favorable organoleptic results and facilitated skinning and filleting processes. The repeating brine solutions continually rinsed the product free of skin mucous and excess blood, enabling safer, more controlled handling during processing. The brine also kept the product chilled during processing which in turn resulted in a firming of the ray meat and a noticeable reduction in drip loss of the finished packaged product. This became evident in the finished product since the ray meat was noticeably lighter in color. Though urea analyses were not performed in this study; it is thought that the urea would be leached to reduced levels similar to those reported by Gordievsknya (1) in shark meat processing. Salt and/or water uptake by the ray meat during brine soaking was not analyzed in this study.

Skinning was easily performed by the Steen skinning machine on the larger, thicker wing sections to be filleted, and also on pieces of body meat. However, thinner sections of the wing could not be skinned effectively by this type of skinning machine. The skin and muscle was too strongly interconnected on many of the thin wing sections, preventing skin/muscle separation. When machine skinning of thin wing sections occurred, the meat integrity was severely affected, resulting in a stringy, ragged product. Therefore, the majority of the thin wing sections were transferred to the filleting station and skinned by hand. Skinning ray wing sections prior to filleting provided structural support to the meat during skinning. This reduced the amount of meat tearing, ragged edges, and stringy flesh. Processing runs of only fillet product forms resulted in lengthy delays at the skinning station. If ray fillets are the chosen product form, additional skinning machines could reduce product backup and maintain a smooth product flow.

Filleting skinned wing strips was conducted with little effort. It was performed by removing flesh from the cartilaginous wing support. Thin strips which could not be skinned by the machine slowed the filleting process. These strips were first filleted, then skinned by hand. Even after hand cutting, the resulting thin fillets were ragged in appearance and did not provide for an attractive fillet product. Preliminary cooking results further demonstrated a loss of meat integrity as fillet thickness decreased. Thin fillets became fragmented and stringy when cooked, while thicker fillets remained firm and intact. These results preclude packaging mixed product forms for marketing efforts. Separation of product into various market forms was most efficiently performed by the fillet cutters after filleting and just prior to packaging. Separating product forms at this point facilitated subsequent handling.

Vacuum packed, heat-sealed packaging provided for an attractive product. However, proper handling during the packaging process is vital for successful results. The ray meat must first be thoroughly drained of brine solution before placed in vacuum bags. This prevents the accumulation of undesirable excessive liquid in the finished, sealed bags. Care must also be taken while placing meat into vacuum bags so the outside of the bags are not soiled. Vacuum packaging in this study required two individuals to drain and place ray meat into bags, and one operator of the vacuum packaging machine. Due to the small size of our laboratory vacuum packaging machine, product flow at this point was limited. A commercial size machine, however, could operate effectively in this type of ray processing, therefore reducing production time. Skin-on steaks produced the most appealing packaged product form (Figure 5). The dark ray meat lends itself better to a steak cut than a fillet cut. The dark flesh, together with the dark ray skin, resembles other highly marketable fishery products as shark and swordfish. Since steaks are cut across muscle fibers, the stringiness associated with ray meat is not perceived, possibly increasing marketability. The coarse muscle bundles of ray meat become exemplified in moderately thin to thin skinned fillets, thus detracting from the appearance. Thicker fillets, however, maintain their integrity through processing and also produce an attractive product form.

The previous grouping of product forms permitted the packaging and subsequent boxing processes to be performed quicker, thus minimizing time product is held unrefrigerated. Extended periods at room temperature allows for the accumulation of drip in the sealed bags, and detracts from product appearance.

Figure 5. Vacuum packed cownose ray steaks. Steak cuts provided the most appealing product form.

Economics

Costs for each input into the process were estimated using the three audit days data. This data was used to estimate costs for the second day's (June 25) processing run for simplicity. On this day 937 pounds of whole ray were purchased and processed yielding 272
pounds of edible flesh for a recovery rate of 29%. (Table 1)

Table 1. Parameters For Cownose Ray Processing and Yield

Ray (Round Weight)	937 lbs.
Edible Flesh	272 lbs.
Waste	665 lbs.
Yield (Edible Flesh)	29.03%
Yield (Waste)	70.97%
Cost (Edible Flesh)	$1.26 per pound

Component costs estimated per unit (pounds of whole ray, poly bags used, and etc.) and then used to cost out the second days entire production. (Number refers to the amount used for production day two.) Cost/Unit refers to the cost of one unit of the component, as defined in Table 2.

Table 2. Process Component Cost

	NUMBER	COST/UNIT
Rays Purchased (lbs. whole wt.)	937.00	$ 0.15
Poly Bags used (each)	225.00	0.14
Direct Labor ($6.00/hr)	11.75	6.00
Ice Used (Tubs)	10.00	5.00
Corrugated Cartons (each)	7.00	2.00
Water ($1.49/100,000 cu.ft.)	0.66	1.49
Sanitizer (1 gallon)	1.00	0.90
Permits/Water Discharge (renewal)	1.00	300.00
Waste (transport/lb.)	665.00	0.04
Salt (40 lb. bags)	0.50	8.00

The cost of processing vacuum packaged cownose ray in fillet or steak form is estimated to be $ 1.26 per edible (finished) pound. (See Table 3.) The prime contributors to cost include the purchase of whole rays (41%), direct labor (20%), processing ice (14%)and the vacuum package bags (9%). With extensive production experience, direct labor use would lessen, as previously noted, the work crew used for the study had very little experience cutting rays. Ice was accounted for "per tub" as the location dictated. In a real operation, the cost of ice would likely be lower and based on weight. The cost of the poly-bags used could be lessened, but it is noted that a cut in bag quality (to an non-oxygen permeable bag of lower ply) would very likely lessen product quality and shelf life.

Table 3. Operational Costs

	COST PER EDIBLE lb.	TOTAL COST	%OF COST
Purchase of Rays	0.5167	140.55	41.0908%
Direct Labor	0.2592	70.50	20.6112%
Salt	0.0147	4.00	1.1694%
Ice	0.1838	50.00	14.6179%
Poly-Bags	0.1158	31.50	9.2093%
Corrugated Cartons	0.0515	14.00	4.0930%
Equipment	0.0059	1.61	0.4707%
Water	0.0037	1.00	0.2924%
Sanitizer	0.0033	0.90	0.2631%
Permits/Water Discharge	0.0042	1.15	0.3362%
Waste(Transport)	0.0978	26.60	7.7767%
Equipment Op. Cost	0.0009	0.24	0.0693%
Total	1.26	342.05	100%

Equipment used for processing included a band saw, skinning machine, a vacuum packager and scales. It was assumed that these were purchased used and were non-dedicated. A work year of 260 operational days and an estimated life of 10 years was used to estimate the capital replacement/depreciation. Capital replacement/depreciation costs for production using this schedule were very low at .47% (Table 4).

Table 4. Capital Replacement/Depreciation

	COST	EXPECTED LIFE YEARS	OPERATING DAYS/YEAR	DEPRECIATION PER DAY*
Band Saw	$ 450	10	260	$0.17
Skinning Machine	250	10	260	0.10
Vacuum Packer	2700	10	260	1.03
Scales	800	10	260	0.31
Total				1.61

* Equipment prices on used equipment, straight line depreciation. It is assumed that this is not dedicated use equipment.

Operational costs for electrically run equipment were estimated using standard commercial rates for the location of the processing plant. (Table 5) Equipment operational costs were a very small part ($ 0.005 per pound at 0.47%) of the overall cost structure.

Table 5. Process Utility Cost*

EQUIPMENT	Kw	AVERAGE HRS.	$kwh	AVERAGE$/Unit
Band Saw	0.80	0.0208	0.085	0.0014
Skinner	0.37	0.1208	0.085	0.0038
Scales	0.11	0.0222	0.085	0.0002
Vacuum Packer	1.30	0.0225	0.085	0.0025
Total Price Per Unit				$0.0079

*(Provided by R. Lane, Virginia Tech, 1992.)

<u>Marketing</u>

To familiarize staff assisting with this project, initial cownose ray tasting/testing was held in house. The in house evaluation revealed that taste and texture were good, however, the appearance of the product was not quite as favorable.

Samples of ray packaged during one of the three audit days was then sent to Baker-Monahan, Inc. (culinary consultants) for evaluation. The culinary experts evaluated the ray and determined that the location of cut of product from the wing of the animal yielded different cooking results. Different cooking recommendations, as well as recipes, were developed for the various cuts. The meat was rated as "excellent, mild flavored seafood." It was suggested that in certain recipes the ray could be substituted for dover sole, flounder, orange roughy, pompano or snapper. See Appendix 1 for detailed culinary consultants' report. Recipes are on file with the Virginia Marine Products Board, Newport News, Virginia.

Restaurant tests at three regional sites revealed mixed results. The restaurants were given the culinary consultants' report and product from the processing audit runs. Chefs were asked to test the product and report via a survey form (Appendix 2), supplemented with interviews.

All sample restaurants indicated that more than sixty percent of their menu offerings and sales volume is in seafood. The restaurants surveyed have annual sales volume in the $1,000,000 to $5,000,000 range. Most indicated that their regular clientele had annual income levels of $ 20,000 or more and were equally divided by sex. Respondents also indicated that 20-30% of their annual seafood offerings are in "non-traditional" species. Each indicated that 100% of seafood purchases were fresh product and that if a reliable source for a quality new product were available they would consider menu addition of that product. Each of the chefs indicated that they were "very willing" to spend time introducing a new product if they felt it had market appeal.

Raw product evaluation of the ray by the chefs was mixed. Appearance was rated as poor to fair, however odor was rated as good to very good. The cooked product was rated similarly. The appearance of the product was again poor to fair. The taste and odor received a rating of good to very good. Texture responses were mixed, from inedible to good.

The indicated acceptable range of purchase price ran from $2.00 to $2.50 per pound. The chefs also felt that it would be a better appetizer selection rather than an entree, indicating a risk minimization option for the diner to allow for the lack of recognition of the product.

A public tasting of cownose ray was held as part of an organized Hampton Bay Days event. (Hampton Bay Days is an annual, family oriented three day outdoor festival event held in Hampton, Virginia with expected attendance exceeding 50,000.) The product was cooked on an electric grill, cut into bite sized portions and served on toothpicks. Several sauces were provided for dipping. Approximately 500 people tasted ray and 232 completed the questionnaires collected. Respondents (207) rated the ray good or very good. Forty-eight responded that they would purchase the product at a grocer, compared with 53 at a restaurant and 83 at both. (Thirty-nine said they would not purchase the product at all.) Ninety-seven people liked the taste best of all characteristics and 18 responded that they disliked the appearance most of all. The majority (165) of the respondents had not heard of cownose ray and only 8 had eaten it before. Most respondants (155) eat seafood once a week or more. See Appendix 3 for complete summarized results.)

A retailer (seafood specialty shop) was given a sample shipment of cownose ray for display sale. (Figure 6) Approximately 20 pounds of product at $1.99 per pound were sold. The retailer reported that the nature and appearance of the item received much attention.

Figure 6. Fresh cownose ray fillets and steaks displayed in retail market case.

CONCLUSIONS

This research has shown that cownose ray can be harvested and processed successfully incorporating only minimal equipment and labor changes for many current harvesters and processors. The cost of processing cownose ray ($ 1.26 per pound) is high for a product with no proven markets and price history. As noted in the discussion, it is suspected that an experienced production line could lower this cost. The nature of the seasonality (very short) and specialized processing line suggests that cownose ray processing would be an acceptable add-on product line for a current seafood processor that would have other duties/uses for labor and equipment. Cownose ray would not be a high profit item, but could be an effective means of maintaining a regular labor force, while contributing to operational costs and marginally to profits.

The lack of established demand for the product indicates that the successful processor of cownose ray would have to be capable of a rather intensive, directed marketing effort. Marketing response was mixed, but did not indicate that the ray would be impossible to sell. Key issues indicated for the marketing of cownose ray included:

- Overcoming the "fresh" only demands of domestic restauranteurs
- Educating foreign buyers about cownose ray as a completely separate and distinct species from skates currently marketed as previously indicated in work by Thomas (6).
- Providing support marketing (recipes, posters, stickers) to assist grocers and restauranteurs in their customer education process.

- Educating chefs and grocers in the proper storage, display and preparation of cownose ray. Preparation is considered essential as the culinary consultants and chefs responded dramatically different based on the cut of ray flesh and the preparation method.

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APPENDIX 1

REPORT ON TESTING OF COWNOSE RAY

Susan G. Coe of Baker-Monahan, Inc. Culinary Consultants tested ten pounds of ray wings to determine various cooking characteristics, develop suggested cooking techniques and create three recipes that could be used by chefs in a broader test market. Recipes and information would be used in a fact sheet to be distributed prior to test marketing on a national basis.

Because of the small quantity of cownose ray available, only ten pounds were used for testing.

CULINARY CHARACTERISTICS

Cooking characteristics and cook texture varied based on the location of the wing on the rays body (the edge wing flap cooked more quickly with a finished texture like flounder, the part of the wing closest to the body had a more muscular texture with the cooking characteristic more like tenderloin). When the same cooking technique was used for both the thinner, less dense wing disintegrated. The thinner edge wing cooked up white and the muscular wing was darker and striated. Both cuts of the wings were excellent, mild flavored seafood. However, with this limited test it appears that the preparation should be different.

COOKING PREPARATION

Edge Wing or Outer Wing

Edge wing would be suitable for any soft flesh fish recipe. It has lean, white meat and a light, delicate flavor. Its firm texture allows great flexibility of preparation from broiling and frying to stuffing and poaching. Wing flaps could be substituted for dover sole, flounder, orange rough, pompano or snapper. The wing portion can be used in a wide variety of preparation styles used for other soft flesh fish filets such as florentine, rolled, stuffed or sauced. Some suggested sauces would be beurre noir, beurre blanc and provencale just to name a few. When this portion is marinated it tends to fall apart. Therefore, any type of marinated recipe is not recommended.

Muscular Wing or Thick Cut

Muscular wing can be cooked like any firm flesh fish as well as some meat preparations. The more muscular wings can be marinated, smoked, grilled, fried, broiled and as an ingredient for a wide variety of pasta, salads, chowders and seafood casseroles. They are excellent marinated and smoked. Muscular wings cook darker with a striation that may need to be camouflaged by a sauce.

APPENDIX 1 (Continued)

CULINARY NOTES

- A gristly piece of cartilage remained on some of the muscular wings. This appeared to be a dividing point between the more muscular portion and softer portion of the total wing.
- Blood continues to leach from the wings. If they are going to be breaded or battered in any way, this has to be done twice to contain the oozing.
- While this is only a focus group of one, the striation and gristle on the more muscular wings was a visual and textural disadvantage to the product.

Attached are the recipes that have been developed.

APPENDIX 2 Representative Sample Survey

NON-TRADITIONAL SEAFOOD SURVEY/FOOD SERVICE

1. On average, how many seafood items (including shellfish) are normally included on your menu?

	Check list of ranges	None	1-30%	31-60%	Greater than 60%
2.	What percent of your menu is seafood items?				✓
3.	Of your total food sales, what percent is seafood?				✓
4.	Of your seafood sales, what percent is in specialty or "non-traditional" species?		✓		
5.	What percent of your finfish purchases are frozen?	✓			
6.	What percent of your finfish purchases are fresh?				✓

7. Please indicate frequency of use or appearance on menu of "non-traditional" seafood products by circling the appropriate number.

0=Never 1=Occasionally 2=Often 3=Very Often 4=Always

Butterfish	0	(1)	2	3	4
Ray	0	(1)	2	3	4
Eel	0	(1)	2	3	4
Shark	0	1	(2)	3	4
Blowtoad (Chicken of the sea)	0	(1)	2	3	4

APPENDIX 2 (Continued)

8. How often do you try new seafood products of any kind on your menu? (Scale as above)

   0 1 2 (3) 4

9. Where do you get new menu/recipe/product ideas? (Check all appropriate answers.)

   ✓ Chef/Cooks | ✓ Cookbooks | ___ Customers
   ___ Distributors | ✓ Trade Press | ___ Wait Staff
   ✓ Seafood Marketing Organizations | ___ Other (please specify)______

ANSWER THE FOLLOWING QUESTIONS BY CIRCLING THE CORRECT ANSWER:

0=Never 1=Not Very 2=Somewhat 3=Likely 4=Very Likely

10. Given your clientele group, how likely would you be to put a nontraditional fish on the menu given a reliable source of the product?

    0 1 2 (3) 4

11. In general, how aware are your customers of non-traditional species of seafood?

    0 1 2 (3) 4

12. How likely would you and/or your chef be to try non-traditional seafood recipes if available?

    0 1 2 3 (4)

13. Which designation best describes your business? (Circle)

    Steak/beef House | Continental | Family Dining
    (Seafood) | Ethnic | Other (Specify)______

APPENDIX 2 (Continued)

14. What are your approximate annual food and beverage sales?

< $100,000 $100,000-$500,000 $500,000-$1,000,000

$1,000,000-$5,000,000 (circled) > $5,000,000

15. How many dining seats do you have? 150

16. On average, how many covers are served (turns) in a day? 450

17. What percentage of your business is:

Lunch 50 % Dinner 50 % Other ____%

18. Describe a "typical" customer:

SEX 50 % Male 50 % Female

INCOME LEVEL
- ___ < $10,000
- ___ $10,000-$20,000
- ✓ $20,001-$35,000
- ___ $35,001-$50,000
- ___ > $50,000

AGE
- ___ < 25
- ___ 26-35
- ___ 36-50
- ___ 51-60
- ___ > 60
- ✓ All Ages

19. Have you had any test market experience previously?

✓ Yes ___ No

Evaluator's Name: ________ , Title Chef

We are interested in the opinions of individuals in your organization who have meal planning or preparation responsibilities. Please feel free to make copies of this page for other evaluators.

1. Thawing

Thaw sealed pouch under refrigeration or cold running water (do not allow to warm). Open the pouch, rinse the meat briefly with tap water and drain.

2. Raw evaluation

Place the ray meat on a white or neutral colored plate and evaluate by indicating your responses on the form labelled "Raw Evaluation". Check one box in each column (appearance, odor and texture). Appearance may include all visual impressions, including color, shape, grain, etc. Raw texture refers to firmness and should be judged by pressing with a finger.

3. Cooked evaluation

Prepare the ray by following one or more of the enclosed suggested recipes or by using your own. Serve small portions covered while still hot and score impressions on the form labelled "Cooked Evaluation". Odor should be judged first, then appearance, flavor and texture (mouthfeel).

Raw Evaluation

Quality	Appearance	Odor	Texture
Excellent			
Very Good			
Good			
Fair		✓	
Borderline			
Slightly Poor			
Poor	✓		✓
Very Poor			
Inedible			

Cooked Evaluation

Quality	Preparation 1				Prep. 2				Prep. 3			
	Appearance	Odor	Taste	Texture	Appearance	Odor	Taste	Texture	Appearance	Odor	Taste	Texture
Excellent												
Very Good												
Good												
Fair	✓	✓			✓	✓	✓		✓	✓		✓
Borderline			✓					✓			✓	
Slightly Poor												
Poor				✓								
Very Poor												
Inedible												

4. Describe each preparation method used in the evaluation (e.g. sauteed, covered with a white sauce)

Preparation 1 Sauteed, marinated in lemon & lime

Preparation 2 Smoked, served with [illegible] French dressing

Preparation 3 Beer battered and fried

5. Please comment on your ratings. For example, if you scored cooked texture "very good", was it because of extra firmness, tenderness or flakiness?. How does cownose ray compare to other, more familiar, species?

Comments:

Raw Unlike any other seafood encountered

Cooked

Preparation 1 Like tough liver or game

Preparation 2 Tasted like pot roast

Preparation 3 Chewy

What would you consider to be a fair and comparable value to you of Cownose Ray in skinless, boneless fillet form?

___ $1.00/LB. or Less ___ $1.25 ___ $1.50 ___ $1.75

✓ $2.00 ___ $2.25 ___ $2.50 or More

All considered, do you see market potential of Cownose Ray as: (check all appropriate answers).

___ Menu Item
___ Daily Special
___ Food Bar Item
___ Special Entree
✓ Appetizer
___ Other (Please Specify)

Other comments, opinions I WOULD LIKE TO TRY THE FRESH PRODUCT, BUT THE FROZEN PRODUCTS TEXTURE AND APPEARANCE WAS UNAPPETIZING, AND ALTHOUGH THE FLAVOR WAS FAIR IT NEEDED TO BE SOMEWHAT COVERED UP TO HIDE THE APPEARANCE.

Thank you for your time, expertise, and valued judgements.

VIRGINIA MARINE PRODUCTS BOARD

APPENDIX 3

COWNOSE RAY EVALUATION

Hampton Bay Days - September 14, 1991

Summary

232 Questionnaires were filled out

OVERALL, HOW WOULD YOU RATE THIS PRODUCT?

Poor: 19
Good: 129
Very Good: 78

WOULD YOU BUY THIS AT A GROCER OR RESTAURANT?

Grocer: 48
Restaurant: 53
Both: 83
Neither: 39
No Comment: 7

WHAT DID YOU LIKE/DISLIKE ABOUT THE RAY?
(Such as taste, texture, appearance, etc.)

LIKE

Taste: 97
Texture: 25
Taste and Texture: 50
No Comments: 48

Special Comments:
Excellent-unique
like chicken
like monkfish
firmness
appearance
steaklike: 2
no bones
fair

DISLIKE

Appearance: 18
Texture: 14
Taste: 15
Too fishy: 5
Too strong: 11
Too dry: 10
Too chewy: 2
Too tough: 3
Too mushy: 1

APPENDIX 3 (Continued)

Special Comments:
Too much fat
Taste like bluefish
May be good marinated
Disliked the idea -- but are always open to new things
Lack of seafood flavor

HAVE YOU HEARD OF CHESAPEAKE OR COWNOSE RAY BEFORE TODAY?

Yes: 62
No: 165
No Comments: 5

HAVE YOU EATEN ANY RAY, SHARK OR SKATE BEFORE TODAY?

Ray: 8
Shark: 105
Skate: 12
None: 121
No Comments: 4

HOW OFTEN DO YOU EAT SEAFOOD?

Never: 12
One a month: 65
Once a week: 97
2 to 4 times a week: 52
More than 4 times a week: 6

EVALUATION OF CATFISH SURIMI PREPARED FROM FRAMES AFTER FILLETING

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World demand for surimi has increased rapidly since its introduction in the late 1970s. By 1990 it had reached 380.6 million pounds, a respectable increase from a decade before (1). At present, an estimated 150 million pounds of analogs are consumed in the United States annually. Recently, however, the whole category of surimi analog products is declining. There is a global shortage of surimi, resulting in tripled surimi price since last year. This is mainly due to the allocated Alaska pollack fishing season in order to protect the stock (2).

New species have been explored as alternatives to Alaska pollack in the Atlantic and Pacific Oceans of the United States during the 1980s. Species explored were red hake, silver hake, croaker, Atlantic menhaden, and Atlantic mackerel in the Atlantic Ocean, and Pacific whiting and arrowtooth flounder in the Pacific Ocean. Due to limited volume of stock, seasonal variation, dark color of the flesh, highly unstable fat content in the flesh, or high level of proteolytic enzymes from parasites in the muscle, commercial scale surimi production from these species has not been successful for the American marketplace.

The aquaculture industry has experienced significant growth in the United States in recent years. Catfish are the leading aquaculture product produced in the United States. The 1991 figure for total catfish processed rose to 395 million pounds from 41 million pounds in 1978 (8). Production and processing technologies for catfish have allowed the industry to deliver a consistently high quality, uniform product to consumers nationwide throughout the year. Yet there is little published information on the growing problems associated with waste disposal and utilization of catfish processing waste.

Therefore, this study was initiated 1) to investigate the feasibility of recovering mince from catfish frames using a mechanical deboner, 2) to optimize surimi processing, and 3) to evaluate gel-forming behavior of catfish surimi.

MATERIALS AND METHODS

Preparation of surimi

Fresh frames, the residual from filleting, were obtained within 24 hr from Delta Pride, Inc. (Indianola Industrial Park, Indianola, MS). Frames were run through a deboner (Model NDX13, Bibun Machine Construction Co. Ltd., Japan) with a drum having perforations 5mm in diameter. The recovered minced meat was washed once, twice, or three times with water, using 1 part fish meat to 4 parts water. The slurry was drained using a rotary screen rinser (Model F32LW, Bibun Machine Construction Co. Ltd., Japan), followed by a strainer (Model RE120, Bibun Machine Construction Co. Ltd., Japan) to remove any residual black skin and bone material. The strained meat was dewatered using a screw press (Model YS200, Bibun Machine Construction Co. Ltd., Japan), blended with cryoprotectants (sucrose, sorbitol, and sodium tripoly-phosphate 4%, 4%, and 0.2%, respectively) in a silent cutter (Model VCM40, Hobart Manufacturing Company, Troy, OH). It was then packed in cryobags, frozen in a plate freezer, and stored at -20℃ until used. A portion of unwashed mince was mixed with cryoprotectants and subsequently frozen and stored at -20℃ until used. Another portion of the unwashed mince without cryoprotectants was also frozen and stored at -20℃.

Color measurement

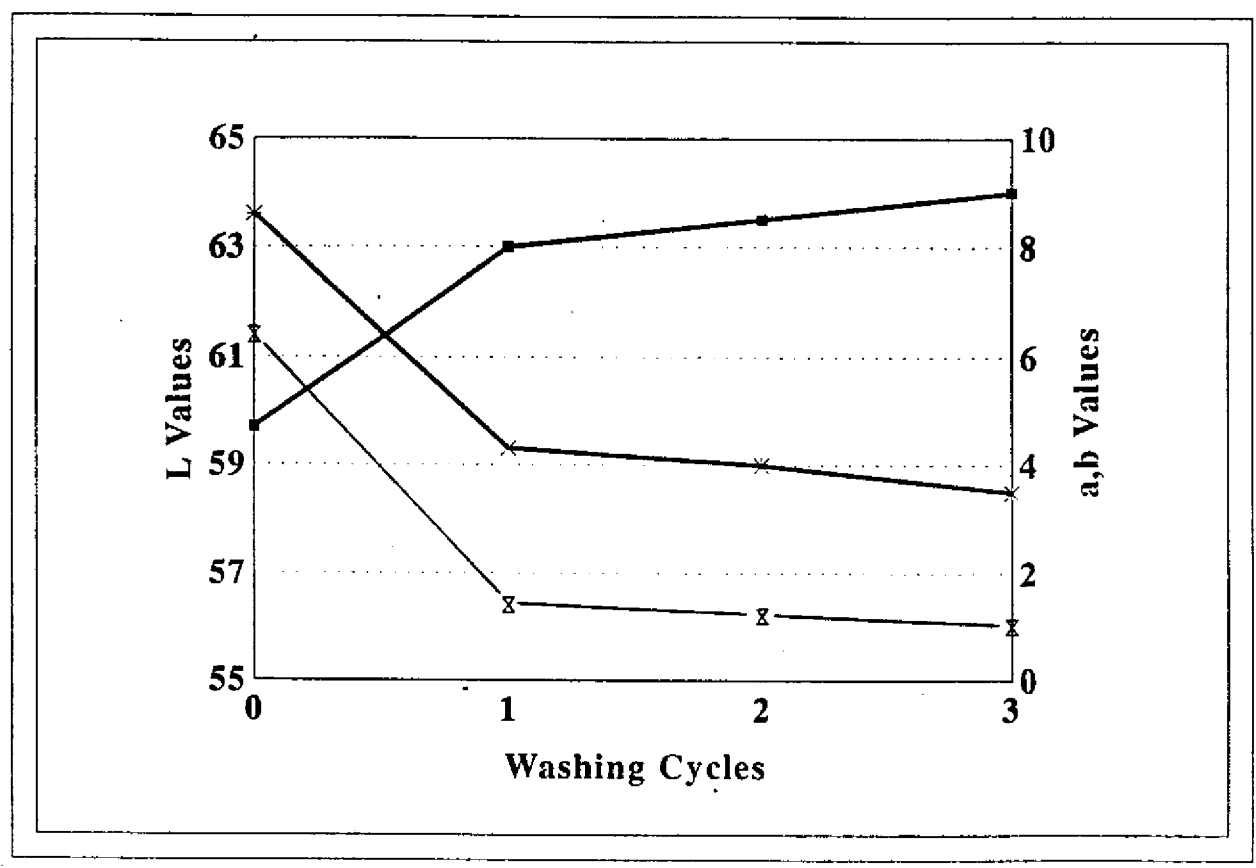
Hunter color values, L (whiteness), +a (redness), and +b (yellowness), were measured using Color Guard System (Model HX-20, Pacific Scientific, Silver Springs, MD) on catfish surimi which had been prepared after 0, 1, 2, or 3 washes. The system was calibrated with an aperture of 2 inches in diameter.

Preparation of heat-induced surimi gels

Surimi gels were prepared according to the procedures used by Kim and Lee (3). The surimi was thawed overnight in a refrigerator and chopped with 2% salt in a silent cutter for 10 minutes, either with or without 6% added starch (NuStar, A.E. Staley Manu-facturing Company, Decatur, IL). The chopped paste was stuffed into a 30mm diameter cellulose casing and cooked at 90℃ for 40 minutes in a water bath. Cooked gels were cooled in running tap water and left at room temperature to equilibrate to room temperature before measurement of textural properties. The unwashed meat either with or without cryoprotectants was thawed and made into gels in the same manner described above. Gels were also prepared with unwashed fresh mince immediately after the deboning and straining process.

Measurement of textural properties

Percent expressible moisture, compressive force, and penetration force were measured as an index of water-holding ability, cohesiveness, and firmness of the gel. An Instron testing machine was used to make these measurements according to the procedures used by Lee (5). Gels (30mm in diameter) were cut into 25mm lengths. The cylindrical gel specimen was then placed on a filter paper and compressed at 90% deformation using a 10cm diameter compression head. Failure point during compression was reported as the compressive force. The moisture collected in the filter paper during compression was converted to percent expressible moisture from each gel specimen. Penetration force was measured at 90% deformation using a probe of 9.5mm in diameter.



-■- L Values (white) -⧗- a Values (red) -✳- b Values (yellow)

Figure 1. Effect of washing cycles on Hunter color values of catfish surimi.

Statistical analysis

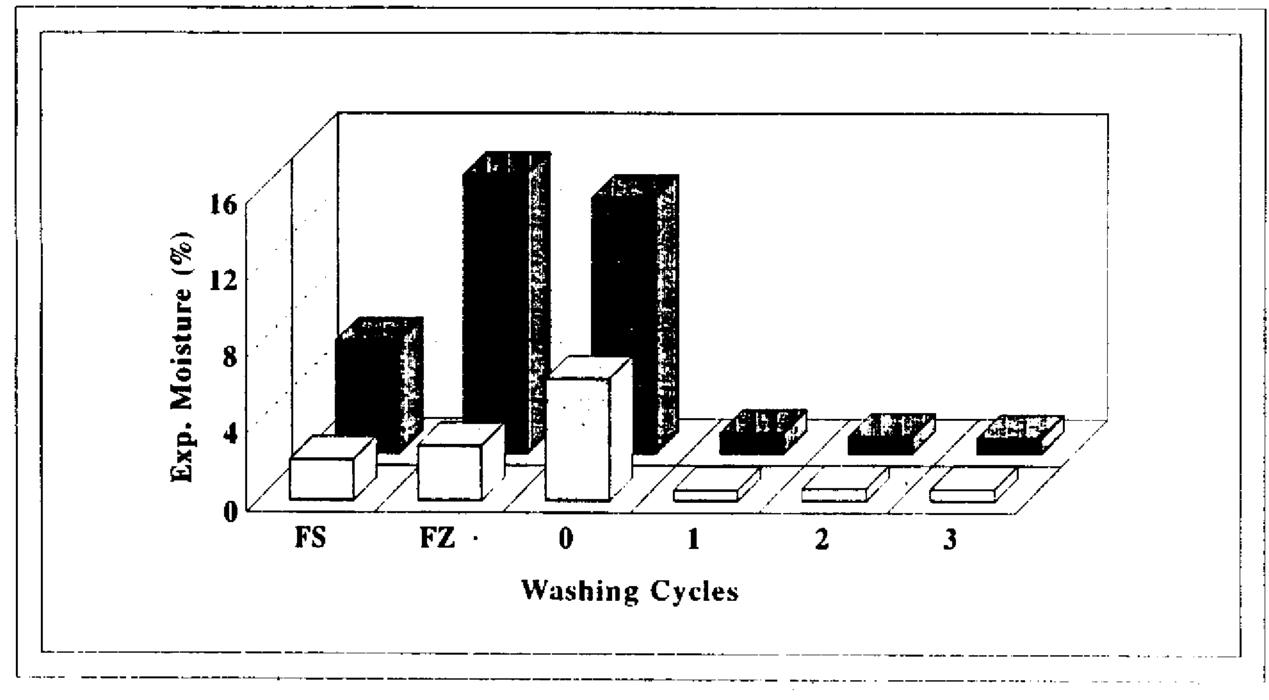
Data were analyzed by the analysis of variance (ANOVA) as described by Snedecor and Cochran (7). Least significant difference test (LSD) was used to evaluate differences between the means whenever the overall F test was found to be significant (6).

RESULTS AND DISCUSSION

Significant differences in Hunter L, a, and b values ($P < 0.05$) were found due to one washing (Fig. 1). However, there were no additional changes ($P > 0.05$) in the color values after two or three washes.

There were differences ($P < 0.05$) in percent expressible moisture between gels prepared with unwashed - fresh (FS) or -frozen (FZ) mince, unwashed-frozen mince containing cryoprotectants (UFMC) and surimi which was washed once, twice, or three times (Fig. 2). Gels prepared with UFMC showed the highest ($P < 0.05$) percent expressible moisture, followed by the gels prepared with FZ and FS. The water-holding ability of a gel is directly related to the gel-network formation (4). The gel prepared with FS demonstrated commercially acceptable water-holding ability with fairly good gel-strength when 6% starch was added. The gel prepared with UFMC was so weak that it did not exhibit commercially acceptable water-holding ability. In contrast, gels prepared with catfish

surimi which was washed once, twice, or three times showed excellent water-holding ability. Firm gel-network formation resulted in little expressible moisture from the gel upon compression at 90% deformation (4). There were no differences ($P > 0.05$) in percent expressible moisture of the surimi gels due to the number of washes.



Gel Preparation:

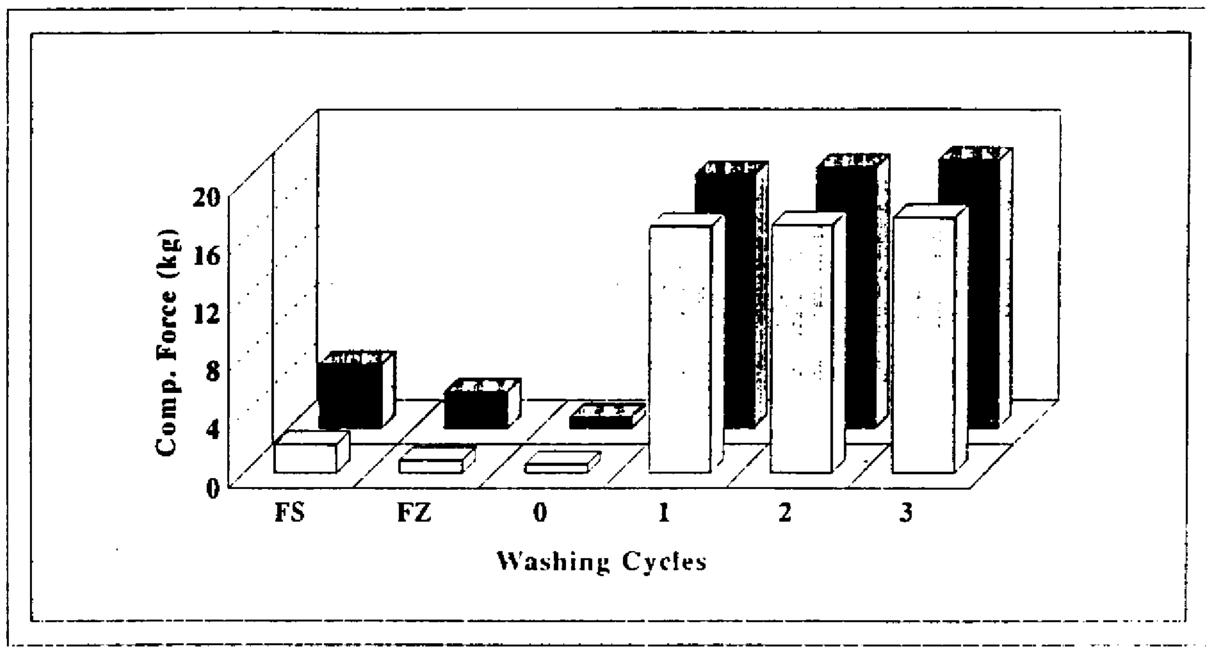
☐ **With** ■ **Without starch**

FS - Unwashed, fresh minced meat without cryoprotectants
FZ - Unwashed, frozen minced meat without cryoprotectants

Figure 2. Effect of washing cycles of minced catfish on percent expressible moisture of the surimi gels prepared with and without 6% added starch.

Gels prepared with UFMC and FZ were much weaker and compressive force was lower ($P < 0.05$) than gels prepared with FS (Fig. 3). Compressive force values for gels prepared with FS was high enough to be commercially acceptable if 6% starch was incorporated. There were no differences ($P > 0.05$) in compressive force values among surimi gels due to the number of wash cycles.

Similarly, as seen for compressive force, substantial differences in penetration force ($P < 0.05$) were found between gels prepared with FS, FZ, UFMC, and surimi (Fig. 4). No differences ($P > 0.05$) in penetration force was found between surimi gels due to the number of wash cycles.

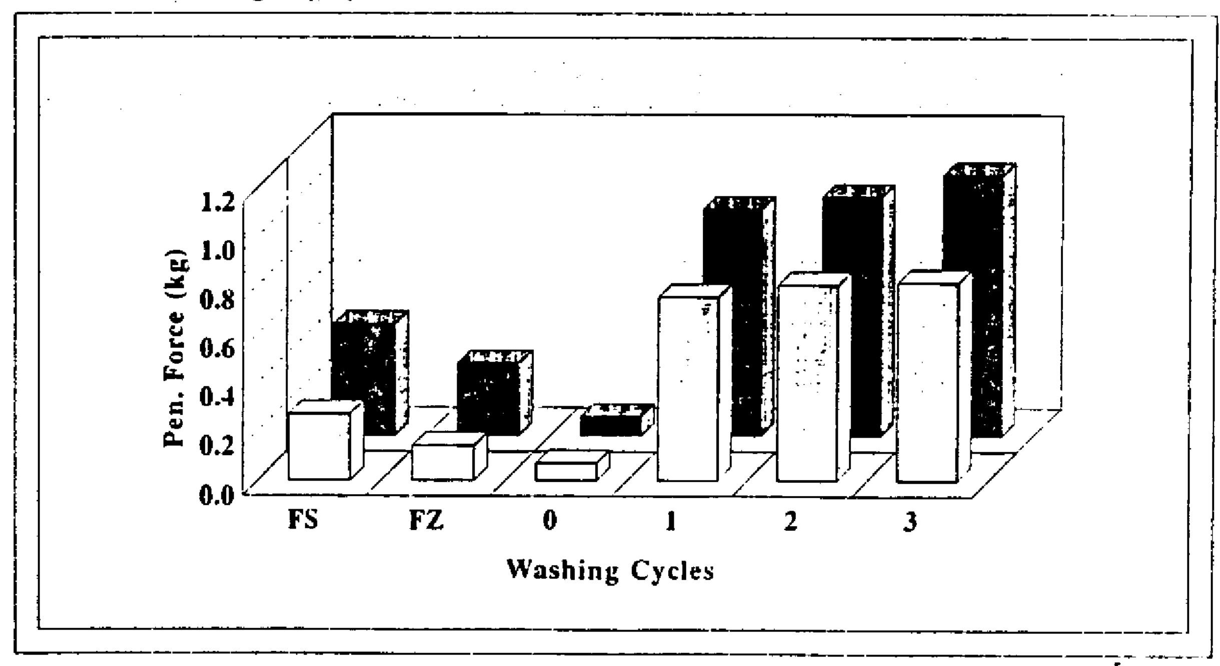


Gel Preparation:

☐ Without ■ With starch

FS - Unwashed, fresh minced meat without cryoprotectants
FZ - Unwashed, frozen minced meat without cryoprotectants

Figure 3. Effect on washing cycles of minced catfish on compressive force of surimi gels prepared with and without 6% added starch.



Gel Preparation:

☐ Without ■ With starch

FS - Unwashed, fresh minced meat without cryoprotectants
FZ - Unwashed, frozen minced meat without cryoprotectants

Figure 4. Effect of washing cycles of minced catfish on penetration force of surimi gels prepared with and without 6% added starch.

CONCLUSIONS

1. There were differences ($P < 0.05$) in Hunter color values and textural properties, such as percent expressible moisture, compressive force, and penetration force between gels prepared with fresh or frozen unwashed mince, frozen-unwashed mince containing cryoprotectants, and surimi.

2. The gel prepared with unwashed fresh catfish mince demonstrated textural properties that were commercially acceptable if starch was incorporated.

3. No differences ($P > 0.05$) in color and textural properties were observed in surimi gels due to the number of washes.

4. Data indicate that catfish surimi has functional properties which are feasible for commercial production of shellfish analogs and other fabricated products.

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RELATIVE IMPORTANCE OF ENVIRONMENTAL FACTORS AMONG IMPEDIMENTS TO SEAFOOD AND OTHER FOOD PROCESSING IN NEW JERSEY

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From 1976 to 1986, the number of seafood processors in New Jersey declined from 43 to 28 and the number of people employed in seafood processing declined from 1599 to 1071 (7). The same downward trend is occurring in other New Jersey food processing sectors (5). State and local policy-makers and governmental officials are concerned about this loss of employment and shrinking market outlets for farmers and fisherman of the state.

To stimulate manufacturing growth, governmental officials encourage the establishment of new enterprises and/or stimulate the expansion of existing food processors. According to Carlton (2), the forces determining changes in plant capacity or level of processing output are clearly different from those shaping location decisions because of the large fixed costs tying down an already existing plant. At some point, a decision may involve whether to expand or build a new plant. However, according to Schmenner, only if compelling problems with on-site expansion exist do manufacturers turn to opening a new plant or to relocating an existing one (8). Factors affecting location decisions of Mid-Atlantic food processors have been identified by Lopez and Henderson (6).

Expansion and contraction decisions can be characterized as production responses to economic signals. That is, they may be driven by changes in the derived demand for the plant's output or changes in the supply of inputs. Expansion reflects an increase in the profitability of a plant as demand or price for the plant's output increases or as per unit cost of production decreases. Expansion could be pursued to achieve economies of scale as well. On-site expansion usually requires more workers and more raw products. Impediments to expansion can be physical constraints such as size of the property, or institutional constraints, such as environmental regulation and zoning. These impediments can delay or even prevent expansion of on-site capacity. Contraction decisions, on the other hand, reflect a lack of profitability or viability of plants. This could be due to a lack of demand for the product results from competition or changes in consumer preferences, or an increase in production cost.

In order to formulate effective public policies for stimulating food manufacturing growth, governmental officials need to understand the underlying factors which affect expansion/ contraction decisions. Certain of these factors are within the purview of public policy. The economic and regulatory impediments

to expansion of existing food processing operations in New Jersey are identified as a contribution to improving the business climate for food processors in New Jersey.

METHODS

A mail survey of New Jersey food processors was utilized to collect information about processor characteristics, production expansion/contraction trends, and the relative influence of various economic and regulatory factors on food processing operations. A list of potential impediments was developed based on factors identified in other food processing studies. Although environmental regulatory factors were included among the business climate factors, they were analyzed in detail in a special section of the mail survey as well. This was done because a previously conducted survey conducted to identify perceived advantages and disadvantages of operating in the state had indicated that environmental factors are of considerable concern to New Jersey food processors (5). The mail survey was pre-tested with eight New Jersey food processors.

The target population was limited to New Jersey food processors which use seafood, vegetables, fruits, eggs, and poultry as raw product because these processors have the strongest linkage to the farming and fishing industries of the state. No complete up-to-date list of these processors existed. Lists of New Jersey food processors were provided by Dun and Bradstreet (4), New Jersey Food Processors Association, New Jersey Department of Agriculture, county economic development offices, and the New Jersey Sea Grant Marine Advisory Service. Firms were separated into three general types of food processors: seafood, fruits and vegetables, and eggs and poultry, based on 4-digit SIC codes and information provided by our sources. A total of 378 firms: 180 seafood, 153 fruits and vegetable, and 45 egg and poultry, were assigned a mail-sort code number and logged. A minimum response rate of 50% was targeted to insure accurate representation of the industry.

The mail survey was conducted using procedures outlined by Dillman (3). The survey, along with a cover letter explaining the intent of the study and assuring anonymity of response, was sent. A week later a "follow up" postcard was mailed to all firms included in the initial mailing thanking those who had returned surveys and urging those who had not to complete and return them. One month from the initial mailing, a certified letter and copy of the mail survey were sent to non-respondents.

The computer data analysis consists of frequency distributions and cross-tabulations utilizing the statistical package SAS. To protect the anonymity of the respondents, data is reported in the aggregate. Recognizing that the importance of factors affecting expansion/contraction choices may vary with the characteristics of particular manufacturing plants, the survey responses are analyzed not only for the entire sample, but for two key characteristics as well: type of raw product processed and size. Type of raw product processed categories include seafood, fruit and vegetable, poultry

and egg, and multiple raw materials. Size was determined by number of employees rather than value of shipments due to the lack and unreliability of sales data available. Consistent with SIC reporting categories, small plants are those with fewer than 19 employees, medium with 20 to 99 employees, and large with 100 or more employees.

RESULTS AND DISCUSSION

The population surveyed, characteristics of the New Jersey food processors surveyed, the trends in expansion/contraction of operations, and impediments to expansion are discussed in turn.

Population Surveyed

Of the 378 firms mailed a survey, a total of 51 firms returned completed surveys after the first letter and/or "follow up" postcard. An additional 47 firms returned completed surveys in response to the certified letter bringing the total response to 98. An additional 173 firms were accounted for: 55 were postal service returns indicating the firm was out of business, and 118 firms indicated that they were non-relevant food processors or not a food processor. A total of 107 firms did not respond.

Assuming that the ratio of relevant processors to non-relevant processors and non-processors among non-respondents is equal to that among respondents, 45% of the 107 non-respondents, or 48 firms, are relevant processors, the total number of relevant food processors is 146, and the 98 survey respondents represent a response rate of 67%. This is most likely a conservative estimate. The Bureau of Census reports the number of New Jersey seafood processors at 16, fruit and vegetable processors at 63, and egg and poultry processors at 12, for a total of 91 food processors (1). Therefore, the coverage of relevant New Jersey food processors was most likely excellent, approaching 100%.

Processor Characteristics

Of the 98 food processors surveyed, 30% process seafood, 42% fruit and/or vegetables, 20% eggs and/or poultry, and 8% more than one of the above types of food products. The size distribution is 29% small, 38% medium, and 33% large-sized firms.

Most are single plants (70%), and the remainder are branch plants or subsidiaries (30%). The majority are primary processors (63%), that is, they use raw seafood and agricultural products as inputs, and the remainder are secondary processors (37%), that is, they use already processed products as their inputs. Most plants do not pretreat their wastewater and have access to municipal sewers (74%). The majority of plants use some degree of automation or are highly automated (63%) and use skilled labor (56%). The remainder use manual production (36%) and unskilled labor (42%). It should be noted, however, that levels of automation and labor skill were subjective. Of the 98 firms surveyed, 36% market their product in

the New Jersey/New York/Philadelphia area, 24% market in northeastern U.S./eastern Canada, and 37% have a national market. Only 3% have a national and international market.

Expansion/Contraction Trends

The trend in processing capacity was assessed by asking New Jersey food processors whether, during the past five years, they had expanded, maintained the same level, or reduced their production capacity. All 98 firms responded to the question. Of these, 64 reported expansion, 22 reported an unchanged level of production, and 12 reported reduction in production.

Although these results indicate a relatively healthy industry, it must be remembered that survey respondents are "survivors" in the existing business climate. In addition to the 34 survey respondents which have not expanded operations in the past 5 years, 55 firms, or 15% of the 378 total firms mailed a survey, were out of business. Although not all of the firms mailed a survey are likely to be relevant food processors, there are also likely to be numerous unaccounted for relevant food processors which have gone out of business in recent years and were eliminated from the lists from which the survey population was drawn. Therefore, the percentage of firms which reported expansion drops to only about 47% when out of business firms are included in the total population.

The change in New Jersey food processing plant capacity is reported by type of raw product processed in Figure 1. Seafood processors exhibited the highest proportion (28%) of plants which had contracted plant production. The proportion of plants which

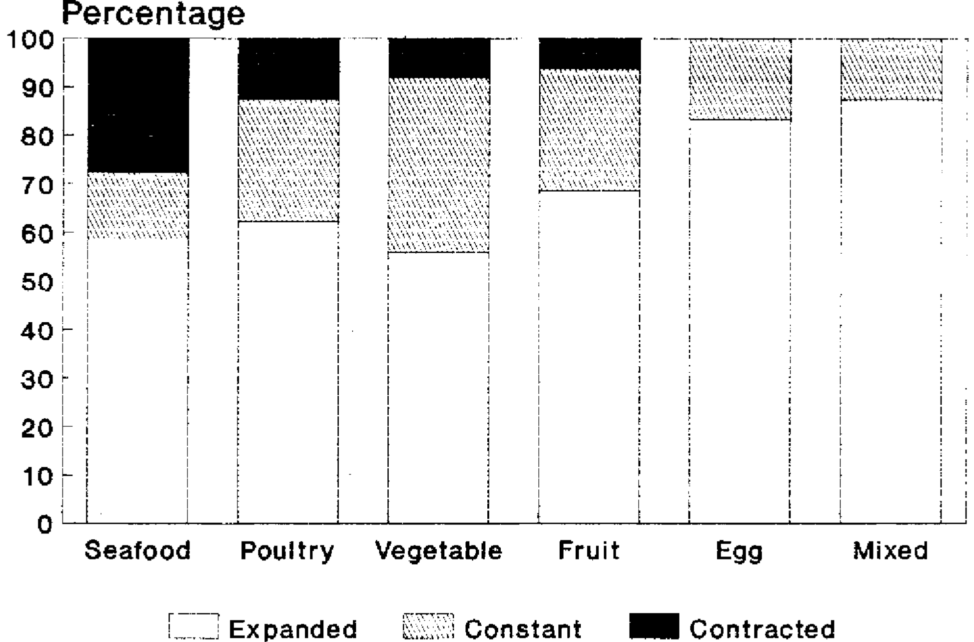

Figure 1. Change in New Jersey Food Processing Plant Capacity by Type of Raw Product Processed.

Table 1. Economic and Regulatory Impediments to Food Processing Expansion in New Jersey, All Responses

FACTOR	RANK	MAJOR IMPEDIMENT	IMPEDIMENT	NOT AN IMPEDIMENT
		Percent		
Shortage of Workers	1	56.9	25.3	16.7
Insurance Costs	2	50.1	39.6	10.4
Cost of Compliance with Environmental Regulations	3	47.3	35.5	17.2
Difficulty of Compliance with Environmental Regulations	4	40.9	38.7	20.4
High Land Costs	5	39.8	23.7	36.6
High Utility Costs	6	36.1	50.0	13.8
Lack of State Development Incentives	7	34.1	30.8	35.1
High Construction Costs	8	31.2	42.0	26.9
Land Use Restrictions	9	29.0	34.4	36.6
High Cost of Labor	10	28.7	52.1	19.1
Local Zoning Laws	11	24.8	44.1	31.2
Shortage of Agricultural or Seafood Products	12	23.6	24.7	51.6
Severe Competition from Other Firms:	13	22.3	40.4	37.2
Limitation of Existing Facilities	14	17.6	30.8	51.6
Union Working Rules	15	15.7	20.2	64.0
Image of the State	16	13.9	24.5	61.7
Adequacy and Cost of Truck and Rail Service	17	12.9	34.4	52.7
Inefficient/Outdated Plant Technology	18	12.1	37.4	50.5
Insufficient Demand for Product	19	6.5	22.6	71.0
Difficulty with Handling Volume of Raw Products	20	4.4	26.4	69.2

remained roughly constant was highest among fruit/ vegetable plants (32%). The proportion of plants which expanded was highest among mixed product (88%) and egg/poultry (70%) processors.

Impediments to Expansion

Survey respondents were asked to indicate the importance of twenty impediments to the expansion of their operation. The range of potential responses were "extreme impediment," "impediment" "minor impediment." The ranking of these factors by descending frequency of "extreme impediment" responses is presented in Table 1. Four impediments were identified by 40% or more of respondents: the shortage of workers, high insurance costs, cost of compliance with environmental regulations, and difficulty of compliance with environmental regulations. Also among the top 10 impediments are high land costs, high utility costs, lack of state development incentives, high construction costs, land use restrictions, and high cost of labor.

Impediments are ranked by type of products processed in Table 2. Seafood processors ranked the difficulty of compliance with environmental regulations 2nd, higher than any other type of food processor. In open ended questions, surf clam and ocean quahog

Table 2. Economic and Regulatory Impediments to Food Processing Expansion in New Jersey, Ranking by Type of Raw Product Processed

FACTOR	SEAFOOD	VEGETABLES	FRUIT	POULTRY	EGGS	MIXED
	Rank					
Shortage of Workers	3	1	1	1	7	1
Insurance Costs	1	8	3	3	1	12
Cost of Compliance with Environmental Regulations	4	2	2	2	4	9-11
Difficulty of Compliance with Environmental Regulations	2	4	5	4	9	9-11
Lack of State Development Incentives	5	10	9	5	11-12	2
High Utility Costs	12	3	11	8	3	7-8
High Land Costs	8	11	7	12-13	2	7-8
Land Use Restrictions	10	12	4	12-13	5	6
Local Zoning Laws	9	17	13	14	6	4-5
High Construction Costs	11	16	12	6	8	9-11
Shortage of Agricultural or Seafood Products	7	15	10	11	14	14-18
Severe Competition from Other Firms:	13	7	8	9-10	13	14-18
High Cost of Labor	14	6	6	7	11-12	19
Inefficient/Outdated Plant Technology	18	5	19	9-10	18-20	4-5
Image of the State	6	20	15	17-19	16	20
Limitation of Existing Facilities	17	14	14	17-19	15	3
Union Working Rules	16	13	17	16	10	13
Insufficient Demand for Product	19	9	18	20	18-20	14-18
Adequacy and Cost of Truck and Rail Service	15	18	16	15	17	14-18
Difficulty with Handling Volume of Raw Products	20	19	20	17-19	18-20	14-18

processors, in particular, indicated concern with the severity of waste water discharge regulations. This concern is significant as sea clams comprise the majority of total fish and shellfish landed in the state and several shucking operations have closed in the past two decades. Seafood processors ranked the image of New Jersey 6th and raw product shortage 7th, higher than any other type of processor. In open-ended questions seafood processors expressed concern regarding marine pollution and media stories which question seafood safety and believe these factors affect the marketability of their products.

When impediments are ranked by size of plant, the shortage of workers is among the top three impediments for all size plants (Table 3). Insurance costs apparently is more of an obstacle for small (1st) and medium (2nd) plants than large (7th). Small plants reported that shortage of raw product (5th), competition from other firms (10th) and insufficient demand for product (15th), higher than medium and large firms. Medium-sized plants ranked limitations of existing facilities (6th) as a major impediment to expansion. The relative importance attached to this factors is note-worthy considering its low overall ranking (16th). Both medium and large plants ranked the lack of state development incentives and land use restrictions higher than did small plants as an obstacle to expansion. Large plants ranked high construction costs (5th) higher

Table 3. Economic and Regulatory Impediments to Food Processing Expansion in New Jersey, Ranking by Size of Plant (Number of Employees)

FACTOR	SMALL (1-19)	MEDIUM (20-99)	LARGE (100+)
	Rank		
Shortage of Workers	2	1	3
Insurance Costs	1	2	7
Cost of Compliance with Environmental Regulations	3	4	2
Difficulty of Compliance with Environmental Regulations	4	7	1
Lack of State Development Incentives	16	3	10
High Utility Costs	9	5	9
High Land Costs	6	8	6
Land Use Restrictions	9	4	4
Local Zoning Laws	7-8	10	12
High Construction Costs	7-8	17	5
Shortage of Agricultural or Seafood Products	5	11	15
Severe Competition from Other Firms:	10	12	14
High Cost of Labor	13	15	11
Inefficient/Outdated Plant Technology	18	16	8
Image of the State	14	14	19
Limitation of Existing Facilities	17	6	16
Union Working Rules	20	13	13
Insufficient Demand for Product	15	18	20
Adequacy and Cost of Truck and Rail Service	12	19	17
Difficulty with Handling Volume of Raw Products	19	20	18

Table 4. Relative Impact of Various Environmental Regulatory Factors on Food Processing Plant Operations in New Jersey, All Responses

FACTOR	RANK	SERIOUS NEGATIVE EFFECTS	SOME NEGATIVE EFFECTS	NO NEGATIVE EFFECTS
		Percent		
Strictness of Solid Waste Disposal Regulations	1	33.0	35.1	31.9
Difficulty of Compliance with Environmental Regulations	2	32.3	33.3	34.4
Capital Expenditures for Pollution Abatement, Including New Equipment	3	29.8	33.0	37.2
Annual Cost to Comply with Environmental Regulations, Including Permits	4	29.5	40.0	30.5
Conflicting Information from State Agencies	5	28.3	38.0	33.7
Difficulty of Identifying Relevant Regulations, Permits and Permitting Agencies	6	25.8	38.7	35.5
Strictness of Water Pollution Regulations	7	25.5	38.3	36.2
Stringency of Enforcement	8	22.6	36.6	40.9
Strictness of Air Pollution Regulations	9	12.8	27.7	59.6

than both medium and small plants. Large plants ranked the difficulty and cost of compliance with environmental regulations as (1st) and (2nd) most significant impediments to expansion.

Environmental Regulatory Factors

New Jersey food processors were asked to scale the degree to which various environmental regulatory factors impacted their plant operations. A 3-point scale: "no negative effects," "some negative effects," and "serious negative effects" was used. The responses were ranked according to frequency of "serious negative effects" responses.

The overall responses are reported in Table 4. Strictness of solid waste disposal regulations was reported to have the most serious negative effect on plant operations. The difficulty of compliance with environmental regulations, capital expenditures for pollution abatement, the annual cost of compliance with environmental regulations, conflicting information from state officials, conflicting information from state agencies, difficulty of identifying relevant regulations, and strictness of water pollution regulations were reported to have serious negative effects by 25 to 32% of food processors surveyed. Strictness of air pollution regulations does not appear to be important. It may be noted that 59 to 70% of the respondents indicated that all the environmental regulatory factors, except air pollution regulations, cause some negative effects or greater.

The relative importance of environmental regulatory factors are summarized by type of product processed in Table 5. Seafood processors report more difficulty with administrative aspects of

Table 5. Relative Impact of Various Environmental Regulatory Factors on Food Processing Plant Operations in New Jersey, Ranking by Type of Raw Product Processed

FACTOR	SEAFOOD	VEGETABLES	FRUIT	POULTRY	EGGS	MIXED
	Rank					
Strictness of Solid Waste Disposal Regulations	5	1	3	4	5	2-3
Difficulty of Compliance with Environmental Regulations	2	6	1	5	1	6-7
Capital Expenditures for Pollution Abatement, Including New Equipment	8	4	4	1	6	4
Annual Cost to Comply with Environmental Regulations, Including Permits	6	3	2	3	2-3	8
Conflicting Information from State Agencies	1	5	8	7	4	1
Difficulty of Identifying Relevant Regulations, Permits and Permitting Agencies	7	2	5-6	9	2-3	5
Strictness of Water Pollution Regulations	3	8	5-6	2	8	6-7
Stringency of Enforcement	4	7	7	8	7	2-3
Strictness of Air Pollution Regulations	9	9	9	6	9	9

Table 6. Relative Impact of Various Environmental Regulatory Factors on Food Processing Plant Operations in New Jersey, Ranking by Plant Size (Number of Employees)

FACTOR	SMALL (1-19)	MEDIUM (20-99)	LARGE (100+)
	------ Rank	------	------
Strictness of Solid Waste Disposal Regulations	2	4	2
Difficulty of Compliance with Environmental Regulations	7	1	3
Capital Expenditures for Pollution Abatement, Including New Equipment	6	6	1
Annual Cost to Comply with Environmental Regulations, Including Permits	9	3	4
Conflicting Information from State Agencies	3	2	6
Difficulty of Identifying Relevant Regulations, Permits and Permitting Agencies	4	5	7
Strictness of Water Pollution Regulations	1	8	5
Stringency of Enforcement	5	7	8
Strictness of Air Pollution Regulations	8	9	9

environmental regulatory compliance than the cost of compliance. They rank conflicting information from state agencies and difficulty of compliance with environmental regulations higher than annual cost to comply with environmental regulations and capital expenditures for pollution abatement.

The relative importance of environmental factors is summarized by size of plant (number of employees) in Table 6. Large plants are more concerned with capital expenditures for pollution abatement than for any other environmental regulatory factor. This factor was more important to large firms than to medium and small plants. Both medium and large plants are more concerned with the annual cost of compliance with environmental regulations than small plants. Conflicting information and the difficulty of identifying relevant environmental regulations and procedures are a greater problem for smaller plants than for larger ones. One explanation may be that larger plants have specialized staff to deal with these problems.

CONCLUSIONS

The ranking of impediments provided by this study identifies priorities for addressing public policies which affect the economic viability of food processing plants in New Jersey. For food processors as a whole and seafood processors specifically, the top ranked impediments to expansion are shortage of workers, insurance costs, cost of compliance with environmental regulations, and difficulty of compliance with environmental regulations. For seafood firms, additional important impediments are image of the state and shortage of raw product.

The negative effects reported by the majority of survey respondents of almost all environmental regulatory factors emphasizes the need for governmental officials to more carefully consider potential impacts on processor operations. Seafood processors indicate that the administrative aspects of environmental regulation compliance have more detrimental effects on plant operations than the costs associated with compliance. The amount of resources required and the level of uncertainty associated with the "hassle" factor of environmental regulation compliance appear to be substantial. Governmental policies and procedures which improve the consistency of information provided, and the efficiency of procedures related to environmental regulatory compliance would improve the business climate for seafood and food processors in New Jersey.

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MANAGEMENT CONSIDERATIONS FOR BY-CATCH IN THE NORTH CAROLINA AND SOUTHEAST SHRIMP FISHERY

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INTRODUCTION

Commercial shrimp by-catch in the Southeast is an issue of rapidly growing concern to fishery managers. Although there is debate over the biological impact of by-catch on finfish stocks, the environmental and sportfishing communities are increasingly demanding action by fishery managers to reduce by-catch in the commercial shrimp fleet. This paper discusses the problem, reviews by-catch biological data, identifies management alternatives including economic considerations, demonstrates clear legal authorities by the National Marine Fisheries Service and North Carolina Division of Marine Fisheries and recommends policy.

In terms of value, the shrimp fishery is the most important fishery in the Southeast. In 1990, over 277 million pounds of shrimp valued at $454 million were landed in the Gulf and South Atlantic regions (National Marine Fisheries Service, 1990). In North Carolina, 7.8 million pounds of shrimp valued at $15.9 million were landed in 1990 (K. West, NC Division of Marine Fisheries, personal communication). Shrimp are harvested almost exclusively by otter trawls, which are a highly unselective type of fishing gear. In the process of shrimping, fishermen catch a variety of other species called by-catch. By-catch is the incidental, usually unwanted, mix of species caught when trawling for another species. The southeastern shrimp fishery by-catch includes several species of juvenile and adult finfishes, crustaceans such as blue and calico crabs and other invertebrates such as jellyfish.

In recent years, interest in by-catch has switched from its potential commercial use to concerns by environmentalists, sportfishermen and fishery managers about its impact on finfish populations. Possible explanations for its emergence as an issue are: (1) perceived and real reductions in catch per unit of effort by sportfishermen who angrily look for

someone to blame, (2) increased political sophistication by sportfishing groups such as the Atlantic Coast Conservation Association who have brought it to the attention of the public, (3) discovery of the issue by national environmental groups such as the Center for Marine Conservation and Greenpeace, (4) association with other destructive commercial fishing practices such as drift gill-netting and pelagic longlining which have received national negative publicity, and (5) development of regional fisheries management plans for finfish which highlight the need for reduction of by-catch in southern shrimp fisheries.

Commercial fishermen are also concerned about by-catch. They do not like to waste a resource and are becoming increasingly concerned about the negative public perception of the by-catch issue. They fear this concern could lead to draconian management measures such as recently occurred in Old Tampa Bay where the Florida Marine Fisheries Commission proposed to the Governor of Florida to close shrimping because it was found that three area skippers produced a 1,000 pound waste catch to produce 92 pounds of shrimp. Among the waste was "more than 100 redfish and at least that many trout" (Tampa Tribune, 4-26-89). During the Spring of 1989, the North Carolina Marine Fisheries Commission received a petition signed by 1,500 individuals to ban trawling and gill-netting in all territorial waters because of the by-catch problem. At the August 14, 1989 meeting, several commissioners voiced concern that the anti-trawl advocates would by-pass the Marine Fisheries Commission and directly seek legislation to ban trawling.

The issue will undoubtedly become more heated over the next several years. Greenpeace among other environmental organizations was successful in having the by-catch issue addressed in the re-authorization of the Magnuson Fisheries Management Act. The Center for Marine Conservation (CMC) has hired a marine biologist to work on the issue, and according to Marydele Connelly of CMC "We're going to be in the by-catch issue in a big way. It's the issue of the '90's." (Cooper, pg. 13, 1990).

With the possible exception of red snapper in the Gulf of Mexico, where there is strong evidence that shrimp trawling has caused population declines and weakfish along the Atlantic coast where recent statistical analysis by Vaughn and Seagraves suggests weakfish by-catch could have a significant impact on attempts to rebuild weakfish stocks, there are few definitive cause and effect studies which conclude that shrimp trawling is creating a decline in finfish populations (Gulf of Mexico Fisheries Management Council 1990, Vaughn et al. 1991). The Gulf and South Atlantic Fisheries Development Foundation is organizing a steering committee comprised of agencies, industry, conservation organizations and Sea Grant to strategically plan, collect data and evaluate gear for the management of by-catch in the southeastern shrimp fishery. One aspect of the GSAFDF's program, which will be conducted through 1993, involves scrutiny of fishery independent and dependent by-catch data. As data collection and analysis is increased it is anticipated that other impacted finfish stocks will be identified.

Even though at present there is no conclusive evidence that shrimp by-catch is causing a biological problem in other fisheries, a good argument can be made that it is a resource management problem because it is so perceived by the public. There is a political adage which states "the public may not be right, but they are correct." In the case of by-catch the public is increasingly calling for a response by fishery managers. Managers must begin now to conduct research on the biological implications of by-catch and to devise management schemes designed to reduce it.

The turtle excluder device (TED) controversy is instructive when looking at the by-catch issue. In 1978 the National Marine Fisheries Service, in recognition of a potential problem they faced regarding their enforcement responsibilities for the Endangered Species Act (ESA), launched a $3.4 million research program with the goal of eliminating the

incidental capture of sea turtles during shrimp trawling. By 1981, NMFS had developed a TED which was distributed to commercial fishermen for voluntary use. Because early designs were considered bulky and unsafe, commercial fishermen largely ignored it. By 1986, the environmental community led by the Center for Environmental Education threatened to sue NMFS for lack of enforcement of the ESA. The National Oceanic and Atmospheric Administration, the parent agency of NMFS, convened an industry mediation team to adopt regulations to reduce turtle capture by 97 %. The resultant regulations developed over the following months were vociferously opposed by many commercial fishermen throughout the Southeast. In the eyes of the fishermen, the public image of NOAA and NMFS were tarnished by the process. Many observers believe the image of the shrimping industry was similarly damaged.

Because none of the finfish species associated with by-catch are presently listed as threatened or endangered, the legal mandate of the ESA does not apply. However, there are several lessons which can be applied to the by-catch issue. First, environmental community concerns were not seriously, enough considered until the situation had polarized. Second, from the perspective of the industry, NMFS conducted much of the initial research in-house, without involving a broad spectrum of commercial fishermen throughout the region in the gear design phase of the program. In hindsight, the lack of widespread fishermen involvement resulted in fewer ideas for gear innovations, and greater opposition to the program because of a lack of a sense of ownership in it. Also, site specific problems associated with the gear were not discovered until the program was in full effect.

As is discussed later, the by-catch issue parallels the turtle controversy. Fishery managers should take seriously the concerns of the public, begin a process of public discussions about the alternatives toward reducing the concerns, and actively involve the commercial fishing community in all phases of developing management alternatives. The GSAFDF's by-catch management program addresses these issues. The Foundation represents industry, but has appointed a steering committee comprised of agency, conservation group and university interests. Early drafts of the research plan include plans for public information and education and wide industry involvement in data collection and gear evaluation efforts.

Biology

Keiser reported 105 species of fish representing 45 families and 15 orders from shrimp trawl samples in South Carolina (Keiser 1976). Although a wide variety of fish appear in the catches usually only a few species characterize the by-catch. In South Carolina, sciaenids represented an average of 60 %; by number of the catch; spot were the most abundant species 30 %. Species composition and abundance vary widely based on seasonality; abundance peaks in May and composition peaks in June in South Carolina. In general, the finfish by-catch is small in size. Mean total lengths of 25 species in the South Carolina study ranged from 6.9 to 18.6 cm. Investigations of the icthyofauna in coastal waters of the South Atlantic Bight by Wenner and Sedberry, showed a similar pattern of seasonal variation in species diversity (Wenner and Sedberry 1989). For example, weakfish were most abundant in the fall, but ranked low in abundance during other seasons.

The by-catch can be substantial in quantity. In the Gulf of Mexico, an estimated 5 billion croaker were killed in 1989 (Nichols, et. al. 1990). Mortality of other popular sportfish is also high, e. g. Nichols estimated that 19 million red snapper and 3 million spanish mackerel were killed that same year by shrimp trawling in the Gulf of Mexico. Keiser reported that between 3,6 and 16.6 million kg of fish were caught incidental to shrimping in South Carolina in 1975. There was a wide range of fish/shrimp weight ratios -

- from 1.3:1 to 136.1:1 --, but with the exception of three months, the average monthly ratios ranged from 1:1 to 3:1. Other investigators have reported finfish catch rates between 2.8 and 18.0 kg per kilogram of shrimp caught (Watson and Taylor 1986). The amount of by-catch has led to growing concerns over the stock affects associated it. For example, a recent report of the mackerel stock assessment panel concluded that, "existing levels of mortality from shrimp by-catch in the Gulf of Mexico and an unquantified level in the Atlantic will reduce stock potential" (Southeast Fisheries Center 1991).

Through the period of the late 1940s to the late 1970s several researchers investigated by-catch, but usually their objective was to determine if there was a market for the by-catch (Keiser 1976, South Atlantic Fishery Management Council 1981). In general, by-catch was not commercially used because (1) few dealers are prepared to handle and ship incidentally caught fish, (2) the expected price is not worth the effort, (3) additional ice which increases costs would be required, (4) additional space for boxes would be required, and (5) culling for fish may delay icing of perishable shrimp (South Atlantic Fishery Management Council 1981).

There is substantial uncertainty within the biological community about the effects of by-catch on fish populations. Intuitively, given the large numbers of reported by-catch mortality, most observers believe there must be population impacts. Proving cause and effect is difficult because even if a by-catch associated fish stock is in decline, other factors e.g., environmental degradation, may be the culprit. At least one study in the mid 1970s concluded that there was no evidence that shrimp trawling activities were depleting stocks of commercial fish (Keiser 1976). Since this time, however, shrimping intensity and effective effort has increased. Commercial fishermen assert the carrying capacity of a given area will only support a certain amount of adult fish, and that they are only killing juveniles which would have suffered other kinds of mortality before reaching adulthood. Further, they believe that the discarded by-catch which does not survive is used by other species in the food chain.

Nevertheless, in recent years a number of estuarine dependent species associated with by-catch have declined in abundance. In particular, the NC Division of Marine Fisheries is presently concerned about the steady decline in summer flounder and weakfish landings in recent years. Recent evidence by Miller (Miller, et. al. 1990) suggests that in lagoonal estuarine systems such as occur in the Pamlico Sound system of North Carolina and many other estuaries in the Southeast, there is no evidence of density-dependent growth or survival. In these systems, the limiting factor is colonization, because the lagoons are poorly connected to the ocean, the source of larvae and juveniles. Because lagoons are typically not saturated by larvae or juveniles, increases in juvenile mortality caused by shrimp trawling should result in adult population reductions, because the system is non-compensatory. Miller has demonstrated through cage studies that the system could support a five fold increase in density of spot without affecting mortality or growth rates. Fishes with relatively slow growth rates, such as summer flounder, should be more affected by by-catch mortality than other species. From his work on lagoonal estuaries throughout the world, Miller believes the evidence is overwhelming in favor of the hypothesis that juvenile finfish by-catch mortality leads to reduced adult stocks.

A variety of questions remain which need further work by biologists to assist fishery managers. What are the inter-species relationships of reducing one species below optimal level? By introducing by-catch mortality are we boosting the population of other fish or scavengers such as blue crabs? Are pelagic fish populations (bluefish, king and Spanish mackerel) higher because adults have less competition for food? Or conversely, are they lower because prey species have been reduced? Because many of the species migrate between states, coastwide stock assessments will be required. Although more biological

work is needed, the kind of work required is complex and will take time to complete. Given the growing concern over by-catch, unless an aggressive by-catch research program is launched soon, fishery managers may be forced to act without the required information. The by-catch program of the GSAFDF identifies the need for a regional research plan, and as of this writing the steering committee has developed a draft research plan scheduled for release and adoption in early 1992.

Management Alternatives

Aside from the two extremes of ignoring the by-catch problem or banning commercial shrimping, fishery managers have several options available to them to reduce shrimp by-catch. These include traditional management measures such as seasonal and area closures and gear restrictions or modifications. Keiser's data show a much higher fish to shrimp catch ratio in May than in other months during the shrimping season (Keiser, 1976). The mean fish to shrimp ratio for May was 6.37 and 8.40 for the 1974 and 1975 seasons, whereas the ratio for the three following months ranged from 2.06-2.28 in 1974 to 2.15-3.36 in 1975. Although inshore waters are presently closed to shrimp trawling in South Carolina, the data suggest that managers in other states open to inshore shrimp trawling could use this kind of data to reduce by-catch. By delaying the opening of shrimp season until June in the South Carolina example, a significant reduction in by-catch is possible. A one month delay may be acceptable to commercial fishermen because average shrimp size and weight should increase from the delay.

Area closures should be considered where there is a high incidence of by-catch. Commercial trawling in North Carolina is prohibited in primary nursery areas during the entire year and secondary nursery areas during part of the year. In lagoonal systems where finfish populations are limited by larval and juvenile supply, additional areas should be considered for closure, while closure of secondary nurseries should be lengthened. Because the shrimp ultimately move out of the lagoons and into the ocean there would still be opportunities for harvest albeit later in the season and at different and in some cases more distant locations. Larger area closures or sanctuaries would have the additional benefit of reducing benthic disturbances and turbidity which would lead to improvements in the epibenthic community. Area closures should be based on whether or not the system is recruitment limited. Closures may be more necessary for lagoonal systems like the Pamlico and Albemarle Sounds, but not needed in drowned riverine inlet systems like the Cape Fear.

If a net design could be developed which is easy to deploy, reduces the by-catch, and maintains shrimp catch, it is likely to be more acceptable to fishermen than area or seasonal closures. Shrimpers would prefer to reduce by-catch because of the additional time required to sort the catch, the damage it causes to the quality of the shrimp, and because the extra weight in the tail bag reduces trawl door spread and fuel efficiency. Also, only a small percentage of the by-catch is of edible size and quality to be marketable (South Atlantic Fishery Management Council 1981).

For many years, shrimpers in the South Atlantic have modified their nets by installing "jellyball shooters" during times of jellyfish abundance. A "jellyball shooter" is a grid- type deflector which shoots jellyfish out an opening in the net. TED designs have developed from the shooters, and use the same deflecting principle. Research undertaken to improve TED designs has led to potential applications with by-catch.

Researchers at the NMFS Pascagoula Laboratory developed a funnel accelerator in front of the TED for the purpose of accelerating shrimp through the grid and past the turtle escape hole in order to minimize shrimp loss associated with TEDs. Recently, researchers at the Pascagoula Laboratory and UNC Sea Grant have experimented with the

accelerator for application to the by-catch problem. Laboratory tests indicated a 20 percent increase in water flow speed directly aft of the funnel and a 20 percent decrease in velocity in area above and below the funnel. Observations with a video camera sewn into the tailbag near the accelerator showed that shrimp were blown to the tailbag but fish moved to the slackwater at the sides of the funnel once past it. These observations have led to current work to evaluate by-catch reduction and shrimp retention rates using by-catch reduction devices (BRDs) that have escape holes for the fish in the slackwater areas at the sides of the funnel. Early results from 60, 1.5 hour tows using three BRD designs off Brunswick, Georgia, although inconclusive, suggest that under the right conditions (daytime with average inshore visibility) a 50 % by-catch reduction without significant shrimp loss can be achieved (Rulifson, et. al., 1991).

This kind of work is only a start. It appears that the escape behavior mechanism for the fish is mostly visual, i. e. BRDs will not work as well at night or in areas of high turbidity. In the Gulf of Mexico, about 90 % of shrimping takes place at night suggesting other designs which stimulate other kinds of escape behavior should be developed. Acoustic, electric, and light signals as well as physical stimuli all show promise for inducing an escape reaction in finfish. If the stimuli are positioned near escape holes, the devices may contribute to by-catch reduction.

In addition to reducing the incidental take of sea turtles, TEDs also function as BRDs. Unpublished data collected by the University of Georgia during TED certification trials on the R/V Georgia Bulldog off Cape Canaveral, FL during daytime hours show sizeable reductions of by-catch. For the seven TEDs presently certified, by-catch reduction ranged from 24 % with the "Georgia Jumper" to 57 % with the NMFS TED (Dave Harrington, University of Georgia Sea Grant, personal communication). During the 1991 shrimp season, investigators at the UNC Sea Grant College Program examined the skimmer trawl for its potential to reduce by-catch mortality. Because the tow time of the skimmer trawl is typically 30-40 minutes as compared with the 90 minute tow times typical of traditional inshore trawls, it was theorized that by-catch mortality should be reduced due to the reduced time the finfish spend in the tailbag and on the culling table. Preliminary results show that both by-catch mortality and abundance is reduced (Jim Murray, UNC Sea Grant, unpublished data).

The key to the success of BRD development will be for fishery managers to involve shrimpers. Their ideas should be solicited and incorporated in the design stage. This step will result in better designs, and the gear will more likely be accepted. The gear must also be tested in many areas and over several seasons. Trawling conditions throughout the Southeast vary considerably and it is highly unlikely that one design will be universally successful.

Economic Considerations

The Magnuson Fishery Conservation and Management Act (FCMA) of 1976 - P.L. 94-265 created eight regional councils to manage domestic and foreign fishing within the 200 mile limit. The function of the councils is to prepare fishery management plans and recommend regulations for each fishery. Plans are designed to produce the optimum yield annually, where optimum yield is defined as that amount of fish which will provide the greatest overall benefit to the nation with particular reference to food production and recreational opportunity. Optimum yield allows fishery managers to allocate fishery resources beyond maximum sustainable yield by considering social and economic factors.

The concept of optimum yield has generally been applied to single species management. When applying optimum criteria in the case of by-catch, inter-species questions arise. Does it make sense, as proposed by the Gulf of Mexico Management

Council in 1989, to ban shrimping in the Northern Gulf of Mexico for ten months in order to revitalize the red snapper population? The shrimp fishery is socially and economically of much greater importance in the Gulf region than the red snapper fishery. Might it not be optimum to sacrifice red snapper for shrimp production? Although there are no easy answers to the question, these kinds of economic and allocative issues must be addressed when considering by-catch management. Biologists must work with economists to develop better bio-economic models in order to assess inter-species trade-offs when managing for optimum.

Legal Authority and Enforcement

Legally, both the federal government through the fishery management councils and the State of North Carolina through powers delegated to the Marine Fisheries Commission, have the authority to manage by-catch. Section 1853 of the FCMA gives the following provisions to any Council and/or Secretary. The Council or Secretary may, designate zones where, and periods when, fishing shall be limited, or shall not be permitted only by specified types of fishing vessels or with specified types and quantities of fishing gear; establish specified limitations on the catch of fish (based on area, species, size, number, weight, sex, incidental catch, total biomass, or other factors), which are necessary and appropriate for the conservation and management of the fishery; prohibit, limit, condition, or require the use of specified types and quantities of fishing gear, fishing vessels, or equipment for such vessels, including devices which may be required to facilitate enforcement of the provisions of this Act. FCMA 16 U.S.C. 1853(b)(2-4) (1976).

Pursuant to the 1990 amendments to the FCMA, the Secretary's authority to implement management measures "to reduce incidental mortality of nontarget fishery resources in the course of shrimp trawl fishing which would restrict the period during which shrimp are harvested or would require the use of any technological device or other change in fishing technology" is prohibited until January 1, 1994. 16 U.S.C. 1854(g)(6)(A). The purpose of the 1990 amendment is to establish by regulation a 3-year program to assess the impact on fishery resources of incidental harvest by the shrimp trawl industry. The amendment also provides for the identification of fish stocks subject to incidental harvest, an assessment of the status and condition of such stocks, and data collection to determine the nature and extent of incidental mortality on such stocks including mortality other than shrimp trawling. 16 U.S.C. 1854(g)(3)(A-C). The amendment also requires the Secretary to commence a program in cooperation with affected interests to evaluate the efficacy of technological devices for the reduction of non-target fishery resources. In effect, Congress provided a moratorium on by-catch management until a three year extensive research program is conducted. A greatly expanded research program is expected during the next two years. Public awareness of the issue and expectations for management action will be heightened, and it is anticipated that potentially dramatic changes in by-catch management may occur in 1994.

In North Carolina, the North Carolina General Assembly delegates law making responsibility to the Marine Fisheries Commission. The statute empowering the Commission (G.S./113-182) states:

> "The Marine Fisheries Commission is authorized to authorize, license, regulate, prohibit, prescribe or restrict all forms of marine and estuarine resources in coastal fishing waters with respect to (1) time, place, character, or dimensions of any methods or equipment that may be employed in taking fish; (2) seasons for taking fish; (3) size limits on and maximum quantities of fish that may be taken, possessed, bailed to another, transported, bought, sold or given away."

Clearly, the Commission has broad powers which could be exercised to affect change related to by-catch in the shrimp fishery. Specifically, these powers could be used to mandate or restrict types of gear, restrict shrimp fishing seasons and limit the size and number of by-catch "taken."

The Division of Marine Fisheries, as a division of the Department of Health and Natural Resources, is charged with the stewardship of the marine and estuarine resources of the state of North Carolina. The Division has responsibility for implementing the laws of the Commission. Its charge is stated in Chapter 3A of the 1990 North Carolina Fisheries Regulations for Coastal Water:

> "It [the Division] is responsible for the maintenance, preservation, protection and development of all marine and estuarine fisheries resources. More specifically, the Division is required to administer and enforce all license requirements and taxes as set out in Article 14 of Chapter 113 of the North Carolina General Statutes to promulgate rules and regulations governing coastal fisheries and enforce them."

Again, it is clear that the Division has the responsibility to implement a broad range of management measures aimed at reducing by-catch, under the auspices of "maintaining," "protecting," and "preserving" "all" fisheries resources.

In a limited way, the Commission has used their powers to limit or reduce by-catch in other non-shrimp fisheries. For example, the use of trawl nets in internal coastal fishing waters except in crab or shrimp trawling is restricted; even in these fisheries it limits possession of finfish to 1,000 pounds. Another provision limits the take of by-catch in the menhaden fishery, while another gives the director of fisheries proclamation authority to establish fishing gear specifications to limit the incidental take of small flounders in the winter flounder fishery. And last, the Commission has made it unlawful to engage in fishing operations for scrap or trash fishing. These examples show that the Commission already has passed laws related to by-catch, and that further regulations related to shrimp by-catch are well within its authority.

Enforcement of seasonal, area or gear restrictions could be readily accomplished through existing federal, state or cooperative federal/state enforcement programs. State and federal fishery enforcement agents already enforce a variety of seasonal and area closures. BRD gear requirements could be enforced as part of the TED inspection program.

Policy Recommendations

The problem of shrimp by-catch is a marine resources management issue which will undoubtedly receive more attention in the foreseeable future. Fish stocks are declining for a variety of reasons, but shrimp by-catch is more obvious and easier to address than many of the other causes. Although more research is needed, fishery managers may not have the time, resources or technical expertise to come up with all the answers before the public demands action. Except for the temporary moratorium imposed on the Secretary of Commerce by the 1990 amendments to the FCMA, the NMFS through the Councils and the NCMFC have a clear legal mandate to require broad restrictions on current practices to conserve the resource. At this time, the various groups affected by by-catch decisions (environmentalists, sportfishermen, and shrimpers) are not exceedingly polarized over the issue and are more likely to negotiate and accept compromise management measures.

In order to achieve an acceptable compromise by diverse often opposing constituencies, good coordination, communication and citizen participation processes are required. Many analysts believe that a fundamental problem with the TED issue was that effective communications by NMFS were not achieved until opposing groups had become

too polarized for compromise. In part, this was because early communications attempts with the commercial fishing industry were ignored by it due to wishful thinking that the problem would go away.

In an effort to learn from these experiences, the GSAFDF has contracted with the NMFS to form a steering committee comprised of Council members, commissioners, sportfishing, environmental, commercial fishing, agency and university interests. The steering committee is responsible for developing a formal plan that describes the by-catch issue and establishes criteria for evaluating options to address the issue. The emphasis of the committee is to develop a practical consensus-based plan through a coordinated partnership of the various groups comprising it. The program developed by the steering committee is intended to fulfill the requirements identified by Congress in the 1990 FCMA amendments.

Similarly, in August, 1991 the NCMFC appointed a scrap fish committee comprised of Commissioners representing both commercial and sportfishermen. In November, the Commission adopted a policy directing NCDMF to establish the goal of reducing by-catch losses to the absolute minimum and to consciously incorporate that goal into all its fishery management considerations. In addition, it gave proclamation authority with prior consent of the Commission to the Director of Marine Fisheries to require BRDs or cod end modifications in trawl nets to reduce the catch of finfish that do not meet size limits or are unmarketable as individual food fish by reason of size. The scrap fish committee was directed to develop draft recommendations for affected fisheries by the Fall of 1992 to take to public hearing by Spring of 1993. Future regulatory changes at the federal and state level will almost certainly be a combination of the policies discussed above.

It is important that both NMFS at the regional level and the NCMFC at the state level seize the opportunity these committees provide, not only because it is within their legal mandate, but because if they don't, sportfishermen and environmental groups may by-pass the Council or Commission and obtain political resolution. The danger with this approach is that politicians may take a radical approach such that the resulting statutes would be highly inflexible. The committee process will be more acceptable to shrimpers if they are active participants.

Summary

Commercial shrimp by-catch has become increasingly conspicuous to the public eye, and is rapidly becoming an issue which must be addressed by fishery managers. There is increasing evidence of by-catch causing declines in finfish stocks, and recent work suggests that for some estuarine systems it may have serious implications for finfish stocks. Fishery managers have several alternatives which can be used to reduce by-catch, including seasonal, area, and gear restrictions. Both federal and state agencies have clear legal authority to implement these measures, although due to provisions in the 1990 FCMA amendments, this authority is presently in abeyance for federal waters until January 1, 1994. A policy is needed which brings together the various stakeholder groups to develop an orderly research, communications, and management program. Recent initiatives at the regional and state of North Carolina level are designed to develop a by-catch policy. It is important these efforts succeed, because if we wait much longer the adversarial groups may become further polarized and compromise will be impossible.

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IMPROVED VALUATION OF CHANGES IN FISHERY HARVESTS: MEASURING EFFECTS ON CONSUMER AND SEAFOOD SUPPLY FIRMS

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Changes in harvests of one or more commercial seafood species may have repercussions on a number of markets. This paper discusses a recently developed technique for valuing the effects of those changes in harvests. The technique to be applied is the general equilibrium (GE) derived demand, as developed by Hicks (9), Diewert (6), and Gardner (8). Analysis of the economic welfare effects -- measurement of costs and benefits via price changes -- follows that of Just and Hueth (12), and is a special case of that discussed by Just, Hueth and Schmitz (13). The analytical base for measurement of welfare effects in horizontally-related markets (e.g., species other than the one being subjected to reduced harvests, and which substitute in production for the given species), and an application to a fishery are discussed in Thurman and Easley (18).

Valuation of changes in harvests is an important component of the regulatory/policy scenario. The allocation of harvests between recreational and commercial fishermen, for example, motivated our investigation of the GE derived demand, and is an important current issue in many fisheries. Managers ask in a reallocation of harvests from one group to another: Who gains and who loses, and by how much? In fact, management councils are required by federal legislation and executive order to estimate the economic effects of regulations.

We may also think of the GE derived demand function as a vehicle for valuing effects of environmental degradation that adversely affect stocks of fish, and consequently harvests. It also provides a vehicle for measuring benefits of, for example, water quality or fish habitat improvement.

The next section introduces the reader to what the GE function measures, and contrasts it with normal valuation procedures. The final section discusses why this valuation procedure may be of interest to seafood technologists, processors and distributors.

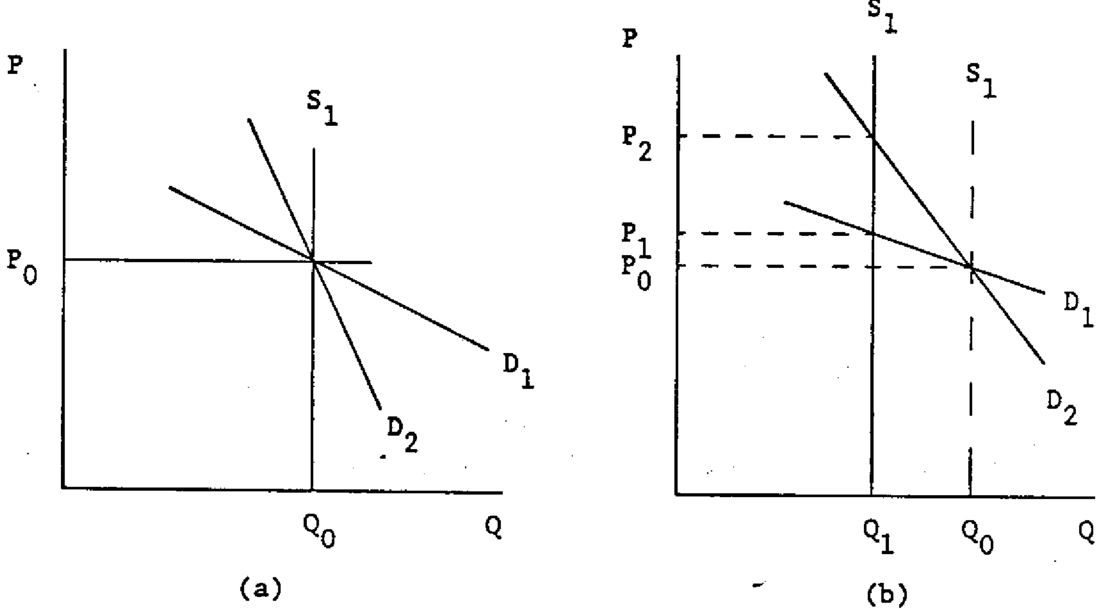
MEASUREMENT OF ECONOMIC EFFECTS OF CHANGES IN COMMERCIAL HARVESTS

The economics literature has concentrated in the past on identifying optimal rates of harvest, i.e., the rate of harvest from a stock that maximizes the present value of net economic returns to the resource. Examples of these models include Clark (4, 5); Anderson (1); Johnson and Libecap (11); and McConnell and Sutinen (15). The emphasis of these and other works has focused on the harvesting sector, but has examined a broad range of harvesting issues such as allowed harvest rates which appear to be suboptimal.

Consumers have received some attention in modeling the demand for seafood products and/or particular species. Some studies have emphasized at-home expenditures for various seafood products, with emphasis on characteristics of consumers which may explain variation in consumption (e.g., Cheng and Capps, 3). Others have examined systems of demand (see review in Emerson, 7). Wang and Kellogg (19) predict wholesale and vessel-level price changes likely as a result of proposed minimum size limits in a lobster fishery.

Economists recognize that costs may be imposed on consumers via higher prices when regulations or other phenomena cause reduced quantities of a given species to be harvested. How much price may rise depends upon the characteristics of the demand function facing the fishery. In panel (a) of Figure 1, for example, we have shown two

Figure 1. Hypothetical Demand



possible demand functions, D_1 and D_2 and vertical supply functions, S_1 and S_2 (supply is assumed fixed in the short run). Suppose this market is in initial equilibrium at P_0 and Q_0 Now suppose allowed harvest is reduced[NoteNum] by regulation to Q_1 (resulting in a new supply function S_1, as shown in panel (b). Note that if the demand for this species is D_1, the price increase is relatively small. In the classic "small fishery" case, in which demand is perfectly elastic (i.e., horizontal), no price change would result. However, if D_2 is our relevant demand, then the new price in this market is P_2 and consumers are much more adversely affected (though producers may gain from the price increase). The point of this illustration is that the characteristics of demand matter a great deal to evaluating the economic effects of policy.

Problems exist, however, in identifying various consumer products and markets through which any given species reaches the ultimate consumer. Not only do we face lack of information on what these products and respective quantities are, we also lack retail

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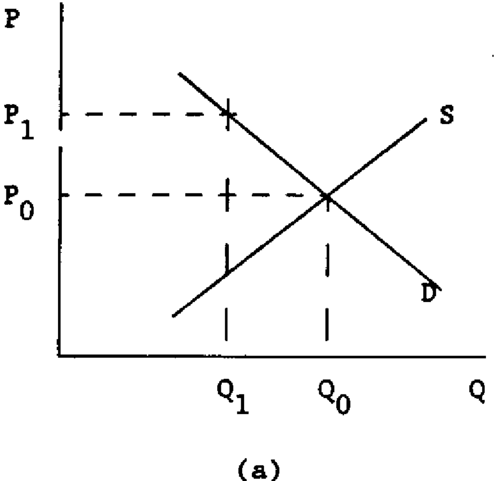
[1]The source of the reduced quantity harvested also could be from some form of environmental change.

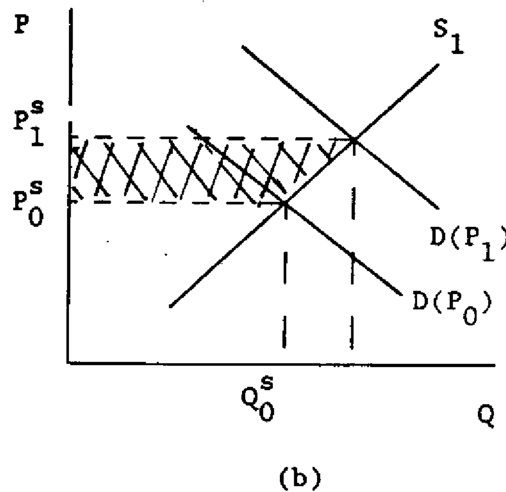
prices in most cases with which we might estimate those demands. Thus we frequently look to derived demand -- the demand for an input into a production process that is derived from the demand for the final consumer product -- to evaluate the effects of a regulation. The characteristics of the demand function facing harvesting firms depend upon the characteristics of the final consumer demand and how those price changes are transmitted between the different marketing levels. Important to this transmission of price changes is the production function(s) of firms processing and distributing seafood products.

The model developed in Thurman and Easley (18) differs from traditional models in fishery economics in that it explicitly treats fish harvested as an input into the production of final consumer seafood products. Thus the vessel-level demand, or derived demand, accounts for both characteristics of consumer demand, and of the production process which converts the input, harvested fish of a given species, into the final consumer commodity(ies).

One of the most important characteristics of the production process influencing derived demand is the degree to which other species are substituted for the one subject to regulation in production of consumer commodities. Suppose several species are considered good substitutes. Then those markets -- referred to as horizontally-related markets -- are likely to be affected by regulations which raise the price of the given species. How this occurs is illustrated in Figure 2. Suppose the markets are in initial equilibrium at $P_0 Q_0$ and $P_0^s Q_0^s$ where the superscript "s" refers to a substitute species. Then suppose a regulation reduces the harvest of the species illustrated in panel (a) to Q_1. Price in this market initially rises to P_1 due to the restriction. What are the effects in the substitute species market? Demand shifts out from $D(P_0)$ to $D(P_1)$, and price rises to P_1^s. Demand for the substitute species shifts as firms substitute away from the now-higher priced, regulated species. Note that as both the price of the regulated and substitute species have risen, the

Figure 2. Illustration of Effects of Regulation of a Single Species on Horizontally-Related Markets





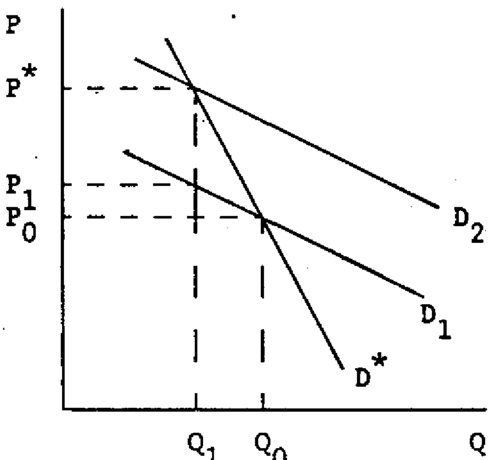
consumer price of commodities produced from these species will also rise, and quantity consumed will be reduced. In fact, it is possible (but unlikely) for the demand in panel (b) to be shifted backwards if reduced meal consumption outweighs the effect of substitution of the relatively lower price substitute input in production. These prices and demands will continue adjusting until new market equilibria are reached in all affected markets. Now, who gains and who loses due to the regulation?

As the price to consumers has risen, the consumer loses, and how much depends upon the price elasticity of demand for the commodity, which can be estimated if retail price-quantity data are available. Producers of the substitute species gain as demand for their input has shifted out. How much they gain depends upon the size of the demand shift, and the characteristics of the supply curve. The shaded area in panel (b) illustrates the size of this gain in our example.

We have not at this point told the full story in panel (a). As the price of the substitute species rises, the demand for the regulated species will also shift out due to the "feedback" from the price change in the market for the substitute species. This may, under certain assumptions, be reinforced by the changes occurring simultaneously in the market for the consumer product.

Figure 3 below illustrates the market for the regulated species after new market equilibria are reached. Our original equilibrium, P_0 Q_0 from panel (a) of Figure 2 is the same, as in P_1. However, due to price changes in other markets, we end up with the new demand curve D_2in this market, and a new equilibrium (P^*, Q_1). The new demand function, D^*, connects the two points of equilibrium, and is the "general equilibrium" (GE) derived demand for this species. It is a GE demand because it explicitly allows prices in related markets to change.

Figure 3. The GE Derived Demand



Regulated Species Market

In contrast to the GE function, the traditional derived demand function holds constant the prices of other input (species) that might substitute for the regulated one. It is this function that is illustrated as D_1, D_2in Figure 3. Were that function to be estimated, changes in economic surpluses along it would approximate changes in consumer well being, and exclude changes in those surpluses in the processing/distribution sector. Thus either more markets must be examined to fully account for the effects of regulation, or we fail to estimate the full costs of regulations.

Changes in economic surpluses estimated with the GE function are shown in Thurman and Easley (18) to estimate economic surpluses in horizontally-related markets (the substitute species), <u>and</u> surpluses in the consumer commodity market. The technique is powerful in that it estimates these economic effects in a single market: The vessel-level market for the regulated species. As Just and Hueth (12) have shown, the technique

estimates economic surpluses for producers in all vertically connected markets, and effects on the final consumer. It does not measure effects on vessels in the regulated market, however.

RELEVANCE OF THE GE DERIVED DEMAND TO THE SEAFOOD PROCESSING/DISTRIBUTION SECTOR

In this section we discuss several issues related to estimation of regulatory and environmental effects on the seafood consuming and processing/distribution sector, including reasons why we believe improved estimates of their costs are important. These issues are summarized as follows:

i) Potential bias in partial equilibrium estimation of regulatory costs and decomposition of GE welfare measures

ii) Improved estimation of benefits to:

  a) Stock enhancement via regulations

  b) Stock enhancement via water quality/habitat improvements

iii) Aquaculture and potential effects on seafood firms

As noted in the previous section, ignoring consumer and producer effects of changes in harvests will likely produce a bias in our estimation of the full costs of regulation. Management plans generally look at expected effects of a harvesting regulation on vessels, and attempt to estimate changes in vessel net revenues, catch per trip or other unit of effort, and numbers of vessels likely to exit the industry, to cite a few examples. Data above the vessel level to support these analyses are generally inadequate to carefully predict effects.

Consider an example the east coast summer flounder fishery. Proposed amendment one to the fishery management plan is discussing potential curtailment of harvests by about 50 percent for the first three years the amendment takes effect (Mid-Atlantic Fishery Management Council, 16). Such actions could involve significant price changes as quantities harvested are reduced by that magnitude. If prices to consumers of flounder commodities are increased significantly, consumers lose. This loss is illustrated below in Figure 4, panel (a), as the cross-hatched area. The reduced quantity of the input summer flounder, and assumed higher price, shift the output supply up. Firms producing the output may also lose as output is curtailed (even though price has risen). In the market(s) for substitutes for summer flounder, panel (b), the effects are ambiguous.[NoteNum]2 As the price of summer flounder rise due to the reduced harvests, firms producing flounder commodities will attempt to purchase more substitute species (Q^{ss}). This effect would tend to shift out demand, and increase profits to SS suppliers. However, as consumption of final flounder commodities is reduced, the output effect serves to reduce the demand for SS, in which case suppliers of the substitute species would lose profits. Whether SS suppliers gain or lose depends upon which of these effects dominate. We assume that the substitution effect dominates, and D^{ss} shifts out in panel (b). Finally, panel (c) illustrates the GE derived demand in the summer flounder market. The significance of this function is that it can be used to measure the loss of consumer surplus

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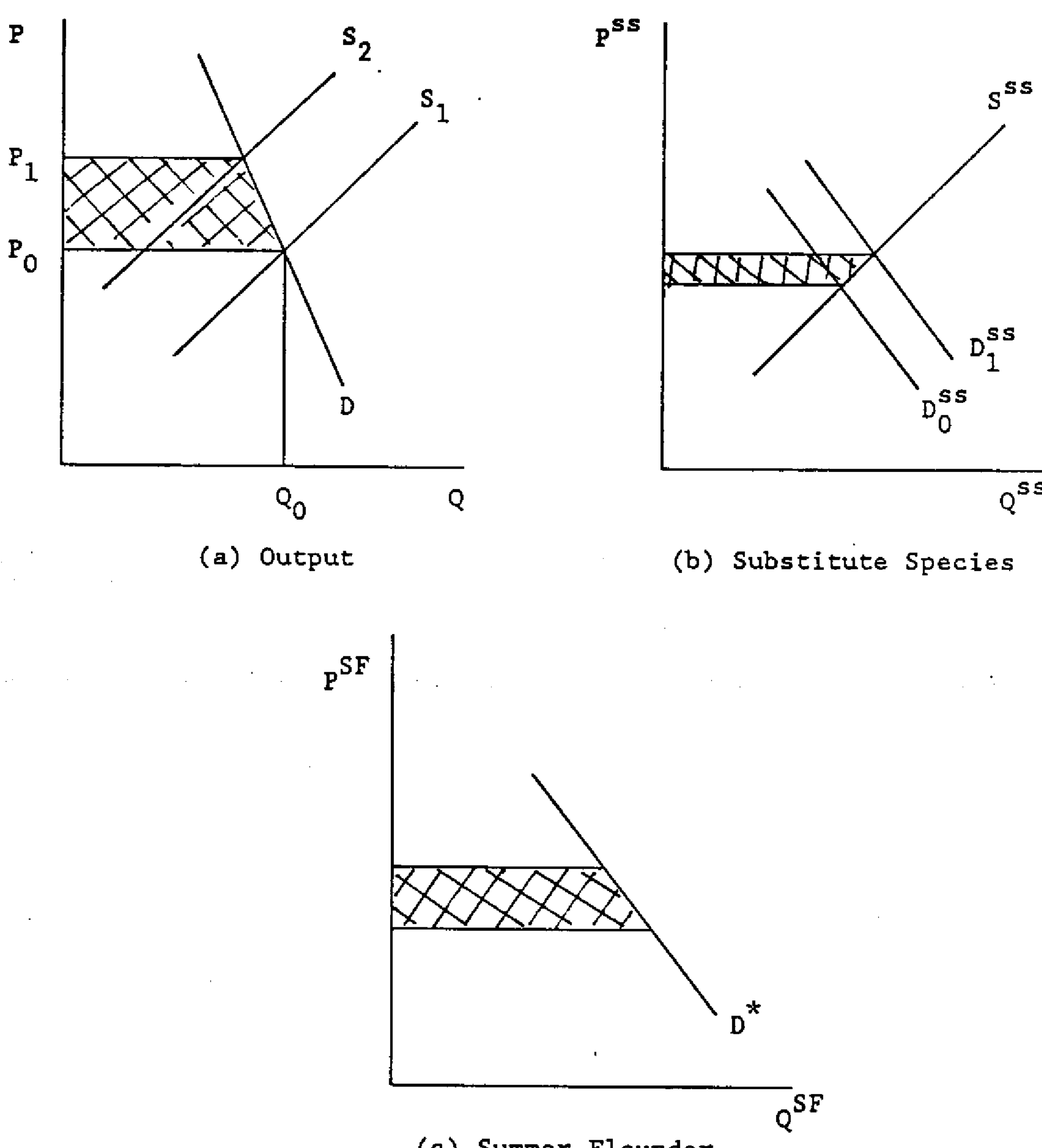
[2] This discussion draws from Thurman (1991).

(as shown in panel a) minus the gain in profits to suppliers of substitute species (as shown in panel b) plus the loss in profits to flounder commodity producers.

Figure 4. The Summer Flounder Output and Input Markets



In Thurman and Easley (18) it is found that GE estimates of welfare losses due to reduced Gulf red drum harvests exceeded the partial equilibrium estimate by 43 percent. The circumstances under which a partial equilibrium estimate of regulatory costs would not be biased is if output-producer effects exactly offset effects on suppliers of substitute species (some of whom may be foreign exporters). The circumstances under which such offsets could occur have not been explored. To fully ascertain these effects in different

markets is, in effect, to decompose the welfare estimate from the GE derived demand function. This is a complex task as we may not understand all the markets (other species, etc.) affected.

ii) Improved Benefit Estimation of Stock Enhancement

Use of the GE derived demand function can improve estimates of benefits to policies that enhance stocks and, consequently, allowable harvests. Two classes of policies are briefly discussed: Regulatory actions of fishery managers and policies designed to enhance water quality/habitat.

Managers are frequently confronted with the problem of reducing harvests in the current period, or for several years, in
order to allow stocks to rebuild for larger subsequent harvests. The GE function provides us with a vehicle for more complete accounting of those costs (of current period reduced harvests). It also could be used to value larger, future harvests from those rebuilt stocks. Managers, when confronted with significant costs of curtailing current harvests, want to know what future benefits (larger future harvests) are worth. The GE derived demand, in conjunction with future stock/harvest predictions, could value the multi-market effects of those larger harvests.

Similar arguments apply to water quality and habitat improvements that could lead to larger stocks of fish and shellfish. We note that most of the estimated benefits to water quality improvements have relied on recreational fisheries and selected other water users as the sources of those benefits. This ignoring of the commercial fisheries sector as a potentially significant source of benefits to water quality improvements may be due to several factors. Among these are: i) a general lack of retail data has ruled out or limited efforts to estimate effects of larger future harvests on consumers; ii) traditional economic models of open-access, common property fisheries have emphasized rent dissipation in the harvesting fleet, and changes in rents due to larger harvests would be the relevant benefit measure.[NoteNum] [3] However, the traditional model suggests that there would be no lasting benefit in the vessel sector to stock enhancement as those rents would be competed away by larger effort; and iii) EPA -- the major funder of environmental benefit studies -- may have assumed that NMFS as the lead fisheries agency has responsibilities for such benefit estimation in commercial fisheries, yet NMFS continues to be primarily a biological-oriented agency.

The GE function provides a technique to estimate such benefits. However, the links between water quality or habitat, stocks, and subsequent harvest may require further refinements to be able to reasonably predict changes in future harvests, hence future net benefits.

iii) Aquaculture and the Wild-Harvest Fishery

Only recently have economists begun to look at interactions between aquaculture and wild harvest of the same or nearly identical species. Bell (2) modeled the Louisiana crawfish industry, and found that the existence of large quantities of privately-owned pond crawfish reduces rent dissipation in the wild-harvest sector. He argues that this occurs because the lower price reduces effort and harvest from the commons. We note that the demand function used was a partial equilibrium function, but separate estimations were

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[3] We note that recent models acknowledge that rent may exist in open-access fisheries. See Johnson and Libecap (1982) and Karpoff (1987).

made for rents to producers. Thus, potential effects on rents to processors and distributing firms are not included (though we have little information on how important this sector is in the crawfish industry).

Johnson (10) also raises questions about interaction between aquaculture and wild-harvest of the same species. His emphasis is on incentives created for different types of regulations, and how those influence rent dissipation in the wild-harvested fishery, or the blocking of a competing aquaculture industry (e.g., the pen-rearing of salmon in Alaska). He also expresses concern that if the wild harvest poses a competitive threat to the aquaculture sector, ". . . there is little incentive for those engaged in captive rearing to protect the natural habitat" (p. 138).

These interactions, and how incentives are affected to preserve and enhance wild stocks are just beginning to be explored. It is obvious that the traditional seafood processing and distributing industry has a strong vested interest in the outcome of these processes.

Following this reasoning for a moment, let us think about interactions between aquacultured fish and wild-harvested seafood in aggregated (across species) terms. If production of aquacultured species grows relative to demand growth or wild-harvested supplies, and those species are viewed as substitutes by consumers, then consumer welfare will grow. Another effect, however, will be to reduce demand over time for wild-harvested species. That in turn will reduce rents to the processing/distribution sector if it is specialized in products from wild-harvested stocks (i.e., if the aquaculture sector develops its own processing/marketing distribution system). To the extent that we may be subsidizing the development of aquaculture, we should be aware of those costs on the wild-harvest sector which are indirectly imposed by market forces. We may also want to encourage utilization of existing processing/marketing/ distribution capacity in the seafood sector where excess capacity exists, and doing so is economically feasible.

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AN ECONOMIC ASSESSMENT OF CHANGES IN THE SOUTHEAST FINFISH PROCESSING INDUSTRY

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INTRODUCTION

The Southeast Region of the United States[1] is recognized for its abundance of fishery resources and its harvests contribute significantly to the U.S. domestic supply of edible seafood products. While it may best be known for its large shellfish harvests-especially shrimp, oysters, and crabs- the region is also a major producer of edible finfish products.

A distinguishing element of the Southeast edible finfish industry relates to the large number of species produced. Several of these species including red snapper, grouper, king and Spanish mackerel, and red drum were the subject of declining catches and/or harvesting restrictions in recent years. Imports of many of these same species through Southeastern ports, however, appear to have increased during the same period (Adams and Lawlor). This increase may be in response to a growing deficit between domestic demand and domestic supply of products harvested in the Southeast.

Southeast U.S. finfish processors use domestic and, to an unknown extent, imported sources of raw material, i.e, unprocessed or semi-processed finfish, in processing activities[2]. The overall goal of this paper is to provide an economic overview of the Southeast finfish processing industry based on available secondary data. Specific objectives and methodology used in the paper are presented below.

OBJECTIVES AND METHODS

Five specific objectives will be accomplished with this paper. They are as follows:

(1) To document the number of Southeast finfish processing plants and changes therein, especially in recent years
(2) To document changes in the quantity and value of Southeast finfish processing activities in total and on a per plant basis
(3) To document stability in the Southeast finfish processing industry and changes therein
(4) To document industry concentration among Southeast finfish processors
(5) To relate changes in the structure of the Southeast finfish processing sector, as outlined in the previous four objectives, with changes in Southeast finfish harvests and imports.

The National Marine Fisheries Service (NMFS), Fisheries Statistics Division, collects and maintains the most complete database on the Southeastern finfish processing industry. This database, which was used to accomplish the aforementioned objectives, includes detailed historical activities on each processing plant (eg. quantity, value, and product form of each species processed) and is collected during end-of-the-year voluntary surveys. The period of analysis is for the years 1973-88, inclusive.

A wide variety of products are produced by finfish processors in the Southeastern United States. To facilitate comparison among species and product forms, all reported products in this study have been converted to round weight based on conversion factors developed by NMFS. Converting the pounds of raw king mackerel fillets into round pounds, for example, was carried out by multiplying the pounds of fillets by the NMFS conversion factor (i.e. round weight = pounds of raw fillet * 2.86). Similar calculations were made for each species and product form.

For purposes of analysis, non-edible processed finfish products and processed catfish have been excluded from this analysis. The list of non-edible products produced from finfish would include such items as fish meals, oils, pet foods, animal feeds, canned croaker, and items for medicinal purposes. Catfish were excluded from the analysis because this study is only concerned with the processing of wild finfish stocks and because the emphasis of the study is on marine fish species. It is noted, however, that some species included in the analysis, such as salmon, may not be harvested in the Southeast but are brought to the region for processing.

SOUTHEAST FINFISH HARVESTS AND IMPORTS

Domestic Harvests

Annual Southeast finfish harvests, excluding menhaden, remained relatively stable during the 1973-88 period when evaluated on a four-year basis (Table 1). The high four-year annual average of 224.2 million pounds during 1985-88, for example, exceeded the low four-year annual average of 195.8 million pounds during 1977-80 by less than 15%.

Though the annual Southeast edible finfish harvests[3] remained relatively stable during 1973-88 when expressed on the basis of poundage, the value of these harvests adjusted for inflation to 1988 dollars, experienced a significant increase (Table 1). For example, the deflated value of edible finfish harvests during 1985-88, averaging $157.8 million annually, exceeded comparable statistics for the 1973-76 period by 50%. Most of the increase in deflated value, as indicated in Table 1, was the result of an increase in the deflated price per pound for the harvest, rather than any long-run increase in the harvest. This increased real price may be the result of several factors including but not limited to (1) increased demand for Southeast edible finfish relative to supply, (2) harvests of more desired, higher priced, finfish species, (3) some combination of the two.

Imports

As noted, imported raw materials, ie, unprocessed or semi- processed finfish products, are also used to an unknown extent by the Southeast U.S. finfish processing industry. While detailed import statistics are not maintained for most finfish species comparable to those harvested in the Southeast, such as grouper and king mackerel, recent analysis by Adams and Lawlor (1989) helps to identify some changes in the Southeast finfish import situation. Overall, the authors found that imports of key species[4] into Southeastern ports of entry increased from 17.4 million pounds (product weight) in 1983 to 70.4 million in 1987 while the number of imported species expanded from 32 to 60. The imports of whole fish, that product form most likely to be used in Southeast U.S. finfish processing activities, grew from less than 20 million pounds to more than 50 million pounds

during the five-year period ending in 1987. Among products of particular interest to Southeast finfish processors, imports of whole snapper increased from about 4 million pounds in 1983 to more than 12 million pounds in 1987, imports of whole grouper increased from less than one million pounds to almost six million pounds, and whole king mackerel imports expanded from just over six-hundred thousand pounds to 1.8 million pounds.

Table 1. Selected Statistics Pertaining to Southeast U.S. Finfish Harvests (Excluding Menhaden), 1973-88, Four Year Averages.

TIME PERIOD	POUNDS HARVESTED	DEFLATED VALUE	DEFLATED PRICE
	(Mill. lbs.)	($ Mill.)	($/lb.)
1973-76 avg.	217.6	105.5	0.485
1977-80 avg.	195.8	116.9	0.597
1981-84 avg.	217.1	132.3	0.609
1985-88 avg.	224.2	157.8	0.704

Source: Unpublished data provided by National Marine Fisheries Service.

FINFISH PROCESSING RESULTS

Number of Processing Plants and Industry Size

As indicated by the information contained in Table 2, an average of 71 finfish processing plants were active on an annual basis in the Southeast U.S. during 1973-76. By 1985-88, the number had risen almost 75%. While an increasing number of plants was evident during each four-year period of analysis, it was especially pronounced through the 1981-84 period. While the average of 123 plants during 1985-88 represented a net increase of 11 from the previous period, this increase was only about one-half that observed in other periods.

Total edible finfish processing activities in the Southeast also increased during 1973-88 when evaluated on the basis of either pounds or value. For example, the total number of pounds processed (round weight) more than doubled during the period of analysis, from 45.6 million annually during 1973-76 to 99.6 million annually during 1985-88. Similarly, the average annual deflated value of the processed product increased about 125%, from $46.1 million to $103.9 million. The information suggests that the largest increase in processing activities occurred between the 1977-80 and 1981-84 periods, when the processed pounds increased by more than 70% and the related value (adjusted for inflation) increased by more than 40%. This time frame coincided with the greatest increase in the number of plants. While processing activities continued to expand beyond the 1981-84 period, they did so at a reduced rate.

Table 2. Number of Southeast Edible Finfish Processing Plants and Related Processing Activities, 1973-88 Four Year Averages.

TIME PERIOD	NO. OF PLANTS	PROCESSED LBS.[a] Total	Per Plant	DEFLATED VALUE[b] Total	Per Plant	DEFLATED PRICE[c]
	#	--- 1,000s ---		-- $1,000s --		$/lb.
1973-76 avg.	71	45,625	640	46,081	647	1.01
1977-80 avg.	90	49,601	550	58,094	644	1.17
1981-84 avg.	112	84,085	749	82,049	731	0.98
1985-88 avg.	123	99,606	811	103,893	846	1.04

[a] Processed pounds have been converted to round weight using NMFS conversion factors.
[b] Deflated value is based on the 1988 Consumer Price Index (i.e., 1988=100).
[c] Derived by dividing the deflated value by processed pounds.

The increased finfish processing activities in the Southeast during the period of analysis was the result of two factors. First, as noted, there was an expansion in the number of plants engaged in finfish processing activities. Second, processing activities per plant grew during 1973-88. Pounds processed per plant, for example, increased from an average of 640 thousand annually during 1973-76 to 811 thousand annually during 1985-88 (Table 2). The deflated value of these processed products, on a per plant basis, increased from $647 thousand annually to $846 thousand annually.

Some finfish processing plants process only finfish products. Others process both finfish and shellfish products. As indicated by the information contained in Tables 2 and 3, about one-third (23 of 71) of the reported Southeastern finfish processing plants during the 1973-76 period produced only finfish products. The other two-thirds (48 of 71 plants) produced both finfish and shellfish products. By 1985-88, however, almost 60% of the reported finfish processing plants processed only finfish products (71 of 123 plants). In general, most of the growth in the number of Southeast finfish processing plants during 1973-88 (71 to 123) appears to be among plants specializing only in the processing of finfish products. Plants of this kind increased from 23 to 71. By comparison, plants that processed both finfish and shellfish species equalled 48 during 1973-76, increased somewhat during the next two four-year periods, and then fell sharply to an average of 52 during 1985-88.

As noted, total Southeast finfish processing activities expanded from 45.6 million pounds to 99.6 million pounds, or a total of 54.0 million pounds, during the period of analysis (see Table 2). Plants processing only finfish products represented about one-half of this total increase while plants processing both finfish and shellfish products contributed the other one-half. On a per plant basis, however, plants producing only finfish products showed no growth in pounds processed. Plants processing both finfish and shellfish products showed significant growth in the pounds of finfish processed (an average of 662

thousand pounds during 1973-76 compared to 1.16 million pounds during 1985-88). Overall, plants that processed only finfish received a higher deflated price per pound for their products than plants that processed both finfish and shellfish products.

Table 3. Selected Statistics Related to Southeast Edible Finfish Processing Activities By Type of Establishment[a], 1973-88 Four Year Averages.

TIME PERIOD	NO. OF PLANTS	PROCESSED LBS.[b] Total	Per Plant	DEFLATED VALUE[c] Total	Per Plant	DEFLATED PRICE
	#	--- 1,000s ---		--- $1,000s ---		$/lb.
Finfish products only						
1973-76 avg.	48	33,015	688	31,772	662	0.96
1977-80 avg.	63	34,985	555	38,241	607	1.09
1981-84 avg.	61	56,893	933	52,157	855	0.92
1985-88 avg.	52	60,320	1160	56,700	1090	0.94
Finfish and shellfish products						
1973-76 avg.	23	12,610	542	14,309	615	1.13
1977-80 avg.	27	14,616	536	19,853	728	1.36
1981-84 avg.	51	27,192	533	29,892	586	1.10
1985-88 avg.	71	39,286	555	47,193	667	1.20

[a] For purposes of these tables Southeast finfish processing establishments have been categorized as to whether they process only finfish products or finfish and shellfish products.
[b] Processed pounds have been converted to round weight using NMFS conversion factors. This poundage reflects only finfish.
[c] Deflated value is based on the 1988 Consumer Price Index (i.e., 1988=100).

Stability

For purposes of this paper stability in the Southeast finfish processing industry was analyzed in relation to entry and exit among its plants[5]. A relatively large degree of entry and exit in relation to the total number of plants would suggest that barriers to entry are minimal. It may also indicate that plants enter and exit in relation to raw material supplies. In years when domestic finfish harvests are good, for example, there may be a large increase in finfish processing plants. In years when domestic finfish harvests are poor, there may be a large decrease in the number of finfish processing plants. One would expect, however, that increased imports could mitigate industry turnover via a more stable supply source.

As indicated by the information contained in Table 4, entry into the Southeast finfish processing industry was common during the period of analysis. During 1974-76, for example, an average of 12 plants entered into finfish processing activities on an annual basis. The number of entering plants continued to expand throughout the study period, equalling 30.8 annually during 1985-88. Overall, the entry rate among plants in the Southeast finfish processing industry averaged about 18% per year during the 1974-76 period (12.0 divided by 68 total plants) and increased to close to 25% per year in each of the three remaining four-year periods.

While a large number of plants initiated finfish processing activities on an annual basis during 1974-88, many also ceased operations. For example, while 12.0 plants initiated finfish processing activities on an annual basis during the 1974-76 period, 15.3 plants ceased these activities. As with the entry rate, the exit rate among Southeast finfish processing plants generally averaged close to 25% annually, with two exceptions. The most recent of these exceptions occurred during the most recent four-year period. During this period the exit rate fell to 15% annually and the total number of plants increased from 99 in 1984 to 149 in 1988.

A comparison of the information contained in Tables 2 and 4 indicates that entering plants had a lower level of finfish processing activities than the more established plants. During 1977-80, for example, entering plants processed 153 thousand pounds of processed product annually compared to 550 thousand pounds among the more established plants. During the most recent four-year period, entering plants processed 40% that of the established plants in terms of pounds, on average, and almost 50% by value.

Processing activities among plants that ceased finfish processing activities also tended to be relatively small, on average, when compared to their more established counterparts. During the most recent four-year period for example, exiting plants averaged 236 thousand pounds of processed product annually compared to 846 thousand pounds for the industry in total.

Concentration

Concentration in the Southeastern finfish processing industry was analyzed on the basis of relative market share of the largest five, ten, and twenty plants. As indicated by the information contained in Table 5, the five largest processing plants consistently accounted for more than 50% of the finfish processing activities throughout the period of analysis, when expressed on the basis of poundage. The concentration among these largest plants, expressed on the basis of value, was somewhat less, however, indicating that plants processing the most product were not necessarily processing the products with the greatest value. The ten largest finfish processing plants represented about 65% to 76% of processing activities depending upon the period of analysis, with the lower concentration occurring during the most recent four-year period. While the largest twenty plants accounted for 90% of finfish production activities during 1973-76, their share had fallen to less than 80% by 1985-88.

Analysis of the data also indicated that four of the ten largest Southeastern finfish plants had been in business since at least 1973, the first year that complete data on all plants was available. Finfish processing activities among these four plants averaged about 6.8 million pounds (round weight) each in 1988, value of $6.26 million. Another three of the ten largest plants had been in operation for at least eight years. Processing activities among each of these three companies averaged close to 4.7 million pounds in 1988, valued at $3.7 million. The remaining three companies had been in operations less than eight years and averaged 2.6 million pounds of finfish processing activities on a per plant basis.

Table 4. Average Annual Entry and Exit Among Southeast Finfish Processing Plants and Related Processing Activities, 1973-88.

TIME PERIOD	TOTAL PLANTS	NO. OF PLANTS		PER PLANT PROCESSED POUNDS [a]		PER PLANT DEFLATED VALUE [b]	
		Entering	Exiting	Entering	Exiting	Entering	Exiting
1973	81						
1974-76	68	12.0	15.3	183	218	175	187
1976	71						
1977-80	90	21.8	13.3	153	131	213	106
1980	105						
1981-84	112	25.0	26.5	240	294	212	219
1984	99						
1985-88	123	30.8	18.3	323	236	407	254
1988	149						

[a] Processed pounds have been converted to round weight using NMFS conversion factors.
[b] Deflated value is based on the 1988 Consumer Price Index (i.e., 1988=100).

Table 5. Estimated Concentration in the Southeast Edible Finfish Processing[a] Industry, 1973-88.

TIME PERIOD	N = 5		N = 10		N = 20	
	Lbs.	Value	Lbs.	Value	Lbs.	Value
	%		%		%	
1973-76	59.5	22.7	75.8	71.5	90.0	88.3
1977-80	51.6	36.8	69.3	61.4	84.7	82.4
1981-84	58.1	36.4	74.7	64.2	86.4	81.8
1985-88	50.6	37.2	64.8	55.1	78.0	71.3

[a] Plants were ranked on the basis of pounds produced (round weight) and values are related to the same group of plants. A ranking by value would likely lead to different results.

DISCUSSION

The final objective of this paper was to relate changes in the structure of the Southeast finfish processing industry with changes in Southeast finfish harvest and imports. Several points in relation to this objective are listed below.

(1) There was significant growth in the Southeast finfish processing industry, measured in terms of pounds processed, despite relatively stable domestic harvests. This indicates processors were (a) increasing the share of domestic finfish harvests that went into finfish processing activities, (b) increasing the use of imports, or (c) some combination of the two.

(2) While the harvest price of edible Southeast finfish increased significantly during the period of analysis the processed finfish price has shown no increasing trend. This may be due to (a) increased use of lower priced, domestically harvested species in processing activities, (b) increased use of lower priced imports, (c) a decline in the processor's marketing margin, or (d) some combination of the above factors.

(3) The exit rate among Southeast finfish processors was relatively low during the 1984-88 period when compared to other periods. This decline may be the result of a more consistent supply brought forth by increased imports since the early 1980s. Additional years of data, however, are needed to further assess this hypothesis

(4) A significant decline in industry concentration was evident during the most recent four year period of analysis. This may be due to the increase in imports which may have allowed smaller plants to expand their processing activities through new sources of supply. Also, the new supply source may help explain the increase in the number of finfish processing plants which, ceteris paribus, will result in a reduction in industry concentration.

ENDNOTES

1. Defined by the National Marine Fisheries Services as the coastal states of North Carolina through Texas

2. Finfish processing in the Southeast U.S. would include, but is not limited to, processing activities such as filleting, dressing, gutting, smoking, breading, salting and canning. The processing of finfish into frozen and canned specialty products is also an important sector of the industry.

3. It is considered for purposes of analysis that total Southeast finfish harvest minus the menhaden harvest equals edible finfish production. In reality this figure likely exceeds actual edible finfish.

4. See Adams and Lawlor for a list of key species. Shellfish species, excluding shrimp, are included in the list.

5. Turnover, or entry and exit, can result from three factors. First, existing plants can add or delete finfish products from their processing lines. Second, plants can change ownership which results in both an exit and entrance. Finally, plants that cease operations are recognized as an exit and new processing facilities are recognized as an entrance.

ACKNOWLEDGEMENTS

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SEA CLAM SOLID WASTE GENERATION IN NEW JERSEY

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Disposal of wastes is a serious and growing problem for surf clam and ocean quahog processors. While the industry is expanding, disposal costs are rising and environmental regulations are becoming more stringent (7).

Sea clams, an inclusive term for both surf clams and ocean quahogs, support an important commercial fishing and processing industry in the United States. New Jersey is a major contributor to this industry. In 1990, national total landings of the two species was 118 million pounds of meats valued at $54 million (9). The state's share of the 1990 national surf clam landings rose from 44% in 1981 to 62%. Similarly, it's share of the 1990 national ocean quahog landings rose from 58% in 1981 to 70% (Table 1).

Table 1. United States and New Jersey Surf Clam and Ocean Quahog Landings (million lbs. of meats)

	Surf Clam			Ocean Quahog		
	United States	New Jersey	NJ/ US%	United States	New Jersey	NJ/ US%
1981	46,100	20,290	44	36,100	20,805	58
1982	49,700	24,420	49	34,800	22,856	66
1983	55,900	24,370	44	35,200	21,284	60
1984	70,200	39,200	56	38,800	21,469	55
1985	72,500	33,160	46	52,000	28,934	56
1986	78,700	36,490	46	45,400	24,218	53
1987	60,700	35,820	59	50,300	24,395	48
1988	63,500	37,150	59	45,900	17,537	38
1989	67,100	42,880	64	51,000	27,040	53
1990	71,733	44,752	62	46,313	32,428	70

Source: U.S. Dept. of Commerce, National Marine Fisheries Service

New Jersey not only lands more sea clams than any other state, but also generates the majority of the waste. In 1991, seven sea clam processors operated in Cape May and Cumberland Counties. Primary processing involves the shucking of clam meats from the shell. Secondary processing involves the production of such well-known products as canned, minced and chopped clams, clam chowder, clam sauce, and breaded clam strips. Two plants engage in shucking only, three in both shucking and further processing, and two in secondary processing only. Four of the plants are relatively small, while three are large national corporations.

Clam shucking produces liquid and solid waste products in addition to edible meats and juice. Liquid effluent contains soluble and suspended solids consisting of non-recovered meat, viscera, and sand (1). Treatment of liquid effluent at the processing plant include screening, aerobic or anaerobic digestion, and settling results in partial recovery of suspended solids. Unrecovered suspended solids are discharged to municipal waste treatment facilities and waterways. The predominant solid waste products are shells with some unrecovered meats attached, and recovered viscera (belly waste). The shells are generally put to productive use on road beds. However, clam viscera is merely disposed of in landfills.

Costs for sending viscera wastes to landfills have risen dramatically. From 1987 to 1991, Cape May's landfill tipping fees, including tax, increased by 115% from $38.80 to $83.50 per ton and Cumberland County's landfill tipping fees increased by 56% from $41.64 to $64.87/ton (Figure 1).

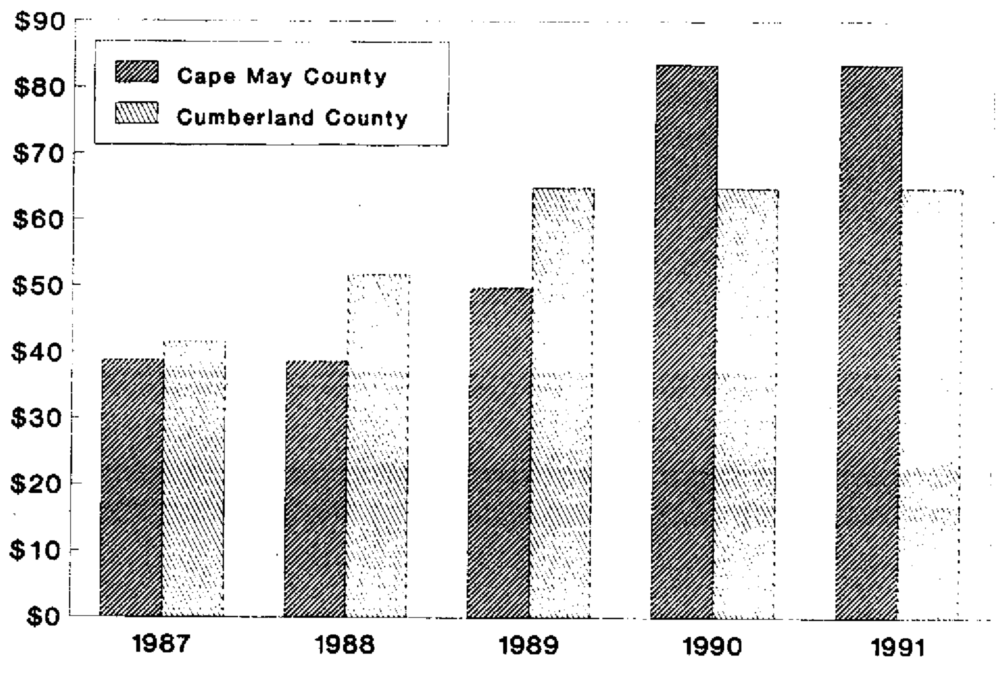

Figure 1.South Jersey Landfill Tipping Fees,1987-91 (includes tax)

An additional solid waste disposal cost is transportation to the landfill. Processors use their own vehicle or hire a hauling company to carry their waste to landfills. Trends in this portion of the total solid waste disposal cost are unavailable because the industry does not divulge contractual information. Nevertheless, the rise in landfill costs alone is a strong incentive for New Jersey clam processors to seek alternative uses for those wastes.

A number of alternative uses for organic sea clam processing waste have been suggested including edible foods and juices (3,8), flavor extracts (2,4), and animal feed (7). This study contributes to the development of these uses and the reduction of the waste disposal problem for sea clam processors by quantifying the amounts of solid sea clam wastes generated annually in New Jersey. An estimate of potential supplies of waste will make possible improved assessments of the technical and economic feasibility of proposed uses.

METHODOLOGY

The quantity of solid wastes generated by sea clam shucking operations in New Jersey is calculated from government landing data and estimates of percent composition (Figure 2). The National Marine Fisheries Service (NMFS) publishes landing data for surf clams and ocean quahogs as "pounds of meats". To provide a basis for determining weights of waste components, these data must be converted to pounds of whole clams. NMFS obtains sea clam landing data from fishermen logbooks which are reported in "bushels of whole clams". Logbook reports are converted into reported landings by use of separate conversion factors for each of the two clam species relating the weight of total meats to the weight of a bushel of whole clams.

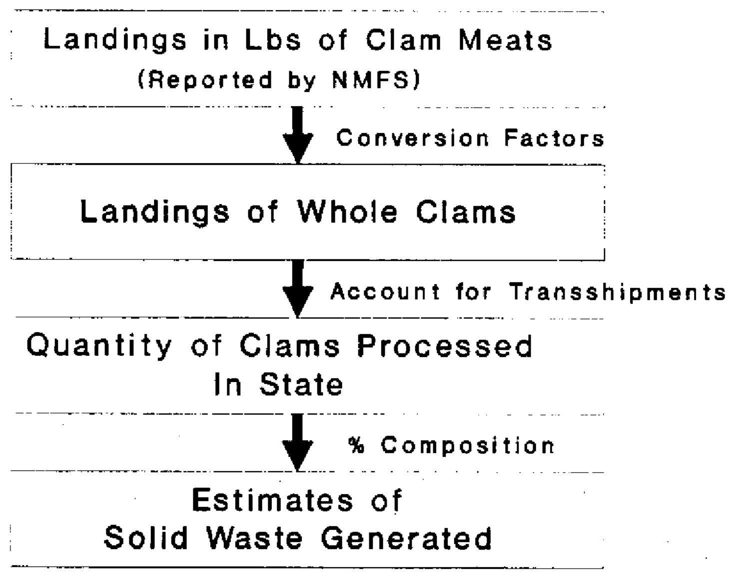

Figure 2. Method of Estimating New Jersey Sea Clam Solid Waste

Reversing the NMFS calculation yields pounds of whole clams landed. NMFS assumes 17 lbs. of meats per 90 lbs. of whole surf clams and 10 lbs. of meats per 80 lbs. of whole ocean quahog. These ratios were determined several decades ago, but continue to be used for consistency.

Landing data is adjusted to take account of transshipment in and out of the state in order to more accurately estimate quantities received by New Jersey processors. Some clams landed in New Jersey are trucked to other states for shucking. These amount must be estimated and subtracted from the previous total. On the other hand, New Jersey processors also receive whole clams landed in other states, so this amount must be added. Although the logbooks supplied to NMFS by fishermen and processors are confidential, NMFS is able to supply the total number of vessels which land sea clams in New Jersey, the number of vessels whose landings are trucked out of New Jersey, and the number of vessels which landed out of state but whose landings are trucked into New Jersey for shucking.

The next step in estimating wasted generated is to separate quantities processed into waste and usable components. Zall and Cho (8) estimated the composition of whole surf clams as 53% shell, 21% recoverable meat, 10% juice and 16% viscera and non-recoverable meat.

Scientific analysis of ocean quahog composition has not been undertaken. Estimates of quahog composition are made based on industry and NMFS expertise. Composition estimates in Figure 4 are obtained through the following procedure. The base calculation is the weight of ocean quahogs per bushel, reported by NMFS as 80 lbs. (William Brey, NMFS - Statistical Investigation, Oxford, MD,
personal communication). In calculations of annual landings, NMFS uses a traditional estimate of ten pounds of meats (recoverable and nonrecoverable) per bushel. The source of this estimate is unknown. Industry reports that approximately 8 lbs. of meats are recovered from a bushel of quahogs leaving 2 lbs. of nonrecoverable meats per bushel by subtraction.

Direct estimates are not available for viscera, juice or shell components. For viscera, however, industry reports that the proportion of viscera to recoverable meats is about the same in ocean quahogs as in surf clams. This proportion can be calculated from Zall and Cho (8) as 0.38. Using this relationship with the estimate of eight pounds of recoverable meats per bushel given above results in the estimate of slightly over three pounds of viscera per bushel of ocean quahogs.

To complete the estimates of ocean quahog composition it is assumed that the amount of juice in ocean quahog per total clam weight is the same as surf clam. Zall and Cho provided this as ten percent. The amount of shell can then be finally calculated by subtraction.

Applying the composition estimates for surf clams and ocean quahog to the respective estimates of weights of whole clams processed in New Jersey results in a calculation of the quantities of wastes generated in 1990.

RESULTS AND DISCUSSION

1990 Landings

In 1990, New Jersey landed 44.8 million pounds of surf clam meats and 32.4 million pounds of ocean quahog meats. These quantities of meats are equivalent to 236.9 million pounds of whole surf clams and 259.4 million pounds of whole ocean quahogs.

Transshipments

In 1990, adjustments for interstate transshipments of surf clams resulted in a 61.6 million lb. net reduction so that 175.3 million pounds of this species were delivered to New Jersey processors. In 1990, 100 vessels landed sea clams in New Jersey. The catches of 28 of these vessels were trucked to processing plants out of state, mainly in Maryland but also Virginia. In addition, the catches of two vessels landed in Long Island were trucked to New Jersey for processing. (Richard Schween, NMFS- Resource Statistics, Silver Spring, MD, personal communications). It is assumed that each vessel delivered a proportionate share of the state landings. The assumption is considered reasonable because NMFS reports that these vessels are typical of the fleet. Therefore, if 28% of New Jersey landings left the state and 2% were replaced by incoming clams, an estimated total of 74% of New Jersey sea clam landings were processed within the state. Because NMFS also reports that out-of-state processors reporting receipt of New Jersey clams are surf clam processors (R. Schween, personal communications), it is further assumed that the

26% reduction applies only to surf clams and that all ocean quahog landed in New Jersey remain in the state for shucking.

Waste Estimates

Estimates of New Jersey production of surf clam and ocean quahog components in 1990 are shown in Figures 3 and 4,respectively. Shucking of surf clams and ocean quahogs produced, 28.0 and 29.2 million pounds, respectively, of organic solid wastes. Combined organic solid wastes from processing the two species amounted to 57.2 million pounds. 1990 shell waste from New Jersey sea clam processing totaled 32.3 million pounds with over 71 percent coming from ocean quahog processing.

Annual Variability in Waste Production

Annual landings of sea clams in New Jersey increased over the period 1981 to 1990 (Table 1). Surf clam landings increased 121% from 20.3 million pounds of meats in 1981 to 44.8 million pounds in 1990. Ocean quahog landings rose 56% from 20.8 million pounds in 1981 to 32.4 million pounds in 1990. The ten-year averages for surf clams and ocean quahog were 33.9 and 24.1 million pounds, respectively.

Year to year fluctuations in New Jersey sea clam landings can be considerable and apparently are due to factors independent of resource availability. For example, landings of surf clam meats rose 61% from 24.4 million pounds in 1983 to 39.2 million pounds in 1984, while total national landings of surf clams increased by only 26%. Likewise, the landings of ocean quahog declined by 28% from 24.4 million pounds in 1987 to 17.5 million pounds in 1988, while total U.S. landings declined by only 12%.

Applying the methods for estimating waste generation described above to annual landing data for the period 1981 to 1990 demonstrates that production of sea clam waste has been highly variable (Table 2). For each type of solid waste, the ten-year high was 220 percent of the low for surf clams and 184 percent for ocean quahog.

Table 2. Estimated Range of Surf Clam and Ocean Quahog Solid Wastes Generated in New Jersey, 1981-1990 (000 lbs.)

Component	High	Low	Avg.
Surf Clams			
Viscera	18,954	8,593	14,338
Nonrecoverable Meats	18,954	8,593	14,338
Shell	125,569	56,932	94,988
Ocean Quahogs			
Viscera	16,214	8,769	12,049
Nonrecoverable Meats	12,971	7,015	9,639
Shell	230,239	124,513	171,089
Combined Total			
Viscera	33,059	16,407	28,679
Nonrecoverable Meats	29,816	14,653	25,436
Shell	341,836	175,118	312,820

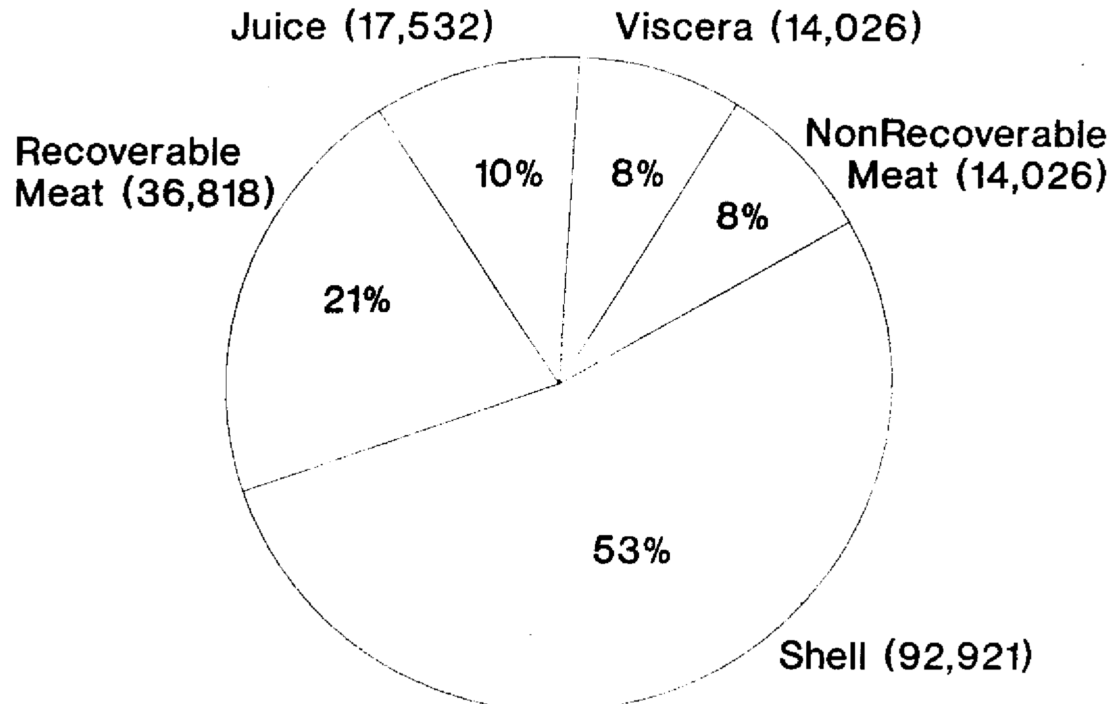

Figure 3. Surf Clam Component Production Estimates for New Jersey, 1990, (000 lbs.)

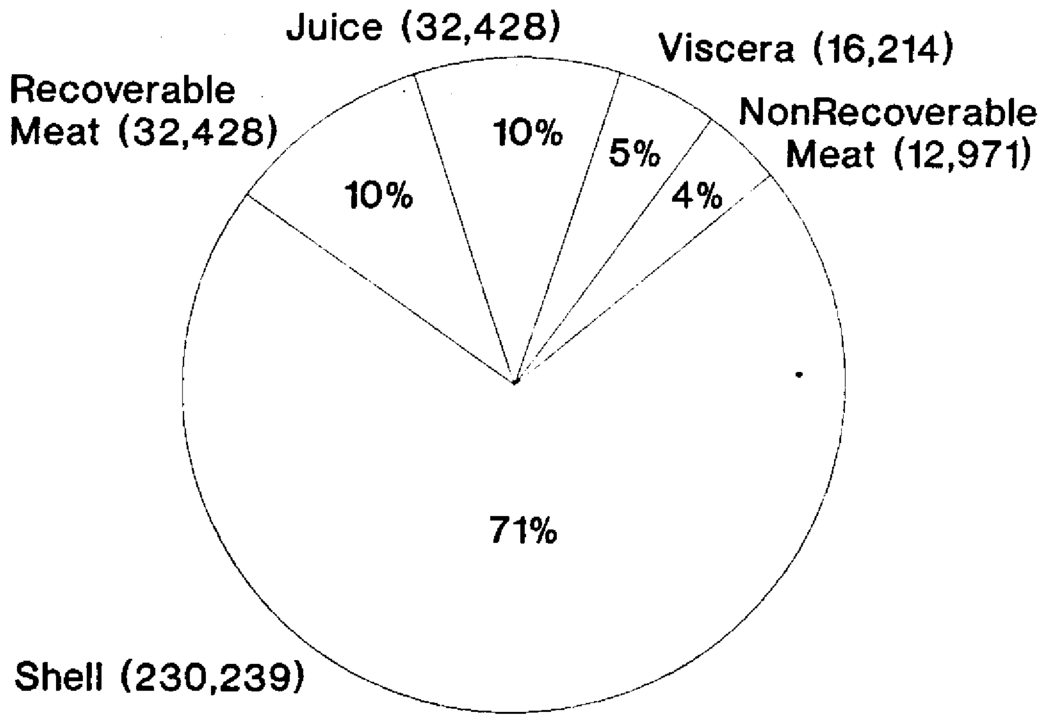

Figure 4. Ocean Quahog Component Production Estimates for New Jersey, 1990, (000 lbs.)

CONCLUSIONS

New Jersey processors are generating an increasing share of total sea clam waste, and there is considerable annual variability in the level of waste production. The waste estimates derived from composition analysis provide maximum quantities produced. The amount of waste potentially available for alternative uses will depend primarily upon the processing and waste recovery technology employed. In the case of clam viscera, there is a considerable difference between production and availability. In 1990, landfills in Cape May and Cumberland Counties reported receiving a total of 3.68 million pounds of viscera, just over one-tenth of the estimated 33.06 million pounds of total viscera produced (J.Jones, Cape May County Municipal Utilities Authority, and A.Brown, Cumberland County Improvement Authority, personal communications). A scientific determination of ocean quahog composition and annual data on transshipments of sea clams would improve these estimates of annual sea clam waste production.

ACKNOWLEDGEMENTS

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IMPLEMENTING FISHERY-BASED COMPOSTING APPLICATIONS: THE NEXT STEP

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INTRODUCTION

Landfills have been and still are the primary means of disposal for seafood processing and fishery related by-products in Florida and in many other states (9, 15). In Florida, landfill space is rapidly being depleted and the cost to close and open new landfill cells is ever increasing. Fishery and seafood processing by-products, because of their high protein content, are extremely malodorous and require extra chemicals and/or soil cover to control their putrification odors. This adds cost and reduces the life expectancy of the landfill cell. One county in the Florida Big Bend region, where seventy (70) percent of the state's blue crab processors are located, spends twenty-five (25) percent of their landfill operating budget on seventeen (17) percent of the waste-stream..."crab garbage" (2).

In the near future, landfills will not be available for disposal of these processing by-products. In Florida, the 1988 Legislature passed a Solid Waste Management Act, which 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 Department of Environmental Regulation-FDER)(4). Although most seafood processors in Florida are relatively small waste generators (less than 3 cubic meters/day (2)), landfills fees are increasing dramatically to eliminate unwanted and often expensive waste streams. Leon County, Florida, for example, implemented a $100/ton fee for blue crab processing by-products.

Many alternative methods of by-product recovery have been examined and used to eliminate wasting by-products, including dehydration (11, 14), land application (5, 16), ensiling (1, 13), composting (6, 7, 8) and anaerobic bioconversion (3, 10). All of these methods, among others, have potential benefits, yet composting may be the best least-cost option for a long term solution. It also has the potential to eliminate several waste streams from landfills. Not only the fishery related by-products, but also other carbon-based materials, such as yard trimmings, brush and some construction debris can be utilizing for composting. In Florida, there is a mandated thirty percent (30%) recycling requirement by the end of 1994. Therefore, several objectives can be met with composting.

There are several entities for which composting applications can be implemented, depending on location and amount of bulk and organic material available. These include the processing plants, marinas and commercial composting operations, in the private sector, and publicly owned facilities, such as, landfills, sewage treatment plants and road departments.

MATERIALS AND METHODS

Three large-scale demonstrations of seafood processing by-products composting were demonstrated in Florida during 1989-1991 (10). Two different mechanical aeration methods were used for: 1) blue crab by-products (Taylor County), and 2) calico scallop viscera (Brevard County). The third demonstration involved static pile methods for blue crab scraps (Wakulla County). Availability of various carbonaceous and nitrogenous by-products in the area were inventoried. Windrows of carbonaceous by-products, primarily wood chips and sawdust, were set up at county landfills. Incoming seafood processing by-products were added to the windrows via a frontend loader and mixed with the bucket or the mechanical aeration equipment. The ratio of carbon to nitrogen was 2:1, by volume. The windrows were turned daily during the mechanical aeration demonstrations. Static pile procedures required that the material be ground with a tub grinder after approximately two months and allowed to reheat and finish composting. Total time from start to finished compost was four (4) months.

RESULTS AND DISCUSSION

When examining composting as an alternative for disposal or recovery of a current fishery related by-product, several factors need to be assessed. A few of the major attributes and limiting factors are discussed below.

ATTRIBUTES

Simple Methods. No matter what method is chosen whether it is open windrow or containment systems, active aeration or static pile, the methods are simple and cookbook in nature. They can be adjusted to scale and are flexible to fit the smallest processor to the largest commercial operation.

Affordable. Because of the simplicity, composting does not require expensive equipment or facilities, to operate successfully. This is dependent on scale, however. The larger the operation, the larger the equipment needed. But this can be minimized by utilizing equipment already purchased for other uses. For example, a front end loader at a landfill can also be used at the compost site; a fork lift or small bobcat used in a seafood plant or marina could be used for composting.

Minimal Labor. Composting does not require excessive manpower. One man working two-three (2-3) hours per day can maintain a large-scale compost operation. In Taylor County Florida, one man was able to handle daily waste inputs of up to ten (10) tons in a 2-3 hour period (Figure 1). This adds to the affordability of the process by reducing labor costs. Efficient composting does require that one person be responsible for coordinating the delivery of bulking materials and organics with the labor schedule, to meet the needs of the compost mix. However, this management task is also a part-time job.

Marketable End Product. Obviously, the attribute that brings the most attention is the one determined by value. In general, seafood-based composts will command a higher price on the market if quality control is maintained. Particularly crustacean-based composts, containing chitin, which have limited nematicide value, as well as soil enhancement properties (10). The fact that the end-product can be sold, thereby providing a means of recovering a portion, if not all, the cost of production, indicates that composting can be a least-cost option for handling these by-products.

FIGURE 1. Composting daily procedures: Wakulla County landfill

```
PROCEDURE                                HOURS OF THE DAY

                     6am  7   8   9   10  11  12n 1   2   3   4   5pm
                     ------------------------------------------------

RECEIVE MATERIALS     **************************************************

STACKING                                                  *****

MIXING                                                    **********

MAINTENANCE                                                   *****

SET-UP RECEIVING                                              *****

GRINDING             **********************************************
                                 (one day per month)

STORAGE                                    ****************
                                              (as needed)
                     ------------------------------------------------
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LIMITING FACTORS

Carbon/Nitrogen Supply. The composting process requires the right mix of ingredients for the micro-organisms to function (6). The general rule of thumb is a two to one (2:1) ratio of carbonaceous to nitrogenous material, by volume. The availability of carbon and nitrogen sources can vary dramatically over the course of the year as seasons change. One must be flexible and ready to substitute other types of carbon or nitrogen to maintain an ongoing composting operation. Certain fisheries, e.g. Calico scallops and spanish mackerel, tend to be seasonal; therefore, composts produced from by-products of these fisheries will also be cyclical. Difficulties in marketing may arise due to an unpredictable supply throughout the year, or inconsistent quality, e.g. NPK, pH, organic content, etc., due to changing ingredients.

Space. Space can be limiting at processing plants or marinas. Commercial operations and landfills generally have plenty of room. A seafood processing plant or marina may have to find a remote site to have enough room to set up windrows or store finished compost. This is not a large problem to overcome, however, space limitations will affect the operational logistics and ultimately the cost for long term composting.

Maintaining Quality. The quality of the finished compost will determine its value on the market. Even though we are taking what is currently thought of as a "waste" by-product and making compost from it, quality control is still required to produce a high quality end-product. Our experience in Florida with blue crab processors was that often the incoming "crab garbage" also contained plastics, metal cans, fish boxes, waxed cardboard boxes, bed springs, garden hoses, trash bags, etc. Needless to say, more time was spent sifting out the unwanted materials than mixing the compost. Landfills and commercial operations will need to screen incoming compost ingredients to maintain quality and reduce time and manpower "cleaning" the compost.

Regulations. The biggest obstacle in Florida's development of compost operations at processing plants, or at private facilities, are the regulations regarding siting and site design criteria. Since the composting industry is just getting started, the regulatory agencies in Florida have set up very cost prohibitive rules without much input from the fledgling industry, or available research data. These strict rules are primarily related to protection of groundwater quality. For example, sites must have concrete, or asphalt, pads under the windrows and have on site leachate collection and removal systems (FL. Admin. Code, Rule 17-709.5). Many of these precautions may be alleviated over time as more information is gained and research conducted on leaching, contaminants, etc. However, at the present time, regulations may limit operations in certain areas. Landfills are not faced with this problem since compost permits can be added to their landfill operation permit.

TECHNOLOGY TRANSFER

If composting technology is simple, manageable, affordable and the end-products marketable, putting these applications into practice in the real world should be relatively easy as well. Unfortunately, this may be easier said than done. Although on a small scale this may be true, on a larger scale there is some question as to where this industry will ultimately fit in.

In Florida, the ultimate goal of the three large-scale composting demonstrations was to turn the demonstrations over to either county governments or private enterprises to be continued as ongoing operations. The two projects that were continued at county landfills (Brevard and Wakulla Counties) were approved by the Florida DER under their landfill operation permits and are successfully planning to proceed with composting. The Taylor

County demonstration was transferred to a private farm in a neighboring county with the assistance of the Suwannee River RC & D Council. However, after two (2) attempts to acquire the necessary permits from Florida DER, each denied for leachate concerns, this operation will be temporarily put on hold (12).

The difficulties in acquiring permits, specifically the costs associated with meeting the compost design criteria and the apparent unwillingness of the permitting agencies to work with the industry, may prevent the private sector from getting involved at the present time. However, the prediction is that a large percentage of the Municipal Solid Waste-stream (MSW) will be composted by 1994 to meet the mandated recycling requirements (4). Composting fishery and seafood processing by-products will complement this pattern, at least in the public sector and hopefully in private sector in the future.

IMPLEMENTATION NEEDS

The following needs assessment is based on the Florida composting demonstrations and results reported in earlier papers. There appear to be four gaps that need filling and this technology will take hold and begin operate on its own.

First, as mentioned above, there is an immediate need for more research-based information on compost leachates and their potential effects on ground and surface waters. This will help the permitting agencies understand what level of contaminants exist in composts, and be better able to determine what site designs are necessary to protect water supplies.

Secondly, there is a need for manuals and guides directed to the audiences mentioned before, that will be producing compost: by-product generators, commercial compost operators, landfill operators, etc. It should include a cookbook of recipes for the novice composter to the experienced. These recipes should describe a variety of inputs with some formulas for producing desired end-products. It should also describe the different compost technologies listing their advantages and limitations. The final product of this conference will begin to solve this need.

Third, a manual describing the uses of the various compost products themselves will be necessary for teaching the consumers how to best apply them. Since these composts are just being developed, studies are needed on how to best use them for vegetables, horticulture, nematacide, etc. or how to use them in conjunction with more traditional soil amendments.

A final need is in the marketing area. With the volume of compost products expected to be produced, moving these products may become a bottleneck. With the higher quality attributes of the seafood-based composts, marketing studies would be a great help in placing these products in the best form and packaging for the consumers to recognize them as a better value. This will ensure a premium price on the market.

CONCLUSION

The future looks bright for the development of fishery-based composting, an idea whose time has come. Composting has been around a long time, but now the time is right to apply this age old practice to the current disposal problems of fishery and seafood processing by-products. The outcome is a natural and cost-effective solution which will produce long term positive results for many businesses and communities.

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CHARACTERIZATION OF WASTES FROM BLUE CRAB PROCESSING FACILITIES

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As environmental legislation becomes more stringent, the blue crab industry is being challenged to develop technically and economically feasible methods to manage their liquid and solid wastes. The blue crab industry is one of the largest seafood processing industries in Virginia. Almost 50 blue crab processing firms are presently certified by the Bureau of Shellfish Sanitation, Virginia Health Department (R. E. Croonenberghs, 1991, Richmond, VA, Virginia Department of Health information bulletin). The industry is economically vital to many communities of the Chesapeake Bay and its tributaries. In 1989, there was a total landing of 206.7 million pounds of blue crab in the United States valued at 81 million dollars. The Chesapeake region produced 89 million pounds of this total (11). Solutions to the waste management problems are essential for the future of many blue crab processing facilities. Some of the solutions will require the application of innovative technology, while others will result in substantially increased production costs.

In the 1970's, the United States Environmental Protection Agency (EPA) developed documents which defined effluent limits for segments of the seafood processing industry. The regulations were then adopted by the states. Compliance with these limits was obtained, in most cases, with minor plant modifications that did not require large capital investments and operating costs. During the last decade, citizen and environmental groups have encouraged more stringent legislation in an effort to achieve a cleaner environment. Individual states have established waste disposal standards that exceed current U.S. EPA requirements.

The restoration of water quality in the Chesapeake Bay has become a goal of states which border the Bay and its tributaries. In Virginia, the State Water Control Board (SWCB) has initiated a vigorous program to remove pollutants from the Bay. As a result, new standards for the disposal of liquid and solid wastes have been established. The new standards, many of which substantially exceed previous standards, have resulted in: the loss of public landfills; highly regulated waste transportation regulations; the inability of municipal systems to accept industrial wastes; waste disposal surcharges; and perhaps most importantly, new liquid effluent standards for discharge into receiving waters. Many blue crab processors have reported serious waste disposal problems as they are unable to consistently comply with their current allowable pollutant discharge limits.

Waste management is difficult for blue crab processing firms for several reasons. In general, crab firms are less capitalized and do not have the economics of scale when compared to some other seafood industries. Treatment systems with the ability to produce the desired effluent quality may not be economically feasible. Chemical, biological and physical treatment may be required to reduce the strength of processing plant effluents

(7,12). These treatment processes will be expensive and require adequate planning to avoid unfavorable economic impact.

Secondly, the location of processing plants also creates problems. Most blue crab processing facilities are located in rural areas on bodies of water. These facilities usually do not have access to municipal waste treatment plants. Land application and lagoon treatment are often not viable treatment options due to the high water table and wetland limitations. Some larger crab plants are located in cities with access to municipal treatment systems. However, these plants are often assessed substantial surcharges unless the concentration of their waste is reduced. There is also a possibility that municipal treatment systems may be forced to reject crab wastes due to the rapid population and industrial growth that is pushing municipal waste treatment systems to maximum operating capacity. Furthermore, plants located in metropolitan areas often lack the space needed for treatment systems. The plants are usually tightly bordered by water, parking lots and/or neighboring industries.

Finally, in Virginia, as the SWCB is in a period of transition in implementing new standards, different crab processing firms are often monitored for different effluent constituents. This discrepancy can even appear in two adjacent crab plants. The inconsistency of the regulations makes it difficult for the industry as a group to address its waste disposal alternatives.

The blue crab processing industry faces serious solid and liquid waste disposal problems. Approximately 14% of the live crab results in food for human consumption with the remainder as byproducts or waste (5). Consequently, processing firms will need to develop in-plant programs that will include: water conservation and recovery processes, improved by-product recovery systems and the development of industrial products from wastes (including foods, feeds, or biologics). The achievement of such a goal presents substantial economic and technical obstacles and may be one of the greatest challenges in the future.

The objective of this project, funded by the National and Virginia Sea Grant Programs, was to characterize waste streams and identify ways to reduce waste quantities and strengths at three blue crab processing facilities. The bulk of this paper deals with characterization of wastes generated during processing, but some treatment studies are presented. The results of additional treatability studies will be presented in later publications.

MATERIALS AND METHODS

Plant processes

The waste streams of each individual process in three blue crab processing plants were characterized. The three plants studied are all located in Virginia. The first plant sits on the Rappahannock River in rural surroundings. The second and third plants are located in downtown Hampton, Virginia. For the purposes of this paper, the processing facilities will be designated plant #1, #2 and #3 respectively. Two trips were made to plants #1 and #3. Three trips were made to plant #2.

The typical steps for processing live blue crabs are shown in Figure 1. The unrefrigerated live crabs are usually delivered by boat or truck to processing plants. The crabs are weighed, then dumped into large stainless steel baskets. During the winter dredging season, the crabs are directed through a tumble spray washer prior to being dumped into baskets. This washing step is essential for dredged crabs because they are covered with sand and grit from being buried in sand.

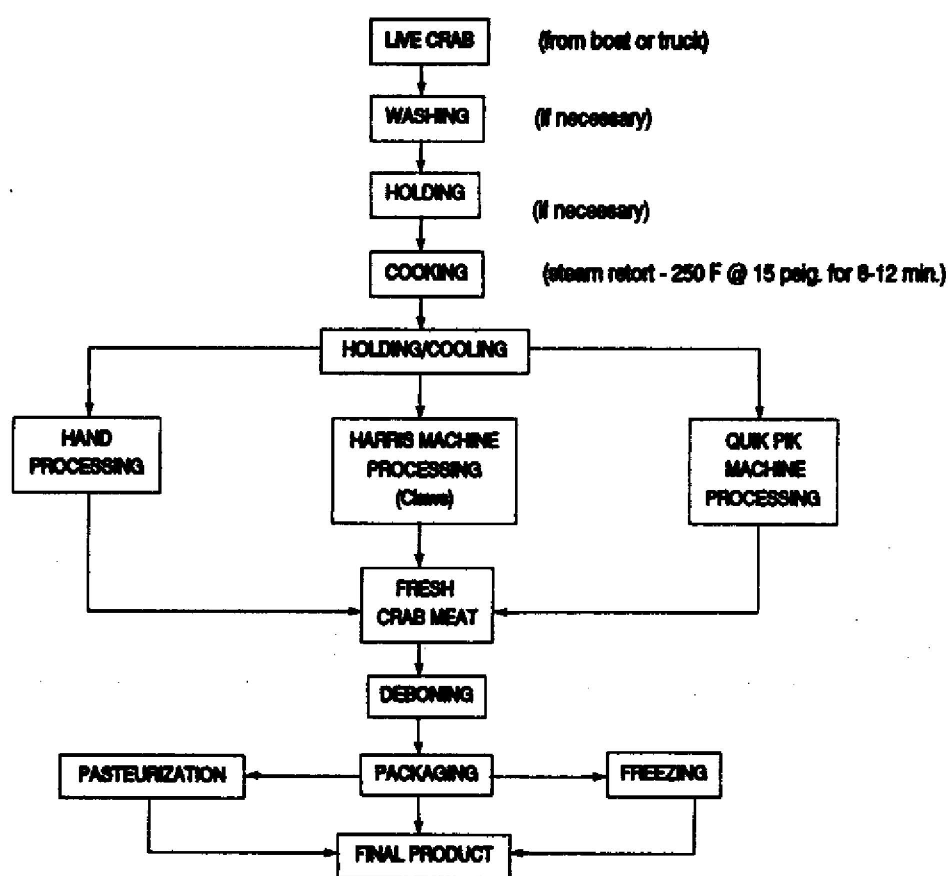

Figure 1 - Flow diagram of blue crab processing.

After washing, the crabs are placed into horizontal or vertical retorts, and cooked by steaming for 7 to 23 minutes at 121°C and 15 psig (9). The main objectives of cooking are to facilitate removal of meat from the shell, give the product a characteristic crab meat odor and flavor, and reduce microbial populations.

Following cooking, the crabs are moved to a room where they are air-cooled to ambient temperatures within thirty minutes. Before the cooled crabs are moved to the cooler (33°F to 40°F), they must be cool enough that steam is no longer rising from them. If cooked crabs were moved immediately to the cooler, steam rising from the crabs would condense on the ceiling of the cooler and drip back down on the crabs. This could potentially contaminate the cooked crabs (10,14).

Two different processes are used to prepare the crabs for removal of the meat (picking): a wet process and a dry process. In the wet process, crabs are backed (carapace removed) and declawed first. The crab bodies are then washed by hand or machine and the meat is removed immediately, or the bodies may be refrigerated overnight. The dry process, most commonly used in the Chesapeake Bay area, does not include the washing step. Each picker backs, declaws, and removes all meat from each crab (4).

Picking meat from the crab is labor intensive and is sometimes supplemented in a few large plants by mechanized picking. The Harris Claw machine combines hammer mills with brine-tank flotation to remove the meat from claws. The Quik-Pik machine uses high speed vibration to remove body meat from the crab (4,13). The Quik-Pik process may also include a bobber which mechanically cuts off the legs, removes the carapace and then removes the viscera from the remaining core using spray jets and brushes. The bobber produces a concentrated effluent.

After the picking process, the meat goes through a deboning step in which any remaining shell fragments are removed from the meat by hand. The deboned meat is then packed fresh, frozen or pasteurized.

In plant #1, crabs are processed by hand picking and the Harris Claw machine. The second plant uses both of these processes but also uses the Quik-Pik machine. The third plant uses only hand picking. In the first and second plants, the following processes were characterized: cooking, hand picking, Quik-Pik (plant 2 only), Harris Claw, and cleanup. In the third plant, only the cooker effluent was characterized. In this paper the cooker effluent will be called "retort water", effluent from the Quik-Pik process will be called "Quik-Pik bobber liquid".

Several different effluent streams were produced in the Harris Claw process. These streams were characterized separately and are named as follows: claw wash reel, shell liquid, brine bath and claw meat conveyor wash. At the start of the process, the claws are usually washed in a tumble spray process resulting in the "claw wash reel" effluent. The claws are then shattered as they pass through a hammer mill and the resulting meat and shell fragments fall into a tank of approximately 70% saturated brine solution. Due to the differing specific gravities between the meat, shell fragments and brine solution, the meat floats to the top of the tank while the shell fragments settle to the bottom. The shell pieces are carried out of the tank by a conveyor where they empty into a perforated receptacle. The shell conveyor carries a large quantity of the brine solution out with the shell fragments and is responsible for the "shell liquid" effluent. The perforations in the receptacle allow the liquid to run out leaving only the shell remaining.

The meat that floats to the top of the tank is carried off by another conveyor belt where it is washed with potable water through a spray nozzle to reduce the salt content.

Meat on the conveyor is also checked by hand to ensure removal of any remaining shell bits. This "claw meat wash water" provided a third effluent stream from the Harris Claw process. The final effluent from this process is the brine bath. The brine tank is filled only once or twice during a processing day, but is continually being refilled as solution spills over in the tank or is carried out by the conveyor belts.

Process flows and volumes

In the process characterizations, effluent flow rates and volumes were determined in the following ways. First, the water line which fed the Harris Claw process at plant #1 had a flow totalization meter. Omega FTB series turbine flow totalization meters were placed on several individual feed lines of the Harris Claw and Quik-Pik process at plant # 2. The effluent volumes produced over a given period of time could be read directly from the meters.

Some effluent streams, such as the shell liquid effluent, could not be read directly from totalization meters. In this case, the time required for the effluent to fill a calibrated five gallon bucket was recorded. When the bucket method was used, several samples were collected during process operations and the results averaged to improve the accuracy of the measurement.

The volume of the Harris Claw brine tanks was determined by measuring their dimensions. This volume was multiplied by the number of times the brine tanks were dumped and refilled during the day. Some solution was removed from the brine tanks by the meat and the shell fragment conveyor belts. This volume was accounted for in the measurements of those respective effluent streams.

Finally, the volume of the retort water was determined in two separate ways. At plants #1 and #2, retort water flowed through pipes which discharged at the edge of the docks. The cooker water was collected by placing a five gallon bucket over the end of the pipe, and then repeatedly transferring the contents of the bucket to a large calibrated receptacle, until the cooking cycle was complete. At plant #3, the pipe exiting the cooker was attached to a fire hose. The fire hose was connected directly to a 55 gallon industrial drum where all the fluids from a cook were collected.

Sample collection, preservation and transport

Samples of each effluent were collected in the following manner. First, a grab sample of a given effluent was collected in a clean, five gallon bucket when a process was operating at full speed. The contents of the bucket were mixed thoroughly and then transferred into 500 ml polyethylene bottles. At this time, the temperature and pH of the sample were taken. Half of the samples were then acidified to a pH of 2.0 or below for preservation. All samples were immediately placed on ice in a barrier container.

Two exceptions to the method of collection were the retort water from the cookers and the brine bath from the Harris Claw process. For the retort water, all the fluids from a cooking cycle were collected in a 55 gallon receptacle. The contents of the drum was mixed thoroughly with a paddle to homogenize the sample. Samples were then transferred to polyethylene bottles by a hand pump. The entire effluent stream from the cook was collected after it was determined that the characteristics of the retort water changed substantially during the cooking cycle and a single grab sample would not be representative.

The samples of the brine bath were collected at the end of the Harris Claw process cycle just before the contents of the brine tank was dumped. The same brine solution was used for the entire processing period and therefore was most concentrated at the end of the cycle.

After all samples had been collected, they were packed on ice and transported by car to VPI & SU's Environmental Engineering Analysis Laboratory in Blacksburg, Virginia. The samples were then placed in a 4 °C refrigerator until all analyses were performed. All tests on non-acidified samples were completed within 48 hours of collection and all tests on acidified samples were completed within 28 days as prescribed by EPA sample preservation methods (6).

Analytical procedures

Effluent samples were analyzed for the following: pH, temperature, chemical oxygen demand (COD), 5-day biochemical oxygen demand (BOD_5), total suspended solids (TSS), volatile suspended solids (VSS), total Kjeldahl nitrogen (TKN), ammonia nitrogen (NH_3N), total phosphorous, oil and grease (O&G) and chloride (Cl). The criteria used in selecting these tests was to include those that are now regulated, those that may be regulated in the near future, and those that might impact treatment works or the environment.

All samples were performed in accordance with Standard Methods for the Examination of Water and Wastewater (1) with the following notations:

- BOD_5 samples were not seeded with the exception of those used in the acidification treatability study. With the acidified samples, the pH was adjusted to approximately 7.0. The samples were then seeded with effluent from a municipal activated sludge facility. Quality control samples made from standard glucose solution were included.

- Following distillation, TKN and NH_3N were determined by titration with 0.02N H_2SO_4

- Chloride was determined by means of ion chromatography (Dionex model 2010i chromatograph) with a cross-linked polystyrene/ divinyl benzene column, flow rate of 2 ml/min and pressure of 1,000 psi.

- Total phosphorus was determined using the ascorbic acid method with persulfate digestion. Absorbance values were determined with a Beckman DU-6 spectrophotometer at a wavelength of 880 nm. . A standard curve was produced for each set of samples.

Treatability studies

Three limited treatability studies were performed on individual effluent streams from blue crab processing plants. In the first tests, settling and filtration of effluent samples were performed. COD, BOD_5 TSS, VSS, TKN, NH_3N, and total phosphorus were determined for each effluent type before settling, after settling and after filtration. To obtain settled values, collected samples were mixed and then allowed to settle at room temperature for one hour. The supernatant from a sample was then analyzed for the above constituents. To obtained filtered values, samples were filtered through Whatman grade 934AH glass-fiber filters. Analyses were performed on the filtrate.

Second, retort water, Harris Claw shell solution and Harris Claw brine bath was acidified to test for removal of COD, $BOD_5$and TSS. The authors noted that when the pH of the effluent samples was adjusted to 2.0 or below with concentrated H_2SO_4 for preservation purposes, coagulation of the contents occurred due to the denaturation of

proteins and perhaps other substances. The coagulated material floated to the top of the sample for the retort water and settled to the bottom of the Harris Claw shell and brine bath solutions.

To test treatability through acidification, the pH of the three samples was decreased to 2.0. The samples were then allowed to sit undisturbed for 24 hours. The respective subnatants and supernatants were then removed and the remaining solution was tested for COD, BOD_5 and TSS. As noted earlier, BOD_5 test samples were increased back up to approximately pH 7.0 and seeded with activated sludge effluent. More testing needs to be done using a range of pH values and settling times to gain a better understanding of the treatment method.

Finally, an aerobic treatment study of the cooker water was initiated using a bench-scale, sequencing batch reactor (SBR). A 12 liter SBR was filled with 10 liters of retort water from plant #1. The reactor was then seeded with 2 liters of return activated sludge from the Blacksburg-VPI Sewage Authority Plant. The contents were reacted for 18 hours. Mixing and aeration were accomplished with lab supplied air through two six inch air stones. Even with two large air stones, aerobic conditions were not maintained. Severe foaming problems also occurred. The initial mixed-liquor suspended solids (MLSS) was 2,440 mg/L. After 18 hours of reaction, the MLSS was 8,360 mg/L. The air was turned off at the end of the 18 hour cycle and the contents allowed to settle for 30 minutes. The supernatant was then analyzed for COD, BOD_5 and TSS. More rigorous studies are planned in the future.

RESULTS AND DISCUSSION

The results of the effluent characterizations from three blue crab processing plants are shown in Table 1. Overall, plant characterizations were done in plant #1 and plant #2. Retort water was also characterized at plant #3. The given flows and volumes correspond to the pounds of finished crab meat product processed during that day of production. The exception is for retort water, which is represented in terms of pounds of live crab per cook.

Table 1 shows that blue crab processing typically produces relatively high concentration, low volume wastewaters. Similar results have been found by other researchers (2,3,7,8,12). For example, effluent concentrations exhibited the following ranges: BOD_5 = 410-28,500 mg/L, TSS = 400-33,400 mg/L, TKN-N = 50-3,400 mg/L, NH_3-N = < 10-330 mg/L and TP = 7-320 mg/L. The total daily process volumes for these facilities never exceeded 20,000 gallons per day (gpd). When no mechanized processes operated during a day, the total effluent volume was typically around 2,000 gpd. The mechanized processes were responsible for the majority of the effluent volume.

The COD values are only slightly larger than the BOD_5 values in most cases and the VSS typically constitutes a very high percentage of the TSS. The BOD_5 was 32-98% of the COD, and the VSS was 19-93% of the TSS. These results imply that the wastewater consists largely of highly degradable organic matter.

The Harris Claw process displayed a great variability in effluent quality between and within the plants. Only plant #2 actively used the crab claw reel washer during the site visits. The variability of this process may be related to when and where a particular batch of crabs was harvested, and whether they were put through the tumble spray washer upon arrival.

Table 1 - Characterization of wastes from three blue crab processing plants

	Trip #	Product (lbs)	Flow (gpm)	Volume (gal)	Temp	pH	COD (mg/L)	BOD-5 (mg/L)	TSS (mg/L)	VSS (mg/L)	Chloride (mg/L)	O & G (mg/L)	TKN-N (mg/L)	NH3-N (mg/L)	Total P (mg/L)
Crab Claw Wash Reel	1	2,380	1.8	650	30	7.5	9,145	>3,740	3,970	3,400	620	-	-	-	-
	2	1,535	2.3	540	-	8.4	2,940	2,770	1,270	1,100	-	-	400	14	42
	3	760	-	390	-	-	15,410	>7,800	10,880	9,500	-	-	2,400	100	135
Claw Shell Liquid	1	-	0.3	-	22	8.4	15,690	15,350	18,340	5,160	112,170	10	-	-	-
	2	1,550	0.5	135	16	8.2	23,280	15,120	25,100	5,420	-	-	2,260	330	190
	1	2,380	1.1	400	30	8.1	13,005	>9,250	-	-	100,160	-	-	-	-
	2	1,535	2.2	520	-	7.9	17,280	13,500	13,740	2,660	-	-	2,240	125	170
	3	760	-	370	-	-	39,680	16,240	33,415	16,390	-	-	3,390	115	280
Claw Meat Brine Bath	1	-	-	230	20	8.3	14,980	7,000	17,460	4,390	143,990	6	-	-	-
	2	1,550	-	230	19	8.2	21,510	15,000	31,540	8,320	-	-	3,060	190	270
	1	2,380	-	220	30	-	-	7,805	-	-	135,020	-	-	-	-
	2	1,535	-	220	-	7.9	17,460	14,000	14,970	4,270	-	-	2,330	110	230
	3	760	-	220	-	-	15,870	8,925	12,670	3,740	-	-	1,775	80	160
Claw Meat Conveyor Wash	1	-	25.4	-	22	8.6	570	265	445	410	3,100	-	-	-	-
	2	1,550	33.5	9,715	19	8.3	650	640	660	540	-	-	150	< 10	7
	1	2,380	8.2	2,950	30	8.2	1,790	1,020	585	355	15,275	-	-	-	-
	2	1,535	8.8	2,070	-	8.2	2,520	2,040	930	480	-	-	370	22	25
	3	760	-	1,800	-	-	2,625	1,790	1,170	775	-	-	390	17	27
Claw Room Cleanup	1	-	7.8	-	22	8.6	3,920	1,260	1,375	630	26,770	-	-	-	-
	2	1,550	-	520	18	8.5	900	-	3,050	2,790	-	-	210	< 10	20
	1	2,380	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	1,535	1.3	120	-	8.5	550	420	590	500	-	-	160	< 10	8
	3	760	-	360	-	-	-	-	-	-	-	-	-	-	-
Quik-Pik Bobber Liquid	1	-	2.3	-	30	7.0	9,755	7,000	5,130	4,490	590	-	-	-	-
	2	750	2.5	960	-	7.7	16,460	12,690	5,440	5,040	-	260	1,030	28	234
	3	490	-	280	-	-	29,655	16,980	12,000	10,860	-	-	2,000	53	321
Quik-Pik Cleanup	1	-	-	-	30	7.4	-	-	1,100	880	510	-	-	-	-
	2	750	-	-	-	7.9	620	410	410	370	-	-	50	< 10	7
	3	490	-	-	-	-	-	-	-	-	-	-	-	-	-
Retort Water	1	1,800 *	-	70 @	91	8.6	32,940	27,350	1,790	1,550	6,770	22	-	-	-
	2	1,800 *	-	72 @	74	7.0	35,240	-	6,200	4,710	-	10	3,940	160	185
	1	1,050 *	-	-	64	6.6	29,000	28,500	1,460	1,305	5,100	-	-	-	-
	2	1,050 *	-	47 @	74	7.1	21,510	17,380	1,010	910	-	50	2,240	70	102
	1&	1,300 *	-	50 @	-	-	31,040	18,780	653	535	-	-	-	-	-
	2&	1,300 *	-	50 @	-	-	23,920	13,720	1,980	1,640	-	-	2,510	130	160
Handpick Cleanup	1	-	-	-	-	9.6	2,750	1,510	2,610	1,975	370	-	-	-	-
	2	-	-	280	18	9.2	4,980	3,075	1,660	1,080	-	-	180	< 10	110
	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	920	-	220	-	-	3,680	2,480	1,240	1,000	-	-	250	10	125

Shaded trip #'s are results from plant #1; unshaded trip #'s are for plant #2; & denotes trips to plant #3.
* denotes pounds of live crab, other weights are of final product; @ denotes gallons per cook.

The Harris Claw shell liquid and brine bath exhibited extremely high chloride concentrations (100,000-144,000 mg/L). The brine bath was kept approximately 70% saturated with sodium chloride to maintain efficient separation of the meat and shell fragments. On one site visit to plant #2, it was noted that 3,440 pounds of salt were used to produce 2,250 pounds of claw meat. The high chloride concentrations are of concern because of the potential toxicological effects to microorganisms in biological treatment systems. The shell effluent and the brine bath contained a large percentage of non-volatile suspended solids due to sand and components of the shell.

The claw meat conveyor wash effluents displayed high variability. The differences result from plant #1's use of four to five times more conveyor wash water to process the same amount of product. However, the loadings produced by each plant for this effluent were approximately the same because the high volume effluent was more dilute (Table 2). In the plants that used the Harris Claw process, the claw meat conveyor wash water produced a greater effluent volume (1,800-9,715 gpd) than any other process. Since this effluent is relatively dilute, it offers potential for use in diluting some of the more concentrated effluents for biological treatment. However, the character of this relatively dilute effluent substantially exceeds permit limits and still possesses chloride concentrations that could be inhibitory/toxic in the wrong environment (3,100-15,275 mg/L).

Representative effluent volumes and concentrations for the Harris Claw cleanup, the Quik-Pik cleanup and hand pick cleanup were the most difficult of any process to obtain. Some of these process rooms used as many as five different hoses during cleanup. The runoff from these hoses often flowed into several different drainage channels. The character of the cleanup water changed continuously during wash down. The quantity of water used for wash down also varied each processing day. This variation was probably due to such factors as the individual doing the wash down, how dirty the process room had become, and the particular plant studied. Typically, grab samples were taken near the beginning of cleanup and the quantity of water used was determined by flow meters or by timing the use of hoses for which the flow rate had been previously determined. Composite sampling methods should be performed to obtain more reliable results in the future.

The Quik-Pik process was used in plant #2 only during days of very high production to support the hand pick operations. The Quik-Pik bobber produced the only substantial effluent stream from this process. The bobber effluent was extremely concentrated (BOD_5= 7,000-17,000 mg/L, TSS = 5,100-12,000 mg/L, TKN = 1,030-2,000 mg/L and TP = 230-320 mg/L) and contributed significantly to the total plant effluent loadings on days when it was used. The bobber effluent was also the only process plant process with significant concentrations of oil and grease (260 mg/L).

The retort water was studied in more detail than other processes because it was used in all plants and it typically produced the majority of pollutant loadings from each plant (Table 2). It should be noted that the values in Table 2 are per cook of live crab. Each of these plants will typically have 6 to 20 cooks per day. This corresponds to a retort water volume of 400-1,000 gallons per day. The cooker water character and volume remained very consistent within and between each plant. BOD_5 concentrations were greatest in the cooker water, ranging between about 14,000-30,000 mg/L. The TKN, NH_3N and total phosphorous values were also very high. The suspended solids values, however, were typically lower than most other processes (TSS = 650-2,000 mg/L).

The temperature of the retort water was high during discharge 64 to 91°C. The high temperature is of concern when discharging to receiving waters. Some processors currently have discharge permits which place limits on the discharge temperature of their

Table 2 - Typical effluent loadings from three blue crab processing plants.

	Trip #	COD	BOD-5	TSS	VSS	Chloride	O & G	TKN-N	NH3-N	Total P
Crab Claw	1	20.8	>9	9.0	7.7	1.4	-	-	-	-
Wash Reel	2	8.6	8.1	3.7	3.2	-	-	1.2	0.04	0.12
	3	66.0	>33	46.6	40.7	-	-	10.3	0.43	0.58
Claw	2	16.9	11.0	18.2	3.9	-	-	1.6	0.24	0.14
Shell	1	18.2	>13	-	-	140	-	-	-	-
Liquid	2	48.8	38.1	38.8	7.5	-	-	6.3	0.35	0.48
	3	161.1	65.9	135.7	66.5	-	-	13.8	0.47	1.14
Claw Meat	2	26.6	18.6	39.0	10.3	-	-	3.8	0.24	0.33
Brine Bath	1	-	6.0	-	-	104	-	-	-	-
	2	20.9	16.7	17.9	5.1	-	-	2.8	0.13	0.27
	3	38.3	21.5	30.6	9.0	-	-	4.3	0.19	0.39
Claw Meat	2	34.0	33.5	34.5	28.2	-	-	7.8	< .52	0.37
Conveyor	1	18.5	10.5	6.0	3.7	158	-	-	-	-
Wash	2	28.3	22.9	10.5	5.4	-	-	4.2	0.25	0.28
	3	51.9	35.4	23.1	15.3	-	-	7.7	0.34	0.53
Claw Room	2	2.5	-	8.5	7.8	-	-	0.6	< .03	0.06
Cleanup	2	0.4	0.3	0.4	0.3	-	-	0.1	< .01	0.01
Quik-Pik	2	175.7	135.5	58.1	53.8	-	2.78	11.0	0.30	2.50
Bobber	3	141.3	80.9	57.2	51.8	-	-	9.5	0.25	1.53
Retort	1	10.7	8.9	0.6	0.5	2.2	0.007	-	-	-
Water	2	11.8	-	2.1	1.6	-	0.003	1.3	0.05	0.06
	2	8.0	6.5	0.4	0.3	-	0.019	0.8	0.03	0.04
	1&	10.0	6.0	0.2	0.2	-	-	-	-	-
	2&	7.7	4.4	0.6	0.5	-	-	0.8	0.04	0.05
Handpick Cleanup	3	7.3	4.9	2.5	2.0	-	-	0.5	0.02	0.25

All units are lbs/day/1,000 lbs product (except for retort water which is lbs/day/1,000 lbs of live crab).
Shaded trip #'s are results from plant #1; unshaded trip #'s are for plant #2; & denotes trips to plant #3.

retort water. The high temperature also complicates biological treatment methods. A cooling step would be required prior to discharge into a biological system.

The three blue crab processing plants studied currently have no liquid waste treatment systems other than screens in some drains and hand sinks to capture large solids. The facilities discharge all effluent overboard into tributary or river water adjacent to the plant. Some dilute, cleanup effluents from plants #2 and #3 discharge to municipal treatment systems.

Certainly, a rigorous study of pretreatment and treatment alternatives for these effluents must be investigated. Some initial treatment studies are presented herein, including settling and filtration of the major process effluents, acidification and coagulation of the more concentrated effluents and aerobic biological treatment of the retort water using a SBR.

Results of the settling and filtration tests are shown in Tables 3 and 4. Settling tests were done to see if sedimentation basins offer promise for reduction of solids, degradable organics and nutrients. Settling appears to work fairly well for effluents that contain high initial solids concentration including the Harris Claw and Quik-Pik process. However, final solids concentrations are still well above current limits (TSS values ranged from 410-12,000 mg/L after settling). One very important result is that settling has very little effect on COD and BOD_5 removal and may not be a viable method for reducing these parameters. Only 3-36% of BOD_5 was removed by settling. Even after filtration through glass-fiber filters, significant concentrations of BOD_5 and nutrients remained (after filtration: BOD_5 = 1,260-17,000 mg/L, TKN-N = 180-3,040 mg/L and TP = 22-270 mg/L). The results suggest that substantial quantities of soluble material are in these effluents, and biological and/or chemical treatment processes are necessary to meet current regulatory standards.

Acidification of samples with high concentrations of soluble organics was examined to determine the potential reduction of BOD_5 and TSS (Figure 2). TSS and BOD_5 of the retort water were reduced by approximately 40% and 30%, respectively. The brine bath and shell liquid had an over 90% reduction in TSS and a nearly 50% reduction in BOD_5 The finished effluents were still well above permit limits (BOD_5 = 7,580-24,850 mg/L and TSS = 480-2,160 mg/L after acidification), but acidification shows promise as a pretreatment method.

The results of aerobically treating retort water in a sequencing batch reactor are provided in Figure 3. The BOD_5 was reduced by approximately 50%. The TSS increased by about 20%, probably the result of newly formed biomass. Due to the high oxygen demand of the retort water, it was difficult to maintain aerobic conditions in the reactor. Aerobic treatment of this waste will require a large oxygen input that may be costly. Also, a significant amount of foam was produced during the process that may complicate aerobic treatment methods.

CONCLUSION AND REFERENCES

The processing effluents from three blue crab processing plants in Virginia were characterized. It was determined that nine types of liquid waste streams contributed the majority of the pollutant loadings from these plants. Most of these effluent streams were highly concentrated. For example, the retort water from the cookers exhibited the following concentrations: BOD_5 = 14,000-29,000 mg/L, TSS = 650-6,200 mg/L, TKN = 2,500-4,000 mg/L, NH_3N = 70-160 mg/L and total phosphorous = 100-185 mg/L. The Harris Claw process produced effluent streams with chloride concentrations exceeding 100,000 mg/L.

Table 3 - Settling and filtration of effluent samples from a blue crab processing plant.

DESCRIPTION	PRODUCT (lbs)	VOLUME (gal)	COD (mg/l)	BOD (mg/l)	TSS (mg/l)	VSS (mg/l)
CLAW REEL WASH	760	388	15,410	>7880	10,880	9,500
SETTLED CLAW REEL WASH			12,335	>3900	2,850	2,450
FILTERED CLAW REEL WASH			8,335	>3900	-	-
SHELL WASTE SOLUTION	760	370	39,680	16,240	33,414	16,386
SETTLED SHELL WASTE SOL.			20,645	12,533	11,971	3,357
FILTERED SHELL WASTE SOL.			17,135	9,553	-	-
SALT SEPARATION SOLUTION	760	220	15,871	8,923	12,667	3,737
SETTLED SALT SEPARATION SOL.			14,080	7,904	7,610	1,700
FILTERED SALT SEPARATION SOL.			11,467	6,867	-	-
CLAW CONVEYOR WASH	760	1,800	2,663	1,792	1,170	775
SETTLED CLAW CONVEYOR WASH			2,137	1,461	430	225
FILTERED CLAW CONVEYOR WASH			2,059	1,322	-	-
BOBBER	490	280	29,655	16,980	12,000	10,860
SETTLED BOBBER			22,938	13,725	5,360	5,240
FILTERED BOBBER			10,240	9,050	-	-
RETORT TRIP #1	1,300 *	45-50 !	31,040	18,780	653	534
SETTLED RETORT TRIP #1			25,858	18,181	624	497
FILTERED RETORT TRIP #1			24,465	17,000	-	-
RETORT TRIP #2	1,300 *	45-50 !	23,921	13,720	1,980	1,640
SETTLED RETORT TRIP #2			21,463	13,000	780	645
FILTERED RETORT TRIP #2			19,497	12,400	-	-
HANDPICK CLEANUP	920	220	3,680	2,482	1,238	1,000
SETTLED HANDPICK CLEANUP			3,128	1,585	452	356
FILTERED HANDPICK CLEANUP			2,508	1,262	-	-

Samples settled for 1 hour; samples were filtered through glass-fiber filters.

* denotes pounds of live crabs.

! denotes per cook of 1300 pounds of live crab.

Retort data from plant #3; other data from plant #2.

Table 4 - Settling and filtration of effluent samples from a blue crab processing plant.

DESCRIPTION	PRODUCT (lbs)	VOLUME (gal)	TKN (mg/l)	NH3-N (mg/l)	TOTAL P (mg/l)	SET. SOL. (ml/L)
CLAW REEL WASH	760	388	2,399	97	133	102
SETTLED CLAW REEL WASH			1,006	110	111	-
FILTERED CLAW REEL WASH			916	96	71	-
SHELL WASTE SOLUTION	760	370	3,391	114	278	196
SETTLED SHELL WASTE SOL.			2,371	128	185	-
FILTERED SHELL WASTE SOL.			2,172	120	140	-
SALT SEPARATION SOLUTION	760	220	1,773	77	157	45
SETTLED SALT SEPARATION SOL.			1,491	76	131	-
FILTERED SALT SEPARATION SOL.			1,305	74	93	-
CLAW CONVEYOR WASH	760	1,800	388	17	27	12
SETTLED CLAW CONVEYOR WASH			269	25	24	-
FILTERED CLAW CONVEYOR WASH			252	19	22	-
BOBBER	490	280	1,998	53	321	72
SETTLED BOBBER			1,435	46	221	-
FILTERED BOBBER			1,025	49	110	-
RETORT TRIP #1	1,300 *	45-50 !	-	-	-	1
SETTLED RETORT TRIP #1			3,069	165	298	-
FILTERED RETORT TRIP #1			3,034	163	289	-
RETORT TRIP #2	1,300 *	45-50 !	2,511	131	157	-
SETTLED RETORT TRIP #2			2,455	130	153	-
FILTERED RETORT TRIP #2			2,277	122	124	-
HANDPICK CLEANUP	920	220	249	10	123	24
SETTLED HANDPICK CLEANUP			218	9	112	-
FILTERED HANDPICK CLEANUP			179	9	91	-

Samples settled for 1 hour; samples were filtered through glass-fiber filters.

* denotes pounds of live crabs.

! denotes per cook of 1300 pounds of live crab.

Retort data from plant #3; other data from plant #2.

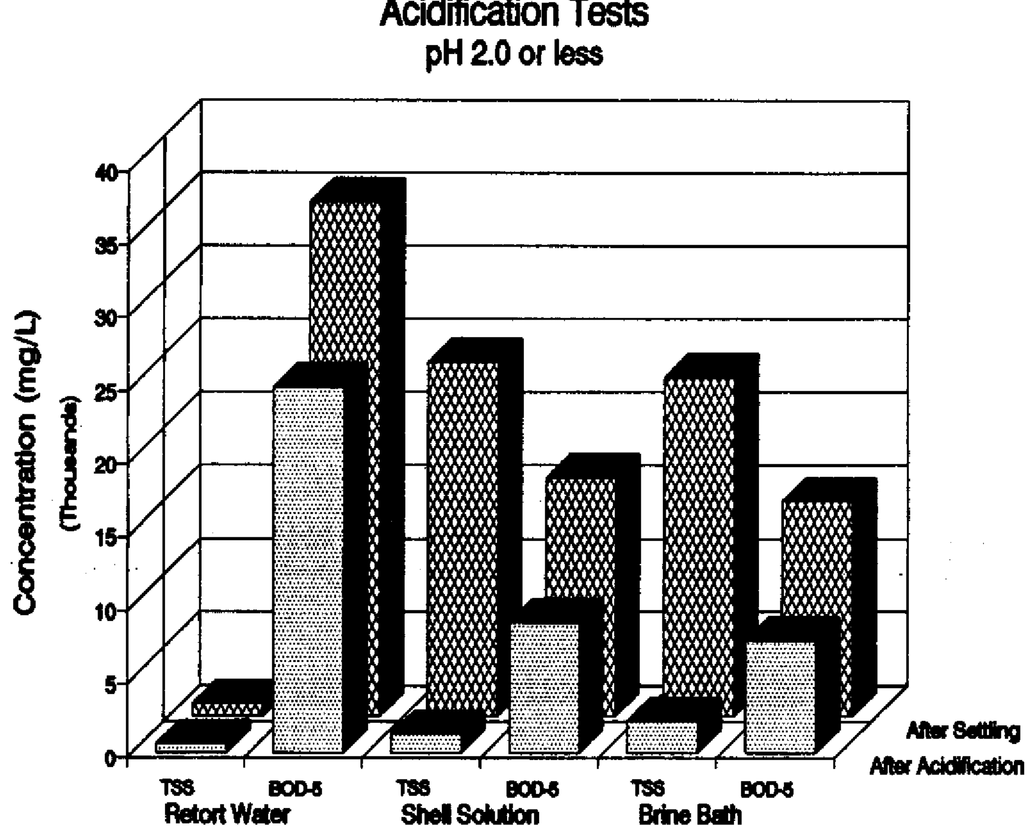

Figure 2 - Removal of BOD_5 and TSS by acidification of previously settled retort water, shell solution and brine bath.

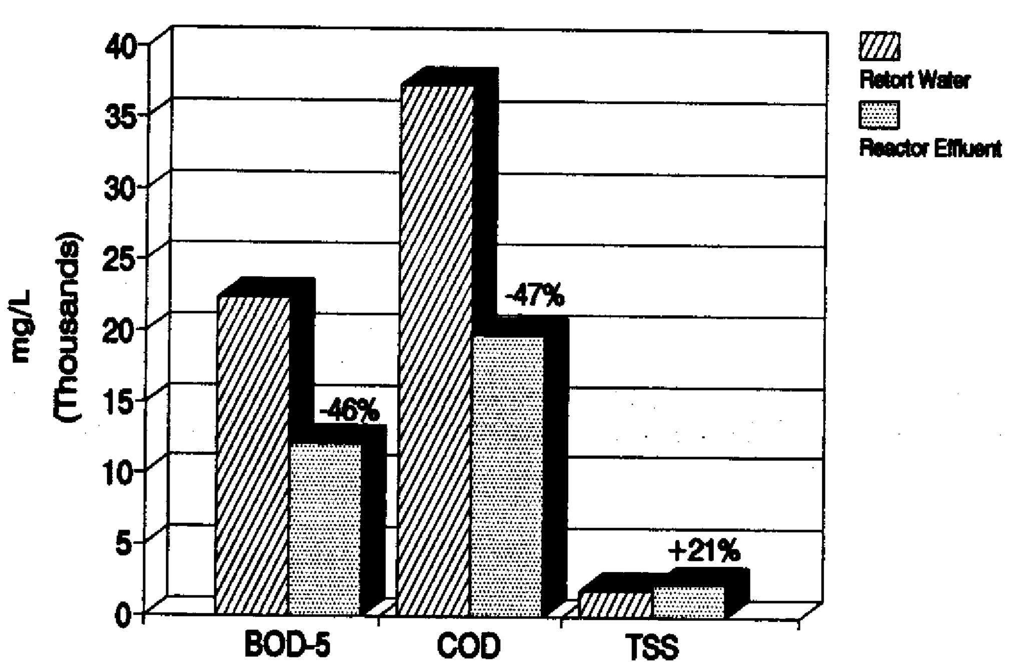

Figure 3 - Aerobic biological reduction of retort water BOD_5 and COD in a sequencing batch reactor.

Settling and filtration studies were done on these effluents. The organics and nutrients in the wastes were highly soluble and very little reduction of COD, BOD_5 TKN, ammonia and total phosphorus was achieved by these methods. Acidification of the concentrated effluents caused some coagulation of the contents. Acidification achieved TSS removals of 40-95%, and BOD_5 removals of 40-50%, thereby showing promise as a pretreatment method. Finally, aerobic treatment of the retort water in a SBR achieved approximately 50% removal of COD and BOD_5 after 18 hours of reaction time. Though the treatment methods above effected some good removals, effluent residuals remained well above permit limits.

Thus, among the waste management problems faced by the blue crab industry are: high organic and nutrient concentrations, high retort water temperatures, potential toxicity to biological treatment systems from brine effluents, intermittent daily and seasonal flow patterns, adequate space for locating treatment systems and low working capital to pay for technically feasible treatment methods.

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COMPARISON OF MANUAL AND AUTOMATED BRINE SYSTEMS FOR BLUE CRAB MEAT RECOVERY

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INTRODUCTION

Brine floatation has been used successfully for the recovery of blue crab meat for over 40 years. The (Harris) machines have provided commercial processors with an economically viable means for recovering crab claw meat (5). The meat is separated from the shell by dropping crushed claws into a dense brine solution. Meat, having a lower specific gravity than brine that is typically maintained at 60 to 70 salometer degrees (i.e., 177 to 210 grams NaCl per liter), floats to the top of the tank where it is directed over a spillway onto a dewatering and inspection belt system. The heavier shell sinks to the bottom of the tank where it is picked up by a conveyor mechanism to be discarded as scrap.

Although providing a cost effective means for recovering claw meat, the machines are virtually unchanged in design and operation from the early models and do have several drawbacks. First, the salt content in the meat increases and is variable which can lead to marketability problems. Secondly, the machines require frequent monitoring and manual adjustments in order to operate correctly. This is because both brine volume and density (sodium chloride content) will vary during operation due to spillage, salt uptake in meats and dilution effects. For adequate separation of meat from shell, sodium chloride content must be maintained at a high enough level to facilitate floating of meat. Therefore, during the course of 2 to 7 hours of operation, brine tanks must be closely monitored and small amounts of fresh or saline water added. In effect, the machines are not "fail-safe." In recent years, attempts have been made to improve the design and efficiency of Harris machines by adding an automated microcontroller to the system. The microcontroller is designed to read the density of the brine solution (i.e., specific gravity) and add automatically either brine or fresh water as needed for maintaining the tanks at predetermined sodium chloride levels (6).

The objectives of this study were: 1) to monitor brine tank composition (total suspended solids, TSS; total dissolved solids, TDS; dissolved protein, DP; sodium chloride, NaCl) of both automated and manually operated systems in order to compare their ability to maintain brine concentration during operations, 2) to monitor the consistency in meat quality produced (defined as percent NaCl and moisture) under both operations; and, 3) to determine if observed meat quality fluctuations are correlated with fluctuations in the brine composition under which they were processed.

METHODS

Observations and sample collection were made at two NC blue crab processing facilities during the summer of 1991. Washington Crab Company (Washington, NC) and Fulchers' Point Pride Seafood (Oriental, NC) were industry cooperators in a NC Pollution Prevention project designed to assess water use patterns and water and wastewater reductions options for crab meat processors using the brine floatation technique.

Brine Composition

Samples were collected from Harris machine operations at the junction of the floatation tank spillway and the dewatering/ inspection belt (Figure 1) every 15 to 20 minutes during 5 hour runs of both automated (microprocessor) and manually controlled systems. The brine was collected in a shallow pan as it spilled into the overflow/reservoir tank. One liter of the brine was immediately transferred to an Imhoff cone and allowed to settle undisturbed for one hour to determine the total settleable solids (TSS) content (7).

The remaining brine solution was measured for density using glass hydrometers (Fisher Scientific, Atlanta, GA) calibrated in salometer units, percent NaCl and specific gravity and for refractive index using a standard hand-held refractometer (American Optical, Buffalo, NY). Temperature of samples was measured with a mercury in glass thermometer and recorded. The microprocessor LED display in salometer units (KelTech, Inc., Roanoke, VA) was recorded during operation of the automated system.

The total dissolved solids (TDS) fraction of the solution (defined as solids passing through a 0.45 micron filter) was obtained by passing representative samples of brine through a series of 5 filtration steps. The procedure is schematically represented in Figure 1 and includes: 1) glass wool wrapped in cheese cloth to remove coarse particulates of shell and meat; 2) Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, England); 3) Whatman No. 54 filter paper; 4) glass fiber filter (Gelman Instrument Co., Ann Arbor, MI); and, 5) 0.45 micron cellulose nitrate membrane filters (Whatman International Ltd, Maidstone, England). Twenty to thirty milliliters of filtrate were collected for each sample taken. Filtrates were placed in clean containers and kept on ice. All samples were refrigerated in the laboratory until analyses could be performed (within 24 hours of collection) for TDS, DP and NaCl.

Total dissolved solids (TDS) content was determined by evaporating a 5 ml aliquot of sample in clean preweighed test tubes at 103-105 C until constant dry weights were achieved. Duplicate analyses were performed for each filtrate. Chloride content in ppm was determined utilizing No. 1176 Quantab chloride titrator strips (Environmental Test Systems, Inc., Elkhart, IN) on samples diluted with distilled, deionized water. Values were corrected for dilution and converted to the corresponding NaCl levels. The Quantab chloride titrator method has been used successfully for determining NaCl content of a variety of high protein foods (8).

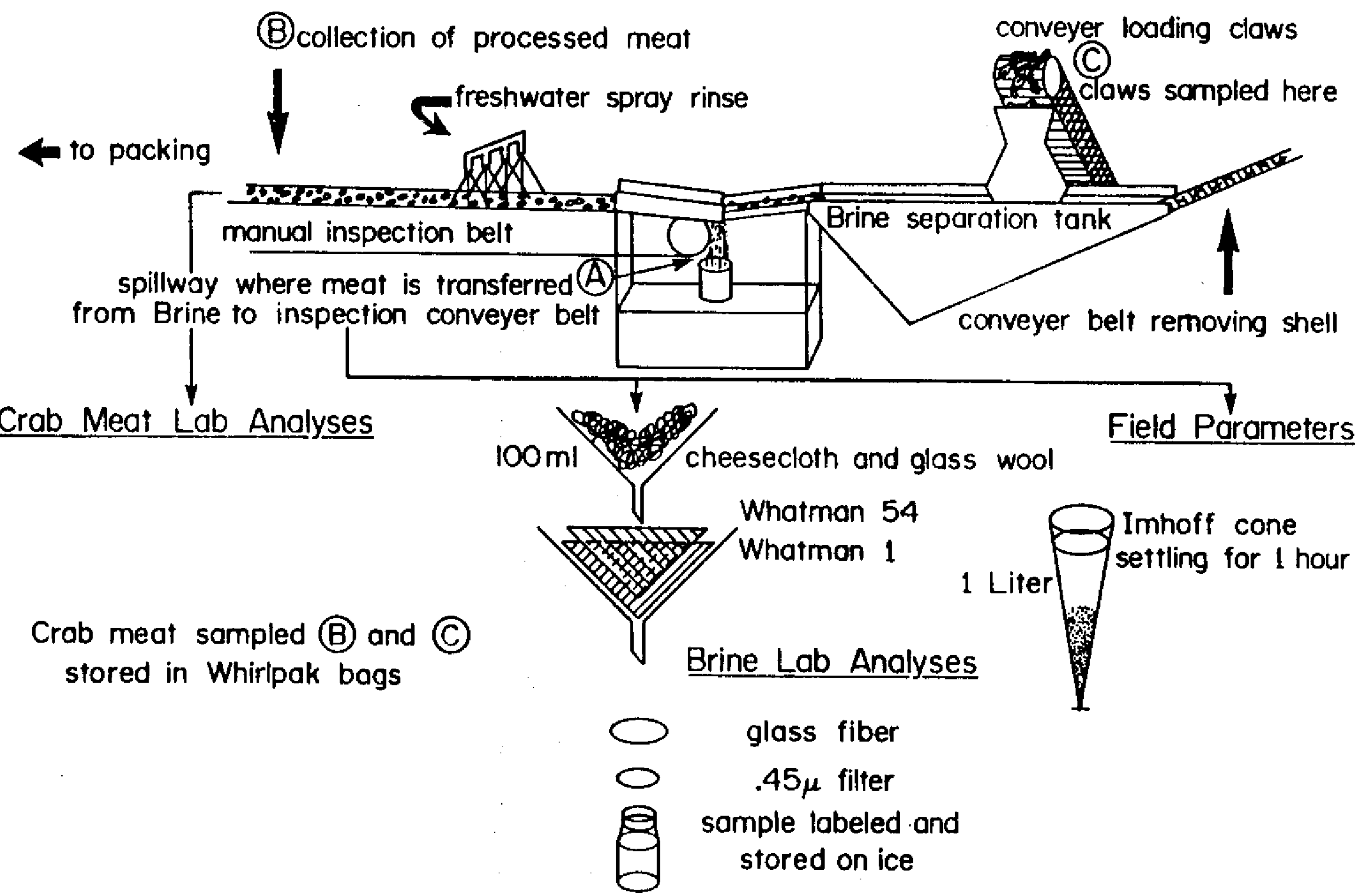


Figure 1. Sample collection for brine, A and meat, B & C and schematic of procedure for total dissolved solids.

Dissolved protein (DP) content of filtrates was measured using the Bio-Rad protein dye binding assay (3). Bovine serum albumin (BSA) was used as a standard protein for calibration. Laboratory tests indicated that normal brine concentrations (ca. 210 g/L) encountered in the floatation tank did not interfere with the Bio-Rad protein assay using the standard procedure given (2).

Crab Meat Composition

Crab claw meat was collected from the inspection line every 15 to 20 minutes during operations. In all instances, effort was made to collect meat samples corresponding in time with the brine samples collected. The samples were placed in Whirlpak bags (Baxter Scientific, Charlotte, NC) and stored on ice for transport back to the laboratory.

During automated runs, unprocessed crab claws were sampled from the incoming conveyer belt midway through the operational period for comparison with meat recovered through brine floatation. During manual operations, unprocessed claws were collected at eight intervals to assess the variability of incoming crab meat quality and to compare with finished product qualities.

On return to the laboratory, meat samples were refrigerated until they could be analyzed the next day for NaCl content using the Quantab chloride titrator procedure (4). The percent moisture content of meat samples was determined in triplicate by weight loss after oven drying at 103 to 105 C for 18 hours (1). In addition, unprocessed and processed meat samples were frozen and sent to North Carolina State University's Department of Food Science for Kjeldahl-protein analyses (1).

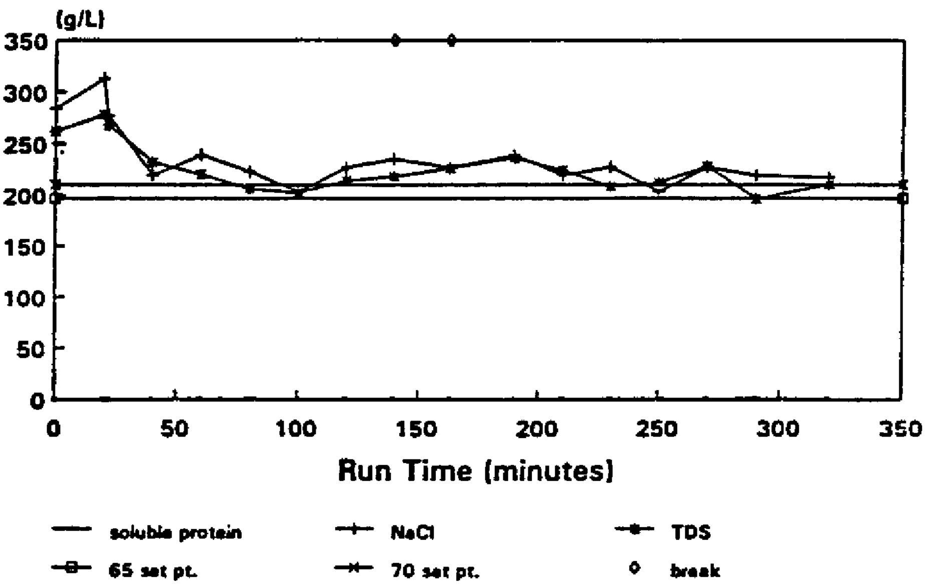
RESULTS AND DISCUSSION

General observations made during automated and manually controlled operations are discussed in greater detail in the preceding paper entitled, "Waste reduction in mechanical blue crab claw processing operations." Specific data collected during experimental runs are summarized below.

Brine Composition

Figures 2b and 3b show the results from density and refractive index measurements taken during manual and automated operations, respectively. In both cases, measurements using refractive index and specific gravity did not provide sufficient resolution to show variability in brine solutions. A NaCl hydrometer showed better resolution. However, the salometer calibrated hydrometer, which is the type commonly used by industry operators, showed the best resolution. The microcontroller LED readings agreed well with the manually determined salometer readings (Figure 3b).

When the LED readings were plotted along with measured TDS levels, good agreement at the beginning and end of the operational period was shown (Figure 3a). However, during the middle of the run, LED readings showed some discrepancy from those measured by the TDS procedure. The LED readings indicated that the tank was being maintained between desired levels while TDS levels actually measured fell below these values.



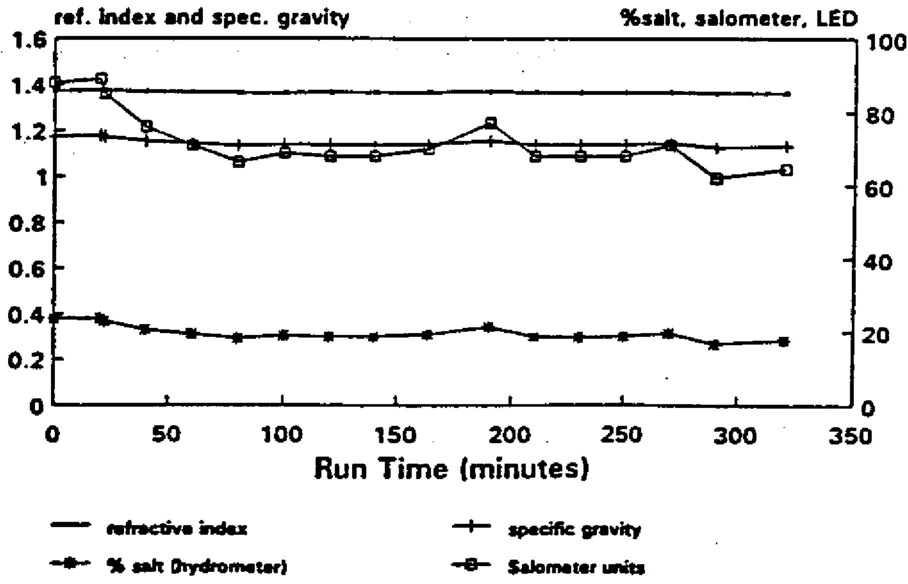
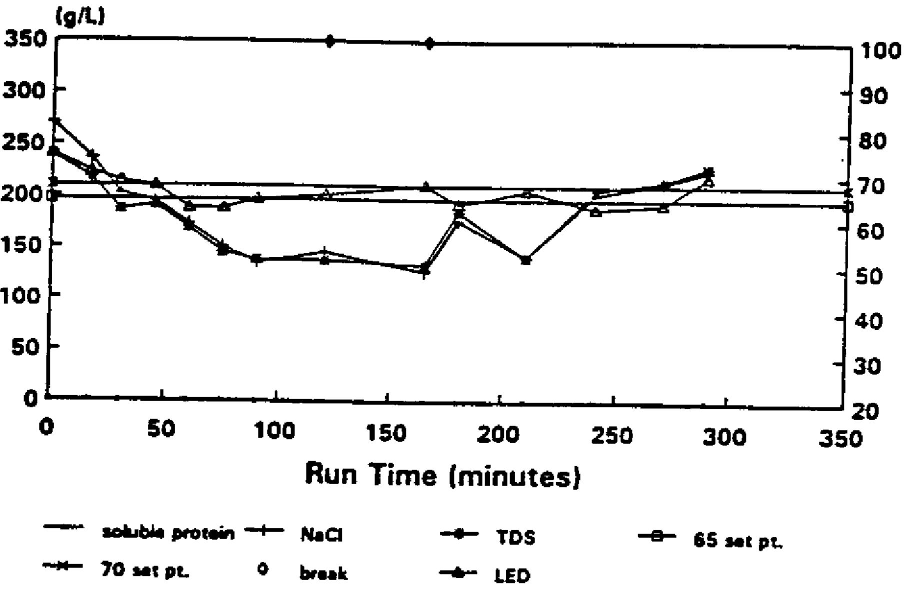


Figure 2 Brine composition (A) and field parameters (B) measured during manual Harris machine operations.



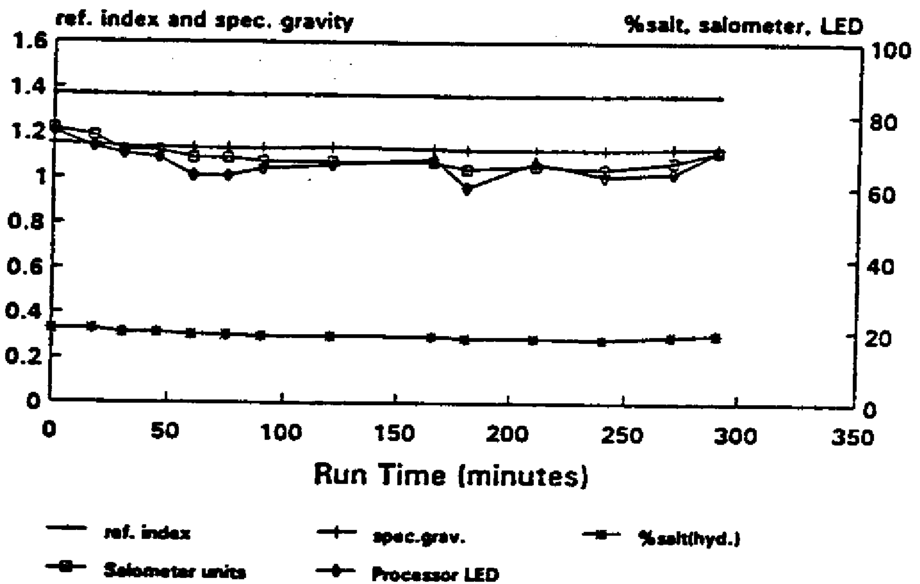


Figure 3 Brine composition (A) and field parameters (B) measured during automated Harris machine operations.

Accounting for this discrepancy would require further investigation although it should be noted that the locations where the two readings were taken differs. The microprocessor sensor (LED) was positioned in a small stainless steel surge tank located adjacent to the main floatation tank. While measured DS levels were made on brine taken at the spillway/inspection belt interface. The TDS and NaCl composition in the floatation tank during manual operations held steady although slightly higher than the desired levels (Figure 2a).

Figures 2a and 3a illustrate that total dissolved solids (TDS) content in the floatation tank can be nearly accounted for by NaCl alone. Dissolved protein (DP) content in the brine does increase with processing time (Figures 4a and 4b). However, protein concentrations never exceeded 2 g/L which is negligible in its affects on the density of a 150 to 250 g NaCl/L solution.

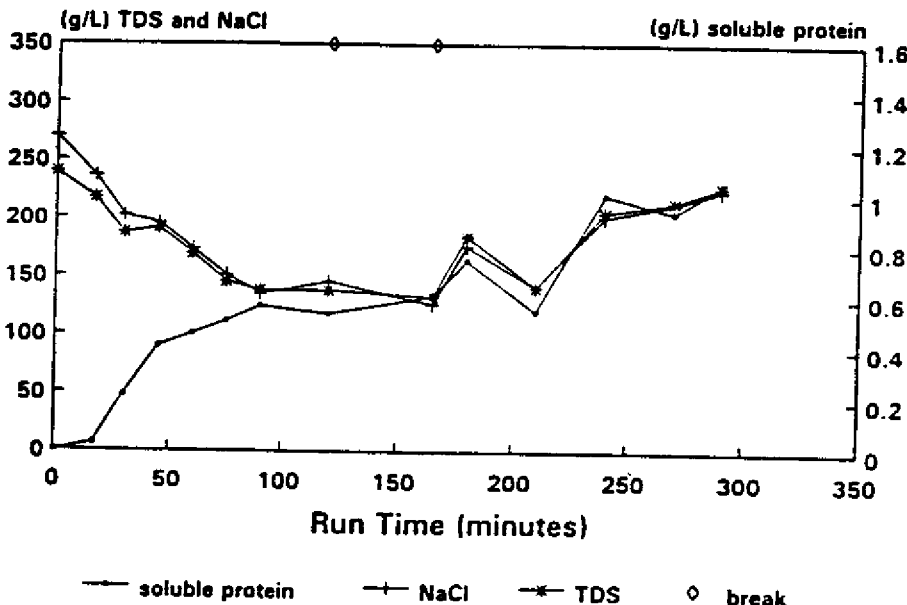
The pattern of increase for DP in the brine over time for both operations paralleled the increase in total settleable solids (TSS) measured (Figures 5a and 5b). Linear regressions (Figures 6a and 6b) of TSS versus DP concentrations gave correlations of 0.90 and 0.84 for automated and manual runs, respectively. This suggests that the amount of settleable solids (consisting largely of small meat particles) building up in the brine over time exerts a direct effect on DP content in the solution.

Crab Meat Composition

During automated and manual operations, the percent moisture of recovered crab meat was constant (Figures 6a and 6b). Meat moisture content increased approximately 5 percent (Table 1). Sodium chloride content of processed claw meat was more variable than moisture (Figure 6a and 6b). The levels approximately tripled from pre-processing levels (Table 1) and was slightly higher over all for meats recovered during the manual process. Recall that the brine in this process was found to be higher in sodium chloride levels (200-250 g/L) compared to the automated process (140-200 g/L). This suggests a direct relationship between brine concentration and the salt content found in recovered meats. However, when mg NaCl/g meat was plotted against brine concentrations (Figures 7a and 7b), no direct correlation was found (R^2 values of -0.15 and as -0.58, respectively).

Table 1. Salt and moisture content of blue crab claw meat.

Sample	System	NaCl (mg/g)	Moisture (%)	NaCl at 80% Moisture	NaCl at 80% Moisture (%)
Unprocessed	Automated	10.14 $\pm$ 0.00	77.99 $\pm$ 0.53	10.4	1.04
	Manual	9.37 $\pm$ 1.64	78.04 $\pm$ 0.84	9.61	0.96
Processed	Automated	29.42 $\pm$ 4.26	84.09 $\pm$ 0.76	27.99	2.8
	Manual	33.99 $\pm$ 4.92	82.55 $\pm$ 1.39	32.94	3.29



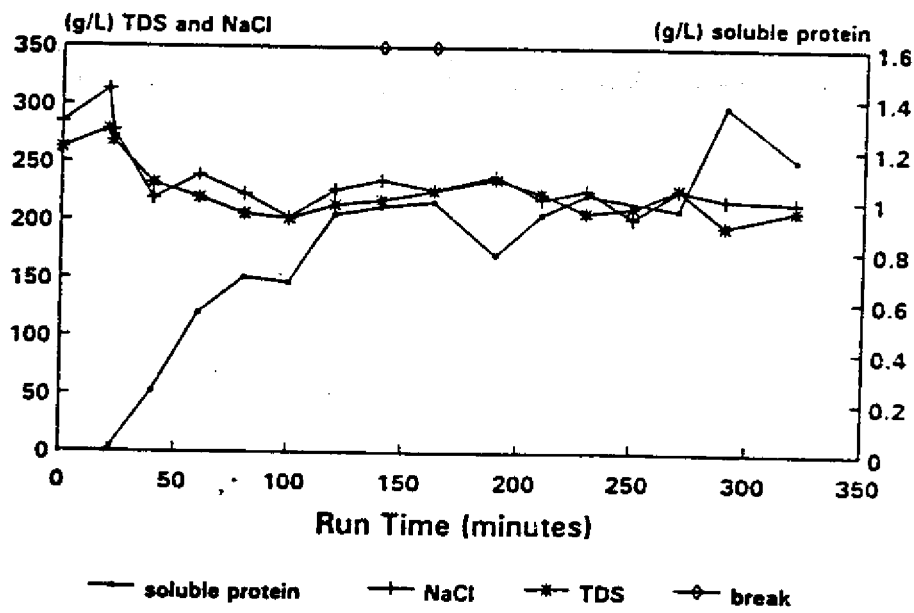
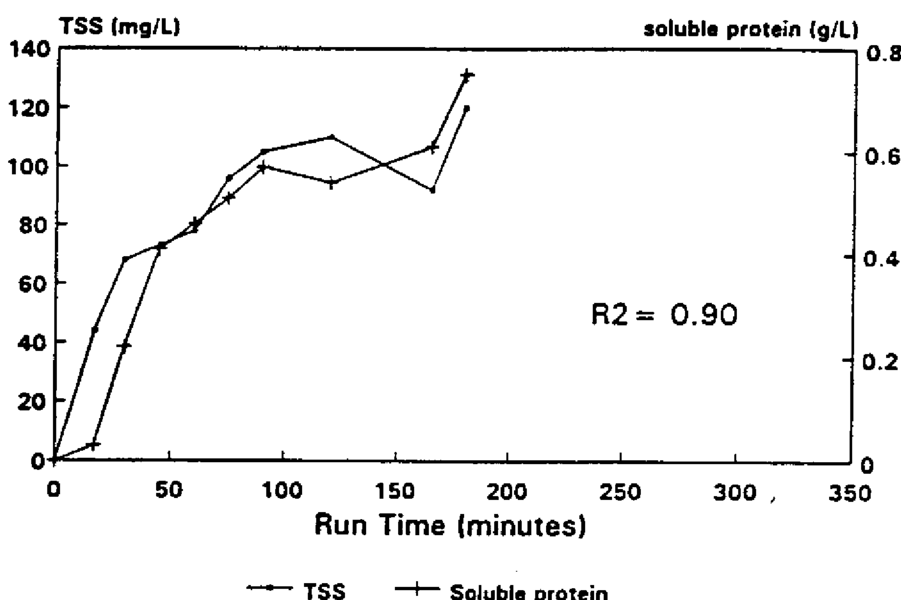


Figure 4. Total dissolved solids (TDS), NaCl and soluble proteins (DS) in automated (A) and manual (B) Harris machine systems.



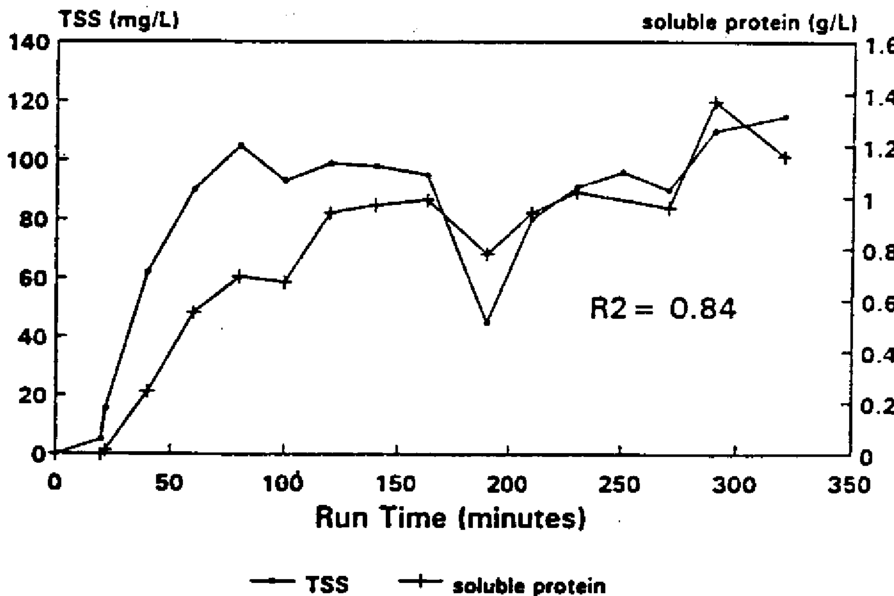
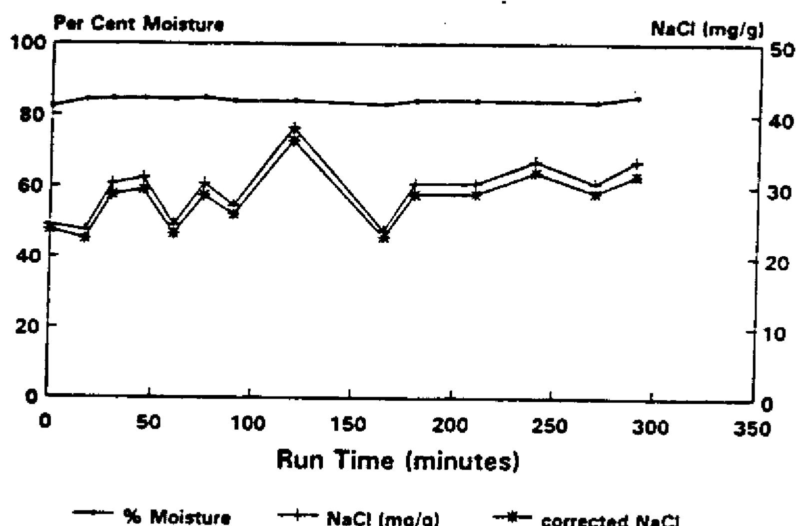


Figure 5. Correlation of total settleable solids (TSS) and dissolved proteins (DS) in automated (A) and manual (B) Hasrris machine systems.



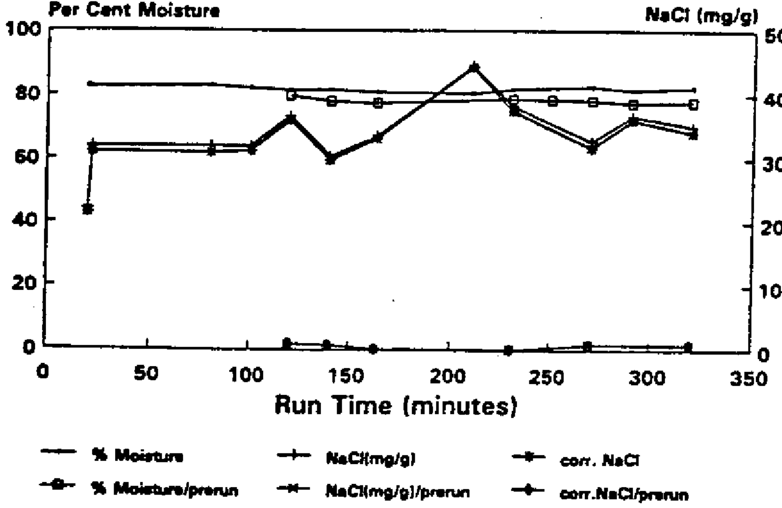
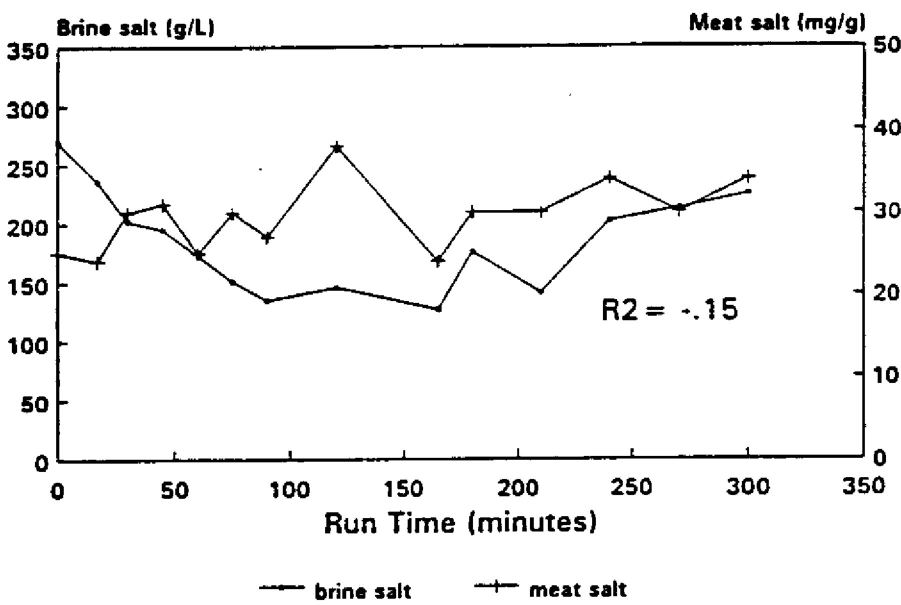


Figure 6. Crab meat qualities during automated (A) and manual (B) Harris machine systems.

Covariance Brine and Meat Salt



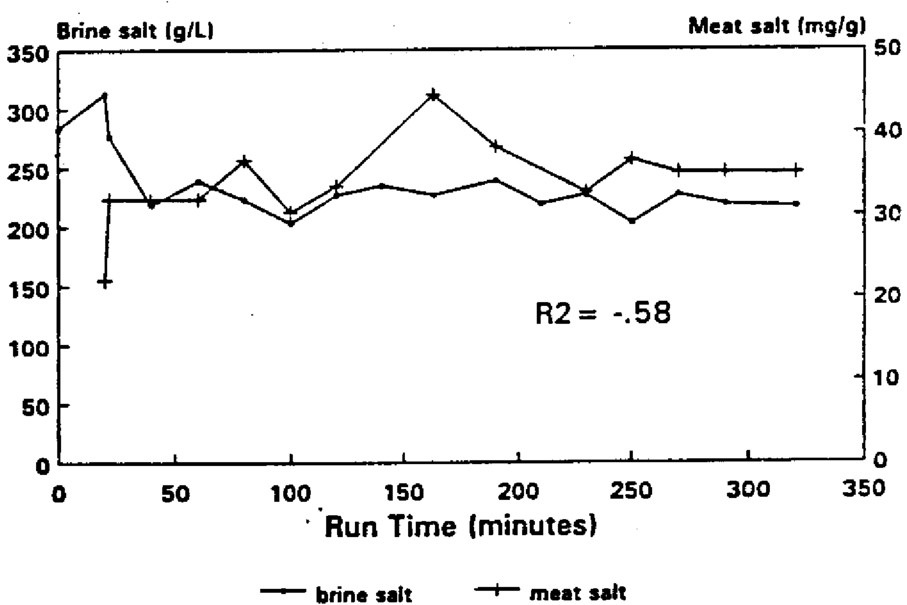


Figure 7. Covariance of crab meat chloride conctent in automated (A) and manual (B) Harris machine systems.

No differences were found in protein content of claw meat processed early and late during manual operations (Table 2). Protein levels for processed meat was found to be 36 percent lower than protein levels for unprocessed meat. Although salt, moisture and protein data suggest no difference in product quality at the beginning and end of processing runs, the meat visually appeared quite different. Meat produced early in the run was lighter in color, contained larger particles and was softer in texture. Meat produced later in the run was dark in color, had smaller particle sizes, was grainier in texture and generally was less appealing.

Table 2. Proximate composition of blue crab claw meat recovered by brine floatation.

Crab Meat	Protein, %	Moisture, %	Protein, % (at 80 % Moisture)	Protein, % Loss
Unprocessed	21.58 ± 0.47	77.94 ± 0.95	22.15	-
Processed (Early)	14.44 ± 0.62	82.70 ± 0.05	13.97	36.9
Processed	14.61 ± 0.25	82.90 ± 0.23	14.10	36.3

CONCLUSIONS

Results suggest that both automated and manually controlled systems for operating Harris machines can adequately maintain the process within desired operating conditions. Whether or not installation of an automated microcontroller would be beneficial to a particular operation depends on the mechanical reliability of the controllers. Cost of the equipment could be assessed in terms of the savings gained from offsetting the need for skilled labor.

Meat quality, particularly salt content, appears to be a function of several variables in addition to salt concentration in the floatation tank. Loading rates and tank circulation patterns both of which pertain to the amount of time that the meat is exposed to the brine are important variables that warrant further study. Seasonal changes in crab meat composition and processing temperature of crab claws are two other variables that processors believe affect recovery efficiency and meat qualities (J. Johnson, Washington Crab Co., Washinton, NC, personal communication, 1991).

Although wastewater proteins (DP) did not seem relatively high compared to the salt content of the brine, amounts were significant in terms of protein losses. Considering the protein and product quality losses incurred during processing, further technological improvements in the Harris machine systems are warranted.

ACKNOWLEDGEMENTS

This project was sponsored in part by the NC Department of Environment, Health and Natural Resources, Office of Waste Reduction, Pollution Prevention Program, the University of North Carolina Sea Grant College Program and the North Carolina Cooperative Extension Service. The authors express their appreciation to Chris Fulcher and Glen Barnes of Fulchers Point Pride Seafood, Inc. and Harold Stephenson and Jim Johnson of Washington Crab Company for use of their facilities and valued assistance. We thank KelTech, Inc. of Roanoke, Virginia for the cooperation and suggestions given. Jutta Malbaum, Reino Korhonen, Joyce Taylor and Robin Lazenby provided technical help and assistance. The use of trade names in this publication does not imply endorsement by North Carolina State University, nor criticism of ones not mentioned.

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ABSTRACTS

FISHERY DISEASES, ENVIRONMENTAL QUALITY AND HUMAN HEALTH

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The environment plays a critical role in the expression of disease in aquatic animals. There is considerable empirical evidence that many diseases in fishery populations are associated with human activity. Some of the best indicators of environmental degradation include shell disease, fin erosion, skin ulcers, and neoplasia. Although these syndromes are clearly associated with polluted environments, the mechanisms responsible for their development are uncertain and a precise cause and effect relationship to anthropogenic factors has yet to be demonstrated. Nonetheless, they serve as useful bioindicators of impacted environments and in some cases, might be indicators of risk to human health from contamination of seafood products.

PATHOGENIC VIBRIOS AND SHELLFISH

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Unlike many of the disease-producing bacteria and viruses that may be present in coastal waters, the presence of bacteria of the genus _Vibrio_ show no correlation with pollution. These bacteria are normal flora in coastal waters and shellfish and occur in high numbers in oysters. At least 11 species of Vibrio are known human pathogens, and infections with most, such as _V. parahaemolyticus_, typically results in mild to severe gastrointestinal disease. Other species, such as _V. cholerae_, have long been involved in worldwide epidemics, and may lead to significant mortality. More recently, _V. vulnificus_ has received increased attention as it, of all microorganisms occurring in coastal waters, is now realized to be the major cause of seafood-related deaths in the United States. This paper will describe the various human pathogenic vibrios, concentrating on _V. vulnificus_. To be discussed will be the clinical aspects and epidemiology of the disease produced by _V. vulnificus_, its "viable but non-culturable" state and our most recent findings regarding its interactions with shellfish, including the possibility that commercial depuration will not be an effective method of removing the potential health hazard presented by this bacterium.

PHAGOCYTIC INTERACTION OF CLAM AND OYSTER HOMOCYTES WITH VIBRIO VULNIFICUS

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The morphology, hematological paramet4rs and behavior of both clam and oyster hemolymph cells were studied when challenged with capsulated, non-encapsulated, and viable but non-culturable *Vibrio vulnificus*. Two general classes of hemocytes (granular and agranular) were found to exist in both the quahog, *Mercenaria campechiensis*, and the eastern oyster, *Crassostrea virginica*. Transmission electron microscopy revealed three sizes of electron dense granules. Both granular and agranular hemolymph cells were capable of colchicine-sensitive pseudopodial movement and spreading. A higher number of non-encapsulated and viable but non-cultured *V. vulnificus* were associated with both clam and oyster hemocytes when compared to the encapsulated *V. vulnificus*. Phagocytosis was serum dependent and heat colchicine sensitive. Temperature increased the *V. vulnificus* hemolymph association. The morphology of these cells as determined by high scanning and transmission electron microscopy showed some similarity to mammalian mononuclear phagocytes.

THE EFFECTS OF IONIZING RADIATION AND HIGH ENERGY ELECTRON BEAMS ON MOLLUSCAN SHELLFISH WITH RESPECT TO VIBRIO VULNIFICUS

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A three to four log cycle reduction in bacterial number was observed upon exposure to oysters to ionizing radiation (^{60}Co_ at 1.0, 2.0 and 5.0 kilograys. The shelf life of irradiated oysters was also monitored. Fify percent of the irradiated oysters were dead within 12, 10 and 7 days at 1.0, 2.0 and 5.0 kilograys of exposure, respectively. Cultures of virulent and non-virulent *Vibrio vulnificus* were quite radiosensitive as no colony forming units could be detected after 0.5 kilogray exposure. Furthermore, survival curves were plotted for virulent and avirulent *V. vulnificus*, and subsequent D_{10}values were calculated.

THE DETECTION AND INTERPRETATION OF CADAVERINE AND PUTRESCINE LEVELS IN FISHERY PRODUCTS AS INDICATORS OF DECOMPOSITION

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The presence of cadaverine and putrescine in decomposed fishery products has been determined for a variety of species, including tuna, mahi mahi, snapper, cod, crab meat, scallops, shrimp and sea trout. The formation of these compounds as a function of decomposition temperature and time has been studied in mahi mahi and has been compared to the amount of histamine formed. The relationship of these compounds to sensory evidence of decomposition will be discussed, and the practical use of the two compounds with respect to quality control and surveillance programs will be addressed.

MODIFICATION OF GLC METHOD: RAPID SCREENING TEST FOR PUTRESCINE AND CADAVERINE

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The gas-liquid chromatographic (GLC) method of Staruszkiewicz and Bond for the determination of putrescine and cadaverine has been modified to provide a rapid screening test for diamines in fishery products. The amines were extracted from fishery products by using 75 percent methanol in water. A dried residue of the hydrochloride salts of the amines was prepared and an internal standard (hexanediamine) was added. Derivatives were made by heating the dried residue with pentafluoropropionic anhydride in ethyl acetate for 30 minutes at 50 C. Toluene was added to the reaction mixture to adjust the ethyl acetate concentration to 30 percent. A 100-ul portion of this mixture was purified on a 30-ml alumina-N solid-phase extraction (SPE) column, consisting of 1,000 mg of neutral alumina. The derivatives were eluted with 8 ml of 30 percent ethyl acetate in toluene. Putrescine and cadaverine were determined by GLC with electron capture detection on a column of 3 percent OV-225 at 175 C. In the range of 1 to 60 ug/g, the results obtained using the SPE columns were comparable to those obtained using the standard glass columns. The SPE columns reduced cleanup time prior to GLC separation from 1 hr to 10 min, which requiring less space and eliminating the washing of glass columns. The differences between SPE columns will also be discussed.

INHIBITION OF HEAT STABLE PROTEASES IN INTACT FISH FILLETS

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The flesh of several fish species, such as Alaskan arrowtooth flounder, can possess high levels of heat-activated proteases. This can limit use of the fish fleshfo surimi, mince or fillet forms because the texture rapidly degrades under all but the most rapid cooking conditions (such as by microwaves). We have previously shown that beef plasma, egg white and one of the components of these materials, alpha-2- macroglobulin (alphalin), effectively inhibit these proteases when incorporated into surimi prepared from several fish species. The present study additionally demonstrated their effectiveness when used to treat intact arrowtooth flounder muscle via vacuum infusion. Beef plasma and alphalin were found to be equally effective in inhibiting proteolytic degradation of the infused arrowtooth flounder muscle, but egg white was much less effective. However, egg white is an effective inhibitor in certain species such as Atlantic menhaden. Use of alphalin has the advantage of broad spectrum effectiveness with no discoloration or flavoring of the flesh.

THE USE OF OZONE TO DEGRADE RED TIDE TOXINS

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Gymnodinium breve toxins were exposed to ozone treatment in both extracted form and in intact whole cells. Samples displayed a three log reduction in the total amount of toxin (PbTx-1, -2, -3,
-5, -7 and -9) recovered after 10 minutes as determined by HPLC analysis. Ozone effectively killed the red tide dinoflagellates when directly contacted ozone and when exposed in a pre-ozonated ASW environment. Both samples, when examined by light microscopy, displayed little difference between the direct and indirect ozone treatments. Reduction in toxin levels directly correlated with reduction of toxicity as observed using by a fish (Cyprinodon variegatus) bioassay.

DISTINGUISHING WILD FROM CULTURED FISH USING DIFFERENCES IN FATTY ACID PROFILES

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This paper provides the results of a two-year cooperative research effort between the National Marine Fisheries Service (NMFS), Charleston Laboratory's Marine Forensics Program, and the South Carolina Wildlife and Marine Resource Division (SCWMRD). The first objective of the research effort was to develop a biochemical method to distinguish wild from cultured fish. The second objective was to use the edible portion of the fish as the test material. This research effort was initiated because of concern by SCWMRD that a successful hybrid striped bass aquaculture industry might result in an increase in the illegal capture of wild striped bass and its hybrids from South Carolina waters for sale to commercial markets. In this study, over 1,500 wild striped bass and its hybrids were collected four times a year over a two-year period, from Lakes Hartwell, Thurmond, Murray, Wateree and Moultrie. Cultured hybrid striped bass and diet samples were collected from the Waddell Mariculture Center in South Carolina and from three commercial aquaculture operations. In addition to visual interpretation of the data, Linear Discriminant Analysis was used to classify fish into wild or cultured categories and collection site. Classification into wild and cultured categories was 100 percent accurate. Classification of fish into collection site categories ranged from 75 to 100 percent accuracy.

ACCELERATION OF LIPID OXIDATION DURING COOKING OF REFRIGERATED MINCED CHANNEL CATFISH MUSCLE

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Minced channel catfish muscle was refrigerated for periods up to seven days. At 0,2,5 and 7 days, preweighed samples were analyzed either before or after baking for fatty acid composition, thiobarbituric acid reactive substances (TBA-RS), fluorescent pigments and tocopherol content. No differences were noted between the raw and cooked product in polyunsaturated fatty acids (PUFA) at all sampling periods. In addition, no loss of PUFA was seen with increasing length of refrigerated storage. Although increases in the amount of TBA-RS and fluorescent pigments were recorded in all cooked products over that of raw product, the amount of increase varied with the length of refrigeration. Larger increased in TBA-RS were found after cooking two-day refrigerated samples than 0 day samples, whereas cooking of five- and seven-day-old samples led to much smaller increases in TBA-RS. In contrast, the largest increases in fluorescent pigment content after cooking were found in the five-day refrigerated samples. No change in the tocopherol content was seen with storage of raw samples. Loss in gammatocopherol upon cooking remained fairly constant (15%) whereas losses of alpha-tocopherol were greater in the two- and five-day refrigerated samples (40%) than in the seven-day refrigerated sample (14%).

CONSIDERATIONS FOR THE DEVELOPMENT OF CRAB PASTEURIZATION PROCESSES

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Spoilage of pasteurized crab meat can be due to a variety of reasons including faulty can seams, temperature abuse and/or the presence of microorganisms able to grow at refrigeration temperatures. An apparently new species of *Clostridium* was found to cause spoilage in pasteurized crab meat. This organism is psychrotrophic and can grow at temperatures as low as 2 C. Spores of this organism were able to survive a pasteurization process with an F16 185 of 85 minutes. Considerations for the development of crab pasteurization processes will be discussed.

ELIMINATION OF LISTERIA MONOCYTOGENES AND EXTENSION OF SHELFLIFE IN FRESH CRAB MEAT BY ATMOSPHERIC STEAM

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Fresh lump meat from blue crab (Callinectes sapidus) was processed in a modified blancher until the internal temperature of monitored lumps reached the designated temperatures of 175°F (80°C) or 185°F (85°C). Control and heated meat placed in eight-ounce containers was stored in ice in a commercial refrigeration unit. Analyses included Listeria count (Oxford agar, 30°C), Staphyloccocus aureus count (Baird-Parker agar, 42°C), aerobic plate count (20°C), texture measurement (Instron), color measurement (Minolta Color-meter, L*a*b*scale), and sensory panel evaluations for appearance, taste, odor and texture. On Day 1 only, samples were also tested for percent moisture content, total coliforms and fecal coliforms. Samples were tested over the apparent shelflife of the meat, determined by a borderline score in sensory evaluation and unaccpetable microbial counts. Steam treatments resulted in the elimination of Listeria monocytogenes, confirmed by inoculated pack studies. Steam treatments also controlled the growth of S. aureus. The processed meat also had a one to four log reduction in aerobic plate counts, thsu extending shelflife by as much as two weeks, upheld by sensory panel evaluations. The use of steam enhanced several sensory attributes and contributed no additional moisture to the meat.

FUNCTIONS OF AND USES FOR PHOSPHATES IN THE SEAFOOD INDUSTRY

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The use of phosphates in muscle food systems has been investigated since the 1950s. Crawford (1980) evaluated the effect of one to two minute dips of 6 percent sodium tripolyphosphate on mechanically peeled shrimp (Pandulus jordani) and determined that the yield was increased by 12 percent. This short-term treatment caused a case hardening of the flesh, which resulted in more efficient separation from the shell and the retention of muscle moisture. The economic impact of short-term phosphate dips to shrimp resulted in greater than $65 million in the first eight years of use to the ex-plant value of the product to Oregon alone. The use of phosphates and blends to other seafoods has expanded. Its importance is directly related to the sensitivity of seafood myofibrillar proteins and denaturation at chill storage temperatures. Retention of water holding capactiy of the muscle is vital to the retention of natural juices and thus prevents economic fraud due to fluid losses during shipment and prior to sale.

ON-BOARD CRYOGENIC FREEZING OF SEA SCALLOPS

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Freezing a product does not improve its quality. It simply maintains the quality of the product which was inherent at the time of freezing. The factors which influence the quality of the final product quality include the raw material, handling prior to freezing, the freezing process, handling after freezing and storage before distribution.

We compared the freezing process, cryogenic with liquid CO_2 vis-a-vis mechanical plate freezing for its effect on product quality. Both prerigor and postrigor scallops were included in the study and quality aspects covering microbiology, sensory, thaw loss, moisture/protein ratio, etc. were studied.

It is on two counts that one estimates value to the processor: the worth of the product and the reduction in loss. On both counts, cryogenic freezing was found to be better than mechanically (plate) frozen scallops.

INTERACTIONS OF ZINC IONS AND SUCROSE FOR CYROPROTECTION OF SURIMI

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Previously a remarkable synergistic effect of zinc ions with carbohydrates in the cyroprotection of certain enzymes was reported by other workers. For example, at 0.6 mM $ZnSO_4$ the level of sucrose needed to protect phosphofructokinase activity could be lowered by two orders of magnitude. Studies were conducted to determine whether a similar effect might be observed in the cyroprotection of surimi by sucrose and other carbohydrates. A model system was employed involving rapid freezing and thawing of actomyosin solutions followed by evaluation of myosin Ca^{2+}ATPase activity as an indicator of protein denaturation. Initial tests with $ZnSO_4$ levels varying 0.05 to 1.0 mM were disappointing in that rapid loss of ATPase activity was effected by increasing levels of $ZnSO_4$ Further investigation with even lower levels $ZnSO_4$ and sucrose showed that in contrast with other reported protein systems there appears to be little if any increase in cyroprotection with addition of $ZnSO_4$ to surimi.

WASTE REDUCTION IN MECHANICAL BLUE CRAB CLAW PROCESSING OPERATIONS

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Pollution prevention through source reduction, water reuse, and recycling has become increasing important to seafood processors in light of stricter environmental regulations, decreased profit margins and greater international market competition. This study focuses on in-plant management practices, process modifications and employee education programs in mechanical operations for recovery of blue crab claw meats. Discussion will focus on how seafood processors can make "pollution prevention pay" and become a more responsible and environmentally aware industry.

RECOVERY AND FOOD UTILIZATION OF SURIMI LEACHWATER PROTEINS

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Earlier this year we reported results of a comparative study of various methods of protein recovery from surimi leachwater, including ultrafiltration, ultrafiltration coupled with diafiltration, precipitacion by pH shifting, and ion exchange. The latter method, which is now commercially used to produce an excellent quality whey protein isolate, has now been scaled up in the pilot plant with comparable recovery rates to earlier bench-scale trials. A progress report will be given on the testing of the protein recovered by ion exchange as a filler material for surimi.