BACTERIA OF SIGNIFICANCE IN THE INTERNATIONAL TRADE OF SHRIMP

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

RESULTS

Spoilage and decomposition
The loss of freshness in shrimp due to the multiplication of bacteria can take two different paths. One occurs at refrigeration temperatures from the metabolism of psychrotrophic bacteria and is commonly referred to as spoilage. The other is the
result of the metabolism of mesophilic bacteria at ambient temperature and is more aptly referred to as decomposition. Both can be minimized through good sanitation and proper time/temperature control.

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

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

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

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

*Salmonella*

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

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

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

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

In one study conducted in the Philippines (23), researchers concluded that brackish water ponds and shrimp taken from them were inherently contaminated with enteric pathogens. *Salmonella* spp. were present in 16% of prawn samples and 22% of mud samples analyzed. In a separate study from the Philippines (13), researchers reported *Salmonella* spp. in zero to 23% of mud samples from ponds, but none from freshly harvested shrimp or from the processing environment. These researchers concluded that salmonellae were not part of the natural flora of the shrimp. Instead, they were the result of contamination from animal manure or nearby human settlements with inadequate sewage disposal. In a report from
Indonesia, salmonellae were isolated frequently from sediment samples taken from ponds, but not from the shrimp itself (21).

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

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

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

*Listeria*

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

Recent surveys report the presence of *Listeria monocytogenes* in various shrimp products throughout the world. *Listeria monocytogenes* was isolated from nine percent of ceviche (lime juice marinated, raw product) samples in Peru (10). The results indicated that *Listeria monocytogenes* could survive short exposure
times (same day of manufacture) in the pH range of 3.8 to 4.8 as a result of the buffering capacity of shrimp. Seventy-five percent of the samples were positive for *Listeria innocua*.

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

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

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

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

Information on the effectiveness of sanitizers on *Listeria* should be evaluated carefully. Many studies are performed with suspended cells. Higher concentrations of sanitizers may be required to inactivate *Listeria* attached to surfaces. Mafu et al. (14) indicated that *Listeria* attached to surfaces were more resistant to sanitizers than those suspended in a media or buffer. They reported sodium
hypochochlorite to be more effective at the recommended manufacturers concentration (200 ppm for nonporous and 800 ppm for porous surfaces) than quaternary ammonium compounds (200 ppm). The researchers also reported that concentrations of two to three times greater are required when applications were at 4°C versus 20°C. These concentrations, however, do not seem to be appropriate for commercial applications. Another report (2) indicated Listeria biofilms attached to chitin are more resistant to chlorine and iodine than to quaternary ammonium compounds. This was true even when higher than normal concentrations and longer contact times were analyzed. Quaternary ammonium compound used at double strength for five times the recommended exposure period was the most effective concentration examined in this study.

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

Vibrio cholera

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

The disease produced by the pandemic O1 cholera causes a devastating loss of body fluids from diarrhea and large numbers of deaths where medical treatment is inadequate or not available. Interestingly, the ancient Chinese used tea as an antidote. Black tea contains catechins and theaflavins which have been shown to be bactericidal (27). Recent research from Japan showed tea extract inhibited the hemolysin activity of V. cholera O1 El Tor, leading the authors to suggest tea as a possible preventive and therapeutic agent against cholera in developing countries.
The non-O1 endemic cases are more similar to "traveler's diarrhea" and may be associated with the consumption of improperly handled seafood, polluted water or general lack of sanitation in undeveloped countries.

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

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

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

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

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

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

The crab meat was returned in the traveler's suitcase and placed in his home freezer (still frosted). The next day, it was thawed in a double boiler; then two hours later, without further cooking it was served in a cold crab salad and as cold crab in
the shell. Over a six hour period, three other individuals consumed the crab and subsequently became ill. Crab from Ecuador was the most probable source of the infection since *V. cholera* O1 type El Tor can survive in crabs boiled up to eight minutes. In this case, the vibrios may have survived the boiling, contaminated the meat during the shelling and then multiplied during storage at ambient temperature.

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

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

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

**DISCUSSION**

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

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

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

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>n</th>
<th>c</th>
<th>m</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>5</td>
<td>2</td>
<td>50/g</td>
<td>500/g</td>
</tr>
<tr>
<td>Thermal tolerant coliforms</td>
<td>5</td>
<td>2</td>
<td>100/g</td>
<td>1000/g</td>
</tr>
</tbody>
</table>

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

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

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

CONCLUSION AND REFERENCES

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

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


SCOMBROID POISONING FROM SEAFOOD

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

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

Since scombroid poisoning is a chemical intoxication, the incubation period is short, usually ranging from an immediate reaction to several hours after ingestion. The duration of the illness is usually a few hours but in some cases, symptoms lasting several days have been reported (2). A range of symptoms can occur during incidents of
histamine poisoning. These vary from rashes and localized inflammation to gastrointestinal effects including nausea, vomiting and diarrhea. A burning sensation of certain body tissues such as the lips has also been reported. Cardiac involvement is also experienced, ranging from headaches to hypotension. Because of the range of effects reported, the potential role of other decomposition metabolites in the mechanism of poisonings has received much attention but without conclusive results.

**Histamine and other suspected agents.**

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

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

Other compounds such as the vasoactive amines tyramine, tryptamine, dopamine, and serotonin have not been demonstrated to be of significance in scombroid poisoning. The possibility that bacterial endotoxins might act in conjunction with histamine and cause a hypersensitivity has not been proven. Geiger (8) suggested that the use of alcohol or specially seasoned foods prepared from spoiled fish might
alter intestinal conditions and facilitate the absorption of histamine. Ferencik (9) postulated three types of toxins; histamine, other active amines, and N substituted compounds with a cholinotropic effect.

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

**Decomposition in seafood.**

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

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

**Levels of Histamine Associated with Poisoning.**

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

**Formation of Histamine in Fish.**

The formation of histamine in tuna and in mahimahi as a function of time and temperature has been studied in two research projects at the University of Hawaii (NOAA Contract 03-6-208-35369 and FDA Contract 223-80-2295 XVIII). The results from these two studies illustrate not only the production of histamine but also its anatomical distribution and the formation and distribution of other compounds such as cadaverine as well. In a study on tuna, the fresh fish contained essentially no free histamine; a maximum of 0.1 mg% was present in any fish section and was frequently lower (13). The formation of histamine during spoilage was studied over a temperature range of 60 to 120°F for periods of time representing those experienced in commercial practice. Rapid production of histamine was found at temperatures of 70°F and higher with an optimum temperature of 100°F. Each fish was divided into five transverse sections for chemical analyses. The characteristic pattern found in the study was a maximum concentration of histamine in the anterior end with decreasing amounts proceeding towards the posterior end. The belly flaps were exceptions to this gradient and usually had histamine levels that were as high as the anterior end. For the first six hours of spoilage, histamine formation was minimal but was 100 times higher by 12 hours of decomposition. During this period of decomposition, the histamine levels in the remaining
sections of fish averaged 5.1 mg%. After 12 hours, histamine increased significantly in all sections of the decomposing tuna. Since this research was conducted, the analyses of many commercial samples of decomposed fish have shown that, on occasion, the maximum concentration of histamine appears in the posterior end of a fillet. Furthermore, the gradient across the loin differs between small and large fish and varies with the time of spoilage. When spoilage occurs at lower temperatures, thus at a slower pace, deterioration is evident throughout the loins. If the fish are damaged (broken flesh, intrusions into the muscle, etc.) the spoilage pattern is also disrupted and fish tend to decompose much more rapidly. A further practical concern in commercial practice is the difficulty of removing body heat from large tuna after capture. The temperature of the tuna can be 8° F above ambient and if many fish are taken at one time it is necessary to place them in prechilled brine water which is circulated to drop the temperature below a point where spoilage may occur. The effect of these variables on the canned product at the consumer’s level is illustrated by the histamine data shown in Table 1 for three different samples of canned tuna. Some cans contain toxic levels of histamine while others have low levels of the amine.

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

<table>
<thead>
<tr>
<th>CAN #</th>
<th>SAMPLE I</th>
<th>SAMPLE II</th>
<th>SAMPLE III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.8</td>
<td>0.6</td>
<td>1.7</td>
</tr>
<tr>
<td>2</td>
<td>15.1</td>
<td>1.0</td>
<td>42.9</td>
</tr>
<tr>
<td>3</td>
<td>15.5</td>
<td>96.0</td>
<td>3.0</td>
</tr>
<tr>
<td>4</td>
<td>12.0</td>
<td>3.0</td>
<td>23.0</td>
</tr>
<tr>
<td>5</td>
<td>7.0</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>6</td>
<td>20.3</td>
<td>2.0</td>
<td>11.0</td>
</tr>
</tbody>
</table>

Research on mahimahi resulted in similar findings. Formation of histamine was a function of the spoilage temperature and the compound was distributed in a gradient across the fillets. In research being concluded at FDA, the formation and distribution of cadaverine and putrescine with decomposition was also determined and correlated with the presence of histamine and odors of decomposition. Putrescine was not generally found at high levels except in an advanced decomposed state. Cadaverine was always found in decomposed fish, even when spoilage temperatures were below 70° F. In the study on mahimahi,
decomposition was studied from iced conditions to 90° F. The results show that cadaverine forms more rapidly than histamine, over a wider temperature range, and is increased over a wider area of the loins than is histamine. It was found that many of the spoilage bacteria were poor histidine decarboxylate formers but frequently produced large amounts of lysine decarboxylate which led to the formation of cadaverine.

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

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

<table>
<thead>
<tr>
<th>SPOILAGE TEMP, °F</th>
<th>TIME OF SPOILAGE</th>
<th>HISTAMINE (mg%)</th>
<th>CADAVERINE (ppm)</th>
<th>PUTRESCIN E (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>14 Days</td>
<td>6.1</td>
<td>24.0</td>
<td>11.9</td>
</tr>
<tr>
<td>50</td>
<td>5 Days</td>
<td>5.9</td>
<td>241.8</td>
<td>28.3</td>
</tr>
<tr>
<td>70</td>
<td>40 Hours</td>
<td>320.5</td>
<td>130.4</td>
<td>22.1</td>
</tr>
<tr>
<td>90</td>
<td>15 Hours</td>
<td>108.5</td>
<td>223.1</td>
<td>41.1</td>
</tr>
</tbody>
</table>

Analytical Methods for the Detection of Scombrototoxic Fish.

Because scombrotoxic fish are produced as a result of decomposition, methods of detection can focus on the physiological activity of the fish, the direct determination of histamine, or the detection of decomposition regardless of the possibility of a toxic threat. The detection of decomposition can be subdivided into sensory techniques, physical changes, and the chemical determination of decomposition metabolites, which includes the detection of histamine. A determination of the histamine-like activity of fish as a measure of its safety is unwieldy, lacking in specificity and not amenable to the commercial environment. In addition, such a test would require that a product already be in an unsafe condition in order for a test to function effectively. Preferably, a test should be sufficiently quantitative to detect problems prior to a hazardous condition and be implemented by any well trained analyst on production samples.
Sensory analyses have been an accepted part of both commercial and regulatory programs for rejecting unacceptable fish. In addition to the usual difficulties with sensory analyses, high temperature spoilage frequently does not produce the typical decomposition odors associated with unacceptable products. Many analysts are trained on spoilage packs prepared under iced conditions which produce a different set of odors. Training analysts on packs spoiled at temperatures above 70°F can improve their performance. However, a significant number of scombrototoxic fish are still passed undetected. Physical changes such as honeycombed tissue are useful but do not appear until the fish are cooked which limits such an approach.

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

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

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

Liquid chromatography offers the opportunity to assay all of the amines of interest in a single run: histamine, cadaverine, and putrescine and be automated (17). Two primary approaches are precolumn derivatization with dansyl chloride and postcolumn reaction with OPA.
Despite the potential advantages of HPLC, this laboratory has collaborated on many proposed procedures with only marginal success. In order to use precolumn dansylation, the fish extract requires removal of amino acids to permit routine quantitation without interfering peaks. Liquid/liquid extractions are, at best, semiquantitative since histamine is very water soluble. In addition to limitations in the chemistry, liquid chromatographic pumps are a frequent source of variations in assays, particularly if buffers are part of a mobile phase as is the case for separation systems using postcolumn OPA reactions. With an improved procedure, HPLC may yet prove useful in confirming the presence of scombrototoxic fish.

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

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

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

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

**Applications of Chemical Data.**

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

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

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

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

<table>
<thead>
<tr>
<th>FROZEN STORAGE (weeks)</th>
<th>SPOILAGE AT 90° F</th>
<th>SPOILAGE AT 70° F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 Hours</td>
<td>24 Hours</td>
</tr>
<tr>
<td>0</td>
<td>2.0</td>
<td>292.0</td>
</tr>
<tr>
<td>24</td>
<td>0.1</td>
<td>85.0</td>
</tr>
<tr>
<td>40</td>
<td>0.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

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

SUMMARY

The potential for scombroid poisoning upon the ingestion of decomposed fish such as tuna and mahimahi has been known for decades. While commercial canned tuna seldom causes illnesses, other
fishery products are frequently responsible for reactions in consumers. The toxic reactions appear to be due to the formation of histamine during decomposition as well as the production of potential synergists such as cadaverine. Because this type of decomposition does not result in large amounts of spoilage odors and the amines are distributed in a heterogenous manner within and between fish in a lot, effective quality control measures require adequate sampling and analysis by highly qualified organoleptic analysts supported by chemical tests.
REFERENCES


USE OF SULFITES AND PHOSPHATES WITH SHRIMP

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Gainesville Florida 32611

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

SULFITES

The sulfite agents most commonly used to treated shrimp are sodium bisulfite (NaHSO₃) and sodium metabisulfite (Na₂S₂O₅). These compounds are sold dry in 50 pound (22.7 kg) bags or plastic tubs distributed through regional fishermen supply firms. These powders are blended with tap water or clean seawater to prepare dip concentrations of approximately 1.25% (weight/weight). Fresh harvested whole or headless shell-on shrimp from trawls, traps or culture ponds are first washed then placed in the sulfite dips for approximately 1 minute. The dip concentration and soak time (1.25% for 1 min.) corresponds to an average residual sulfite level (measured as liberated SO₂) of 100ppm (100 parts SO₂ per one million parts of edible shrimp). This residual level has been demonstrated to effectively prevent the formation of shrimp "black-spot" or melanosis. Melanosis is the development of black pigments that can adversely discolor the shrimp shell and meat.

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

Sulfites block the enzyme activity and can bleach some of the developed melanin. In the United States the sulfite compounds are approved as 'GRAS' substances ("generally recognized as safe") based on their previous use and prior sanction by the U.S. Food and Drug Administration (FDA) in 1956. More recent FDA regulations have specified the legal residual
sulfite level allowed on the edible portion of shrimp is 100ppm (SO₂). A 10ppm residual level is the detectable amount that determines the requirements to label treated product. Recommended package labeling on treated product should state "- ingredients: shrimp, sulfites used as a preservative". Product with residuals greater than 100ppm are considered adulterated and should not be sold. The international Codex Alimentarius recommendations for residual limits on shrimp are also 100ppm on raw products and 30ppm on cooked shrimp.

Residual sulfite limitations are necessary to prevent possible adverse health consequences associated with exposure to sulfur dioxide (SO₂). This gas has been the cause of severe respiratory and anaphylax (m.s.) reactions to foods with elevated residues. This problem is rare and has been more commonly associated with asthmatic consumers hypersensitive to SO₂ exposure. The more problematic foods have been carbohydrate based such as certain potato products, salads and fruits. There is only minor concern for health consequences due to consumption of sulfited shrimp, a protein based food that can bind the SO₂ residual. Likewise, the legal residual limits provide an additional safety guideline. A rare, but more serious concern can involve fishermen and other users of the sulfite compounds. If the dry powders become wet during storage or if the dip concentrations are improperly made too strong, these situations can release toxic levels of SO₂ gas. If this occurs in enclosed or poorly vented areas the workers could inhale a lethal dose.

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

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

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

PHOSPHATES

Use of phosphates with shrimp was initially based on commercial practice with hams, poultry and other meats. The primary benefit was to retain moisture loss that was otherwise lost during subsequent processing, chilling, freezing, frozen storage, thawing, refrigeration and cooking. The initial compound of choice was a brand of sodium tripolyphosphate (\(Na\times3P\timesO\times6\)). Overtime additional phosphates and phosphate blends were introduced for variable effects and applications (Table 1). The blends combine various phosphates and other approved food ingredients which may influence the pH (acidity) and/or antimicrobial attributes of the mix.
Some of the larger corporate suppliers of these phosphates are listed in Table 1. These substances were all reaffirmed as ‘GRAS’ ingredients by an FDA review published in the December issue of the 1979 federal register.

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

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

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

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

ACKNOWLEDGEMENT

Our continuing research and technology services with sulfites and phosphates used in seafoods has been supported in parts by the National Fisheries Institute, Florida Sea Grant College Program and Gulf and South Atlantic Fisheries Development Foundation, Inc. through distribution of federal Saltonstall-Kennedy funds.
Table 1. Some GRAS phosphates agents used to influence moisture in muscle foods

<table>
<thead>
<tr>
<th>Monosodium phosphate</th>
<th>Sodium acid pyrophosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hexametaphosphate</td>
<td>Dipotassium phosphate</td>
</tr>
<tr>
<td>Sodium metaphosphate, insoluble</td>
<td>Dipotassium phosphate</td>
</tr>
<tr>
<td>Sodium tripolyphosphate</td>
<td>Potassium tripolyphosphate</td>
</tr>
<tr>
<td>Sodium pyrophosphate</td>
<td>Potassium tripolyphosphate</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Moisture Content in scallop meat</th>
<th>Product Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>less than 80%</td>
<td>Considered a 'scallopf' and can be labelled as such</td>
</tr>
<tr>
<td>greater than 80% up to 84%</td>
<td>Considered a 'scallopf product and must be labelled as such*</td>
</tr>
<tr>
<td>greater than 84%</td>
<td>Illegal, adulterated scallop subject to seizure</td>
</tr>
</tbody>
</table>

* The 84% moisture content was considered equivalent to "25% water added scallop product". This is the recommended label statement.
*Clostridium botulinum* Type E Outgrowth and Toxin Production in Vacuum-Skin Packaged Shrimp

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(706)542-2286
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Due to the increasing consumer demand for high quality, convenient seafood products, more interest has been directed towards new packaging methods that will extend the shelf-life of seafood products, as well as maintain quality under refrigerated temperatures (4). Vacuum packaging may be the means of meeting this consumer demand. However, the use of this packaging method may be limited in commercial application in fresh seafood storage because of the possible increased risk from the growth and toxin production by *Clostridium botulinum* (10). This concern is due to the fact that: (a) *C. botulinum* type E spores which are widely distributed in marine environments, are capable of growth at temperatures as low as 3.3°C to 4°C; (b) the reduced oxygen environment in vacuum-packaged products may eliminate bacterial competition allowing *C. botulinum* growth at slightly abused storage temperatures; and (c) the possibility that noticeable spoilage of seafood products may not precede toxin production (10).

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

**MATERIALS AND METHODS**

**Spore preparation**

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

**Experimental design**

White and brown shrimp (*Penaeus* spp.) were harvested from the Georgia coast near the University of Georgia Marine Experiment Station in Brunswick, GA. Shrimp were deheaded, held at 0-3°C, and transported to the laboratory for use the following day. Shrimp were inoculated with a mixed pool of four strains of *C. botulinum* type E spores (Beluga, Minnesota, G21-5, 070) obtained from the Food and Drug Administration, Washington, D.C. Shrimp were inoculated by dipping them into a Butterfield's phosphate buffer solution containing log₁₀ 6-7 *C. botulinum* spores/ml (17). After a 30 minute exposure, the shrimp were placed on a sterile screen and the excess liquid was allowed to drain over a 30 minute period. The dipping procedure allowed for a target spore load on the shrimp of log₁₀ 3-4 spores/g. Controls samples were dipped the same way except the solution did not contain *C. botulinum*. Oxygen barrier film (Trigon Intact skin packaging film), designed for the RM331 Mark III Mini Intact Machine (Trigon National Corp., Redmond, WA) was used to vacuum-skin package the shrimp. Packages were prepared film-to-film with a sealing temperature of 120°C for 20 seconds. Packaged shrimp were stored at either 4°C for 21 days or 10°C for 15 days. For the 4°C storage, samples were analyzed at 3 day intervals. Samples held at 10°C were analyzed on 0, 1, 2, 3, 6, 9, 12, and 15 days. For each storage temperature, three replications of both inoculated and control samples were performed.
Most Probable Number procedure for *C. botulinum*

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

Psychrotrophic enumeration

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

Toxin analysis

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

Spoilage endpoint

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

RESULTS AND DISCUSSION

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

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


2. BAKER, A. and C. GENIGEORGIS. 1990. Predicting the safe storage of fresh fish under modified atmospheres with respect to Clostridium botulinum toxigenesis by modeling length of the lag phase of growth. J. Food Sci. 53:131-140.


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

<table>
<thead>
<tr>
<th>DAY</th>
<th>CONTROL</th>
<th>INOCULATED</th>
</tr>
</thead>
<tbody>
<tr>
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</table>

<sup>a</sup> the number of positive toxic shrimp samples.

<sup>b</sup> the total number of shrimp samples tested for toxin.

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

<table>
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<th>INOCULATED</th>
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</tr>
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</tr>
</tbody>
</table>

<sup>a</sup> the number of positive toxic shrimp samples.

<sup>b</sup> the total number of shrimp samples tested for toxin.