Distribution of Dungeness Crab Meat to Retail Stores and Restaurants in Hermetically Sealed Five Pound Cans and the Risks of *Clostridium botulinum* Toxin Production

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Seafood and seafood products packaged in hermetically sealed containers without additional treatment (such as thermal processing) to inactivate bacterial pathogens, continues to be a concern to microbiologists and regulatory agencies. Based upon the uncertainty of temperature control of the product at refrigerated temperatures during distribution and in some cases at the consumer level, ready-to eat products are especially of interes because they do not receive a heating step prior to consumption.

Recently, the practice of packaging five pound quantities of picked Dungeness crab meat in No.10 cans and hermetically sealing them under vacuum and the distribution of this product to retail stores and restaurants has been questioned because of the potential of outgrowth and toxin production by the bacterium *Clostridium botulinum* in this packaging environment. The packaging of the crab meat in cans and hermetically sealing the cans under vacuum however continues to be a preferable method for the distribution of crab meat for the following reasons: (1) in seasons or production periods when there is an abundance of the crab mea the cans of product are frozen for later markets. The vacuum protects the product from oxidative and other quality changes during frozen storage; (2) the cans provide a durable packaging material during frozen, refrigerated and ice storage and distribution of this very expensive product; and (3) most companies have the equipment for the cans.

The purpose of this paper is to provide background information and research data that can be of help in evaluating the safety of the current packaging practices used by the Dungeness crab industry.

Background Information

In the 1970's, my colleagues and I at the National Marine Fisheries Servic visited several Dungeness crab processing facilities to learn more about th distribution of crab meat in hermetically sealed cans. We were told that sometime before the 1950's , that they distributed the crab meat in No. 10 cans with a loose lid that was applied by hand

Inspectors were concerned that the loose lids would permit bacteria from the melting ice to enter the cans and contaminate the crab meat. These processors therefore began hermetically sealing metal lids onto the cans. The distribution of Dungeness crab meat in No.10 hermetically sealed cans has been the practice of the industry for over 40 years without any reported illnesses from *Clostridium botulinum* . As will be discussed later in this text, the safety of this product is undoubtedly related to the rapid growth of the psychrotrophic spoilage bacteria in the crab meat, even at ice temperatures. As a result the crab meat has a short shelf- life and spoilage occurs before *Clostridium botulinum* can produce its neurotoxin.

In order to evaluate the microbiological safety of a process and food product, we must first understand: (1) the characteristics of the bacterial pathogen or pathogens envolved; (2) the characteristics of the product, how it is processed, packaged and distributed; (3) barriers that prevent the bacterial pathogen from growing during storage and distribution of the product; (4) the shelf-life of the product, which relates to the growth of spoilage bacteria and spoilage of the product; and (5) in some cases the competitive inhibition of the bacterial pathogen by the spoilage bacteria.

Characteristics of *Clostridium botulinum*.. Foodborne botulism is a neuroparalytic disease that is caused by the ingestion of neurotoxin produced by the bacterium *Clostridium botulinum* during its growth in a food product. This bacterial species includes a very heterogenous group of strains that are divided into types A through G on the basis of the antigenic specificity of the neurotoxins that are produced. Some strains produce a mixture of neurotoxin and are designated as Types Ab,Af, and Bf. Types A,B,E,and F have caused the majority of human botulism outbreaks, whereas types C and D are usually involved in animal and avian botulism. Type G, the most recent type to be isolated, has not been involved in botulism outbreaks.Types A,B,E,and F can be further divided into two different groups based upon their biochemical and physiological characteristics.

Group 1 consists of the proteolytic types A,B, and F, and group 2 consists of the nonproteolytic types B,E, and F. Members of group 1 attack complex proteins, and their growth is usually accompanied by off odors. They have a minimum growth temperature of 10°C.(50°F.), which is a desirable characteristic for refrigerated foods, and they are inhibited in foods below pH 4.6. The strains of this group are the most heat resistant and will grow and produce toxin in foods containing 8-9% water-phase salt. Foods with water activity (aw) below 0.93 are inhibitory to their growth.

Members of group 2 on the other hand, are the most heat-sensitive types and are inhibited by 5-6% water-phase salt.

They will grow in foods with aw of 0.96 or higher and are inhibited below pH 4.6. They do have the undesirable characteristic of growing at refrigeration temperatures as low as 38°F (3.3°C.) and they are nonproteolytic (Schmidt et al. 1961; Eklund et al. 1967a; Eklund et al. 1967b). Because of their nonproteolytic characteristic, their growth in foods cannot be detected by off-odors and off-flavors. A dramatic example of this lack of correlation between spoilage and toxicity was observed during a type E outbreak involving 16 people in 1963. None of the victims detected any off-odors in the implicated food, and only three observed any unusual flavors.

Conditions Necessary for Botulism Outbreaks.

In order for foodborne botulism outbreaks to occur, the following conditions must be met:

1. A food must be contaminated with *Clostridium botulinum* spores or vegetative cells from the environment. Based upon the numerous investigations on the geographical distribution, it is clear that type E is the prevalent type in most freshwater and marine environments in the North American continent. With the exception of type G, the other types, however have also been occasionally isolated from the aquatic environments. In light of this evidence, it would be incorrect to accept the position that under any given circumstances, *Clostridium botulinum* would not be present in any raw product.
2. The processing treatment must be inadequate to inactivate the *Clostridium botulinum* spores, or the product must be contaminated after processing. Even though the nonproteolytic strains B,E,and F. are the most sensitive types to heat, studies have shown that they will survive processes such as smoking, when the internal temperatures exceed 180°F.(82.2°C).
Procedures have been developed to inactivate the nonproteolytic types using pasteurization temperatures.
3. The food must support the growth and toxin production of *Clostridium botulinum* when storage temperatures exceed 38°F (3.3°C). On the basis of conditions 1 and 2, we must always assume that raw products may be contaminated and that they will survive most semi-preservation food process. Condition 3 is therefore the most important in preventing botulism outbreaks which do not receive treatments to inactivate the spores. Storage of the finished product at temperatures below 38°F.(3.3°C) is probably the most important factor in controlling *Clostridium botulinum* growth in foods preserved short of sterlization.
4. Since foodborne botulism results from the ingestion of food which contains preformed botulinal neurotoxin, the food must be acceptable to the

consumer and consumed without further cooking or after insufficient heating to inactivate the neurotoxin. The type of food causing botulism depends upon the degree of organoleptic changes which are caused by the spoilage bacteria and the degree of spoilage which is acceptable to the consumer. This is especially of importance when the insidious nonproteolytic types B,E, and F are involved and where spoilage bacteria do not participate in spoilage Figure 1 illustrates how processing of fishery products can affect potential botulism problems. If foods are sterilized (right side of figure) by thermal processing or other methods, they can be safely stored at ambient temperatures. The canning industry , for example, has had an excellent record for producing billions of cans of thermally processed foods with only a few botulism problems. We are very much aware, however, of what can happen if the foods are underprocessed or are recontaminated with *Clostridium botulinum* after processing. They then enter the danger zone as illustrated in Figure 1 (Eklund 1992).

At the other end of the scale, eviscerated raw fishery products have historically never been implicated in human botulism outbreaks. This undoubtedly is related in part to the fact that most raw products are cooked before consumption, but more importantly to the endogenous spoilage bacteria which cause a rapid deterioration and spoilage of raw fishery products. A great part of these bacteria are psychrotrophic pseudomonads which produce obvious spoilage before a public health hazard can occur from botulism. These spoilage bacteria greatly outnumber *Clostridium botulinum* , grow rapidly at temperatures of 0°C(32°F.), and often effectively compete with *Clostridium botulinum* for nutrients. In some cases, they inhibit the growth of this pathogen or the proteolytic enzymes of the spoilage bacteria will inactivate the neurotoxin, especially the neurotoxin of the nonproteolytic types B,E, and F. In some specific cases the spoilage bacteria consume the oxygen, creating favorable anaerobic conditions for *Clostridium botulinum* growth. The danger zone in Figure 1 is therefore entered when we destroy or inhibit the spoilage bacteria in an effort to extend the storage life of a product and the preservation method is inadequate to inhibit or inactivate *Clostridium botulinum*.

Characteristics of Dungeness Crab Meat, and the Processing, Packaging and Distribution of Finished Product.

Dungeness crab (*Cancer magister*), is a hard shell crab harvested from the Pacific Coast of the United States and delivered alive to processing facilities. Cooked/steamed Dungeness crab has the following range of proximate composition: 76.3-78.1% moisture, 17.6-20.8% protein, 0.9-1.3% fat 1.4-

3.0% ash, and 0% carbohydrate as determined by difference. There are however small amounts of glucose ribose, etc. This product compostion will change slightly after processing. For example, the salt level of the finished crab meat will range from 1.0-1.5% salt (1.28-1.9% water phase salt). Higher concentrations of salt in the finished product are unacceptable to the consumer. Figure 2 shows the flow diagram of the processing steps, storage and distribution used for Dungeness crab meat. When crab meat is picked and marketed in this form, the carapace (shell cover), gills, intestines, and hepatopancreas are removed during the butchering step. This exposes all the areas where contamination could occur to the direct contact of the cooking water. The butchered crab are placed in baskets and the baskets immersed in cooking water. The crab initially lower the temperature of the water. The wate is heated usually by live steam and when it reaches a rolling boil 212°F(100°C) or a specified temperature, the crab are held in the boiling water for 9-10 minutes or at a water temperature of 205°F(96.1°C) for 16 minutes. As will be discussed later, this markedly reduces the incidence of the type E spores. After the cooking step, the basket of crab are transferred to cooling water and then transferred to the picking room. In some cases when the number of crabs is not sufficient to hire the personnel to pick the crab, or in cases where the volume is too large, the cooked crab sections are frozen and thawed later for picking. After the picking step, the crab meat is transferred to brine flotation tanks to remove small pieces of shell that enter the product during the picking operation The crab meat is then immersed in running fresh water to lower the salt concentration. After rinsing, the product is sometimes transferred to a dark room equipped with a ultra violet light as an additional inspection step to remove remaining shell fragments. The crab meat is then packed in C-enameled No.10 cans (5 pounds per can) and several ounces of water added. A vacuum is pulled on the cans and they are hermetically sealed. After labeling, the cans are chilled to below 38°F (3.3°C.) and held on ice or refrigerated until distribution. Distribution to the retail stores and restaurants is usually on ice. In talking with managers in the fish marketing section of several retail stores, I have learned that the cans of crab meat are delivered on ice and held on ice in the refrigeration room of the stores until they are opened and the crab meat transferred to the display counters. The managers indicated that the temperature of the product is checked four times during delivery and they were well aware of the short shelf-life of the product. In some seasons, depending on the volume and price of the crab meat, the processing plants freeze the cans of crab meat and market them at a later date.

Barriers that Prevent *Clostridium botulinum* from Growing and Producing Neurotoxins.

Even though the butchered Dungeness crab are cooked, the finished product will contain 10,000 or more total aerobic bacterial colony forming units per gram. These spoilage bacteria enter the finished product during the picking operation. As a result, Dungeness crab meat will spoil within 9 to11 days at ice temperatures, 4-5 days at 50°F (10°C) and 24-36 hours at 77°F (25°C). As discussed earlier, the finished product contains approximately 1.3-1.9% water phase salt. This is not high enough to inhibit the spoilage bacteria or the growth of *Clostridium botulinum* . Therefore, the barriers against the outgrowth and toxin production by this pathogen is temperature control. The rapid growth of the spoilage bacteria, their competition with *Clostridium botulinum* type E,and the short shelf-life of the product however also play a very important role in the safety of this product.

Incidence *Clostridium botulinum* in Live Crab, Cooked Crab, and Crab Meat

Prior to the incidence studies, the laboratory enrichment procedure used to detect *Clostridium botulinum* was tested for its sensitivity in demonstrating the presence of this bacterium in crab meat. Crab meat (50 or100 grams) was inoculated with either 10 or 100 type E spores and incubated anaerobically in enrichment broth for 3 days at 30°C or 5 days at 25°C and the supernatant fluid was assayed for the neurotoxin. These preliminary studies demonstrated that low numbers of *Clostridium botulinum* type E could be detected if it was present in crab meat.

In studies by Eklund and Poysky (1970), *Clostridium botulinum* type E was detected frequently from marine sediments and live Dungeness crab harvested from these areas.When live crab were delivered to a processing plant, 18 were tested for the presence of *Clostridium botulinum*.. Type E was shown to contaminate 28% of the gill samples and 44% from the crab shells. An additional 18 whole crab (not butchered) from the same batch were tested after the cooking step. *Clostridium botulinum* could not be demonstrated from the gills, shell or intestines of the 18 cooked crab. These studies indicated that the cooking step played an important role in the reduction of type E spores from the crab (Table 1).

In additional studies, Eklund and Poysky (1965) failed to demonstrate the presence of *Clostridium botulinum* from 94 swab samples of surfaces of tables and equipment in the picking room and from 4 samples of brine. Type E also could not be detected from 66 samples (100 grams each) of picked crab meat collected throughout several processing days (Table 2).

In similiar studies by Lerke and Farber (1971), *Clostridium botulinum* could not be found in 74 crab meat samples collected over a 6 month period. Later they failed to detect *Clostridium botulinum* from 130 samples of shrimp and crab meat. In each of these studies, Type E spores could be detected if spores were inoculated at levels of 1 and 10 per gram of crabmeat.This study also demonstrated that the laboratory procedure used in their studies was sensitive enough to detect type E, if it had been present in the crab meat or shrimp samples.

These studies therefore indicate that the incidence of *Clostridium botulinum* is very low in picked crab meat and that the spoilage bacteria greatly outnumber this pathogen. In spite of the absence of *Clostridium botulinum* in crab meat in the previous studies, there always is a possibility tha the product occasionally can be contaminated through cross contamination or when crab sections are frozen and thawed out later for picking. During the thawing process, the crab sections do not reach temperatures that are necessary to inactivate bacterial pathogens.

Studies on the Spoilage of Crab Meat and Outgrowth and Toxin Production by Clostridium botulinum.

The previous studies do indicate that the incidence of *Clostridium botulinum* is very low in picked crab meat. However, as discussed in the earlier section sections of this paper, we must always assume that this bacterium can occasionally be present in a product. Based upon this possibility, several laboratories have studied whether crab meat would spoil before *Clostridium botulinum* could produce its neurotoxin.

In studies at the National Marine Fisheries Service Laboratory (data presented at Pacific Fisheries Technologists meeting, 1977) freshly picked Dungeness cral meat was packaged in oxygen permeable or vacuum packaged in oxygen impermeable bags. The crab meat in oxygen permeable bags was inoculated with known numbers of *Clostridium botulinum* type E or type A spores and th openings of the bags were tied off. Crab meat packaged in oxygen impermeable bags were vacuum sealed (to simulate hermetically sealed cans) and the spores were inoculated through a rubber septum attached to the packaging film with a special adhesive.The crab meat had an initial aerobic bacterial plate count of 44,000 and 73,000 colony forming units per gram. The crab meat for both packaging materials was inoculated with 10, 100, 1000, and 10,000 spores of either type A or Type E per 50 gram sample. Samples inoculated with type E were stored at either 12°C (53.6°F.) or 25°C.(77°F.) and assayed for botulinal neurotoxin after various periods of storage. Type E neurotoxin was assayed both trypsinized and untrypsinized

The use of trypsin is essential to the detection of type E neurotoxin especially if it grows and produces toxin at refrigerated temperatures.

Samples inoculated with Type A spores were incubated only at 25°C. Type A neurotoxin was assayed only in the untrypsinized state. Type A is proteolytic and will therefore activate its own toxin with its proteolytic enzymes.

Results from Type E Inoculated Pack Studies. Crab meat inoculated with type E and packaged in oxygen impermeable films were nontoxic after 6, 13 and 20 days of storage at 12°C.(53.6°F) even when the inoculum level was 10,000 spores per 50 gram sample (Table 3). 12°C was selected as a high refrigeration abuse temperature. After 6 days at 12°C., spoilage bacteria had produced strong spoilage odors and all of the samples were very spoiled. This was the first sampling period. When the crab meat was stored at 25°C(77°F) in oxygen impermeable bags, all of the samples were grossly spoiled at 3 days, the first day of sampling. Crab meat will usually spoil after 24 hours at this temperature. The detection of type E neurotoxin was not detected until after 13 days and only in samples that were inoculated with 10,000 spores per 50 grams. (Table 4).
When type E was inoculated onto crab meat and packaged in oxygen permeable bags and stored at 25°C., all of the samples were grossly spoiled after 3 days,the first sampling time. Type E neurotoxin however was not detected in any of the samples after either 3 or 6 days of storage (Table 5). Spoilage odors were slightly different, but very obvious, for product packaged in oxygen impermeable as compared to those in oxygen permeable bags.

Results from Type A Inoculated Pack Studies. Type A will not grow below 10°C.(50°F.) and very slowly at 12°C.(53.6°F). Samples inoculated with type A spores therefore were stored only at 25°C.(77°F.). The results from the studies in which the crab meat was packaged in oxygen permeable films is summarized in Table 6. None of the samples inoculated with type A contained detectable type A neurotoxin after 3 days of temperature abuse, even when the spore inoculum was 10,000 spores per 50 grams. At this time all of the samples were grossly spoiled. Type A neurotoxin was detected after 6 days at 25°C, but only when the spore inoculum level was 10,000 per 50 grams. Samples inoculated with 10, 100, and1000 spores were nontoxic after 6 days. All of the 6 day samples contained very strong spoilage odors.

Data from the type A inoculated crab meat samples stored at 25°C and vacuum packaged in oxygen impermeable films are summarized in Table 7. After 3 days of storage the product was grossly spoiled and all of the samples irrespective of the inoculum level (10 to 10,000 spores) were nontoxic. Type A neurotoxin was detected after 6 days when the inoculum level was 1000 and 10,000 spores, but all of the samples inoculated with 10 and 100 spores were nontoxic. After 11 days at 25°C, all of the samples with an inoculum of 10 to 10,000 contained type A neurotoxin. These 11 day data are relatively unimportant in that the product was grossly spoiled after 3 days, the first sampling period.

Results of Inoculated Pack Studies from Other Laboratories. In 1971, Lerke and Farber studied the growth and toxin production of *Clostridium botulinum* type E in Dungeness crab meat after the product had been packaged in oxygen impermeable mylar-polyethylene pouches and heat pastuerized. In these studies the spoilage flora had been inactivated. Type E neurotoxin was detected after 30-40 days at 40°F.(4.4°C) in the pasteurized product inoculated with 50,000 spores per 6 ounce of sample,but the crab meat was only slightly sour after 45 days. In comparison, when the unpasteurized crab meat was inoculated with the same number of spores they became toxic 27 days after the product was spoiled. At a storage temperature of 50°F., the crab meat was spoiled 14 days before type E produced its neurotoxin. These studies therefore again emphasize the importance of the spoilage flora in competing with *Clostridium botulinum* and also causing spoilage before neurotoxin is produced.

In 1996, Harrison et al. studied the risk of *Clostridium botulinum* type E toxin production in blue crab meat packaged in four different commercial type containers. The containers were sealed by: (1) snapping the lid on by hand; (2) sealed by placing a lid onto the container and then placing a polyethylene seal around the top edge of the lid; (3) aluminum top cups were sealed by placing the metal lid on the cup and sealing with a can sealer; and (4) "tamper evident" cups were sealed with tamper-evident pull tabs and snapping the lid on the container by hand. The blue crab meat was inoculated with 1000 and 10,000 spores per gram (50 gram samples) prior to sealing the containers and the samples were incubated at 4°C.(39.2°F) and 10°C(50°F). During storage, the oxygen concentration decreased and the carbon dioxide concentration increased in the containers at the storage temperature 10°C.(50°F.). The greatest accumulation of carbon dioxide was in the aluminum top and tamper evident containers that were sealed in a manner that was less likely to allow exchanges of gases.

Nonetheless, there was no difference in the spoilage of the product in any of the four different containers. Spoilage was evident after 6 days at 10°C and 9 days at 4°C. Botulinal neurotoxin was not detected in any of the samples held at 4°C for 21 days or in samples held at 10°C. for 15 days.

Summary

The packaging of picked Dungeness crab meat in No. 10 cans and hermetically sealing them under vacuum for distribution to retail stores and restaurants has recently been questioned because of the potential dangers from the bacterium *Clostridium botulinum.*. This packaging procedure however is beneficial to the crab industry in that the sealing of the cans under vacuum protects the product from oxidative and other undesirable changes during frozen storage and the durability of the cans protects the product during frozen and refrigerated storage and distribution. In addition, most companies have the equipment for cans.

The current practice of marketing picked Dungeness crab meat in five pound quantities in No.10 hermetically sealed cans has been followed by the Dungeness crab processors for over 40 years without any illnesses from the bacterium *Clostridium botulinum.*. The data presented in this paper indicate that this is undoubtedly related to the low incidence of *Clostridium botulinum* in the finished crab meat product, which are markedly outnumbered by spoilage bacteria. These spoilage bacteria grow rapidly at refrigerated temperatures, and produce spoilage odors and flavors resulting in a short shelf-life of the product even at 0°C.(32°F). These spoilage bacteria also compete with *Clostridium botulinum* for nutrients and in some cases even inhibit its growth. The safety of this packaging procedure is also related to the fact that the processors realize that the crab meat has a short shelf-life and the cans of crab meat are therefore rapidly chilled and held refrigerated or iced. This is a practice that we also observed in processing facilities in the 1970's. The fact that these large cans are sold to retail or restuarants which remove the product from this vacuum environment before it is purchased by the numerous consumers has undoubtedly contributed to the safety of this method of marketing crab meat.
Previous studies have shown that *Clostridium botulinum* type E can be isolated frequently from the shell, gills and intestines of the live Dungeness crab. These same studies also showed that when type E was present in a high percentage of the live crab, it could not be detected from even the whole crab (not butchered) after cooking.

Crab scheduled to be picked and sold as crab meat are butchered. This process removes the carapace, gills, intestines and hepatopancreas and exposes the surfaces where type E can contaminate the crab. Additional studies have shown that type E could not be demonstrated from swabs of table tops and equipment in the picking area and several hundred crab meat samples collected over different processing days. This indicated that there is not a high incidence of type E spores in picked crab meat.

Psychrotrophic spoilage type bacteria are introduced during the picking operation and their populations usually are in the range of 10,000 or more colony forming units per gram.Based on the incidence studies, these spoilage bacteria greatly outnumber any possible *Clostridium botulinum* contamination of the crab meat, grow rapidly at temperatures of 0°C to 30°C.(32°to86°F.), and cause rapid spoilage of the crab meat.

Even though there is a low incidence of *Clostridium botulinum* in crab meat, there are occasions when it might be present. Inoculated pack studies therefore have been made to determine whether this pathogen could produce its neurotoxin before the crab meat spoils.

Studies have shown that *Clostridium botulinum* type E did not produce neurotoxin in crab meat stored at 12°C(53.6°F) in oxygen impermeable films during a 20 day storage period even when 10,000 type E spores were inoculated. These same samples had an initial bacterial population of 44,000 and 73,000 per gram and were very spoiled after 6 days, the first sampling period.

At a gross abuse storage temperature of 25°C.(77°F) type E did not produce neurotoxin in crab meat vaccum packaged in oxygen impermeable bags until after 13 days and only when a high inoculum of 10,000 spores was used. These same samples were very spoiled after 3 days of storage,the first sampling period.

In another study, 50,000 type E spores were inoculated into Dungeness crab meat that had previously been heat pasteurized to destroy the spoilage flora. Type E neurotoxin was produced in 30-40 days at 40°F. Unpasteurized crab meat inoculated with the same number of spores became toxic 27 days after the crab meat was spoiled. These studies therefore emphasize the importance of the spoilage bacteria.

Similiar results were obtained when blue crab meat was sealed in containers with different gas permeability. The blue crab meat spoiled (6

days at 10°C,and 9 days at 4°C) during the same period of time in all packaging containers held at either 4° or 10°C. but type E did not produce toxin during 21 days at 4°C.(39.2°F) or 15 days at 10°C(50°F).
Type A, was used as the inoculum in one of the studies. Neurotoxin was not detected in the inoculated Dungeness crab meat until after 6 days of storage at 25°C. in oxygen permeable bags and only when 10,000 spores were used. Crab meat vacuum packaged in oxygen impermeable bags became toxic after 6 days when the inoculum level was 1000 and 10,000 spores, but not at the lower inoculum levels of 10 and 100 spores. These products were grossly spoiled after 3 days, the first sampling period.

In visits to several retail stores, I learned that they receive the crab meat in No.10 cans and the cans are delivered to them on ice and held on ice until opened. They also know that crab meat is a very expensive product and that the shelf-life is longer if the product is held at the colder temperatures.

Results from recent studies of two different lots of crab meat have shown that Dungeness crab meat with an intitial total aerobic bacterial plate count of 17,000 and 18,000 colony forming units per gram spoiled after 10-11 days at ice temperature, 4-5 days at 50°F(10°C) and within 24-36 hours at 77°F (25°C). The crab meat was packaged in films with high oxygen and carbon dioxide permeability and also in barrier films with very low gas permeability without vacuum. Spoilage was comparable in both packaging systems. These preliminary studies indicate that the crab meat has about the same shelf-life at the different temperatures as it did in the earlier studies.

Recommendations

The following recommendations are made to assist the Dungeness crab industry to continue to maintain its excellent public health safety record with regards to *Clostridium botulinum* .

1. All cans must be labeled to store below 38°F (3.3°F). See examples on page 23. This reminds the customer that this product is not sterilized and is perishable. In fact, it might be advisable that the label or a leaflet or other advisory form state that crab meat is an extremely perishable product and for safety reasons they should store the product below 38°F. See example on page 24.
2. All cans whether delivered or picked up by the customer, be transported on ice. This provides obvious evidence that the product is cold. This also increases the shelf-life of this expensive product.
3. To assist customers and address food safety concerns, the processor should supply a plastic lid that can be placed over the end of the each can when it is

opened. An advisory should accompany each shipment of cans from the processing facility. This advisory should state that the crab meat must be stored below 38°F(3.3°C) and if the product cannot be held on ice by the customer that the can be opened and fitted with the plastic lid. This immediately exchanges the gases in the can with the ambient atmosphere, permits greater gas exchanges in the can while it is in storage, and also increase: growth of spoilage bacteria if the cans are exposed to higher temperatures. This is a conveince to the stores and also increases safety of the product. When I mentioned this to the managers of fish sections within the retail stores, they were very much in favor of the practice. This practice addresses two of the concerns discussed earlier in this text. First, the concern of delivering the crab meat in hermetically sealed cans and the possible storage of the cans above 38°F.(3.3°C), and the second concern of bacteria contaminating the crab meat from ice water when a loose lid is used while the cans are stored on ice.

4. If the crab meat is frozen in cans and marketed later, the product must be thawed under refrigeration temperatures of 38°F.(3.3°C) or lower. If other thawing methods are used, the crab meat should not exceed 38°F. If it is thawed at the customer level, the can should be opened and fitted with the plastic lid before it is thawed. Extra precautions should be followed in the handling of cans of crab meat that have been sealed under vacuum.

5. Studies should continue to determine whether the total aerobic bacterial plate counts of the finished product have changed significantly since the original studies. If there has been a marked reduction in the bacterial populations, then studies should be made to determine the spoilage patterns of crab meat hermetically sealed in cans with and without vacuum and additional inoculated pack studies with *Clostridium botulinum* should be considered.

Suggested HACCP Critical Control Points

(Refer to Figure 3)

1. **Cooking**- To reduce the incidence of *Clostridium botulinum* type E and inactivate other nonsporeforming bacterial pathogens.
2. **Label**- labeling of cans- to store below 38°F(3.3C)- to control the growth of *Clostridium botulinum*. and other pathogens and to emphasize the importance of refrigeration to the customer. Labeling of products, such as storing smoked fish below 38°F,has undoubtedly contributed to the 35 year safety of this product.
3. **Refrigerate Below 38°F(3.3°C) or Ice Chill**- lower temperature to inhibit growth of nonproteolytic *Clostridium botulinum*.
4. **Ship on Ice**-to control growth of *Clostridium botulinum* and other pathogens.

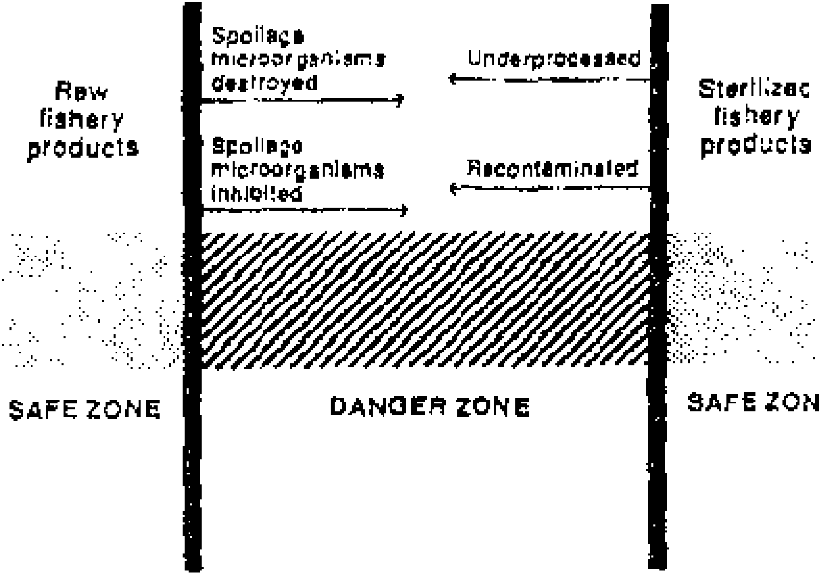
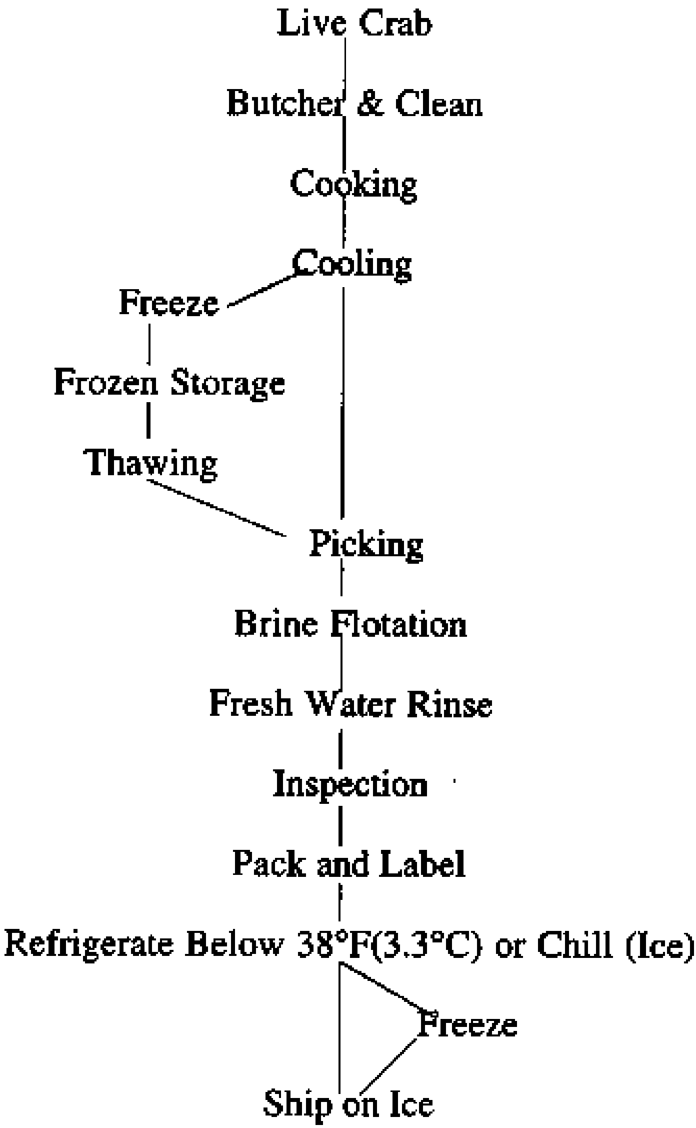


Figure 1. Relationship of preservation procedures to botulism problems in fishery products stored above 38°F (3.3°C

<u>**Refrigerated Dungeness Crab Meat**</u>



igure 2. Flow Diagram for Processing Dungeness Crab Mea

Table 1.--Occurrence of *Clostridium botulinum* in whole Dungeness crab before and after the boiling tanks.

Sample tested	No. samples	% samples contaminated with type E
Uncooked		
Gills	18	28
Shell	18	44
Cooked		
Gills	18	0
Shell	18	0
Intestines	14	0

Only Type E was detected in the samples.

Table 2.--Incidence of *Clostridium botulinum* in Dungeness crab processing plants.

Sample tested	No. of samples	No. of samples contaminated with type E
Swabs of equipment	94	0
Brine solution	4	0
Picked crab meat	66	0

-All samples taken beyond boiling tanks in the processing plant picking area.
-Brine solution centrifuged to collect bacteria and dilute the salt concentration.

Table 3. Outgrowth and toxin production by *Clostridium botulinum* type E (strains Beluga and Saratoga) in picked Dungeness crabmeat. Vacuuum packaged in oxygen impermeable bags and stored at 12°C.(53.6°F.)

Spore inoculum per 50 gm.	6 Days		13 Days		20 Days	
	Spoilage	Toxicity	Spoilage	Toxicity	Spoilage	Toxicity
10	4	0/2	4	0/2	4	0/2
100	4	0/2	4	0/2	4	0/2
1000	4	0/2	4	0/2	4	0/2
10,000	4	0/2	4	0/2	4	0/2

-Toxicity 0/2 =Number of samples containing type E neurotoxin/ number of samples tested.

-Spoilage 0-4 ranking 0 = fresh. 4= very spoiled

Table 4. Outgrowth and toxin production by *Clostridium botulinum* type E (strains Beluga and Saratoga) in picked Dungeness crabmeat. Vacuuum packaged in oxygen impermeable bags and stored at 25°C. (77°F.)

Spore inoculum per 50 gm.	3 Days		6 Days		13 Days	
	Spoilage	Toxicity	Spoilage	Toxicity	Spoilage	Toxicity
10	4	0/2	4	0/2	4	0/2
100	4	0/2	4	0/2	4	0/2
1000	4	0/2	4	0/2	4	0/2
10,000	4	0/2	4	0/2	4	1/2

-Toxicity 0/2 = Number samples containing type E neurotoxin/ number of samples tested.
-Spoilage 0-4 ranking 0=fresh. 4= very spoiled

Table 5. Outgrowth and toxin production by *Clostridium botulinum* type E (strains Beluga and Saratoga) in picked Dungeness crabmeat. Packaged in oxygen permeable bags and stored at 25°C. (77°F.)

Spore inoculum per 50 gm.	3 Days		6 Days	
	Spoilage	Toxicity	Spoilage	Toxicity
10	4	0/2	4	0/4
100	4	0/2	4	0/4
1000	4	0/2	4	0/4
10,000	4	0/2	4	0/4

-Toxicity 0/2 = Number samples containing type E neurotoxin/ number of samples tested.
-Spoilage 0-4 ranking 0=fresh, 4= very spoiled

Table 6. Outgrowth and toxin production by *Clostridium botulinum* type A (strains 62A and BIG4) in picked Dungeness crabmeat. Packaged in oxygen permeable bags and stored at 25°C.(77°F.)

Spore inoculum per 50 gm.	3 Days		6 Days	
	Spoilage	Toxicity	Spoilage	Toxicity
10	4	0/2	4	0/4
100	4	0/2	4	0/4
1000	4	0/2	4	0/4
10,000	4	0/2	4	2/4

-Toxicity 0/2 =Number of samples containing type A neurotoxin/ number of samples tested.

-Spoilage 0-4 ranking 0 = fresh. 4= very spoiled

Table 7. Outgrowth and toxin production by *Clostridium botulinum* type A (strains 62A and BIG4) in picked Dungeness crabmeat. Vacuuum packaged in oxygen impermeable bags and stored at 25°C.(77°F.)

Spore inoculum per 50 gm.	3 Days		6 Days		11 Days	
	Spoilage	Toxicity	Spoilage	Toxicity	Spoilage	Toxicity
10	4	0/2	4	0/2	4	2/2
100	4	0/2	4	0/2	4	2/2
1000	4	0/2	4	2/2	4	2/2
10,000	4	0/2	4	2/2	4	2/2

-Toxicity 0/2 =Number of samples containing type A neurotoxin/ number of samples tested.
-Spoilage 0-4 ranking 0 = fresh, 4= very spoiled

Refrigerated Dungeness Crab Meat

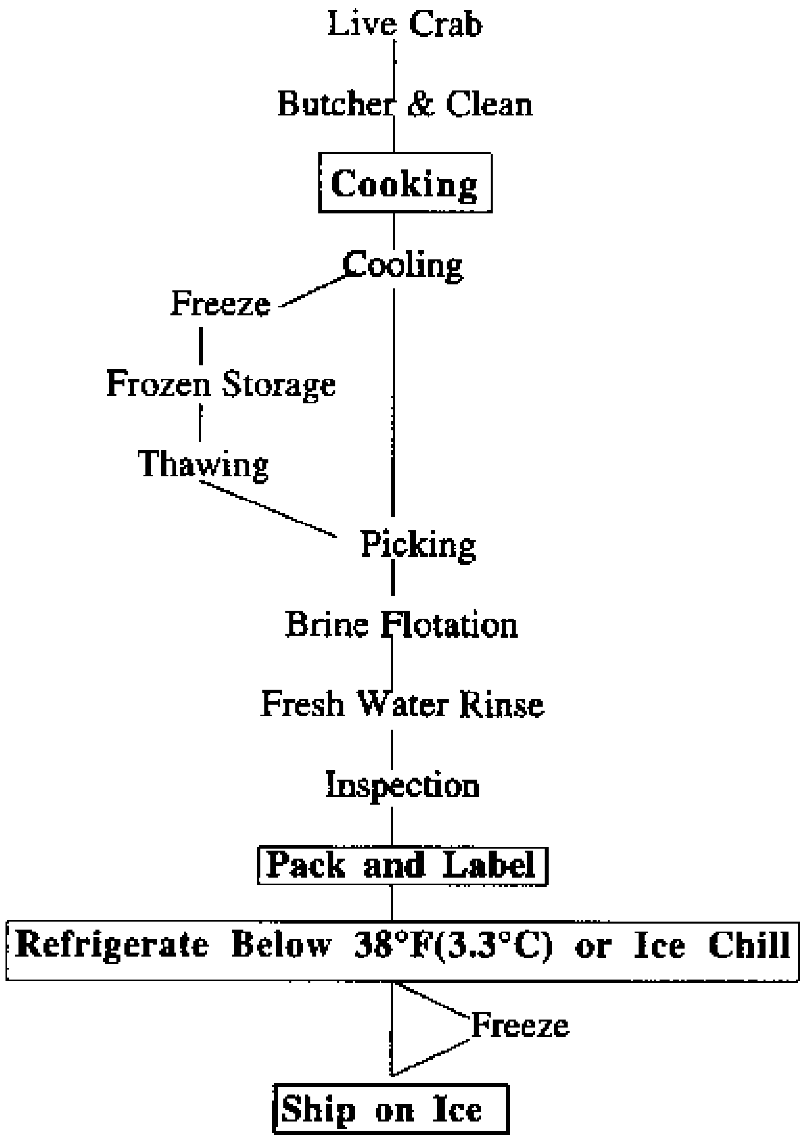
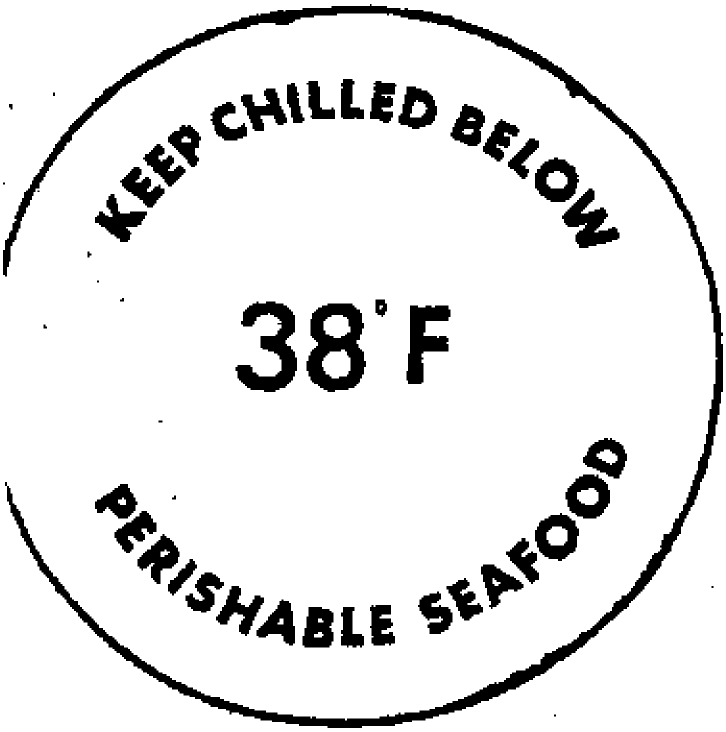


Figure 3. Flow Diagram for Processing Dungeness Crab Meat.
Bold Type and Boxed Areas are Suggested HACCP Critical Control Points for Refrigerated Crab Meat.



CAUTION - PERISHABLE SEAFOOD
38°F
KEEP CHILLED BELOW

This label could be placed on cans of crab meat to inform buyers, especially new buyers, about the perishability of this product and refrigeration requirement for public health safety

Caution

This can of crab meat is extremely perishable.

For Public Health Reasons

Store on ice or under refrigeration at temperatures below 38°F(3.3°C)

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