Survival of Vibrio Cholerae, Biotype E1 Tor, Serotype Inaba in Seafoods.

Arthur Hinton Jr
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SURVIVAL OF VIBRIO CHOLERAE, BIOTYPE E1 TOR, SEROTYPE INABA IN SEAFOODS

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A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

Food Science

by

Arthur Hinton, Jr.

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M.S., University of Kentucky, 1979

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ABSTRACT

The D values of \textit{Vibrio cholerae} in oyster and shrimp meat homogenate were determined at 49°, 54°, 60°, 66°, 71°, 77°, and 82°C. In oyster homogenates, the D values in minutes were 4.7 at 49°C, <0.35 at 54°C, <0.35 at 60°C, <0.33 at 66°C, <0.30 at 71°C, <0.29 at 77°C, and <0.24 at 82°C. In shrimp homogenates, the D values in minutes were 9.17 at 49°C, <0.43 at 54°C, <0.39 at 60°C, <0.32 at 66°C, <0.31 at 71°C, <0.30 at 77°C, and <0.28 at 82°C.

Whole oysters, inoculated with \(10^6\) \textit{V. cholerae}/gram, were cooked in boiling water (100°C) or in hot oil (191°C) for one minute. Whole shrimp, inoculated with \(10^6\) \textit{V. cholerae} were cooked in boiling water (100°C) or in steam (100°C) for 10 minutes. \textit{Vibrio cholerae} was not isolated from the cooked oysters or shrimp.

Sterile and unsterile shrimp, oyster, crab, and crayfish meat homogenates were inoculated with \(10^7\) \textit{V. cholerae}/g. The homogenates were treated with 0, 25, 50, or 100 krad and stored at 4°, 0°, or -8°C for 21 days. No \textit{V. cholerae} were isolated from any of the homogenates treated with 100 krad. \textit{Vibrio cholerae} was able to survive longer in the sterile oyster, crab and crayfish homogenates stored at 4° and 0°C than in the unsterile homogenates stored at the same temperatures. The organism was able to survive longer in the unsterile shrimp homogenates stored at 4° and 0°C than in the sterile homogenates stored at the same
temperature. At -8°C, survival time was usually longer in the unsterile seafood homogenates than in the sterile seafood homogenates stored at -8°C. Due to the retardation of competitive inhibition the organism was usually able to survive longer in the unsterile homogenates stored at -8°C than in the unsterile homogenates stored at 4° or 0°C.
INTRODUCTION

Contaminated seafoods have been responsible for cholera epidemics in several countries. There have also been some *Vibrio cholerae* infections in the United States in recent years. Contaminated crabs which were improperly cooked or stored were responsible for 11 cases of the disease in Louisiana in 1978. The public's fear of the disease caused economic hardship to Louisiana's seafood industry.

*Vibrio cholerae* has been isolated from water and seafood taken from the Chesapeake Bay and the Gulf of Mexico. The organism has also been isolated from waters which were free of fecal contamination. This may indicate that *V. cholerae* is a free living organism, and that the coliform test may not be useful for determining if shellfish beds are free of the organism.

*Vibrio cholerae* is a relatively fragile organism. Those foods which serve as vectors for the organism are usually consumed raw or partially cooked. Adequate cooking is an effective means for destroying the organism in foods.

The F.D.A. may soon allow foods to be treated with ionizing radiation doses of less than 1 Mrad. Irradiation would not only extend the shelf life of seafoods, but could also be used to destroy pathogens in the food. Pasteurization doses of irradiation have little effect on the organoleptic qualities of food. The longer shelf life provided by irradiation would allow the shipment of chilled fresh seafoods for greater distances.
Little information has been published on the thermal or irradiation resistance of *V. cholerae* 01, biotype El Tor. The purpose of the following study was to:

1. Determine the D values of the organism in oyster and shrimp homogenates.
2. Determine the effect of various cooking methods on the organism in oysters and shrimp.
3. Determine the effect of low doses of ionizing radiations on the organism in shrimp, oyster, crab, and crayfish homogenates.
4. Determine the effect of storage at 4°, 0°, and -8°C on survival of the organism in shrimp, oyster, crab, and crayfish homogenates stored for 21 days.
VIBRIO CHOLERAE

General characteristics. *Vibrio cholerae* is a member of a large family of water bacteria. It was first described and named by Pacini in 1854 (2). Koch isolated and described the organism in Egypt in 1883. It was one of the first bacterial agents to be implicated as the causative agent of a disease (56,63).

The organism is a small, curved Gram negative rod. It has a single polar flagellum which propels the organism in darting-like movements in liquid environments (61).

It is a facultative anaerobe. The organism can grow between a pH range of 6.0 and 9.6, but its optimum range is between 7.6 and 8.6 (52). The optimum temperature for the growth of *V. cholerae* is between 30° and 35°C, but it can grow at temperatures ranging from 15° to 42°C. It can also grow at sodium chloride concentrations between 0 and 6%.

Classification. *Vibrio cholerae* serogroup 01 is the causative agent of Asiatic cholera. The species is currently divided into two biotypes, El Tor and classical (19). *Vibrio cholerae*, biotype El Tor may be differentiated from *V. cholerae*, biotype cholerae (also known as the classical biotype) by its resistance to Murkejee's Group IV bacteriophages, its ability to agglutinate chicken red blood cells and by its resistance to polymixin B (24,42,43,52).

The El Tor biotype also has greater resistance to environmental stresses and produces a lower morbidity
mortality rate in people infected with the organism (20,21,23,52). The El Tor biotype was not identified as the causative agent of cholera until 1962, but since that time it has been responsible for most of the outbreaks of the disease (62).

The biotypes of \textit{V. cholerae} 01 may be classified as one of three serotypes depending on the presence of three somatic antigens (a, b, and c). The organism may be classified as Ogawa, Hikojima, or Inaba depending on the amount of each antigen present (33,52). Serotypes may be identified by placing a suspension of the bacteria into the appropriate antiserum, and checking for agglutination. Organisms which fail to agglutinate in any of the \textit{V. cholerae} 01 antisera are known as non-agglutinable vibrios (NAG) or non-cholera vibrios (NCV) (39).

The non-cholera vibrios are morphologically and physiologically similar to \textit{V. cholerae} 01 (39). They are also capable of producing a less severe form of the disease (60). The organism has been responsible for epidemics of enteritis in Malaysia, Czechoslovakia, and Sudan (15). It was also responsible for a food infection in Louisiana during 1972. The vector was raw oysters.

The exact nature of the relationship between \textit{V. cholerae} 01 and the non-cholera vibrios is not yet fully understood. It has been hypothesized that changes in the biotype and serotype of \textit{V. cholerae} may be caused by genetic mutations or phage-mediated transductions (16). Changes in
serotype have been reported in individual patients and during epidemics (54,62). The acquisition and loss of agglutinability by the organism has also been reported (15). Theoretically, if a *V. cholerae* 01 organism lost all of its somatic antigens due to a change in its genome, it would be transformed to a non-cholera vibrio (62).

**The disease.** Cholera is one of the most terrifying diseases because it strikes suddenly and can involve whole villages or cities. History indicates that the disease has plagued mankind for thousands of years. It has been endemic along the river systems of India for centuries (26). Six pandemics of the disease, each lasting about 10 years, occurred between 1817 and 1923. The seventh pandemic started in 1961 in Celebes, Indonesia. Due to better sanitation and public health measures most technologically advanced countries have been relatively free of the disease since early this century. Today the disease remains endemic in India and parts of Asia (26).

Cholera is a fecal-oral disease. The infection is acquired by ingesting water or food contaminated with the organism. Incriminated foods include raw or partially cooked oysters, shrimp, crabs, and mussels. The ingestion of approximately $10^{11}$ cells is required to produce the disease (9,52). The large inoculum which is required is one of the factors which make person-to-person transmission of the disease uncommon (62). Since the organism is sensitive to low pH's, the gastric acid of the stomach provides some
protection against the disease (1). Individuals whose stomach acidity has been decreased are more susceptible to the disease (44,45,62).

If the organism survives the conditions of the stomach, it passes into the small and large intestines where it can multiply. It does not spread beyond the gastrointestinal tract or penetrate the epithelial layer, but it remains adhered to the intestinal mucosa (61).

Symptoms include a feeling of fullness in the abdomen and loss of appetite. The patient may vomit and pass large quantities of liquid stools. First the stools are brown, but later the stools take on a "rice-water" appearance. The rice-water stools consist of mucous, epithelial cells, and large numbers of vibrios. Patients may lose more than a liter of stools per hour for many hours during the diarrhea. The patient develops cramps in the limbs and abdomen and may go into shock. Death, which occurs due to the effects of dehydration, occurs in approximately 60% of the cases if the patient is not treated (26,61).

These symptoms occur in the worst cases of the disease. There may also be subclinical infections where the host shows no sign of illness. The classical biotype has an attack rate of 1 in 10, and the El Tor biotype has an attack rate of 1 in 25 to 100 (52).

The profuse diarrhea occurs as a result of the secretion of the cholera enterotoxin which stimulates the activity of adenyl cyclase (14,27,28,29,46,59). This enzyme
catalyzes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) \( (11,22,55,61) \). The high concentration of cAMP causes the cells of the intestinal wall to secrete chloride ions and water into the intestine. It also causes a decrease in the absorption of the sodium ions, which also enhances fluid loss \( (52) \).

**Isolation of the organism.** The organism may be viewed directly in the patient's stools with the aid of a dark field microscope \( (61) \).

The isolation of *V. cholerae* is based on the rapid growth of the organism at alkaline pH's in the presence of available oxygen \( (58) \). *Vibrio cholerae* can outgrow most of the contaminating organisms under these conditions and can be isolated from the pellicle of liquid alkaline media after six to eight hours of incubation at 35°C. The organism may also be isolated on selective media such as thiosulfate-citrate-bile salts-sucrose (TCBS) agar \( (40,41,49) \). Suspect vibrios may be identified by biochemical and serological test and a test for enterotoxin production.

**Treatment and control of the disease.** When cholera patients are given prompt treatment, the mortality rate may be reduced to less than 1% \( (26) \). Treatment involves replacement of fluids and electrolytes lost due to the diarrhea. The continuous intravenous injection of fluids containing sodium, potassium, bicarbonate, and chloride produces dramatic recovery. The patient regains warmth and alertness within minutes, and recovery is complete within
four to seven days (26).

Intravenous injections require large quantities of sterile fluids, skilled personnel and special facilities. By including simple sugars and certain amino acids in the rehydration fluid, the transport of sodium and water across the intestinal wall is increased. Therefore, fluids may be administered orally after the initial intravenous rehydration (26). This method is especially useful in less developed areas of the world.

Tetracycline may also be used to shorten the duration of the disease. The antibiotic alone is not a cure, however, since the patient's fluid losses must be replaced for recovery to occur (26).

Cholera is controlled by adequate water sanitation and proper sewage disposal (61). The relative lack of the disease in the more developed countries may be attributed to these practices. The disease can still occur in developed countries when lapses in food or water sanitation allows the food or water to become contaminated with the organism before being consumed.

Recent cholera outbreaks. Since the 1960's seafood products have been increasingly incriminated in cholera outbreaks. Contaminated raw shrimp were responsible for an outbreak of the disease which occurred in the Phillipines during 1961 and 1962 (16). Raw seafood was also responsible for the 1973 cholera epidemic in Naples, Italy. There were 25 deaths reported among the 278 confirmed cases (1).
The cholera epidemic of 1974 in Portugal was caused by the consumption of contaminated shellfish which were consumed raw or partially cooked (6). Commercially bottled water was also implicated in the epidemic. There were 2467 confirmed cases of the disease and 48 deaths. *Vibrio cholerae*, biotype El Tor, serotype Inaba was isolated from 42% of the shellfish sampled during the epidemic.

Before 1973, no cases of the disease were reported in the U.S. since 1911. In Port Lavanca, Texas a case of the disease was reported in 1973 (62). Another case was reported in Montgomery, Alabama in 1977 (8). The source of the organism was not determined in either case, but both patients had histories of eating large quantities of raw oysters.

In August and September of 1978, eight cases of cholera and three asymptomatic cholera infections were reported in southwest Louisiana (7,37,38,57). Contaminated crabs taken from Gulf Coast marshes were responsible for the infections. The crabs were collected between Mud Lake (west of Cameron) and Vermilion Bay (south of Abbeville). *Vibrio cholerae* either survived the cooking or recontaminated the cooked crabs when they were returned to their original container for storage. Studies showed that the organism could survive boiling for eight minutes (but not 10 minutes) in crabs (7). The crabs' shells were red and the meat was firm after eight minutes of boiling. This indicates that shell color should not be used to determine if the crabs have been adequately
cooked.

Although crabs prepared by commercial establishments were not implicated in the outbreak, the consumer reaction caused economic hardship to Louisiana's seafood industry (48). After the outbreak the United States Food and Drug Administration (U.S.F.D.A.) conducted a two month surveillance of Louisiana live crabs and other seafoods intended for interstate shipment (39). *Vibrio cholerae* 01 was not isolated from any crab or oysters samples, but it was isolated from one shrimp sample and two of the water samples tested.

Two unrelated cases of the disease were reported in Texas in 1981 (35). One of the patients died from complications due to the illness. Also in 1981, 17 cases of the disease were reported on an offshore oil rig located south of Port Arthur, Texas (10,25). The source of the organism was not identified in any of these cases.

All of the above infections were caused by *V. cholerae*, biotype El Tor. All of the cases in the U.S. were caused by the Inaba serotype. During the pandemic the El Tor biotype seemed to have migrated from Asia to Europe, and finally to North and South America (16).

Ecology of *V. cholerae*. *Vibrio cholerae* thrives in salty, alkaline environments (26). The organism has been isolated from the Chesapeake Bay and from estuaries and sewers of Louisiana (12). Since the organism has been discovered in the absence of human disease, it may be able
to survive and multiply in the natural environment (4,53). *Vibrio cholerae* 01 has also been isolated from oysters taken from estuarine waters of Florida (30). This area had a low fecal coliform count and there was no prior evidence of sewage contamination. These results indicate that there may be a need to reevaluate the effectiveness of the National Shellfish Sanitation Program (NSSP) standards and guidelines for classification of shellfish waters.

Non-cholera vibrios are found in surface waters and shellfish throughout the world (15). Studies indicate that it is ubiquitous in estuarine waters and is not necessarily associated with domestic sewage (16). It has been isolated from shellfish and water samples in the Gulf Coast. The organism has also been isolated from the waters, shellfish, and sediment of the Chesapeake Bay (34). The non-cholera vibrios should be considered potential pathogens since the exact relationship between them and *V. cholerae* 01 is not known (34). Some of the non-cholera vibrios have also been shown to produce enterotoxin and cause acute gastroenteritis (62).

**THERMAL TREATMENT OF FOODS**

*Mild heat treatments.* Heat is applied to foods during several processing operations. These operations include cooking, scalding, pasteurizing, blanching, and canning. Heating is a relatively efficient means of food preservation because it destroys microorganisms and inactivates enzymes in the food.
Cooking is usually the last heat treatment that the food receives before it is consumed. The United States Department of Agriculture (U.S.D.A.) recommends that foods be heated until an internal temperature of 63°C is attained (3). This destroys most vegetative cells, but spores may survive. Viruses may also survive cooking. Cooking oysters inoculated with polioviruses in boiling milk for eight minutes or baking for 20 minutes at 121.5°C did not destroy the virus (18). Since cooking may not eliminate all microorganisms in the food, the food must be held above 50°C or below 4°C to prevent growth of potential pathogens (3).

The main objective of pasteurization is to destroy certain pathogens in specific foods. Many spoilage organisms are also destroyed, therefore, the shelf life of the food may be extended. Pasteurization is used for those foods which are adversely affected by more severe heat treatments. It employs the mildest heat treatment possible which will guarantee the destruction of pathogens and toxins (47). Usually, the foods are heated to between 60° and 85°C for a few seconds to an hour (3). Liquid eggs, dairy products, alcoholic beverages, and crabmeat are some of the products which are pasteurized today.

**Effect of heat on bacteria.** Vegetative cells of most bacteria are destroyed by temperatures 10° to 15°C above its optimum growth temperature (3). Different organisms differ in their heat resistance, but 10 minute at 100°C can destroy all vegetative cells.
The heat resistance of bacteria is affected by the conditions under which it was grown, the nature of the suspending media, and the methods used to enumerate survivors (3). Bacteria which are grown at higher temperatures usually have greater heat resistance than organisms grown at lower temperatures. The water activity, pH, and composition of the suspending media also affect heat resistance. Bacteria are more susceptible to heat in foods with high water activities. Microorganisms also have greater heat resistance near pH 7.0, than at a pH above 8.0 or below 6.0. Proteins, carbohydrates, and fats in the suspending media protect bacteria from the lethality of the heat. Some bacteria may only be injured by a particular heat treatment and may require special conditions to recover. Therefore, the type of recovery media used could determine the number of organisms enumerated after the bacteria had been exposed to a heat treatment (3).

The primary lethal event in the thermal inactivation of microorganisms has not yet been fully determined. Theories suggesting breaks in the bacteria's deoxyribonucleic acid (DNA), damage to the cytoplasmic membrane, denaturation of the cell's protein, or lesions in the ribonucleic acid (RNA) have been proposed. Denaturation of proteins involved in cellular respiration or multiplication is usually cited as the cause of death due to heat. However, at lower temperatures which cause sublethal injury, leakage of the cytoplasmic membrane and RNA degradation have been linked to
bacterial death (51).

**Determination of D values.** When a population of bacteria is exposed to lethal heat, generally a logarithmic survival curve is produced (3). The number of organisms surviving the heat treatment is proportional to the initial number of cells in the population. The thermal death time is the time required at a given temperature to destroy all bacteria in a suspension of cells containing a known number of microorganisms. The decimal reduction time or D value of a microorganism is the time required to reduce the microbial population by 90%. The D value of a microorganism varies with the species, strain, temperature, and other factors which effect the heat resistance of the organism (3). The z value is defined as the number of degrees of temperature required to produce a ten fold change in a D value.

When heat is applied to the outside of a container of food, the food near the center of the container is the last to reach the processing temperature. This area is known as the cold point of the container. Heat sensing thermocouples may be used to measure the time required for the container's cold point to reach the processing temperature. While the temperature of the container is rising to the processing temperature (come-up time) the heat produces a certain amount of lethality. One unit of lethality is defined as the heat equivalent to one minute at a given temperature. The lethality or lethal rate of the temperatures which the container passes through during the come-up period may be
calculated with the following formula: lethal rate = 1/[antilog \( T - IT \)/z], where \( T \) is the processing temperature, \( IT \) is the temperature of the homogenate, and \( z \) is the \( z \) value of the organism being studied. A curve can be constructed from the lethal rate of each temperature of the come-up time. The total area under the lethal rate curve is divided by one unit of lethality to give the total lethality of the come-up time (47).

After the homogenate is heated for a known amount of time at a given temperature the following equation may be used to determine the \( D \) value of the organism: \( D = \frac{t}{\log a - \log b} \), where \( t \) is the time (corrected for come-up), \( a \) is the initial number of cells present, and \( b \) is the number of cells remaining after time \( t \) (3).

IRRADIATION OF FOODS

Ionizing radiations. Irradiation has been one of the most intensively studied processes in food technology. The U.S. government has spent approximately $80 million dollars since 1953 to study the process (5).

Irradiation may be used on a variety of foods. It can be used to inhibit sprouting in tubers, to delay ripening of fruits, to destroy insects and their larvae in grain, to destroy parasites in meats, to destroy spoilage microorganisms and extend a product's shelf life, and to prevent or control foodborne illnesses (3).

In some cases, it may be safer to use irradiation than current methods of food preservation. Irradiation reduces
the need for nitrites in cured meats. It can also be substituted for the chemical spray ethylene dibromide which is used to kill insects on fresh produce. This chemical is believed to be a carcinogen and the Environmental Protection Agency is expected to ban its use in July 1983 (5).

Irradiation is considered to be a "cold process" because only a slight temperature rise occurs in the food during the process. The food may therefore retain more of the appearance, taste, and quality characteristics of fresh raw foods. Depending on the number of spoilage and disease organisms killed by the process, properly packaged foods may be stored for years without refrigeration (32).

The packaging required for irradiated products is simpler than that required for most heat processed foods. The cost of irradiating is also comparable to the cost of heat processing (50).

Ionizing radiations contain enough energy to produce ion pairs by dislodging electrons from their atomic orbits. The collisions with atoms in the food result in free electrons and positively charged residues. Collisions may also break chemical bonds between atoms to produce free radicals (47). Alpha particles, beta particles, gamma rays, and neutrons are all ionizing radiations. Gamma rays are the most widely used ionizing radiation for food preservation because of its high penetrating power and its inability to induce radioactivity. Gamma rays from cobalt-60 or cesium-137 may be used for food irradiation,
but cobalt-60 is the most accepted radiation source because of its availability and price (3).

**Food preservation.** Today, the term most frequently used to indicate the dose of irradiation a food has received is the rad. The rad is based on the amount of energy the food absorbs. One rad is equivalent to the absorption of 100 ergs/gram of product (17). Dosage may be measured with a dosimeter such as cobalt glass which changes color in proportion to the dose received.

Foods may be pasteurized or sterilized, depending on the amount of radiation they receive. During pasteurization irradiation or radurization, the food is treated with the maximum amount of irradiation which will produce no changes in the food. Doses for radurization range from 100 to 1000 krad (3). These doses normally kill 90 to 95% of the microorganisms present, but resistant vegetative cells and spores may survive. Radurization may be combined with refrigeration to double the shelf life of a perishable food (31). The psychrophilic pseudomonads, which are the main spoilage organisms of proteinaceous foods stored at cold temperatures, are very sensitive to irradiation (3). Those organisms which survive irradiation are less metabolically active so they grow slower than the food's normal microflora (31). The foods that rank highest for radurization are shrimp, fish, blue crab, and strawberries because they are highly perishable and have a high value (3).

**Radicidation** is an irradiation treatment designed
specifically to rid food of pathogens (31). Examples include treating fish to destroy *Vibrio* spp. and treating meat and poultry to destroy *Salmonella*. Doses may be as low as 250 krad for fresh products or 500 krad for frozen or dried products.

The food irradiation process which renders a food commercially sterile is known as radappertization (31). The minimum radiation dose to achieve commercial sterility is based on the resistance of spores of *Clostridium botulinum*. The 12 D concept is also applied in radappertization. A dose of 4500 krad is generally applied to radappertized foods.

High doses of radiation may produce undesirable flavors, odors, and colors in the food. Trained personnel can detect flavor changes in food treated with 50 krad (3). Even untrained personnel can detect flavor changes in some foods treated with 200 krad. This effect varies with different foods. Cured meats may be treated with high doses without effecting their quality, but flavor changes in milk are produced by low doses. These off flavors and odors tend to disappear during storage (17). Undesirable changes may also be minimized by adding free radical acceptors to the food, by irradiating under anaerobic conditions, or by eliminating moisture from the food or freezing it before irradiation (3).

Even doses used for radappertization are not sufficient to destroy enzymes in food. Enzymes are five to 10 times
more resistant to radiation than the most resistant bacteria. Doses required to inactive enzymes would destroy the quality of the food. The process of thermoradiation involves applying heat to the food to destroy the enzymes before the food is irradiated. Radappertization treatments are therefore usually applied to partially cooked or cooked meats, poultry, and fish products. The combination of heat and radiation effect the quality of food less than if either method was used alone to sterilize the food (3).

Effect on microorganisms. Foods may be preserved by ionizing radiations due to the destruction of spoilage microorganisms in the product. Death of the microorganisms is due to lesions in the nuclear DNA produced by ionizing radiation (31). Indirect effects such as the formation of ion pairs and free radicals, and the reaction of these free radicals with other molecules in the food also alter microorganisms, enzymes, and food constituents (47). When the ionizing radiations pass through water, hydrogen and hydroxyl radicals are produced. These radicals may react with other molecules to produce hydrogen peroxide, hydrogen gas, or peroxide radicals. The use of free radical scavengers, irradiating in a vacuum, or irradiating food after freezing or drying will improve the sensory qualities of the food, but these treatments also offer protection to microorganisms and enzymes in the foods (3).

Species of microorganisms differ in their resistance to ionizing radiations. Gram negative bacteria are generally
more sensitive. Some of the least resistant bacteria are species of Pseudomonas, Proteus, Escherichia, and Vibrio (3). Salmonella serotypes differ widely in radiation resistance, therefore E. coli will not be a useful indicator of Salmonella contamination in irradiated foods. Staphylococcus aureus and fecal streptococci are also relatively resistant. Micrococcus radiodurans is noted for its ability to resist high doses of irradiation. Endospores of some strains of Bacillus cereus are highly resistant to ionizing radiations, but the most resistant endospores are those of C. botulinum type A (31).

Wholesomeness of irradiated foods. Recommendations by the Joint Expert Committee on Wholesomeness of Irradiated Foods which was convened by the World Health Organization concluded that any food irradiated with an average dose of 1 Mrad or less is safe for human consumption and should be approved without further testing. The committee delayed recommendations on higher doses until more data are available (32).

In the U.S. the safety and wholesomeness of irradiated foods have been investigated by the Office of the Surgeon General of the Department of the Army, the Atomic Energy Commission, the National Bureau of Standards, the F.D.A. and others (47). The irradiated foods have been found to be as nutritious as their heat processed counterparts. Irradiation produces no significant reduction in the quality of proteins, lipids, carbohydrates, or mineral constituents,
and its effect on vitamins is comparable to that of other food preservation methods (32).

Significant levels of toxic or carcinogenic substances were not produced when the foods are irradiated with F.D.A. approved dosages from approved sources. Neither did irradiation contribute harmful levels of radioactivity to the treated foods. The sterilized and pasteurized foods were also safe from a microbiological standpoint.

Irradiated foods can also be made very acceptable. Irradiated pork loins stored at room temperature for a year were as acceptable as fresh pork loins to several hundred consumers (17).

Present status of food irradiation. In 1963, the F.D.A. granted the Army approval to use ionizing radiation to sterilize bacon. In 1968, the F.D.A. denied an Army request to irradiate ham due to certain toxicological observations made by F.D.A. scientists on the Army's data. Shortly afterwards, the F.D.A. revoked its approval of the irradiated bacon on the grounds that it needed further proof of safety (47).

The F.D.A. has approved the use of 20 to 50 krad to kill insects and destroy larvae in wheat and wheat flour. It has also approved the use of up to 10 krad to inhibit sprouting in potatoes and the sterilization of certain packaging materials with X-rays (47). Irradiated meats were eaten by Apollo astronauts on the moon and by American astronauts and Russian cosmonauts during the Apollo-Soyuz
space flight in 1975 (32). Radiation sterilized meats were also included in the diet of astronauts on the Columbia space shuttle. Special hospital meals sterilized by irradiation have been approved for use by patients requiring sterile foods due to impaired immune systems. In Canada, up to 750 krad have been approved for controlling *Salmonella* in frozen poultry, and poultry may be treated with 300 krad in the Netherlands.

In the near future the F.D.A. is expected to approve the use of up to 1 Mrad to treat foods (5). In March 1981, the F.D.A. published an Advanced Notice of Proposed Rulemaking (ANPR) which outlined the possible actions available to deregulate irradiation for processing human foods (36). After the rules are issued for public comment, the next step will be to publish the final regulations for the process.

The final success of irradiation will depend on the cost, labeling requirements, consumer acceptance and the fulfillment of a perceived consumer need (32).
REFERENCES


Thermal Death Time of Vibrio cholerae in Oysters (*Crassostrea virginica*)

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INTRODUCTION

As of 1981, 31 non-laboratory acquired *Vibrio cholerae* 01 infections have been reported in the United States since 1973 (11). Prior to 1973, no cases of the disease had been reported in the U.S. since 1911. The source of the cholera organism could not be identified in some cases (4,11,13,24), however, seafood is often implicated as the source of the organism. A patient in Florida ingested a large quantity of raw oysters less than a week before the onset of cholera-like symptoms (17); but the *V. cholerae* 01 isolated from the patient's feces was non-toxigenic (11). Crabs which were eaten after being improperly cooked and/or stored were implicated in the 11 cases of cholera in Louisiana (14).

In addition oysters and other shellfish have been previously implicated in cholera epidemics in Portugal (3) and Italy (2,18). The consumption of raw oysters contaminated with non-01 *V. cholerae* is also responsible for several cases of gastroenteritis in the U.S. each year (15). In a recent outbreak, all patients who had non-01 *V. cholerae* isolated from their feces reported eating raw oysters within 72 hours of the onset of symptoms.

The natural flora of market oysters is predominated by the psychotrophic Gram negative asporogenous rods of the Pseudomonas/Vibrio group (5). Since the oyster is a filter feeding shellfish, it can concentrate bacteria from the water passing through and over its gills, which results in
natural contamination (19). The cultivation, processing, and packaging procedures for market oysters subjects them to other sources of contamination (6).

V. cholerae is able to survive in salty, alkaline environments such as estuaries (12), and it has been isolated from the waters of the Chesapeake Bay (5) and the Gulf Coast (21). The organism has also been isolated from shellfish and crustaceans taken from the Chesapeake Bay. The disease can be controlled by employing proper sewage disposal, insuring that drinking water systems are free of the organism, and avoiding hazardous dietary habits (e.g. eating raw seafoods) (2,14,25).

Although it is generally recognized that V. cholerae is a very fragile organism (12,14), relatively little information has been published on the thermal resistance of V. cholerae 01. The purpose of this study was to determine the thermal resistance of the organism in packaged fresh oyster meat.

MATERIALS AND METHODS

Organism. Vibrio cholerae 01 (Biotype El Tor, Serotype Inaba) Louisiana Strain #5875 was obtained from the Department of Microbiology at Louisiana State University. The organism was stored at room temperature on peptone salt agar slants and transferred to fresh media at 4-6 week intervals.

Preparation and inoculation of the oyster homogenate. Packaged oysters were purchased from local supermarkets and
were blended in a Waring blender at high speed for two minutes. The pH of the homogenate was adjusted to 6.0 with 1.0 molar NaOH, if necessary.

The *V. cholerae* culture was grown in nutrient broth for 20-24 hours at 35°C and added to the homogenate to yield a final concentration of approximately $10^6$ organisms/g (21). The inoculated homogenate was thoroughly mixed by blending at high speed for one minute.

**Preparation of thermal death-time tubes.** Pyrex glass tubing with an outer diameter of 13 mm was cut into 15 cm lengths and heat sealed at one end with an oxygen-acetylene torch.

Four grams of the oyster homogenate were placed into the tubes with a sterile animal force feeder. The tubes were heat sealed with the oxygen-acetylene torch in lengths of 10 cm.

**Determination of the come-up time.** The come-up time of the homogenate was determined at each temperature. Three tubes were connected to copper-constantan thermocouples and immersed in a water bath set at the required temperature. The time required for the temperature of the samples to rise from room temperature to the treatment temperature was recorded on a Leeds & Northrup Speedomax multipoint recorder. The time required for the slowest heating tube to reach the treatment temperature was used as the come-up time for all other experiments at that temperature. The timing of heat treatments was begun at the end of the come-up
period. The lethal effect of the heat during the come-up time was determined mathematically (1,23).

**Endpoint determination.** Tubes containing the oyster homogenate were immersed into a water bath heated from 49°C (120°F) to 82°C (180°F) at approximately 5°C intervals. The endpoint was determined by heating inoculated samples at 5-10 minute intervals. One sample was removed at each interval and cooled in an ice-water bath for 30 seconds. The samples were tested for the presence of *V. cholerae* by an alkaline peptone water (APW) enrichment (24) and isolation on thiosulfate citrate bile salts agar (TCBS) (Difco). Typical *V. cholerae* colonies were confirmed biochemically and serologically (24). Cultures which produced the proper reactions on Klieger's iron agar (BBL) and lysine iron agar (Difco) and were agglutinated by Bacto *Vibrio cholerae* antiserum, Polyvalent and Inaba, (Difco) were considered to be *V. cholerae*.

Sets of 15 tubes were then heated at one minute intervals above and below the time which produced negative results in the preliminary one tube tests. The time period after which no *V. cholerae* was recovered from any of the 15 tubes of heated inoculated homogenate was defined as the endpoint.

The control sample consisted of inoculated homogenate which underwent the same treatment as the other experimental samples, except no heat treatment was applied. This sample was included to verify that viable *V. cholerae* was recovered
from the homogenate during each experiment.

**Inoculated pack studies.** Whole packaged oysters were injected with a 20-24 hour *V. cholerae* culture, grown at 35°C in nutrient broth. A syringe was used to inject $10^6$ organisms/g into the oysters.

Fifty gram samples of the injected oysters were cooked using one of two methods. One group of oysters was cooked in a liter of hot oil (190.5, 375°F) for one minute, and the other group was cooked in a liter of boiling water for one minute.

After cooking, the oysters were blended in 450 ml of APW and incubated at 35°C for eight hours. Isolation and identification of *V. cholerae* was repeated as described above. A control sample consisting of inoculated oysters was treated in the same manner as above, except the oysters were not cooked.

**RESULTS AND DISCUSSION**

**Recovery of *V. cholerae* from the homogenate.** Death of the organism was defined as the inability of the cells to form colonies on TCBS after enrichment in APW; therefore, it was important that a large percentage of the live organisms be recovered from the homogenate. The Most Probable Number (MPN) technique was used to enumerate *V. cholerae* in the inoculated, unheated homogenate. The results indicated that relatively large percentages of the organisms were isolated. In three separate trials, 94, 44, and 125% of the organisms were recovered.
Endpoints. *V. cholerae* was not recovered from any of 15 tubes containing the inoculated homogenate which were heated for 34 minutes or more after reaching 49°C (120°F) (Table 1). At this temperature, 4.67 minutes were required for the temperature of the homogenate in the tubes to rise from room temperature to the processing temperature. The time required to reach the processing temperature of 49°C was equivalent to heating the homogenate at that temperature for 2.66 minutes (Table 1). Therefore, although the tubes containing the homogenate remained in the water bath for a total of 38.67 minutes, this was actually equivalent to heating the tubes for 36.66 minutes at 49°C (Table 2).

*V. cholerae* was not recovered from the homogenate heated for one minute after reaching the temperatures from 54°C to 82°C. The corrected endpoints at these temperatures ranged from 2.76 to 1.84 minutes (Table 2). The minimal heating time used in these studies was one minute. However, no organisms could be recovered from any of the thermal death time tubes after one minute of heating at 54°C or above. This implies that the true endpoints for these temperatures were less than one minute. Therefore, the true corrected endpoints were less than those calculated, which also meant that the calculated D values were greater than the true D values.

D values. D values for each temperature were calculated using the corrected endpoints. The values ranged from 4.71 minutes at 49°C to less than 0.24 minutes at 82°C.
Heat treatments which yield from 4 to 7 D values are generally considered adequate for pasteurization (21). The amount of time required to produce a 7 log cycle reduction of the V. cholerae population in the oyster homogenate was calculated by multiplying the time required for one log cycle reduction by seven (Table 2).

V. cholerae is very heat sensitive. It has been reported that the organism is killed after 10 minutes at 55°C (22). That study was done on classical biotypes, but El Tor biotypes are more resistant than classical biotypes (20).

Many factors influence the thermal resistance of bacteria (10). V. cholerae #5875 has a D value of 1.70 minutes at 49°C in peptone water, and a D value of 8.15 minutes at the same temperature in crab meat homogenate (21). The D value of the organism was greater in the crab and oyster meat homogenates than in the peptone water. The presence of colloidal substances such as proteins and fats may enhance the resistance of V. cholerae in the meat homogenates (8).

V. cholerae #5875 is more heat resistant in crab meat homogenate than in oyster meat homogenate. The lower pH and the presence of some inhibitory substances in oyster meat (9) may contribute to the greater susceptibility of the organism in the oyster meat than in the crab meat. Generally, cells are most heat resistant in a substrate that
is at or near neutrality (8). Acid and alkaline conditions decrease the heat resistance of bacteria, but a change in pH toward acidity causes a greater reduction of heat resistance than a corresponding increase in alkalinity. In this study the pH of the oyster homogenate was 6.0. Oysters with a pH between 5.9 and 6.2 are considered to be "good" (6), but the pH of fresh crab meat is between 7.2 and 7.4 (7). The viability of V. cholerae is reportedly less in an environment with a pH between 5.5 and 6.2 than it is in an environment with a pH greater than 6.2 (16).

Inoculated packs. It has been reported that cooking oysters in hot oil (190.5°C, 375°F) or in boiling water for one minute will destroy $4.0 \times 10^5$ Vibrio parahaemolyticus cells injected into the body of an oyster (19). The ability of these methods to destroy $10^6$ V. cholerae organisms/g in oysters was investigated in this study.

The oysters cooked in hot oil weighed from 5.50 to 24.95 g (Table 3). The weight of the oysters was important because it determined the rate of heat penetration to the cold point of the oysters. The internal temperature of an oyster weighing 6.26 g is greater than 100°C (212°F) after one minute in hot oil, while the temperature of an oyster weighing 19.67 g is approximately 79°C (174°F) (19). V. cholerae was not recovered from any of the three samples of inoculated oysters cooked in the oil, but the organism was isolated from the uncooked inoculated sample.

The results obtained when inoculated oysters weighing
6.81 to 19.61 g were cooked in boiling water are shown in Table 4. The internal temperature of an oyster weighing 6.26 g is 88°C (190°F) after one minute in boiling water, and the internal temperature of an oyster weighing 19.67 g is approximately 71°C (160°F) (19). The results are similar to those obtained when the inoculated oysters were cooked in the hot oil. Both methods proved to be effective for destroying $10^6 \text{V. cholerae/g}$ of oyster meat.

Raw oysters were implicated in the recent cholera outbreaks which occurred in Italy (18) and in Portugal (3). By cooking the oysters properly, the risk of a \textit{V. cholerae} infection is substantially lowered or completely removed, even though the oysters may have been contaminated with the organism.
REFERENCES


Table 1. Endpoints for *V. cholerae* and come-up times in oyster homogenates.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Endpoint (min.)</th>
<th>Come-up Time (min.)</th>
<th>Lethality of Come-up Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>34\textsuperscript{a}</td>
<td>4.67</td>
<td>2.66</td>
</tr>
<tr>
<td>54\textsuperscript{b}</td>
<td>1</td>
<td>3.50</td>
<td>1.76</td>
</tr>
<tr>
<td>60\textsuperscript{b}</td>
<td>1</td>
<td>3.83</td>
<td>1.74</td>
</tr>
<tr>
<td>66\textsuperscript{b}</td>
<td>1</td>
<td>3.17</td>
<td>1.61</td>
</tr>
<tr>
<td>71\textsuperscript{b}</td>
<td>1</td>
<td>3.83</td>
<td>1.37</td>
</tr>
<tr>
<td>77\textsuperscript{b}</td>
<td>1</td>
<td>3.83</td>
<td>1.32</td>
</tr>
<tr>
<td>82\textsuperscript{b}</td>
<td>1</td>
<td>3.50</td>
<td>0.84</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Endpoint based on no recovery from 15 tubes containing a total of 6.0 x 10\textsuperscript{7} organisms.

\textsuperscript{b} True D values obtained at these temperatures are less than those given due to the minimal test period of 1 minute.
Table 2. Corrected endpoints and D values for \textit{V. cholerae} in oyster homogenate

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Corrected Endpoints (min.)</th>
<th>D values</th>
<th>7D values</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>36.66</td>
<td>4.71</td>
<td>32.98</td>
</tr>
<tr>
<td>54(^{a})</td>
<td>2.76</td>
<td>0.35</td>
<td>2.48</td>
</tr>
<tr>
<td>60(^{a})</td>
<td>2.74</td>
<td>0.35</td>
<td>2.46</td>
</tr>
<tr>
<td>66(^{a})</td>
<td>2.61</td>
<td>0.33</td>
<td>2.35</td>
</tr>
<tr>
<td>71(^{a})</td>
<td>2.37</td>
<td>0.30</td>
<td>2.13</td>
</tr>
<tr>
<td>77(^{a})</td>
<td>2.32</td>
<td>0.29</td>
<td>2.09</td>
</tr>
<tr>
<td>82(^{a})</td>
<td>1.84</td>
<td>0.24</td>
<td>1.65</td>
</tr>
</tbody>
</table>

\(^{a}\)True D values obtained at these temperatures are less than those given due to the minimal test period of one minute.
TABLE 3
Survival of *V. cholerae* in Oysters Cooked in Oil at 190°C (375°F) for One Minute

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight of Oysters (grams)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.11-24.95</td>
<td>-&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>5.59-13.96</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>6.89-14.35</td>
<td>-</td>
</tr>
<tr>
<td>Uncooked</td>
<td>5.40-10.65</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>No *V. cholerae* recovered from the sample.

<sup>b</sup>*V. cholerae* recovered from the sample.
**TABLE 4**

Survival of *V. cholerae* in Oysters Cooked in Water at 100°C (212°F) for One Minute

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight of Oysters (grams)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.88-12.79</td>
<td>-(^a)</td>
</tr>
<tr>
<td>2</td>
<td>6.81-9.80</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>11.16-19.61</td>
<td>-</td>
</tr>
<tr>
<td>Uncooked</td>
<td>5.40-10.65</td>
<td>+(^b)</td>
</tr>
</tbody>
</table>

\(^a\)No *V. cholerae* recovered from the sample.

\(^b\) *V. cholerae* recovered from the sample.
Determination of the Thermal Death Time of Vibrio Cholerae in Shrimp (Penaeus Setiferus)

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INTRODUCTION

Since 1973 several cases of *Vibrio cholerae* 01 infections have been reported in the United States (6). Seafood was implicated as the source of the organism in some of these cases, and it has been responsible for cholera epidemics occurring in other countries (1,2,3).

Cooked crabs were implicated in 11 cases of the disease which occurred in Louisiana during 1978 (10). Authorities suspect that the organism either survived the cooking or that the cooked crabs were recontaminated when they were returned to their original container for storage. After this outbreak, hundreds of samples of fresh Louisiana crabs, oysters, and shrimp were examined. *V. cholerae* was isolated from shrimp caught in a canal near one of the sites where the implicated crabs were caught (2).

Although it is generally recognized that *V. cholerae* is a very fragile organism (9), relatively little information has been published on the thermal resistance of *V. cholerae* 01. The purpose of this study was to determine the thermal resistance of the organism in shrimp meat.

MATERIALS AND METHODS

Organism. *Vibrio cholerae* 01 (Biotype El Tor, Serotype Inaba) Louisiana Strain #5875 was obtained from the Department of Microbiology at Louisiana State University. The organism was stored on peptone salt agar slants at room temperature and transferred to fresh media at 4 to 6 weeks intervals.
Preparation and inoculation of shrimp homogenate. Fresh shrimp were purchased from local seafood stores. Three parts peeled shrimp and one part distilled water were blended at high speed in a Waring blender until smooth.

*V. cholerae* grown in nutrient broth for 20 to 24 hours at 35°C was added to the homogenate to yield a final concentration of approximately $10^6$ organisms/g (13). The inoculated homogenate was thoroughly mixed by blending at high speed for one minute.

Preparation of thermal death time tubes. Pyrex glass tubing with an outer diameter of 13 mm was cut into 15 cm lengths and heat sealed at one end with an oxygen-acetylene torch.

Four grams of the shrimp homogenate were placed into the tubes with a sterile animal force feeder. The tubes were heat sealed with the oxygen-acetylene torch in lengths of 10 cm.

Determination of the come-up time. The come-up time of the homogenate was determined at each temperature. Three tubes were connected to copper-constantan thermocouples and immersed in a water bath heated at the required temperature. The time required for the temperature of the samples to rise from room temperature to the water bath temperature was recorded on a Leeds and Northrup Speedomax multipoint recorder. The time required for the slowest heating tube to reach the water bath temperature was used as the come-up time for all other experiments at that temperature. The
timing of the heat treatments was begun at the end of the come-up period. The lethal effect of the heat during the come-up time was determined mathematically (15).

**Endpoint determination.** Tubes containing the shrimp homogenate were immersed into a water bath heated from 49°C (120°F) to 82°C (180°F) at approximately 5°C (10°F) intervals. The endpoint was bracketed by heating inoculated samples at 5 or 10 minute intervals. One sample was removed at each interval and cooled in an ice water bath for 30 seconds. The samples were tested for the presence of *V. cholerae* by an alkaline peptone water (APW) enrichment (16) and isolation of thiolsulfate citrate bile salts agar (TCBS) (Difco). Typical *V. cholerae* colonies were confirmed biochemically and serologically. Cultures which produced the proper reactions of Klieger's iron agar (Difco) and were agglutinated by Bacto *Vibrio cholerae* antiserum, Polyvalent and Inaba (Difco), were confirmed as *V. cholerae*.

Sets of 15 thermal death time tubes were then heated at one minute intervals above and below the time which produced negative results in the preliminary one tube tests. The time period after which no *V. cholerae* was recovered from any of the 15 tubes of heated homogenate was defined as the endpoint.

The control sample consisted of inoculated homogenate which underwent the same treatment as the other experimental sample, except no heat treatment was applied.
Inoculated pack studies. Fresh peeled shrimp were injected with a 20 to 24 hour *V. cholerae* culture grown at 35°C in nutrient broth. A syringe was used to inject $10^6$ *V. cholerae* into the shrimp.

Fifty gram samples of the injected shrimp were cooked using one of two methods. One group of shrimp was cooked in a liter of boiling water for 10 minutes, and the other group was cooked in steam (100°C, 212°F) for 10 minutes.

After cooking 25g of the shrimp were blended in 225 ml of APW and incubated at 35°C for eight hours. Isolation and identification of *V. cholerae* were repeated as described above. A control sample consisting of inoculated shrimp was treated in the same manner as above, except that the shrimp were not cooked.

RESULTS AND DISCUSSION

Recovery of *V. cholerae* from the homogenate. Death of the organism was defined as the inability of the bacteria to form colonies on TCBS after enrichment in APW; therefore, it was important that a large percentage of the live organisms be recovered from the homogenate. An average of 70% of the organisms was recovered from the unheated homogenate with the Most Probable Number (MPN) technique.

Endpoints. *V. cholerae* was not recovered from any of 15 tubes containing the inoculated homogenate heated for 69 minutes or more after reaching 49°C (120°F) (Table 1). At this temperature, 4.17 minutes were required for the temperature of the homogenate in the tubes to rise from room
temperature to the temperature of the water bath. This was equivalent to heating the homogenate at that temperature for 2.16 minutes (Table 1) and produced a total heating time of 71.16 minutes (Table 2).

*V. cholerae* was not recovered from the homogenate heated for one minute after reaching the temperatures from 54°C to 82°C. The corrected endpoints at these temperatures ranged from 3.34 to 2.20 minutes (Table 2). The minimal heating time used in these studies was one minute. However, no organisms could be recovered from any of the thermal death time tubes after one minute of heating at 54°C or above. This meant that the true endpoints for these temperatures were less than one minute. Therefore, the true corrected endpoints and the calculated D values were less than their calculated values.

**D values.** D values for each temperature were calculated using the corrected endpoints. The values ranged from 9.17 minutes at 49°C to less than 0.28 minutes at 82°C (Table 2).

Heat treatments which produce a 4 to 7 log cycle reduction of the bacterial population are generally considered adequate for pasteurization (13). The amount of time required to produce a 7 log reduction of the *V. cholerae* population in the shrimp homogenate was calculated by multiplying the time for 1 log cycle by 7 (Table 2).

*V. cholerae* is very heat sensitive. It has been reported that the organism is killed after 10 minutes at
55°C (14). That study was done with the classical biotype, not with the more resistant El Tor biotype however (12).

Many factors influence the thermal resistance of bacteria (5). At 49°C V. cholerae #5875 has a D value of 1.70 minutes in peptone water, 4.72 minutes in oyster meat homogenate (8), and 8.15 minutes in crab meat homogenate (13). The D value of the organism was greater in the meat homogenates than in the peptone water. The presence of colloidal substances such as proteins and fats may enhance the resistance of the organism in the meat homogenates (4).

V. cholerae #5875 is more heat resistant in shrimp meat homogenate than in the oyster and crab meat homogenates. This may be related to the pH of the meat homogenates. Generally, bacteria are most heat resistant in a substrate that is at or near neutrality (4). Acid and alkaline conditions decrease the heat resistance of bacteria, but a change in pH toward acidity causes a greater reduction in the heat resistance than a corresponding increase in alkalinity. The pH of the shrimp homogenate was between 7.2 and 7.9. The pH of fresh crab meat is between 7.2 and 7.4, and the pH of the oyster homogenate was 6.0 (8). The viability of V. cholerae is reportedly less in an environment with a pH of 5.5 to 6.2 than it is in an environment with a pH greater than 6.2 (11).

Inoculated packs. The shrimp which were steamed weighed between 2.1 and 21.7 g (Table 3). The weight of the shrimp determined the rate of heat penetration to the cold
point of the shrimp. The temperature of shrimp weighing 7 g or less is 100°C after cooking for two minutes in 100°C steam (7), but the temperature of shrimp weighing 13.6 g is 96.67°C after the same time. No *V. cholerae* was recovered from any of the shrimp cooked for 10 minutes with 100°C steam (Table 3).

The results obtained when inoculated shrimp weighing 2.8 to 23.2 g were cooked in boiling water are shown in Table 4. It requires approximately four minutes in 100°C water for the internal temperature of a 10 g shrimp to reach 100°C (7). More time would be required for heavier shrimp. The results of cooking the shrimp in boiling water for 10 minutes were the same as those obtained by cooking the shrimp with steam. Both methods were effective for destroying $10^6$ *V. cholerae*/shrimp.
REFERENCES


TABLE 1

Endpoints of *V. cholerae* in shrimp meat homogenates and come-up time

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Endpoint (min.)</th>
<th>Come-up Time (min.)</th>
<th>Lethality of Come-up Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>69</td>
<td>4.17</td>
<td>2.16</td>
</tr>
<tr>
<td>54b</td>
<td>1</td>
<td>4.34</td>
<td>2.34</td>
</tr>
<tr>
<td>60b</td>
<td>1</td>
<td>3.02</td>
<td>2.02</td>
</tr>
<tr>
<td>66b</td>
<td>1</td>
<td>2.51</td>
<td>1.51</td>
</tr>
<tr>
<td>71b</td>
<td>1</td>
<td>2.39</td>
<td>1.39</td>
</tr>
<tr>
<td>77b</td>
<td>1</td>
<td>2.34</td>
<td>1.34</td>
</tr>
<tr>
<td>82b</td>
<td>1</td>
<td>2.20</td>
<td>1.20</td>
</tr>
</tbody>
</table>

*Endpoint based on no recovery from 15 tubes containing a total of 6.0 x 10^7 organisms. (4 x 10^6 organism/tube)*

*True D values obtained at these temperatures are less than those given due to the minimal test period of 1 minute.*
TABLE 2

Corrected endpoints and D values for *V. cholerea* in shrimp homogenate

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Corrected Endpoints (min.)</th>
<th>D values</th>
<th>7D values</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
<td>71.16</td>
<td>9.17</td>
<td>64.02</td>
</tr>
<tr>
<td>54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.34</td>
<td>0.43</td>
<td>3.00</td>
</tr>
<tr>
<td>60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.02</td>
<td>0.39</td>
<td>2.73</td>
</tr>
<tr>
<td>66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.51</td>
<td>0.32</td>
<td>2.24</td>
</tr>
<tr>
<td>71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.39</td>
<td>0.31</td>
<td>2.17</td>
</tr>
<tr>
<td>77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.34</td>
<td>0.30</td>
<td>2.10</td>
</tr>
<tr>
<td>82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.20</td>
<td>0.28</td>
<td>1.96</td>
</tr>
</tbody>
</table>

<sup>a</sup>True D values obtained at these temperatures are less than those given due to the minimal test period of one minute.
TABLE 3
Survival of *V. cholerae* in shrimp steamed at 100°C (212°F) for 10 minutes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight of Shrimp (grams)</th>
<th>Results</th>
</tr>
</thead>
</table>
| 1      | 2.10-3.99                | -
| 2      | 3.30-13.66               | -      |
| 3      | 4.46-21.74               | -      |
| Uncooked | Not weighed           | +      |

*a* No *V. cholerae* recovered from sample.

*b* *V. cholerae* recovered from sample
TABLE 4
Survival of *V. cholerae* in shrimp cooked in water at 100°C (212°F) for 10 minutes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight of Shrimp (grams)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.80-3.80</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>4.30-11.30</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>4.30-23.20</td>
<td>-</td>
</tr>
<tr>
<td>Uncooked</td>
<td>Not weighed</td>
<td>+</td>
</tr>
</tbody>
</table>

*No *V. cholerae* recovered from sample.

*V. cholerae* recovered from sample.
Low Dose Gamma Irradiation of Vibrio cholerae in Shrimp (*Penaeus setiferus*)

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INTRODUCTION

The recent outbreaks of *Vibrio cholerae* infections in the United States have caused public health officials to become more aware of the possibility of the pathogen causing sporadic cases of the disease in this country. The source of the organism could not be identified in most of the cases, but crabs which had either been improperly cooked or stored were implicated in 11 cases of the disease occurring in Louisiana (7). During subsequent surveillances, *V. cholerae* Serotype Inaba was also isolated from a shrimp sample taken from waters located close to one of the sites where some of the contaminated crabs were caught (4).

Members of the *Vibrio* genera are destroyed by relatively low doses of irradiation (2). Irradiation doses of less than 1000 krads are considered as pasteurization levels (3). Doses of 100 to 1000 krads destroy 90 to 99% of the spoilage microorganisms present in food (2). Because *Vibrios* are so susceptible to irradiation, pasteurization doses may be used to destroy any *V. cholerae* which may be present and to extend the refrigerated shelf life of the shrimp by destruction of spoilage microorganisms.

Since fresh shrimp are so perishable and have a high market value, it is considered to be an excellent candidate for pasteurization levels of irradiation (2).

In the following experiment, the effect of pasteurization levels of irradiation on *V. cholerae* in shrimp homogenate were determined. The irradiated
homogenates were placed in cold storage, and the number of *V. cholerae* surviving were determined over a 21 day period.

MATERIALS AND METHODS

**Organism.** *V. cholerae* 01 (Biotype El Tor, Serotype Inaba) Louisiana strain #5875 was obtained from the Department of Microbiology at Louisiana State University. The organism was stored at room temperature on peptone salt agar slants and transferred to fresh media at 4 to 6 week intervals.

**Preparation of the shrimp homogenate.** Fresh shrimp were purchased from local seafood markets. The unsterile shrimp homogenate was prepared by blending three parts peeled shrimp with one part sterile saline in a Waring blender to form a smooth paste. The sterile shrimp homogenate was prepared in the same manner, except that the shrimp was sterilized at 121°C for 15 minutes before blending with saline.

**Preparation of inoculum.** On two consecutive days *V. cholerae* was transferred to fresh peptone salt agar (PSA) and incubated for 18 to 24 hours at 35°C. The organism was washed from the PSA with 1 ml of peptone water (PW), and 0.1 ml of the suspension was transferred to tubes containing 10 ml of PW. These tubes were incubated at 35°C for 18 to 24 hours. After incubation the *V. cholerae* suspension was centrifuged at 8000 rpm for 10 minutes in a Sorvall Superspeed RC2-B centrifuge. The supernatant was discarded and the bacteria were suspended in 10 ml of saline. This
washing procedure was repeated twice more, and the bacteria were then suspended in 1 ml of saline. A population of $1 \times 10^9$ *V. cholerae*/ml was produced by placing a sufficient amount of the suspension in a saline solution to produce an absorbance ($A_{650}$) of approximately 0.46 on a Bausch and Lomb Spectronic 70 spectrophotometer. The inoculum was added to the shrimp homogenate to produce a final concentration of $1 \times 10^7$ *V. cholerae*/g. The inoculated samples were placed in 250 ml Nalgene bottles, packed in ice, and transferred to the Nuclear Science Center at Louisiana State University.

**Irradiation of the samples.** The Nalgene bottles containing the samples were placed in a water tight chamber which was filled with ice and sealed. The chamber was lowered into the pit to expose the samples to the cobalt 60 source which emitted 1650 rads/min. The samples were exposed to 25, 50, or 100 krads. The bottles were then removed, packed in ice and transferred to the laboratory for analysis and storage. Control samples were treated in the same manner, except the irradiation treatments were omitted.

**Enumeration of V. cholerae.** Samples were examined for the presence of *V. cholerae* after 0, 7, 14, and 21 days of cold storage (4°, 0°, -8°C).

*Vibrio cholerae* in the sterile and unsterile homogenates was enumerated by the MPN technique. The MPN tubes which contained 10 ml of APW were incubated at 35°C for six to eight hours before streaking into
thiolsulfate-citrate-bile salts-sucrose (TCBS, Difco) plates. After incubating the plates at 35°C for 18 to 24 hours, typical *V. cholerae* were removed for further examination. The colonies were confirmed as *V. cholerae* by biochemical tests on Klieger's iron agar (Difco) and lysine iron agar (Difco) and serological test with Polyvalent and Inaba Bacto *Vibrio cholerae* antiserum (Difco).

Irradiation of *V. cholerae* in sterile and unsterile shrimp homogenates and storage of the homogenates were performed in triplicate. The number of *V. cholerae* surviving was calculated by determining the average number of *V. cholerae* recovered from three samples subjected to the same irradiation treatment and cold storage.

**RESULTS AND DISCUSSION**

**Effect of irradiation.** In the unsterile and sterile shrimp homogenates each 25 krads produced a 2 to 3 log reduction in the *V. cholerae* population (Figure 1). In the unsterile homogenate the original population of $5.6 \times 10^6$ *V. cholerae*/g was reduced to $4.6 \times 10^4$ *V. cholerae*/g by the 25 krads treatment. The unsterile homogenate treated with 50 krads of irradiation contained only $8.8 \times 10^1$ *V. cholerae*/g. Similar results were produced in the sterile homogenate. The original population of $1.2 \times 10^7$ *V. cholerae*/g was reduced to $1.2 \times 10^5$ by 25 krads, and $4.4 \times 10^1$ *V. cholerae*/g were recovered from the homogenate treated with 50 krads. No *V. cholerae* were recovered from any homogenates treated with 100 krads.
The shelf life of fresh shrimp irradiated with 500 krads and stored at 3.3°C are extended to five weeks as compared to a shelf life of only one week for unirradiated shrimp stored under the same conditions (1). The refrigerated shelf life of shrimp treated with 750 krads ranged from 8 to 10 weeks. When the shrimp are cooked before being irradiated with 500 krads and placed in cold storage the shelf life is extended to 18 weeks (11).

Since no V. cholerae were recovered from the inoculated shrimp homogenates treated with 100 krads, higher pasteurization doses similar to the ones described above should not only increase the shelf life of the shrimp by reducing its natural microflora, but the irradiation treatments should also remove the risk of any possible V. cholerae infection if the shrimp had become contaminated with the organism.

**Effect of time.** Vibrio cholerae was able to survive for 21 days in the unirradiated homogenates and in some of the irradiated homogenates placed in cold storage. In the sterile and unsterile homogenates which were not irradiated and stored at 4°C there was a 1 to 2 log decrease in the number of V. cholerae/g recovered each seven days (Figures 2 and 3). In the unsterile homogenate the population decreased from $5.6 \times 10^6$/g V. cholerae on day 0 to $2.1 \times 10^4$/g on day 21, and in the sterile homogenate the number of V. cholerae recovered decreased from $1.2 \times 10^7$/g on day 0 to $8.6 \times 10^3$/g on day 21. Other researchers have
also reported a progressive decline in the number of \textit{V. cholerae} recovered from refrigerated shrimp homogenate inoculated with the organism (9).

Over a period of 21 days there was also a decrease in the population of \textit{V. cholerae} in the homogenates treated with 25 krads and stored at 4°C. There was only a 1 to 2 log reduction in the number of \textit{V. cholerae} recovered from the sterile and unsterile homogenates after 21 days of storage.

The trend toward a decrease in the number of \textit{V. cholerae} recovered per unit time was also evident in the homogenate treated with 50 krad and stored at 4°C (Figure 2). After day seven the number of \textit{V. cholerae}/g present decreased from $3.9 \times 10^2$ to $1.43 \times 10^1$ on day 21. In the sterile homogenate no \textit{V. cholerae} could be recovered on or after day 14.

Over the 21 day period of storage the number of \textit{V. cholerae}/g also decreased in the homogenates stored at 0°C, but the organism was recovered from most of the homogenates on day 21. In the unsterile homogenates treated with 0 krad or 50 krad there was approximately a 1 log reduction in the \textit{V cholerae} population after 21 days (Figure 4). The unsterile homogenate treated with 25 krad showed approximately a 3 log decrease in its \textit{V. cholerae} after 21 days.

In the sterile homogenates treated with 0 or 25 krad there was a 3 log reduction in the number of \textit{V. cholerae}/g
present after 21 days (Figure 5). On day 14 and day 21 no *V. cholerae* were recovered from the sterile homogenate treated with 50 krad.

*Vibrio cholerae* was also able to survive for 21 days in most of the homogenates stored at -8°C. The number of organisms recovered from the unsterile shrimp on day 21 was very close to the number recovered on day 0 (Figure 6). The results were similar in the homogenates treated with 0 krad or 25 krad.

During the storage of the sterile shrimp homogenate at -8°C there were decreases in the number of *V. cholerae*/g recovered. In the sample which was not irradiated the initial population of $1.2 \times 10^7$ *V. cholerae*/g decreased to only $1.4 \times 10^3$ *V. cholerae*/g after 21 days at -8°C (Figure 7). In the sample treated with 25 krads the *V. cholerae* population decreased from $1.2 \times 10^5$/g on day 0 to $3.1 \times 10^3$ on day seven. For the remaining 14 days however, there was only a slight decrease in the number of *V. cholerae* present. Of the $4.4 \times 10^1$ *V. cholerae*/g in the sterile homogenate treated with 50 krads, none could be recovered on day 14 or day 21 (Figure 7).

**Effect of temperature.** The temperatures used did not produce large differences in the number of *V. cholerae* recovered from the homogenates. In most instances the number of organisms recovered form homogenates receiving the same treatments, but stored at different temperatures varied by 1 log cycle or less.
These results indicate that *V. cholerae* is able to withstand cold temperatures for weeks. Even at -8°C the organism could be recovered from some homogenates after 21 days. It has also been reported that *V. cholerae* can survive in sterile shrimp homogenate for at least 14 days at -20°C (9). No *V. cholerae* were reportedly recovered from this frozen homogenate after 21 days.

**Survival in sterile and unsterile homogenates.** Freshly caught shrimp contain approximately $2.5 \times 10^4$ organisms/g (5). After 16 days of storage on ice the microbial population increases to $1.2 \times 10^7$/g. The normal flora of shrimp consist of coryneforms, *Pseudomonas*, *Micrococcus*, *Moraxella*, *Lactobacillus* and *Bacillus* (12). There are also several other species of bacteria in the shrimp's microflora which are resistant to 100 krad of irradiation (6). *Pseudomonas* species tend to dominate in fresh shrimp stored under refrigeration (12), but *Achromobacter* comprises 82% of the microflora of shrimp stored on ice for 16 days (5). *Vibrio cholerae* in the unsterilized homogenates had to compete with these organisms to survive. In most instances however, more *V. cholerae* were recovered from the stored unsterile shrimp homogenate than from the stored sterile shrimp homogenate. Perhaps the environmental conditions in the unsterile shrimp allowed the *V. cholerae* to survive, despite the growth of the shrimp's microflora during storage. Shrimp is one of a few products whose pH increases as it spoils. The pH of spoiled shrimp is between 7.3 and
8.5 (2). The optimum pH range for the growth of \textit{V. cholerae} is between 7.6 and 8.6 (10). The higher pH in the spoiling unsterile shrimp homogenates was probably responsible for the greater survival of the organism in these homogenates.

More \textit{V. cholerae} were recovered from unsterile samples stored at -8°C for 21 days than from the unsterile homogenates stored at the higher temperatures. Possibly the \textit{V. cholerae} were able to survive better at this temperature than the natural microflora of the shrimp. Less competition at -8°C may have been the reason for the greater number of \textit{V. cholerae} recovered from the unsterile homogenates stored at this temperature.

Greater survival in the unsterile homogenates stored at -8°C than in the sterile homogenates stored at -8°C may be due to the inability of the denatured proteins to hold as much bound water as the native proteins (8). Native proteins interfere with the rate of crystallization of the water in the food and therefore minimizes the loss of water from the bacterial cell. The loss of water would produce denaturation of the bacterial proteins and death of the cell.

The homogenates treated with 50 krad received the largest amounts of irradiation of any of the homogenates from which \textit{V. cholerae} could be recovered. The \textit{V. cholerae} population of the unsterile homogenates remained stable or decreased slightly during the 21 day storage period, but no
V. cholerae were recovered from the sterile homogenates (treated with 50 krad) on day 14 or day 21. Perhaps a large percentage of the organisms which survived the 50 krad treatment were injured. If the environmental conditions in the unsterile shrimp homogenates allowed recovery of the organisms, but conditions in the sterile homogenate did not, this would explain the longer survival time in the unsterile homogenate treated with 50 krad compared to the sterile homogenate treated with 50 krad.

The results indicate the V. cholerae is able to survive short term cold storage in shrimp tissues. Pasteurization doses of irradiation may be used to render shrimp free of the pathogen, however.
REFERENCES


Figure 1. Effect of low dose gamma radiation on Vibrio cholerae in shrimp homogenates. MPN values are averages of 3 replications.
Days of storage

Figure 2. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated unsterile shrimp homogenates stored at 4C. MPN values are averages of 3 replications.
Figure 3. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated sterile shrimp homogenates stored at 4°C. MPN values are averages of 3 replications.
Figure 4. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated unsterile shrimp homogenates stored at 0°C. MPN values are averages of 3 replications.
Days of storage

Figure 5. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated sterile shrimp homogenates stored at 0°C. MPN values are averages of 3 replications.
Figure 6. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated unsterile shrimp homogenates stored at -8°C. MPN values are averages of 3 replications.
Figure 7. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated sterile shrimp homogenates stored at -8°C. MPN values are averages of 3 replications.
Low Dose Gamma Irradiation of Vibrio cholerae in Oysters (Crassostrea virginica)

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INTRODUCTION

Most oysters are distributed as fresh or chilled products (7). Due to the short shelf life of fresh oysters, they can not be marketed great distances from their source. Oysters can be treated with pasteurization doses of irradiation (100 to 1000 krad) to reduce their microbial population and thereby extend their shelf life. Oysters treated with 200 krad and stored in crushed ice are still acceptable after 21 days, but unirradiated oysters stored under the same conditions spoiled (7).

Live oysters take in their own weight in water every two hours, therefore, they may become contaminated with pathogenic microorganisms in their environment. There have been 31 cases of Vibrio cholerae infections reported in the United States since 1973 (4). Although oysters have not been definitely implicated as the source of the organism in any of these cases, contaminated oysters have been responsible for cholera epidemics occurring in Portugal (2) and in Italy (1).

The purpose of this study was to determine the effect of pasteurization levels of irradiation on V. cholerae in oyster meat and the effect of cold storage on the survival of the organism in the oyster meat.

Materials and Methods

Preparation of oyster homogenate. Fresh oysters were purchased from local seafood markets. The oysters were drained of excess fluid and placed in a Waring blender. The
homogenate was prepared by blending the oysters for one to two minutes at high speed. The pH of the homogenate was adjusted to 6.0 with NaOH if necessary. The sterile homogenate was prepared in the same manner, except the oysters were sterilized at 121°C for 15 minutes before blending.

The *V. cholerae* 01 inoculum was prepared and added to the homogenate as described in the Materials and Methods section of Low Dose Gamma Irradiation of *Vibrio cholerae* in Shrimp (*Penaeus setiferus*) (5).

**Irradiation and storage of the samples.** Homogenate samples were placed in 250 ml Nalgene bottles and treated with 0, 25, 50, or 100 krad as previously described (5).

After irradiation the samples were stored at 4°, 0°, or -8°C for 21 days. The number of *V. cholerae* surviving the irradiation treatment and the cold storage were enumerated at 0, 7, 14, and 21 days (5).

**Irradiation of *V. cholerae* in sterile and unsterile oyster homogenate samples** was performed in triplicate. The number of *V. cholerae* surviving the irradiation treatments and the cold storage was calculated by determining the average number of *V. cholerae* recovered from three samples subjected to the same treatments.

**Results and Discussion**

**Effect of irradiation.** It has been reported that the refrigerated shelf life of raw oysters can be extended by
using pasteurization doses of irradiation to reduce the natural microbial flora of the oysters (7). Oysters treated with 200 krads and stored in crushed ice are still acceptable after 21 days. The total plate counts of the fresh oysters were reduced by 99% (7).

Data illustrated in Figure 1 show that pasteurization levels of irradiation are also useful for reducing the number of *V. cholerae* in oysters contaminated with the organism. In the unsterile homogenates the number of *V. cholerae* present was reduced from $7.23 \times 10^6/g$ to $1.57 \times 10^2/g$ with 25 krads of irradiation. No *V. cholerae* were recovered from the unsterile samples treated with 50 or 100 krads.

In the sterile homogenate the *V. cholerae* population was reduced from $6.53 \times 10^6/g$ to $1.07 \times 10^1/g$ with 25 krads of irradiation (Figure 1). No *V. cholerae* were recovered from the sterile samples treated with 50 or 100 krads.

**Effect of time.** In most cases the *V. cholerae* population of the homogenate decreased during the 21 day cold storage period. In the unsterile homogenate stored at 4°C, the initial decrease in the number of *V. cholerae* was followed by an increase in the number of *V. cholerae* recovered (Figure 2). The original population of $7.23 \times 10^6/g$ decreased to $9.3 \times 10^3/g$ on day seven, but increased to $1.3 \times 10^6/g$ on day 14. No *V. cholerae* were recovered from the homogenate on day 21, however.

The unsterile homogenates treated with 25 krads and
stored at 4°C exhibited the same pattern as the unirradiated samples. There were $1.57 \times 10^2/g$ recovered from the homogenates on day 0, but no cholera organisms were recovered from the unsterile homogenates stored for 21 days at 4°C.

*Vibrio cholerae* was able to survive for 21 days in the unirradiated sterile homogenates and in the homogenates treated with 25 krad and stored at 4°C (Figure 3). There were $6.53 \times 10^6 V. cholerae/g$ recovered from the unirradiated sterile homogenate on day 0, and $1.11 \times 10^6/g$ were recovered on day 21. A survival pattern very similar to this is seen in sterile oyster homogenates inoculated with $10^6 V. cholerae/g$ and stored at 7°C for 21 days (9). In the sterile homogenates treated with 25 krad, $1.07 \times 10^1 V. cholerae/g$ were recovered on day 0, and $1.33 \times 10^0 V. cholerae$ were recovered on day 21.

*Vibrio cholerae* was not able to survive for 21 days in the unsterile homogenates which were stored at 0°C (Figure 4). In the unsterile homogenates which were not irradiated $7.23 \times 10^6 V. cholerae/g$ were recovered on day 0, but no *V. cholerae* were isolated from the homogenates on day 21. The *V. cholerae* population of the unsterile homogenates treated with 25 krad also declined during storage at 0°C. As in the unsterile homogenates stored at 4°C, there was a decrease in the *V. cholerae* population between day 0 and day 7, and an increase in the population between day 7 and day 14. Apparently the changing environmental conditions in spoiling
oysters stored at 4°C or 0°C become more favorable for the survival of _V. cholerae_ between days 7 and 14. These conditions may be related to a change in the dominant microorganisms in the oyster's microflora during spoilage as discussed below.

In the unirradiated sterile homogenates stored at 0°C, _V. cholerae_ was able to survive for 21 days (Figure 5). The 6.53 x 10^6 _V. cholerae/_g recovered on day 0 decreased to 9.3 x 10^5 _V. cholerae/_g on day 21.

In the sterile homogenate treated with 25 krad and stored at 0°C, the _V. cholerae_ population decreased from 1.07 x 10^7/_g on day 0 to 1.33 x 10^6/5 on day 7. No. _V. cholerae_ were recovered from the homogenate on day 14 or day 21.

Results were similar for the sterile and unsterile homogenates stored at -8°C (Figures 6 and 7). There was a progressive decrease in the _V. cholerae_ population of each homogenate. In the unsterile samples, the number of _V. cholerae_ recovered decreased from 7.23 x 10^6/_g on day 0 to only 1.23 x 10^2/_g on day 21. In the sterile homogenate the population decreased from 6.53 x 10^6/_g on day 0 to 2.9 x 10^4/_g on day 21.

In both the sterile and unsterile homogenates treated with 25 krad and stored at -8°C, no _V. cholerae_ were recovered on day 7, 14, or 21.

**Effect of temperature.** In most cases, there were relatively small differences in the number of _V. cholerae_
recovered from the homogenates which were treated in the same manner, but stored at different temperatures. In the sterile homogenates the values usually differed by less than 2 log cycles. More *V. cholerae* were recovered from the sterile homogenates stored at 4°C than at any other temperatures. There were also more *V. cholerae* recovered from the sterile homogenates stored at 0°C than from those stored at -8°C. This indicates that as the temperature was decreased from 4°C to -8°C there was a decrease in the number of *V. cholerae* surviving the cold storage. It has been reported that no *V. cholerae* could be isolated from sterile oyster homogenates inoculated with $10^6$/g of the organism and stored at -20°C for 21 days (9).

Up to day 14 the results obtained in the unsterile homogenate were similar to those seen in the sterile homogenates. After this time more *V. cholerae* were isolated from the unsterile homogenates stored at -8°C than from the unsterile homogenates stored at 4°C or in ice. Possible reasons for this are discussed below.

**Survival in sterile and unsterile homogenates.** After seven days of storage more *V. cholerae* were usually recovered from the sterile homogenates than from the unsterile homogenates. No *V. cholerae* could be recovered from any of the unsterile homogenates stored at 4° or 0°C for 21 days.

In the sterilized homogenates, the oyster's natural microflora had been destroyed and the enzymes which were
present had been denatured. Members of the genera *Lactobacillus* and *Pseudomonas* dominate the microflora of fresh oysters and are primarily responsible for the spoilage of the product (11). Even though the number of microorganisms present is greatly reduced by 100 krad, lactobacilli persist as the dominant microorganism. There are also glycolytic enzymes systems present which can produce acid from the oyster's glycogen (3). Most enzymes are resistant to even sterilization doses of irradiation.

The pH of unirradiated oysters and oysters treated with 200 krad decreases from 6.4 to 5.9 after 21 days of storage in crushed ice (7). Even in oysters treated with 630 krad and stored at 5°C for 14 days the pH decreases from approximately 6.0 to 5.78 (3).

The lower pH produced by the microbial and enzymatic activity in the unsterile homogenates stored at 4°C and 0°C was probably responsible for the decline of the *V. cholerae* population after day 14. The optimum pH range for the growth *V. cholerae* is between 7.6 and 8.6 (10), and the viability of the organism is reported to be less in an environment with a pH between 5.5 and 6.2 than in an environment with a pH greater than 6.2 (8).

In the unsterile homogenates stored at -8°C however, the survival of *V. cholerae* was only slightly less than in the sterile homogenates. The colder temperatures (-8°C) may have retarded the growth of the natural flora and the enzymatic activity in the oysters and allowed the *V.*
cholerae to survive for a longer period of time.
REFERENCES


Figure 1. Effect of low dose gamma radiation on *Vibrio cholerae* 
#5875 in oyster homogenates. MPN values are averages 
of 3 replications.
Figure 2. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated unsterile oyster homogenates stored at 4°C. MPN values are averages of 3 replications.
Figure 3. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated sterile oyster homogenates stored at 4°C. MPN values are averages of 3 replications.
Figure 4. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated unsterile oyster homogenates stored at 0°C. MPN values are averages of 3 replications.
Figure 5. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated sterile oyster homogenates stored at 0°C. MPN values are averages of 3 replications.
Figure 6. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated unsterile oyster homogenated stored at -8°C. MPN values are averages of 3 replications.
Figure 7. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated sterile oyster homogenate stored at -8°C. MPN values are averages of 3 replications.
Low Dose Gamma Irradiation of *Vibrio cholerae* in Crabmeat (*Callinectes sapidus*)

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INTRODUCTION

Pasteurization doses of irradiation may be used to increase the refrigerated shelf life of whole crabs and crabmeat. When stored at 1°C the shelf life of precooked jumbo crabs is extended to 72, 96, and 111 days after being treated with 93, 230, and 450 krad, respectively. The unirradiated crabs stored under the same conditions spoiled in 16 days (8). Unirradiated crabs stored at 7°C spoiled in only two days (9).

Crabmeat treated with 250 krad and stored 3.3°C was acceptable after four weeks, while treatment with 500 krad extended the shelf life to five weeks. The unirradiated crabmeat spoiled in one week (8).

Irradiation increases the crabmeat's shelf life by reducing the number of spoilage bacteria present. Pasteurization levels of irradiation can destroy up to 95% of the spoilage organisms in the microflora (2).

Besides the non-pathogenic organisms which occur as part of the crab's natural microflora, pathogens may be present if the water from which the crabs were taken is contaminated. Contaminated crabs were responsible for 11 cases of Vibrio cholerae infections in Louisiana in 1978 (4).

Food preservation methods must not only control the growth of spoilage microorganisms, but must also destroy any pathogens present in the food. The purpose of this study was to determine the effect of pasteurization doses of
irradiation on *V. cholerae* in unsterile and sterile crabmeat and to measure the survival of *V. cholerae* in unirradiated and irradiated crabmeat placed in cold or frozen storage.

**MATERIALS AND METHODS**

**Preparation of crabmeat homogenate.** Fresh crabmeat was purchased from local seafood markets. The unsterile crabmeat homogenate was prepared by blending two parts crabmeat with one part sterile saline in a Waring blender to form a smooth paste. The sterile crabmeat homogenate was prepared in the same manner, except that the crabmeat was sterilized at 121°C for 15 minutes before blending with saline.

The *V. cholerae* 01 inoculum was prepared and added to the homogenate as described in the Materials and Methods section of Low Dose Gamma Irradiation of *Vibrio cholerae* in Shrimp (*Panaeus setiferus*) to contain a final concentration of 10⁷ *V. cholerae*/g (3).

**Irradiation and storage of the samples.** Homogenate samples were placed in 250 ml Nalgene bottles and treated with 0, 25, 50, or 100 krad as previously described (3).

After irradiation the homogenate samples were stored at 4°, 0°, or -8°C for 21 days. The number of *V. cholerae* surviving the irradiation treatment and the cold storage were enumerated at 0, 7, 14, and 21 days (3).

Irradiation of *V. cholerae* in sterile and unsterile crabmeat homogenates and storage of the homogenates were
performed in triplicate. The number of *V. cholerae* surviving was calculated by determining the average number of *V. cholerae* recovered from three samples subjected to the same irradiation treatments and cold storage.

**RESULTS AND DISCUSSION**

**Effect of irradiation.** Pasteurization levels of irradiation were effective for destroying approximately $10^7$ *V. cholerae*/g. In the unsterile homogenates the original population of $5.18 \times 10^6$ *V. cholerae*/g was reduced to $1.43 \times 10^3$g with 25 krad (Figure 1). No *V. cholerae* were recovered from the homogenates treated with 50 krad or 100 krad.

In the sterile homogenates, the number of *V. cholerae* recovered was reduced from $3.07 \times 10^6$g to $5.0 \times 10^0$g with 25 krad. No *V. cholerae* were recovered from the sterile homogenates treated with 50 krad or 100 krad.

**Effect of time.** Results in the unsterile homogenates treated with 0 krad and 25 krad were similar (Figure 2). In the unirradiated unsterile homogenates stored at 4°C the number of *V. cholerae* recovered decreased from $5.18 \times 10^6$/g on day zero to $8.87 \times 10^5$/g on day seven (Figure 2). The *V. cholerae* population decreased further to $1.07 \times 10^5$/g on day 14, and no *V. cholerae* were recovered on day 21. In the homogenates treated with 25 krad, $1.43 \times 10^3$/g were recovered on day seven. No *V. cholerae* could be recovered on day 14 or day 21.

*Vibrio cholerae* was able to survive for a longer period
of time in the sterile homogenates stored at 4°C (Figure 3). The number of *V. cholerae* recovered from the unirradiated sterile samples on day zero \((3.07 \times 10^6/g)\) decreased to \(1.82 \times 10^5/g\) on day 21.

The number of *V. cholerae* recovered from the sterile homogenates treated with 25 krad and stored at 4°C also decreased with time. There were \(5.0 \times 10^0\) *V. cholerae/g* isolated from the homogenates on day zero. The population decreased to \(1.33 \times 10^0\) *V. cholerae/g* on day 14, and no *V. cholerae* were recovered from the homogenates on day 21.

Although there was a constant decline in the *V. cholerae* population, the organism was recovered from the unsterile unirradiated homogenates stored at 0°C for 21 days (Figure 4). The *V. cholerae* population decreased steadily from \(5.18 \times 10^6/g\) on day zero to \(9.13 \times 10^3/g\) on day 21 in the unirradiated homogenates. In the unsterile homogenate treated with 25 krad the number of *V. cholerae* decreased from \(1.43 \times 10^3/g\) on day zero to \(8.0 \times 10^2/g\) on day 14. No *V. cholerae* were recovered from the homogenates on day 21.

The unirradiated sterile crabmeat stored at 0°C showed only a small decrease in its *V. cholerae* population during the 21 day storage period (Figure 5). On day zero, \(3.07 \times 10^6\) *V. cholerae/g* were isolated from the crabmeat. On day seven and day 14 approximately \(4.5 \times 10^5\) *V. cholerae/g* were recovered. The number of *V. cholerae* decreased slightly to \(1.69 \times 10^5/g\) on day 21. In the sterile homogenates treated with 25 krad the original *V.*
cholerae population was reduced from $5.0 \times 10^0/g$ on day zero to $4.0 \times 10^0/g$ on day seven. No *V. cholerae* were recovered from the homogenates on day 14 or day 21.

*Vibrio cholerae* was also able to survive for 21 days in the unirradiated unsterile homogenates stored at $-8^\circ C$ (Figure 6). The initial population of $5.18 \times 10^6$ *V. cholerae*/g decreased to $1.15 \times 10^4/g$ on day 21. In the unsterile homogenate treated with 25 krad a small increase in the *V. cholerae* population was noted between day 7 and day 14. After 21 days no *V. cholerae* were recovered from the homogenates.

In the unirradiated sterile homogenates stored at $-8^\circ C$ there was a large decrease in the initial *V. cholerae* population of $3.07 \times 10^6/g$ on day zero to $3.35 \times 10^2/g$ on day seven (Figure 7). The number of *V. cholerae* recovered decreased further to $1.93 \times 10^1/g$ on day 21. On day 0 there were $5.0 \times 10^0$ *V. cholerae*/g isolated from the homogenates treated with 25 krad. Only $1.33 \times 10^0/g$ were recovered on day seven, and none were recovered on day 14 or day 21.

Effect of temperature. In the sterile homogenates stored at $0^\circ C$ or $4^\circ C$ there was very little difference in the number of *V. cholerae* recovered from homogenates receiving the same irradiation treatments. In all cases there was less than a 1 log difference in the number of organisms recovered from the sterile homogenates exposed to equal doses of radiation and stored at $0^\circ C$ or $4^\circ C$. There were fewer *V. cholerae* recovered from the sterile homogenates
stored at -8°C, however. While the V. cholerae population in the homogenates stored at 4°C or 0°C remained relatively constant (Figures 3 and 5), there was a large decrease in the V. cholerae population of the homogenates stored at -8°C. After 21 days the number of V. cholerae recovered had decreased by over 5 log cycles. This indicates that V. cholerae is adversely affected by freezing temperatures under certain conditions. It has been reported that no V. cholerae were recovered from sterile crabmeat homogenates inoculated with 10^6 of the organisms/g after 21 days of storage at -20°C (7).

Up to day 14 there were also few differences in the number of V. cholerae recovered from the unsterile homogenates stored at 4°, 0°, or -8°C, though fewer organisms were recovered from the homogenates stored at -8°C than at the other temperatures. On day 21 however, more V. cholerae were recovered from the homogenates stored at -8°C than from the homogenates stored at 4° or 0°C. In fact, no V. cholerae were recovered from the unsterile homogenates stored at 4°C for 21 days. The probable reason for this is discussed below.

**Survival in sterile and unsterile homogenates.** Because commercially prepared crabmeat is picked from the shells after the crab has been boiled for approximately 20 minutes, the microflora is similar to that of a semiprocessed food (9). Fresh crabmeat contains an average of 4.0 x 10^5 microorganisms/g (1). When the crabmeat is stored at 2° to
for 12 to 15 days, the natural microflora increases to between $2.0 \times 10^9/g$ and $1.2 \times 10^{10}/g$. During storage the dominant microflora also changes. Some researchers have reported that Moraxella, Pseudomonas, and Acinetobacter are the predominant bacteria present in crabmeat (5). Shiflett, et al. reported that in crabmeat treated with up to 100 krad, 99% of the surviving organisms were Achromobacter, and that Achromobacter was able to survive refrigerated storage (9). (Members of the Achromobacter group have now been renamed as either Moraxella or Acinetobacter). The V. cholerae in the unsterile homogenates had to compete with the natural flora of the crabmeat to survive.

The effect of competition was most evident in the unsterile homogenates stored at the warmest temperature (4°C). In the unirradiated unsterile samples stored at 4°C, no V. cholerae could be recovered on day 21. In the homogenates treated with 25 krad, no V. cholerae were recovered on day 14 or day 21. In the sterile homogenates however, V. cholerae was recovered from the unirradiated homogenates on day 21, and from the homogenates treated with 25 krad on day 14.

More V. cholerae were also recovered from the unirradiated sterile homogenates stored at 0°C than from the unirradiated unsterile homogenates stored at 0°C.

No V. cholerae were recovered from the sterile homogenates treated with 25 krad and stored at 0°C for 14 days, and no V. cholerae were recovered from the unsterile
homogenates treated with 25 krad and stored at 0°C for 21 days. There were fewer *V. cholerae* in the sterile homogenates on day 0, however.

More *V. cholerae* were recovered from the unsterile homogenates stored at -8°C than from any of the other unsterile homogenates. The lower temperature may have retarded the growth of the natural microflora of the crabmeat. Less competition allowed the *V. cholerae* to survive for longer periods of time.

Fewer *V. cholerae* were recovered from the sterile homogenates than from the unsterile homogenates stored at -8°C. The sterilization treatment to which the sterilized homogenates were subjected may have destroyed substances (e.g. proteins) in the fresh crabmeat which protected the *V. cholerae* from the harsh effects of the freezing.

The native proteins in the unsterilized homogenates have a greater capacity for binding water than the denatured proteins in the sterilized homogenates (6). Native proteins may therefore effect the rate of crystallization during freezing and allow the bacteria to survive for a longer period of time.

The results indicate that *V. cholerae* can survive for some time in crabmeat under certain conditions. The organism can be destroyed in crabmeat by using pasteurization levels of irradiation.
REFERENCES


Figure 1. Effect of low dose gamma radiation on *Vibrio cholerae* #5875 in crabmeat homogenates. MPN values are averages of 3 replications.
Figure 2. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated unsterile crabmeat homogenates stored at 4°C. MPN values are averages of 3 replications.
Figure 3. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated sterile crabmeat homogenates stored at 4°C. MPN values are averages of 3 replications.
Days of storage

Figure 4. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated unsterile crabmeat homogenates stored at 0°C. MPN values are averages of 3 replications.
Figure 5. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated sterile crabmeat homogenates stored at 0°C. MPN values are averages of 3 replications.
Figure 6. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated unsterile crabmeat homogenates stored at -8°C. MPN values are averages of 3 replications.
Figure 7. Survival of Vibrio cholerae #5875 in unirradiated and irradiated sterile crabmeat homogenates stored at -8°C. MPN values are averages of 3 replications.
Low Dose Gamma Irradiation of Vibrio cholerae in Crayfish Meat (Procambarus clarkii Garard)

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INTRODUCTION

Although freshwater crayfish may be found in every state in the United States, Louisiana grows, processes, and consumes 90% of all crayfish produced for human consumption (6). Today the demand for crayfish in Louisiana and the surrounding Southern states is increasing (3). Most commercially processed crayfish is marketed as hand peeled tailmeat (2). The tailmeat may be frozen, but it is usually stored at low temperatures above freezing. When the crayfish meat is properly handled, it has a shelf life of approximately one week (6).

The refrigerated shelf life of most seafoods can be increased by using pasteurization levels of irradiation. Doses of 100 to 1000 krad destroy 90 to 99% of spoilage organisms present (1). A longer shelf life would mean that fresh crayfish could be stored for longer periods of time and marketed at greater distances from its source.

Crayfish are harvested from rivers, bayous, swamps, lakes, or shallow ponds built especially for crayfish production (6). If the water from which the crayfish is taken is contaminated, there is a chance that the crayfish meat may harbor pathogens. Crabs taken from contaminated waters were responsible for 11 Vibrio cholerae infections in Louisiana in 1978 (5). Microorganisms of fecal origin have been isolated from crayfish waters, whole crayfish, and crayfish tailmeat (3).

The purpose of this study was to determine the effects
of pasteurization levels of irradiation and cold storage on
the survival of *V. cholerae* in unsterile and sterile
crayfish homogenates.

**MATERIALS AND METHODS**

**Preparation of crayfish homogenate.** Packaged fresh
crayfish tailmeat was purchased from local seafood markets.
The unsterile crayfish homogenate was prepared by blending
two parts crayfish meat with one part sterile saline in a
Waring blender to form a smooth paste. The sterile crayfish
homogenate was prepared in the same manner, except that the
crayfish was sterilized at 121°C for 15 minutes before
blending with saline.

The *V. cholerae* inoculum was prepared and added to the
homogenate as described in the Materials and Methods section
of Low Dose Gamma Irradiation of *Vibrio cholerae* in Shrimp
(*Penaeus setiferus*) to produce a final concentration of $10^7$
*V. cholerae*/g (4).

**Irradiation and storage of the samples.** Homogenate
samples were placed in 250 ml Nalgene bottles and treated
with 0, 25, 50, or 100 krad (4).

After irradiation the samples were stored at 4°C, 0°C, or
-8°C for 21 days. The number of *V. cholerae* surviving the
irradiation treatment and the cold storage were enumerated
at 0, 7, 14 and 21 days respectively (4).

Irradiation of *V. cholerae* in sterile and unsterile
samples was performed in triplicate. The number of *V.*
cholerae surviving the irradiation treatments and the cold storage was calculated by determining the average number of V. cholerae recovered from three samples subjected to the same treatments.

RESULTS AND DISCUSSION

Effect of irradiation. In the unsterile homogenate each 25 krad dose of irradiation produced a 1 to 3 log reduction in the number of V. cholerae/g recovered (Figure 1). The unsterile unirradiated homogenates' initial V. cholerae population of 4.3 x 10^6/g was reduced to 1.66 x 10^5/g by 25 krad. Only 8.01 x 10^2 V. cholerae/g were recovered from the homogenates treated with 50 krads. No V. cholerae were recovered from the unsterile homogenates treated with 100 krad.

In the sterile homogenates, V. cholerae seemed more susceptible to the irradiation than in the unsterile homogenates. There were 3.67 x 10^6 V. cholerae/g recovered from the homogenates which were not irradiated, but only 4.4 x 10^0/g were recovered from the homogenates treated with 25 krad. No V. cholerae were recovered from the homogenates treated with 50 or 100 krad.

Effect of time. Vibrio cholerae was not able to survive for 21 days in the unsterile homogenates stored at 4°C (Figure 2). The organism was able to survive for 14 days in the unsterile homogenates treated with 0, 25, or 50 krad. After 14 days there was a large decrease in the V. cholerae population.
On day 0, $4.3 \times 10^6 \textit{V. cholerae}/g$ were recovered from the unirradiated unsterile homogenates, and on day 14 $5.6 \times 10^6$ were recovered. No \textit{V. cholerae} were recovered from the unirradiated unsterile samples on day 21. The number of \textit{V. cholerae} recovered from the unsterile homogenates treated with 25 krad decreased from $1.66 \times 10^5/g$ on day 0 to $3.87 \times 10^4/g$ on day 14 before decreasing to 0 on day 21. The unsterile homogenates treated with 50 krad showed results similar to those obtained with the homogenates treated with 0 krad or 25 krad. Though there was a decline in the \textit{V. cholerae} population on day 7, approximately $8.0 \times 10^2 \textit{V. cholerae}/g$ were isolated from the homogenates on day 0 and on day 21. No \textit{V. cholerae} were recovered from the homogenates on day 21.

After 21 days there was only a slight decrease in the \textit{V. cholerae} population of the sterile crayfish homogenates treated with 0 krad or 25 krad and stored at 4°C (Figure 3). On day 0, $3.63 \times 10^6 \textit{V. cholerae}/g$ were recovered from the unirradiated sterile homogenates. There were $6.0 \times 10^6 \textit{V. cholerae}/g$ recovered on day 7. The population of the cholera organisms declined to $3.13 \times 10^6/g$ on day 14 and declined further to $9.11 \times 10^5/g$ on day 21.

The survival pattern of the organism in the sterile homogenate treated with 25 krad was similar to the survival of the organism in the sterile unirradiated homogenate. The \textit{V. cholerae} population numbered $4.4 \times 10^0/g$ on day 0 and $1.33 \times 10^0 \textit{V. cholerae}/g$ were recovered on day 21.
Vibrio cholerae was able to survive for 21 days in the unsterile homogenates stored at 0°C (Figure 4). In the homogenates treated with 0 krad or 25 krad the V. cholerae population decreased approximately 1 log cycle over the 21 day storage period. There were $4.3 \times 10^6$ V. cholerae/g recovered from the unsterile unirradiated homogenates on day 0, and $6.25 \times 10^5$/g were recovered on day 21. The number of V. cholerae recovered from the unsterile homogenates treated with 25 krad decreased from $1.66 \times 10^5$/g on day 0 to $4.86 \times 10^4$/g on day 21. In the homogenates treated with 50 krad the V. cholerae population decreased from $8.01 \times 10^2$/g on day 0 to $5.0 \times 10^0$/g on day 21.

The V. cholerae also appeared to survive well in the sterile crayfish homogenate stored at 0°C (Figure 5). The initial population of $3.63 \times 10^6$/g in the unirradiated homogenate decreased slightly to $1.16 \times 10^6$/g after 21 days of storage. The V. cholerae population of the homogenate treated with 25 krad decreased from $4.40 \times 10^0$/g on day 0 to $1.33 \times 10^0$/g on day 7. On day 14, $3.0 \times 10^0$ V. cholerae/g were recovered, and $2.67 \times 10^0$ V. cholerae/g were recovered on day 21.

There were relatively small changes in the V. cholerae population of the unsterile homogenates stored at -8°C for 21 days (Figure 6). On day 21, $1.14 \times 10^6$ V. cholerae/g were recovered from the unirradiated unsterile homogenates which contained $4.3 \times 10^6$ V. cholerae/g on day 0. The number of V. cholerae recovered from the homogenates treated
with 25 krad decreased from $1.66 \times 10^5 \text{g}$ on day 0 to $9.2 \times 10^3 \text{g}$ on day 21. On day 0 there were $8.01 \times 10^2 \text{V. cholerae/g}$ recovered from the homogenates treated with 50 krad, and $8.15 \times 10^2 \text{V. cholerae/g}$ were recovered on day 21.

There was a decline in the number of \text{V. cholerae} recovered from the sterile homogenates stored at -8°C for 21 days (Figure 7). The \text{V. cholerae} population of the unirradiated homogenates decreased from $3.63 \times 10^6 \text{g}$ on day 0 to only $8.5 \times 10^2 \text{g}$ on day 21. On day 0, although $4.4 \times 10^0 \text{V. cholerae/g}$ were recovered from the sterile homogenates treated with 25 krad, no \text{V. cholerae} were recovered from these homogenates after 14 or 21 days of storage.

**Effect of temperature.** The survival of \text{V. cholerae} in unirradiated and irradiated sterile homogenates stored at 4°C or 0°C was very similar. The organism was able to survive for 21 days in the sterile homogenates stored at both temperatures. In some cases there were slight increases and decreases in the \text{V. cholerae} population, but the number of organisms recovered during storage never varied more than 1 log cycle from the number of organisms recovered on day 0.

The organism did not survive as well in the sterile homogenates stored at -8°C. The number of \text{V. cholerae} recovered from the frozen homogenates declined by over 3 log cycles during storage at -8°C. The homogenates treated with 25 krad contained $4.4 \times 10^0 \text{V. cholerae/g}$ on day 0, but no \text{V. cholerae} were isolated from the homogenates on day 14 or
Up to day 14, there was only a slight difference in the number of *V. cholerae* recovered from the unsterile homogenates stored at 4°, 0°, or -8°C. Usually the number of organisms recovered from the unsterile homogenates stored at the different temperatures differed by less than 1 log cycle.

On day 21, no *V. cholerae* were recovered from the unsterile homogenates stored at 4°C, but the organism was recovered from the homogenates stored at 0° or -8°C. The possible reason for this is discussed below.

Survival in unsterile and sterile homogenates. In fresh crayfish meat *Micrococcus*, *Staphylococcus*, and *Alcaligines* are the predominant genera of microorganisms present (2). After eight days of storage at 0°C, *Pseudomonas* becomes the dominant genera, but after eight days at 5°C *Achromobacter* becomes the dominant genera. At the end of the spoilage of the fresh crayfish meat, *Pseudomonas* and *Achromobacter* groups account for almost 100% of the microbial population.

In the sterile homogenates the natural microbial flora and the naturally occurring enzymes of the crayfish meat had been destroyed. The *V. cholerae* placed in the unsterile crayfish meat had to compete with the crayfish's natural microflora to survive. Fresh crayfish meat has a total aerobic plate count (TPC) of 1.6 x 10^5 organisms/g. After 24 days of storage at 0°C, the TPC increases to 3.2 x 10^9/g,
and after 24 days of storage at 5°C the TPC increases to $2.8 \times 10^9$ organisms/g (2).

In the unirradiated sterile and unsterile homogenates stored at 4°C the survival pattern of *V. cholerae* was similar up to day 14. On day 21, $9.11 \times 10^5$ *V. cholerae*/g were recovered from the unirradiated sterile homogenates, but no *V. cholerae* were recovered from the unirradiated unsterile homogenates. Similar results were seen in the sterile and unsterile homogenates treated with 25 krad. At 4°C, the psychrotrophic microorganisms in the crayfish's natural microflora were able to grow. This competitive inhibition was probably responsible for the decline in the number of *V. cholerae* recovered from the homogenates.

*Vibrio cholerae* were able to survive for 21 days in unsterile homogenates stored at 0° or -8°C. The lower temperatures retarded the growth of the psychotrophs which outgrew the *V. cholerae* at 4°C.

There was a large difference in the survival of *V. cholerae* in the sterile and unsterile homogenates stored at -8°C. The *V. cholerae* population of the unsterile homogenates remained relatively stable during the 21 day storage period, but there was a large decrease in the number of *V. cholerae* from the sterile homogenates after 21 days of storage. The proteins of the sterile homogenates had been denatured by the sterilization treatment. Native proteins are able to provide more protection to microorganisms during freezing because they have a greater effect on the rate of
crystallization in the food (7). Native proteins bind more water molecules and reduce the rate of crystallization. Less dehydration of the bacterial cells is produced and the microorganisms can survive longer in the food.

These results indicate the *V. cholerae* can survive for some time in crayfish tissues stored at low temperatures. Pasteurization doses of irradiation may be successfully used to destroy this pathogen in crayfish.
REFERENCES


Figure 1. Effect of low dose gamma radiation on *Vibrio cholerae* #5875 in crayfish homogenates. MPN values are averages of 3 replications.
0 krad-Z
25 krad-W
50 krad-F
100 krad-I

Figure 2. Survival of Vibrio cholerae #5875 in unirradiated and irradiated unsterile crayfish homogenates stored at 4°C. MPN values are averages of 3 replications.
Days of storage

Figure 3. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated sterile crayfish homogenates stored at 4°C. MPN values are averages of 3 replications.
Days of storage

Figure 4. Survival of Vibrio cholerae #5875 in unirradiated and irradiated unsterile crayfish homogenates stored at 0°C. MPN values are averages of 3 replications.
Figure 5. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated sterile crayfish homogenates stored at 0°C. MPN values are averages of 3 replications.
Figure 6. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated unsterile crayfish homogenates stored at -8°C. MPN values are averages of 3 replications.
Figure 7. Survival of *Vibrio cholerae* #5875 in unirradiated and irradiated sterile crayfish homogenates stored at -8C. MPN values are averages of 3 replications.
SUMMARY

*Vibrio cholerae* is a relatively fragile organism. Though seafoods have been implicated in several cholera outbreaks, the seafood had been consumed raw, partially cooked, or was recontaminated after cooking. Low levels of heat or ionizing radiation may be used to destroy large numbers of the organism in seafoods.

The D values of the organism range from 4.7 at 49°C to less than 0.24 at 82°C in oyster homogenates. In shrimp homogenates the D values range from 9.17 at 49°C to less than 0.28 at 82°C. The lower D values in the oyster homogenates is probably due to the homogenate's lower pH.

No *V. cholerae* were recovered from oysters cooked for 1 minute in boiling water (100°C) or hot oil (191°C) or from shrimp cooked for 10 minutes in boiling water (100°C) or steam (100°C).

Low doses of ionizing radiation are also effective for destroying large numbers of *V. cholerae* in shrimp, oyster, crab, and crayfish homogenates. No *V. cholerae* were recovered from shrimp, oyster, crab, or crayfish homogenates treated with 100 krad of ionizing radiation.

Probably, due to the lack of competitive inhibition, the organism usually survived for longer periods of time in the sterile oyster, crab, and crayfish homogenates than in the unsterile homogenates stored at 4° or 0°C. Survival of the organism was slightly greater in the unsterile shrimp homogenates than in the sterile shrimp homogenates stored at
4° or 0°C. This was probably due to the unsterile homogenate's higher pH.

Survival of *V. cholerae* was also usually greater in the unsterile homogenates stored at -8°C than in the unsterile homogenates stored at 4° or 0°C. The lower temperature may have retarded the growth of the seafood's natural microflora and the activity of enzymes in the homogenates.

Survival in the unsterile homogenates stored at -8°C was also greater than in the sterile homogenates stored at -8°C. Native proteins of the unsterile seafood homogenates may have provided the organism with more protection against the lethal effects of freezing than the denatured proteins of the sterile homogenates.
VITA

The author was born in Aliceville, Alabama on February 1, 1957. Upon graduation from Aliceville High School in May 1974, he entered the University of Alabama in Tuscaloosa, Alabama. In May 1978, he was awarded a B.S. degree in Microbiology and a Commission in the United States Army.

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