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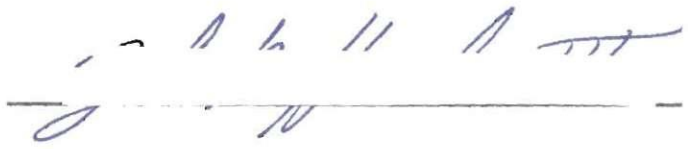

AN EVALUATION OF THE USE OF CARBON DIOXIDE GAS IN WET SHRIMP STORAGE

A THESIS

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In Partial Fulfillment

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Master of Science in Industrial Engineering

By

John Richardson Hardee III

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

ABSTRACT

AN EVALUATION OF THE USE OF CARBON DIOXIDE GAS
IN WET SHRIMP STORAGE
(97 pages)

By

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In recent years there has been increasing interest in the development of better methods of preserving shrimp at sea. The incentive for such development has been twofold: first, a need for furnishing the consumer with a higher quality product in an expanding market and, second, a need for the reduction of spoilage as a factor which limits shrimping operations. Among many research projects there are reports of a bacteriostatic effect caused by an atmosphere of carbon dioxide. The effect of such an atmosphere had been investigated under laboratory conditions, but apparently it had never been applied to the commercial storage of shrimp. Since preservation of shrimp with a carbon dioxide atmosphere seemed to have the possibility of being compatible with present commercial practice and of being more economical than other methods, an experiment was proposed whereby two lots of shrimp would be stored under simulated commercial conditions, except for a carbon dioxide atmosphere surrounding one lot;

the rates of spoilage in air and carbon dioxide atmospheres, respectively, could then be compared.

Before such an experiment could be made, a means of measurement of spoilage of shrimp under field conditions had to be obtained. An experimental method for obtaining such a measure had been proposed by researchers at Louisiana State University. Laboratory trials revealed that such a method could be used if it were altered somewhat and calibrated against a known standard. Supplies of materials were prepared with which a daily measure of spoilage could be obtained throughout the proposed experiment.

Two storage boxes were built in which commercial storage conditions aboard shrimp boats could be simulated. Shrimp were taken directly from the sea, headed, placed in the storage boxes with ice in a normal commercial manner and stored for three weeks. During the storage period an atmosphere of carbon dioxide was maintained in one of the storage boxes. Each day an approximate bacteria count was obtained for the shrimp in each box by removing a sample of six shrimp, washing this sample in a previously prepared jar of sterile distilled water, inoculating tubes of medium with the wash water, incubating the inoculated tubes of medium, and measuring the length of time required for the medium to change color (time for color change being related to bacteria count).

Because of a difficulty encountered in the regulation of the flow of carbon dioxide from supply cylinders, there

was some question as to whether or not a loss of flavor and texture of the shrimp exposed to the carbon dioxide atmosphere had been caused by mere exposure to the gas itself. Therefore, shrimp were stored a second time and three separate conditions of atmosphere duplicated: air, intermittent flow of carbon dioxide and continuous flow of carbon dioxide.

It was found that a carbon dioxide atmosphere does have a measurable bacteriostatic effect, but mere exposure to such an atmosphere causes shrimp to lose flavor and change in texture upon cooking. Therefore, such a storage method is undesirable for commercial practice. However, the testing procedure developed for use in this experimentation proved a practical means of measuring bacterial activity in the field. Because the test proved sensitive and faithfully reproduced acceptable results, it is recommended that consideration be given for its use in establishing quality control techniques in the seafood industries.

CHAPTER I

INTRODUCTION

Growth of the Shrimp Business.--There have been three major phases in the development of the shrimp business in this country. The first phase began several years after the beginning of this century. The second came during the depression years. The third and present phase was entered after World War II.

Two ingredients were required for the first successful shrimping: a net and power with which to drag the net along the ocean floor. Sometime after the turn of this century these two ingredients came together. A modern fishing vessel of the time supplied the latter ingredient, if it was equipped with a diesel engine, while the otter trawl supplied the other ingredient. A man in search of a new product brought the two together.

At first the very nature of shrimping was that of individuality. A fisherman found he could earn a living, and more, by catching shrimp. In time he was able to pay for his boat. After that, if he continued his hard work, he began to pay for a second boat. In taking this step it was possible for the fisherman to obtain a vessel which he regarded as better adapted to shrimping. It is upon this process that the growth and development of the shrimp

business was based. The result was that the fisherman existed as a free agent. He and he alone dictated the time and place for shrimping, the type of boat, and the kinds of equipment. His compensation was determined by the amount of shrimp he returned to port.

It is sometimes conceded that shrimping first occurred near the port of Fernandina, Florida, during the first ten years of this century. From there it spread both up and down the Atlantic coast. As the business developed it was found that the most profitable practice consisted of shrimping in waters as far south as New Smyrna, Florida, during the winter months then moving to Georgia or even North Carolina waters during the summer months.

During this period, the first phase of growth, a shrimping voyage rarely lasted beyond one day. A boat would leave the dock in the early hours of morning. As the sun began to rise the net would be placed in the water. During the late morning and early afternoon hours the net would be pulled along the ocean floor. When necessary it would be taken out of the water, emptied, and returned. In the late afternoon the boat would return to port. The shrimp obtained were processed that same afternoon or evening. If sold on the local market they were available for consumption the next day. If sold on the New York market they were available for consumption in several days, depending upon transportation. It was a rare occurrence when shrimp were offered to the

consumer as long as one week after they had been caught.

The shrimp trawler of 1930 was the result of years of trial and experience. Each feature included in the vessel had been proved to be successful by the men who used it. The hull was about 30 feet long with a mast placed in the center. Behind the mast was an open deck which was clear except for a hatch leading to the storage space for shrimp and crushed ice. In front of the mast was a small cabin containing the steering gear and engine control. Directly beneath this cabin was the engine room, containing a diesel engine of approximately forty horsepower. In the forward portion of the hull there was room for a small cooking stove and one or two bunks, which were used for overnight trips between ports.

At this time the greatest problem of a man in the shrimp business was that of finding shrimp. The boats and equipment had been proved to be practical. With a small amount of crushed ice, preservation of the shrimp was no great problem. Each operator, whether he was working towards his first boat or his tenth, found himself involved with the law of supply and demand. The demand for shrimp was expanding then and has continued to do so in the present market.(19)[1] The supply of shrimp, however, is regulated by nature. To all appearances it was nature that brought

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[1] Numbers in parenthesis refer to references listed in the bibliography.

about a balance. By the year 1935 the number of shrimp reproduced in the Atlantic waters were almost enough to support the number of boats operated.

It was the solution to this problem of supply that brought about the second phase of growth and development. The beginning of this phase is marked by those few persons who were the first to shift the base of their operations from the Atlantic coast to the Gulf of Mexico. A map of the two regions will show the cause of the changes that took place. On the Atlantic coast there is a series of ports in no instance more than fifty miles apart. Each of these ports is easily accessible to the ocean. There was no port so accessible to the waters off Louisiana where shrimp were found in large numbers in 1935. The only port available was that of Morgan City-Berwick, Louisiana.

Although this port possessed the rail and highway connections necessary for the movement of shrimp to market, it was inland. To reach an area where shrimp were found the boats had to move about twenty miles down the Atchafalaya River and then about ten more miles across the Atchafalaya Bay. It was no longer feasible for the boats to return to port each night. Experience proved that a cruise of ten to fourteen days was practical. The limiting factor was found to be the spoilage of shrimp.

In Louisiana, the same process by which a fisherman had become the owner and operator of several boats elsewhere

was repeated. Here a new fleet of boats, adapted to the changed need, was gradually produced. The new trawler was larger, being about 50 or 60 feet long. The rear of the cabin was enlarged to contain quarters for the captain. Otherwise each feature remained substantially the same except for size. One story of this movement to Louisiana and the resulting changes is found in the Morgan City Review (21).

As the end of the first phase of growth is marked by an increase in the number of boats operated, so is the second phase. World War II served to prevent natural expansion for a time. At the end of the war there was a ready supply of funds available for the purchase of new boats. A large part of this money came from those who had been engaged in the shrimp business. However, much of it came from outside sources. For the first time large amounts of capital were lured by the past successes of shrimping. In the space of a few years the number of boats operated was nearly doubled. Figure 1 is a picture of one of these boats.

The third phase of growth was caused by another search for supplies of shrimp. This time the search moved in all directions. Some boats returned to the Atlantic coast and searched as far north as New Jersey. Eventually shrimp were found off the Florida west coast. This caused boats to be based in both Key West and Tampa. Boats searching in the other direction moved down the Texas coast. In the southwestern areas of the Gulf, shrimp were found as far south as

Figure 1. Shrimp Trawler in Brownsville, Texas, Shrimp Basin

the Yucatan Peninsula of Mexico. Thus, Brownsville, Texas, became a shrimping port because it provided access to both Texan and Mexican waters.

Among the problems encountered in this third phase were range and crew comfort. These were solved as before by increasing the size of the boats. A greater problem was that of spoilage. It became necessary to freeze shrimp as they were landed in order that they reach the consumer in good condition. Moreover, the problem of preserving shrimp at sea came to be recognized as a real problem.

Preservation by storage in crushed ice has been and still is the most common method of keeping shrimp in a marketable condition while at sea. Most shrimp trawlers have a hold which is divided into compartments as shown in Figure 2. One or more of these compartments is filled with crushed ice in the preparation for a trip. When shrimp are to be stored, a layer of ice of six to twelve inches depth is placed at the bottom of one of the empty compartments. Shrimp and ice are placed in a mixed manner on top of this bottom layer. Care is taken to keep a layer of ice along the sides of the compartment. At the top of the compartment another layer of ice is placed. In this way the shrimp are completely surrounded by melting ice during the whole period of storage on the boat. Figure 3 shows shrimp so stored being unloaded from a boat.

Experience has shown that shrimp may be kept in the

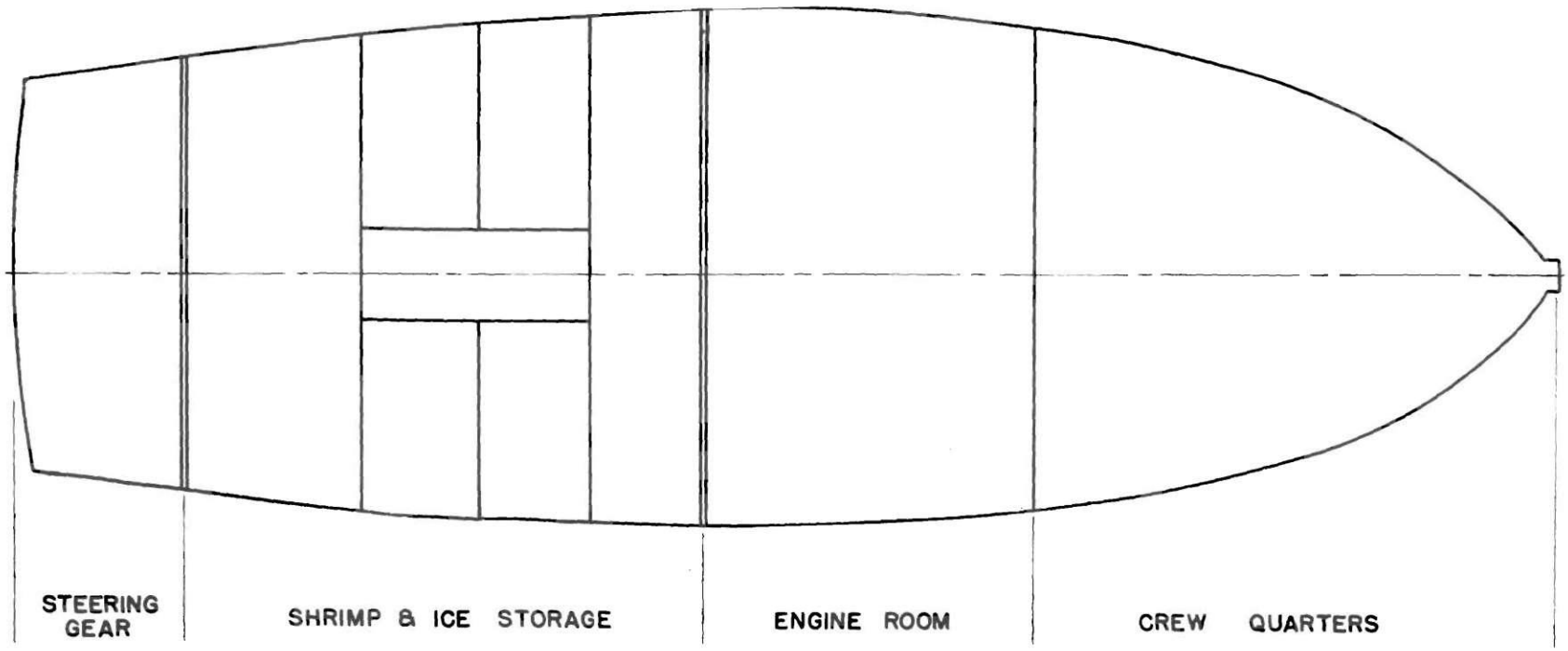

Figure 2. Typical Shrimp Trawler Hull Arrangement

Figure 3. View into Shrimp Trawler Hlld as Shrimp are Unloaded

above manner not much longer than 14 days. Thus, when boats are operating at extreme range, as in the Bay of Campeche, Mexico, shrimp must be transferred every few days to a boat which is returning to port. As a result there have been several studies of the shrimp storage problem in recent years.

Studies Concerned with Shrimp.--One of the first detailed studies of deterioration of shrimp was reported by Green, Holmes and McCleskey (17). This work provides a good introduction to the problem because it was "undertaken to obtain and record bacteriological data on shrimp from the time caught until marketed as a fresh or frozen product." (17, p. 365) In accomplishing this task, the authors used laboratory procedures to obtain data of the numbers and kinds of bacteris found on shrimp stored under different conditions. Their findings confirmed the commercial conclusion that iced shrimp may be kept longer with the heads removed. They recommend thorough and frequent washing of each lot of shrimp to reduce the number of bacteria thereon. A similar study with similar results was reported by Campbell and Williams (5).

The phenomenon of black spotting--occurrence of pronounced black bands or spots where the shell segments of shrimp overlap--has caused much interest and concern to persons handling shrimp. Fieger reported the cause of this condition as oxidation in 1951 (16). Since that time other studies of the same subject have been reported (1, 2, & 15).

Alford and Fieger found, "The shrimp stored under anaerobic conditions in cracked ice did not develop black spots even after ten days of storage, whereas the control developed them in the usual manner." (1, p. 219). Their experimentation with sodium bisulfite and propylene oxide indicated that such antioxidants would control black spotting. The difficulty is that such chemicals are either uneconomical or impart an objectionable taste, odor or color to shrimp. The most promising chemical, sodium bisulfite, will turn shrimp yellow when used in too great a concentration (36).

The idea of storage of shrimp in refrigerated brine solutions has been investigated mainly at the Marine Laboratory, University of Miami, Florida (20 & 23). Mingledorff experimented with a commercial development of this idea and evolved a system for the immersion freezing of shrimp at sea (27). In the use of this system, shrimp are placed in wire baskets in lots of about fifty pounds. The baskets are then placed in a tank of circulating brine which has been refrigerated to nearly zero degrees Fahrenheit. After ten or fifteen minutes the shrimp are frozen and are removed from the brine tank. They are then placed in cardboard boxes in the refrigerated hold of the boat. With this method each shrimp is frozen individually and remains separate so long as it is frozen.

Crowther (8) and Dassow (9) also advocate the installation of equipment aboard boats for the freezing of shrimp at

sea. From the practical view of the commercial shrimper freezing shrimp at sea is undesirable for two reasons. First, the capital investment in equipment and insulation of the hold is large. The shrimper would rather risk his money on two boats since freezing equipment adds nothing to the quantity of shrimp caught. Second, the technical skill involved in the operation and maintenance of freezing equipment is not immediately available in a shrimping crew at sea.

Since the development of antibiotic compounds there has been interest in the possibility of their application to the preservation of foodstuffs. Miller (26) describes the use of Aureomycin with fresh poultry. This method of washing or dipping fresh poultry in a low concentration water solution of antibiotic is the first that has been authorized for commercial use by food and drug officials. Farber (13) reports a similar method of washing used to test antibiotic use with fish and shrimp.

As applicable to shrimp, an antibiotic offers a practical means of controlling spoilage when it is dispersed in the ice in which shrimp are stored. When the ice melts a continual supply of fresh antibiotic is released. The difficulty of making a block of ice having a uniform concentration of antibiotic is described by Upham (35). Furthermore, "Aureomycin ice increases the shelf life of fresh shrimp, but its use presents a serious difficulty. The bivalent-ions used to chelate the antibiotic to the carrier, catalyze

apparently the formation of black spot." (22).

Studies of Seafood.--One of the latest summations of the techniques of commercial fishing is found in Butler (3). Basically these techniques may be considered as problems of materials handling. The commodity may be handled either wet or frozen, but the complication of spoilage is added with the passage of time. "The offering of fishery products to the consumer that are just barely acceptable is not a goal to strive for. Rather it's a fish, oyster or shrimp product that retains a maximum of the characteristic aroma, flavor, texture and general palate satisfaction." (3, Part 1, p. 80). To this end much research on foodstuffs in general has been devoted.

Short and Bartlett (32) and later Staph and Woolrich (34) studied the heat content of shrimp together with other foods. It was noted that "undiluted water has a freezing point, but food has a freezing range." (34, p. 1088). As applied to shrimp, it was found that at a temperature of twenty degrees Fahrenheit almost ten per cent of the mass was unfrozen (32, p. 24). This means that even at temperatures well below that of melting ice there is a considerable portion of a shrimp in the unfrozen state and, hence, capable of supporting bacterial activity. Kiser reported that "studies on bacterial flora of fish have consistently shown that the organisms found in and on fish are able to multiply at low temperatures." (25, p. 257).

An excellent review of research on the spoilage of seafood is found in Reay and Shewan (30). In simpler terms, spoilage has been found to be characterized by a combined simultaneous growth of bacteria and chemical breakdown. The microorganisms cause chemical breakdown and the chemical products provide food for the microorganisms. It has been found that in animal life bacteria exist on the outside surfaces and in the digestive tract while the animal is alive. The flesh and other parts of the animal are, by comparison, sterile so long as the animal is alive. Once the animal dies there is no deterrent to the free growth and multiplication of bacteria. Hence, they are able to progress from the skin and digestive tract throughout the flesh.

The growth pattern of microorganisms has three distinct phases: first, a lag phase where it is believed that the bacteria grow in size with little or no increase in number; second, a log phase where growth and multiplication occur logarithmically; third, a maximum stationary phase where the bacteria maintain their number but no longer increase that number. The time interval for this growth pattern is altered by temperature. At low temperatures the growth interval may be increased to a period of days or weeks. At an optimum-growth temperature this interval may be compressed into several hours, in which case the phases of the growth pattern are likely to be indistinguishable.

There are two more or less separate classes of bacteria:

those found on the land and those found in the sea. The marine varieties, as mentioned before (25, p. 257), are capable of activity at temperatures below the freezing point of water. This partially explains why seafood will spoil much more rapidly than other foods.

Attempts at controlling spoilage must be directed toward the limitation of the three factors involved; action by bacterial, chemical or mechanical means. "All the existing evidence goes to show that bacterial activity is by far the most important factor in spoilage of wet fish." (31, p. 49). Among the methods of controlling spoilage that have been investigated there is mention of the use of carbon dioxide gas (30 & 31). This "has been found to be an effective bacterial inhibitor at concentrations exceeding about 40 per cent when used in conjunction with normal stowage in ice." (30, p. 389)

Studies of the Use of Carbon Dioxide.--The noted reviews of the subject of the spoilage of seafood (30 & 31) both mention the action of carbon dioxide. They also list several studies which have been made (6, 7, 18, 24 & 33). Among the earlier of these is what Killeffer emphasized was a preliminary report (24). He says:

> It is especially interesting to note that the effect on cultures has not been simply to restrain the growth of aerobic bacteria and permit free growth of anaerobes, as one might expect to be the normal outcome of replacing air by another gas (24, p. 142).

This was written in 1930 and was designed to create interest in research on the use of carbon dioxide. As a result there were several reports of such research between 1930 and 1935.

Callow (4) experimented with pork and bacon. Moran, Smith and Tomkins (28) were interested in mould growth on meat. Coyne (6 & 7) published two reports concerned with fish. Haines (18) and Stansby and Griffiths (33) also investigated fish. In all of these works there is general agreement that carbon dioxide gas inhibits the growth of bacteria beyond what might be expected when air is replaced by an inert atmosphere. In a recent study Durbin (10) found that growth of bacteria at toxic levels of carbon dioxide is delayed and may be interpreted as a straight line function rather than as a logarithmic function. All of these studies might be termed the investigation of a laboratory curiosity for "The hitch, as yet, lies in the proper application under conditions feasible in commercial practice." (24, p. 142)

In discussing the use of carbon dioxide on fish Coyne says:

> The concentration of carbon dioxide which gives optimal results appears to be about forty to sixty per cent. Below these limits bacterial growth is less completely inhibited but no great advantage is gained by higher concentrations. In fact, some of the experiments suggest that softening of the flesh occurs to a greater degree in the higher concentrations, although this is not marked (7, p. 24T).

While Reay and Shewan in reviewing the use of chemical agents

in general say:

> The more effective of these preservatives, in concentrations that would stand a chance of being specially permitted (by food and drug authorities), delay the onset of putridity by at most about five to seven days; but the fish, during this period of extension, although not repulsive in odor or flavor, are frequently very soft in texture and of poor, unattractive quality. This criticism applies equally to the use of carbon dioxide (30, p. 389).

Speaking for the Food and Agriculture Organization of the United Nations, Rieman and Bramsnaes point out the fields of research involving fish which are of most immediate interest (31). Among these is mentioned carbon dioxide. Specifically, they say: "Owing to the possibilities of such treatment being cheaper than icing and considering the labor involved in icing, it is felt that practical trials with carbon dioxide already initiated should continue." (31, p. 57) With this in mind it was felt that some experimentation should be carried out whereby the use of carbon dioxice gas for the preservation of shrimp at sea would be evaluated.

Formation of the Experimental Problem.--Previous research with carbon dioxide has been carried on in laboratories. This work gave evidence of a definite bacteriostatic effect gained when air was replaced by carbon dioxide gas. This effect might be expected to add five to seven days to the storage life of shrimp. No report of research with shrimp has been discovered. If the assumption is made that a carbon dioxide atmosphere will add approximately one week to the storage life of shrimp, there are the practical aspects

of actual use that must be considered.

The use of carbon dioxide gas aboard a shrimp trawler could be more economical than either freezing or the use of antibiotic chemicals since both the fixed and variable costs would be lower.[2] Replacement of air in the hold of a boat would be relatively simple because carbon dioxide is nearly one and one half times as heavy as air. Provision would have to be made for the draining of water from the hold into the bilge. No special skill would be required of the boat crew, if mere replacement of atmosphere was all that was required. Some means would have to be devised whereby each compartment of the hold could be sealed as it was filled with shrimp and ice. This would allow the change of atmosphere in only those compartments containing shrimp and enable the crew to work in the remainder of the hold without breathing apparatus or ventilation of the entire storage area. Thus, the installation and use of carbon dioxide would be relatively simple and seems to possess no practical difficulty such as alteration of the boat hull.

The advantage of an additional week of storage life for shrimp aboard a boat would mean that the length of the shrimping voyage would be more limited by the endurance of the crew than by spoilage of shrimp. It also appears logical

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[2]Economically, carbon dioxide cylinders aboard a boat could possess an additional advantage through a reduction of insurance rates, if they were connected as fire extinguishers.

that shrimp, if kept under bacteriostatic conditions for such periods of time as are now commercial practice, would be of better quality when they reached the consumer. From an economic standpoint, the advantages of better quality and better boat utilization could be measured.

What type of experiment would determine the justification of the assumed bacteriostatic effect caused by a carbon dioxide atmosphere? If shrimp were brought into a laboratory the results might prove inconclusive because of the age of the shrimp at the time the experiment was begun. An installation of equipment in the hold of a shrimp trawler would stop the production of that trawler for the duration of the experiment. As a compromise it was decided that storage boxes could be made which would simulate the conditions aboard a trawler. With two identical boxes, one could be used as a control in which normal commercial conditions are simulated. The other box could then be connected to a supply of carbon dioxide and the atmosphere inside changed at will. Samples of shrimp taken from each box, when compared, would give a measure of the effect of the carbon dioxide atmosphere. This measure would then indicate the commercial feasibility of actual use of the gas.

A positive finding in such an experiment would be of tremendous importance to those people who make up the shrimp business. This opinion is based upon the literature mentioned and the author's knowledge, background and interest in

normal stowage in ice as presently practiced.

The crew of a shrimp boat is entirely capable of using carbon dioxide gas at sea. They would be required to place an air tight partition in front of each compartment as it was filled with shrimp and ice and to open a valve to allow gas to flow into the filled compartments.

The author believes that the crew of a shrimp boat would put a partition in place and open a valve, if they were shown to benefit from such actions. By contrast, they would not make periodic analyses, keep detailed records and the like because that is not their nature. One difficulty experienced with brine freezing systems is that the crew would not maintain the brine at sufficient salinity while at sea.

Because there is a possibly low installation cost and a low cost of use, it might be sound economic practice to use carbon dioxide in the preservation of wet shrimp at sea. Through the method of scientific investigation it is possible to evaluate such use and point to a decision which would either implement a new procedure or cause concentration on research of other possibilities.

CHAPTER II

DEVELOPMENT OF TEST PROCEDURE

Methods of Determining Spoilage.--In the preparation for an evaluation of the use of carbon dioxide in wet shrimp storage, it was first necessary to find a means of comparing shrimp samples. If like portions of the same lot of shrimp were to be stored under identical conditions except for atmosphere, a daily comparison of samples from each portion would indicate the day by day effect of that atmosphere. Comparison of the degree of spoilage of daily samples would consequently indicate the relative amount of preservation caused by the change of atmosphere, but the quantative measurement of spoilage is difficult.

Spoilage is an irreversible action and in different foods it manifests itself in different ways. Therefore, no uniform criteria may be applied by which spoilage is measured. In the observation of frozen foods we might want to measure moisture loss as an indicator of spoilage, but this involves only a mechanical condition of spoilage. In observation of a fatty food we might want to measure rancidity or oxidation of the fatty substances as an indicator of spoilage, but this involves mainly a chemical condition of spoilage. Generally speaking a measure of bacterial activity has been found to be a more sensitive indicator of spoilage than any

other method (14).

The most common measure of bacterial activity is the counting of numbers of bacteria (12). This requires laboratory facilities and techniques. It usually involves the dilution of a sample with pure water until only a few bacteria may be expected to be found in a given volume. A measured volume of this water is then placed in a flat surfaced dish containing bacterial nutrients. The dish is then placed in an incubator for 48 to 72 hours. At the end of that time it is assumed that the individual bacteria in the original sample were separated in the process of dilution. The incubation period allows these to grow and multiply to the extent that they become visible spots in the dish. The number of these spots or colonies multiplied by the degree of dilution represents the number of bacteria present in the original sample.

Bacterial activity may be measured indirectly by chemical means. That is, if the chemical products of bacterial activity are known, the amount of these products present in a sample represents the bacterial activity that has taken place in that sample. One of the chemical products is the hydrogen ion. Thus, one of the simpler measures proposed is a measure of pH (11).

In a recent article, Novak, Fieger, and Bailey discuss some of the problems of measurement and propose two methods for approximation of bacterial counts in shrimp and oysters.

> Lack of laboratory facilities or technically trained personnel in seafood industries instigates the development of fast scientific tests requiring only minor equipment and simple application of fundamental chemical knowledge. Standard plate count procedures requiring 48 to 72 hours are definitely too time consuming prior to processing and packaging many foods. Quick methods are essential to determine if it is safe to continue processing a product, or whether it should be rejected. Such tests have been developed, and are here described, for the rapid approximation of bacterial counts in shrimp and oysters. These tests should be applicable to other food products having counts within certain ranges.
>
> One method involves growth of the microorganisms in a carbohydrate-containing medium; the rapid formation of acid, which is proportional to the number of bacteria present in the added sample, furnishes the indicant for the test and the basis of measure. Acid formation is readily measured by observing the color change in an indicator added to the medium.
>
> Another method found to be successful is a modification of the methylene blue reductase test. It differs from the milk test in that rapid counts on numerous other products can be made by employing a single medium. Sterilized, nonfat dry milk solids in solution serves merely as a medium, and is not the source of any microorganisms. In this determination, time required for reduction of methylene blue in a synthetic medium by bacterial reductases is measured. The length of time required is an indication of the number of bacteria present in an added sample (29, p. 66).

It appeared that one of the two above tests could be adapted for use in the proposed experiment. Therefore, preliminary testing was carried on in a laboratory in anticipation of the development of a procedure whereby daily shrimp samples could be compared and their approximate degree of spoilage recorded.

Preliminary Testing.--Before any preliminary tests could be made, it was first necessary to construct an incubator. Since it was anticipated that the incubator would be used in a field test which might involve its use on a shrimp boat at sea, portability and reliability were prime considerations. A test tube rack in a water bath appeared to be the most satisfactory arrangement.[3] Such a device was constructed using a rectangular tropical fish aquarium and a fifty watt aquarium heater with thermostat. Once adjusted this arrangement maintained a temperatore of 95 degrees Fahrenheit within one degree. Its convenience and operation were entirely satisfactory.

Both of the tests recommended by Novak, Fieger and Bailey (29) require the preparation of a medium in test tubes. A volume of five ml. (milliliters) is placed in individual tubes. These tubes are then capped and sterilized. In use an equal volume (five ml.) of sample is added to the medium in the tube. The tube is then placed in an incubator and maintained at a temperature of 95 degrees Fahrenheit until the color of the contents changes. The length of time required for this color change is proportional to the number of bacteria in the inoculating sample. If the number of bacteria in the sample is small, the time required for color change is great. If the number of bacteria in the sample is

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[3]A description of the incubator constructed and used for all the tests herein described is found in the Appendix.

large, the time required for color change is small.

A first test was made to gain experience and determine which of the two media would be the best for use. Glassware was washed according to standard laboratory practice. A quantity of the milk-containing medium was mixed. Fifty test tubes containing five ml. each were prepared. These were plugged with cotton, the top wrapped with heavy kraft paper and sterilized in an autoclave for fifteen minutes. When these tubes had cooled it was noted that some of the milk mixture had curdled. A similar quantity of the acid production medium was mixed and tubes prepared as above.[4] After these preparations were completed it was found that the pH meter had been improperly calibrated. It was thought that this was the cause of the curdling of the milk medium. The error did not appear to be great enough to warrant new solutions for this first test.

The first test consisted of the inoculation of three tubes of each medium. The inoculating samples were all taken directly from the standard E. coli culture, No. 10536, maintained by the Georgia Tech Engineering Experiment Station. The three samples were: one ml., two ml. and one bacteriological loop--that volume which can be contained in a

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[4]For detailed information concerning the formulae, preparation procedure, and use of these media the reader is referred to the original article (29). The formula used in the preparation of the acid production medium used in the course of the experiments described in Chapters III and IV is found in the Appendix.

four mm. (millimeter) diameter loop of fine wire. After eight hours none of the tubes containing the milk medium had changed color appreciably. Those tubes containing the acid production medium required the following times for the color change: 130 minutes for the two ml. sample, 180 minutes for the one ml. sample and 300 minutes for the one loop sample. It was noted that all of the samples were taken directly from the culture where they were in a state of growth. In the transfer to the test tubes this growth was undisturbed. Therefore, in this case the time measured is not directly proportional to the number of organisms present.

Observance of the color change in the acid production medium pointed out advantages in its use. The medium when prepared is a deep green color. When a tube is inoculated this color is diluted according to the volume of the inoculant, but not changed appreciably in hue. As the color change begins a slight amount of yellow may be observed to be almost superimposed upon the green. As the color change progresses the combination of green and yellow produces a straw color. Further progression of the color change results in a predominate yellow color with only a slight amount of green. End of the color change occurs when the tube is all yellow. This progression of color change was later found to require approximately one hour, regardless of the number of bacteria in the inoculating sample. Moreover, within one hour after the end of the color change, the precipitate which

has formed at the bottom of the tube rises to the surface of the liquid in the tube. The facts, that this color change is progressive, that it requires an estimable length of time, and that the colors green and yellow are easily distinguishable by the human eye proved of great advantage in the later use of the acid production medium.

After preparation of a new lot of each medium a second test of the milk medium was made. This time the pH meter was correctly calibrated. The milk medium did not curdle upon sterilization. Performance was the same, however. The change of color that occurred was indefinite.

As prepared, the milk medium tubes are a blue color. When this medium tube is inoculated the dilution results in a lighter shade of blue. The color change that occurs is a bleaching of blue to white. Slight shades of blue superimposed upon white are not readily distinguishable by the human eye. Because it appeared that results obtained with the milk medium could be questionable, it was decided that the acid production medium would be used exclusively.

One further test was deemed necessary before there was assurance that the acid production medium would provide the information desired in the proposed experiment with carbon dioxide. It is known that carbon dioxide in the presence of water forms carbonic acid. Could this by itself cause a false color change? When a small piece of dry ice was placed in a tube of medium the color changed immediately.

In order to check this action on shrimp, two pounds of shrimp were placed in a metal box with water ice in a manner similar to commercial storage. Small amounts of dry ice were placed at the top of the box for about 24 hours so that there would be a carbon dioxide atmosphere in the box and surrounding the shrimp. Two hundred ml. of distilled, deionized water were placed in each of two milk bottles. These were plugged with cotton and sterilized in an autoclave. Two samples of six shrimp each were taken from the box and placed separately in the milk bottles. The bottles were then shaken for two minutes. One bottle was held in the left hand, the other in the right, in order that the degree of agitation be the same. In this manner the shrimp of each sample were thoroughly washed. An inoculant of five ml. of the wash water was placed in each of three tubes of the previously prepared medium. These tubes were shaken to mix the medium and the inoculant and then placed in the water bath incubator. Time of inoculation was recorded as the beginning of the period required for color change.

The wash water in the second milk bottle was aerated for twenty minutes. Air was first bubbled through a solution of potassium hydroxide, then passed through a wad of cotton and into the wash water in the milk bottle. The potassium hydroxide was used to remove traces of carbon dioxide from the air. The cotton was used as a filter to prevent airborne bacteria from entering the wash water. This aeration

was an attempt at removal from the wash water of any carbon dioxide that had been carried into the milk bottle with the shrimp sample.

After aeration, an inoculant of five ml. of the wash water was placed in each of three tubes of medium, as before. The tubes containing the non-aerated sample required 430 minutes for the color change. Those containing the aerated sample required 423 minutes. Because of the closeness of these times it was hoped that there would be little difficulty caused by the carry over of absorbed gas. At any rate it was apparent that the bacterial action in the test tubes was of sufficient magnitude to overshadow a relatively minor condition.

A duplicate test was made concurrently with that described above. All the conditions described were the same except a volume of 100 ml. of wash water was used. In this test the non-aerated sample required 377 minutes for the color change while the aerated sample required 355 minutes. The difference in the times recorded for this test and the one above was caused by the difference in the volume of wash water used. It was thought that a sample of six shrimp washed with 200 ml. of water was more adequate.

It is recommended by Novak, Fieger and Bailey (29) that 40 grams of shrimp be diluted with 200 ml. of sterile, pure water and pulverized for two minutes in a Waring Blendor in the preparation of a sample. A sample of six shrimp

usually weighs 80 or more grams. The size of the sample and the method of preparation were changed in order that the test be more suitable for field use. The use of individual containers of sterile water and simple agitation have many practical advantages over the sterilization and use of Waring Blendor jars. A large volume of sterile, pure water would be difficult to maintain under field conditions. The daily cleansing and sterilization of Waring Blendor jars could be prohibitively involved if attempted outside the laboratory. Since it is known that a large part of the bacteria associated with spoilage are found on the outside surfaces and in the digestive tract of shrimp and fish (30), it was thought that simple washing of sample shrimp would be sufficient. In a communication with Dr. Fieger it was found that the procedure he recommended was not standardized. Furthermore, it was reasoned that simple washing of samples would eliminate the placing of nutrient portions of shrimp in the tubes of medium and prevent a variation in the amount of nutrients available during incubation. Therefore, it was decided that 200 ml. of distilled, deionized water could be put into each of a number of standard home canning jars and the jars sealed and sterilized in the laboratory. These jars of water could then be used as they were needed. Thus, a degree of uniformity and reliability could be added to the proposed experiment.

Calibration of Medium.--Once it was decided that the acid

production medium could be used in the proposed experiment, it was necessary to provide a means of interpreting data obtained through its use. In their proposal of the use of this medium Novak, Fieger and Bailey relate their data to a specific number of organisms per gram of shrimp. Since the test procedure was altered in such a way that the weight of the shrimp sample could vary, the published data could not be used with a great degree of confidence. It was also felt that the medium should be calibrated in such a way that the calibration could be reproduced in a laboratory. For this reason the standard E. coli culture mentioned previously was used in calibration of the acid production medium.

Five samples were prepared from the coli culture for the calibration test. Sample 1 consisted of ten ml. of culture diluted in 100 ml. of sterile, distilled, deionized water. Succeeding samples were similarly 0.1 dilutions of the preceding sample. An inoculant of five ml. of Sample 1 was placed in each of three tubes of medium. This was repeated for each of the remaining samples. The times required for color change were recorded and are presented in Table 1. All three tubes of each set changed color concurrently. After the tubes were inoculated, standard bacteriological plates were made from Samples 1, 3 and 5. These plates were also made in triplicate sets. The actual counts presented in Table 1 are the average of the counts recorded for the three plates in each set.

Table 1. Record of Calibration Test

Sample Number	Minutes Required for Color Change	Actual Plate Count in Number of Organisms per Milliliter	Average Plate Count Assumed for Purposes of Calibration in Number of Organisms per Milliliter
1	163	6.2×10^7	5.0×10^7
2	206	-	5.0×10^6
3	321	5.0×10^5	5.0×10^5
4	380	-	5.0×10^4
5	450	4.2×10^3	5.0×10^3
Six month average count of culture		4.4×10^8	

It was evident that the acid production medium gave an essentially linear response over a wide range of numbers of organisms. In order to define a reference line for the later conversion of time for color change into numbers of organisms, the average counts found in the right hand column of Table 1 were assumed. These points are plotted in Fugure 4. The conversion line was drawn using the plots for Samples 1 and 5. For comparison the dotted line in Figure 4 is included. It is a line drawn through and extrapolated from the data published by Novak, Fieger and Bailey (29).

Definition of Test Procedure.--Once experience had been gained with the preceding tests and the medium calibrated, a data sheet could be prepared and the daily testing procedure defined. A sample data sheet is included as Figure 5. A sufficient quantity of this data sheet was reproduced for use in the later experimentation. Most of the items included in the data sheet are self explanatory. The format is such that entries could be made as the daily test procedure was followed.

A step by step listing of the daily test procedure is found below:

1. Note the temperatures in and near the test boxes.

2. Observe the condition of the water drains. If clogged, clean out.

3. Check the flow of gas into the test box. Record the pressure reading. Observe this throughout the day.

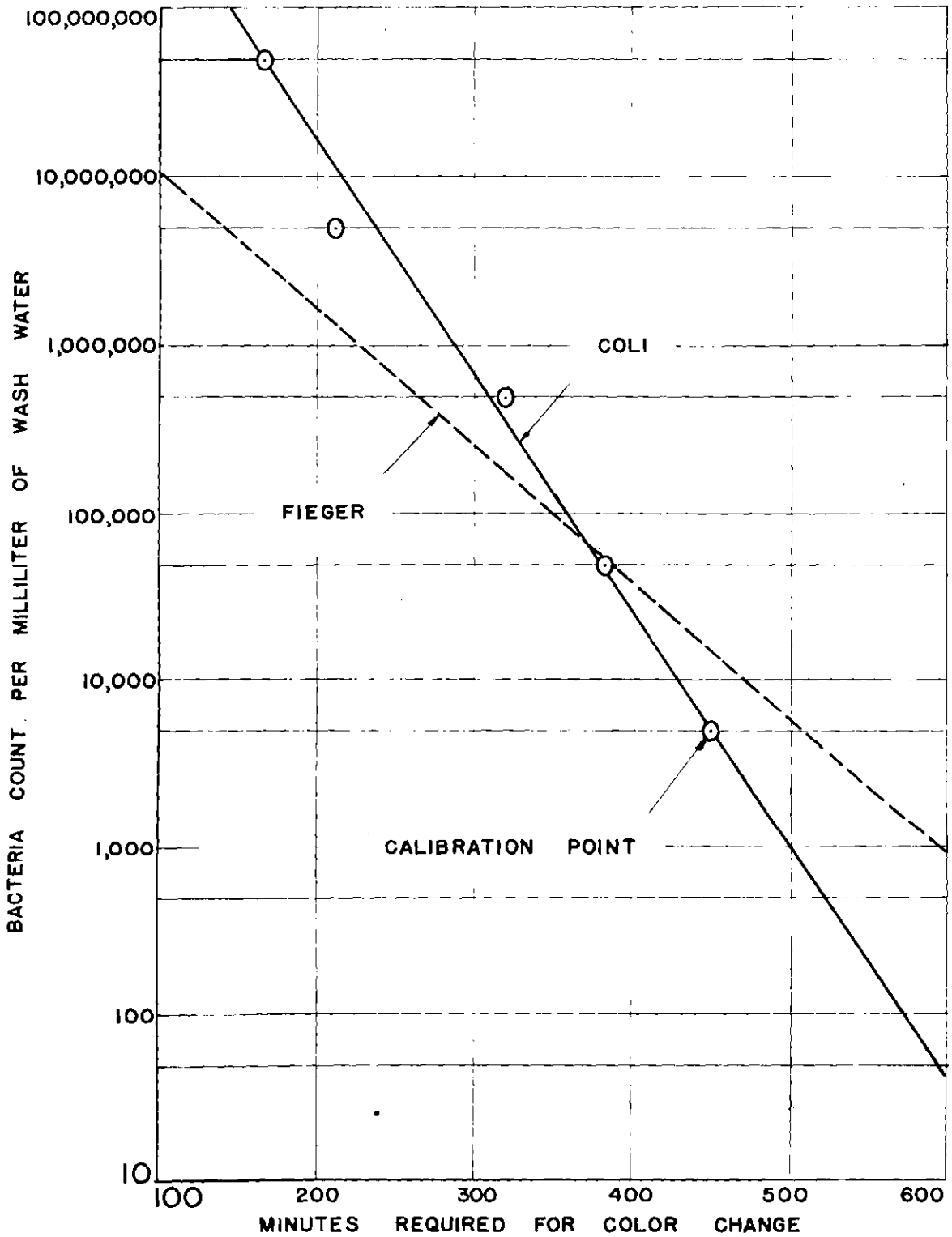

Figure 4. Medium Response Curves

DATA SHEET

EVALUATION OF CO_2 ATMOSPHERE FOR WET SHRIMP STORAGE

DATE July 18, 1956 TEST NO. 1 SHEET 12 OF 21

AMBIENT AIR TEMPERATURE NEAR BOXES (°F) 76 WET, 78 DRY, TIME 625A M.

STORAGE BOX READINGS:

	CONTROL	TEST
1. TEMPERATURE (°F)	MAX 71, MIN 67, AVG 70	MAX 71, MIN 69, AVG 70
TIME ABOVE TAKEN	100 P.M. 625A.M	100 P.M. 625A.M
2. WATER DRAIN CONDITION	OK, 625A.M	OK, 625A.M
3. PRESSURE (INCHES WATER)	3 3/4 UPPER, 3 1/2 LOWER, 1/4 DIFFERENCE	

WATER BATH READINGS:

4. TIME TUBES IN BATH FOR PREWARMING 630A.M.
5. TEMPERATURE (°F) MAX 95, 640A.M. MIN 93, 620A.M. AVG 95

SAMPLE READINGS: (SAMPLE CONSISTS OF 6 SHRIMP TAKEN FROM CENTER OF SHRIMP IN BOX, PLACED IN WASH JAR WITH 200 ML. STERILE WATER AND SHAKEN FOR 2 MINUTES. 5ML. WASH WATER IS PLACED IN TUBE OF MEDIA.)

	CONTROL	TEST
6. TIME TAKEN FROM BOX	630A M.	630A M.
7. VISUAL CONDITION	good*	good*
8. CENTER OF SHRIMP (INCHES FROM BOTTOM)	20	20
9. WEIGHT OF SHRIMP (GMS.)	99	99

10. TUBE BATH LOCATION	2	3	4	5	6	7
11. TIME IN BATH A.M	637	637	637	640	640	640
12. COLOR CHANGE BEGINS A.M	1030	1030	1030	1255	1255	1255
13. CHANGE CONTINUES	1100	1100	1100	115	115	115
14. CHANGE CONTINUES	1120	1120	1120	140	140	140
15. COLOR CHANGE ENDED A.M	1145	1145	1145	205	205	205
16. TOTAL MINUTES FOR CHANGE	308	308	308	445	445	445
17. COUNT PER ML. (COLI)	4.5×10^5	4.5×10^5	4.5×10^5	5.6×10^3	5.6×10^3	5.6×10^3
18. COUNT PER ML. (FIEGER)	2.3×10^5	2.3×10^5	2.3×10^5	1.7×10^4	1.7×10^4	1.7×10^4

19. REMARKS:

Control 3.5 oz 27/lb.
Test 3.5 oz 27/lb.

* Some Black Spot on Control.

830A Boxes re-iced. Ice was melted from sides + Top. Placed 300# each box.

Figure 5. Sample Data Sheet

4. Place six tubes of medium in the water bath at least 15 minutes prior to removal of shrimp from the boxes. This prevents a variation in the initial temperature of the medium from day to day.

5. Periodically observe the temperature of the water bath to make sure it is constant. This prevents variation in the incubation temperature from day to day.

6. Obtain the jars of sterile water marked for use this day.

7. Place the one marked, "T" on the test box.

8. Loosen the cap of the jar marked, "C" and place it on top of the control box.

9. Open the control box.

10. Observe the height of the mass of shrimp and ice in the box.

11. Remove six shrimp from the approximate center of this mass.

12. Place the shrimp in the water jar marked, "C".

13. Tighten the cap and place the jar on top of the control box.

14. Close the control box.

15. Loosen the cap of the jar marked, "T".

16. Open the test box and remove six shrimp in the same manner as for the control sample. Place these in the jar marked, "T".

17. Close the test box and record the time the samples were taken.

18. Note the visual condition of the samples. Observance of an unusual condition might require cooking and tasting of the samples.

19. Note the height of the center of the shrimp in each box. If this falls below 18 inches the re-icing procedure is to be followed.

20. Shake the jars containing the shrimp samples for two minutes, holding the control sample in the left hand and the test sample in the right hand, and moving the forearms together in an up-down motion.

21. Remove the wrappings from the top of the six tubes of medium in the water bath and open a package of sterile pipettes.

22. Draw five ml. of the wash water in the control jar into a sterile pipette.

23. Remove the cotton plug from the tube of medium at location 2 in the water bath and inoculate this tube with the wash water in the pipette. Replace the cotton.

24. Using the same pipette and method inoculate the tubes at locations 3 and 4 with wash water from the control jar.

25. Record the time of inoculation as item 11 on the data sheet.

26. Draw five ml. of the wash water in the test jar into a second sterile pipette.

27. Remove the cotton plug from the tube of medium at

location 5 and inoculate this tube with the wash water in the pipette. Replace the cotton.

28. Using the same pipette and method inoculate the tubes at locations 6 and 7 with wash water from the test jar.

29. Record the time of inoculation as item 11 on the data sheet.

30. Remove the tubes from the water bath two at a time and shake to mix the medium and inoculant. Replace in the same bath locations.

31. Weigh the samples and record this as item 9 on the data sheet.

32. Periodically observe the color of the tubes in the bath. Compare their color with that of the green standard. When a yellow color is noted in one of the tubes record the time as item 12 on the data sheet.

33. Record item 13 when yellow and green are about equal in one of the tubes.

34. After item 13 has been recorded for a tube compare its color with that of the yellow standard. Record item 14 when there is only a slight amount of green left in a tube.

35. Record item 15 when the color in a tube is equal to that of the yellow standard.

36. Record item 16 as the difference in time of items 11 and 15.

37. Make note of any circumstance which has not been mentioned as item 19.

In the development of this procedure care was taken to insure that there would be a uniformity of results, since contamination or a variation in temperature could cause error. Much consideration was also given to the human element and because of this it was felt that it was necessary that the color change be observed and recorded through comparison with definite color standards.

Preparation of Medium and Wash Water.--The final step taken to insure a uniformity of results, insofar as the measure of spoilage is concerned, was the preparation of sufficient quantities of tubes of medium and jars of wash water for the entire testing period. It was estimated that two tests of 30 days each might be required. Therefore, 120 pint wide mouth mason canning jars were obtained for the wash water and three gross of 19 x 150 mm. test tubes were obtained for the medium. Sufficient pipettes were not available because of their cost and it was planned that they could be sterilized in the oven of a kitchen stove.

All the jars, caps and tubes were washed in hot water containing detergent using a sponge and test tube brush to scrub the inside surfaces. They were rinsed a minimum of eight times with hot free flowing tap water. They were rinsed once again with distilled water on the day preceding the actual filling and allowed to drain dry in an upside down position. Thus, all containers were uniformly clean prior to filling.

On June 2, 200 ml. of distilled deionized water was placed in each water jar. As each jar was filled it was capped and a retainer ring screwed on loosely. All of the water used came from the same source. The jars were sterilized in an autoclave in three lots of forty jars each. Each lot was allowed to remain in the autoclave at pressure for thirty minutes. As the jars began to cool upon removal from the autoclave, the cap on each was depressed inward with an audible click. After this indication of adequate sealing was observed the retainer ring on each jar was tightened and the jar placed in the original cardboard case.

On the day preceding the actual filling of the test tubes, the dry ingredients for the medium were measured according to the formula shown in the appendix. This formula is that for the acid production medium developed and published by Novak, Fieger and Beiley (29) multiplied by a factor of 22 to give the volume desired. On June 2 at about nine a.m. the liquid ingredients of this formula were measured and added to the dry ingredients. One large container was used in the mixing. The color indicator, bromthymol blue, required almost two and one half hours to dissolve even with the careful application of heat. Once this was dissolved the medium was a deep clear green color, the same as the second lot mixed for the preliminary tests. The pH of the medium was adjusted to 7.1 as specified. The calibration of the pH meter was carefully checked on the day preceding and again

just prior to its use. Into each of 432 test tubes, five ml. of the medium were measured using an automatic burette. As each tube was filled the top was plugged with a wad of cotton and wrapped with a three inch square of kraft paper, held in place with a rubber band. The tubes of medium were sterilized in two lots at 1:00 p.m. and 2:15 p.m. For sterilization they were placed in the autoclave and remained at pressure for fifteen minutes. After the tubes had cooled, the top of each was wrapped with a six inch square of polyethylene sheet, held in place with a rubber band. This was done to prevent evaporation of the medium in the tubes during storage as had happened with previously prepared tubes.

Color standard tubes were also prepared. Six green standard tubes were made by diluting tubes of five ml. of medium with five ml. of distilled deionized water. Six yellow standard tubes were made by first adjusting the pH of a small quantity of medium to 6.0, placing five ml. of this in each tube and diluting it with five ml. of distilled deionized water. These color standard tubes were capped and sterilized in the same manner and along with the others. The facilities of the Bacteriological Laboratory of the Georgia Tech Engineering Experiment Station were used in the above preparations.

For transportation to Brownsville, Texas, where the experimentation was performed, the cases of water jars were wrapped with kraft paper. The tubes of medium were placed

in three cardboard boxes. On the top and bottom of the boxes was placed a bed of shredded paper. Between each tube was placed a paper towel. Each box of tubes was wrapped with kraft paper and carefully marked to prevent tipping of the contents, wetting of the cotton plugs and spoilage of the medium. Along with the tubes of medium the color standard tubes were packed. Also, eighteen tubes of medium from the lot mixed for, and used in, the calibration test were protected with polyethylene and packaged with the others.

In addition to the tubes of medium and water jars, ten five ml. pipettes were rinsed with distilled water, wrapped with kraft paper in packages of two, and sterilized in the autoclave for anticipated use during the first few days of testing while at sea on a shrimp boat. This completed the laboratory preparations. What remained to be done was the construction of storage boxes and the testing of shrimp stored under simulated commercial conditions.

CHAPTER III

SIMULATED COMMERCIAL STORAGE OF SHRIMP

Storage Boxes and Equipment Used in Test.--The storage boxes used in this test were designed to approximate the conditions in a side compartment of the hold of a shrimp boat. It is in the side compartments that shrimp are usually stored for the longest periods of time. The approximate dimensions of one of these compartments are four feet width, five feet depth and six feet height. The shape is not completely rectangular since one edge follows the contour of the boat hull, but any horizontal cross section is essentially a rectangle. It was thought that a rectangular box approximately two feet wide, two feet deep and four feet high could be used to duplicate the essential conditions of storage. A box of larger size would have been wasteful of shrimp and construction materials; while a box of smaller size might not have provided the crushing effect of a large mass of shrimp and ice.

The two storage boxes were constructed concurrently. The surfaces, except for the front, of each box were cut from a single 4 by 8 feet sheet of 3/4 inch marine fir plywood. Fabrication was such that the inside dimensions were: $23\frac{1}{4}$ inches deep, $22\frac{1}{2}$ inches wide, 48 inches high. Weldwood waterproof glue was used at all joints. In addition, $1\frac{1}{4} \times 10$

wood screws were spaced about four inches apart along all edges to insure a continuous binding of the glue. A system of inner partitions was built from 1 x 6 inch lumber inside the front edge of each box to facilitate loading.[5] The front door of each box was made of 3/16 inch tempered masonite stiffened with 1 x 3 inch lumber. Before the doors were hinged all surfaces of each box were painted with two coats of white marine deck enamel of the same type used in the holds of shrimp boats. A foam rubber gasket was fitted along the front edge of each box after painting. With the gasket in place, the door on each box was hinged so the gasket was compressed as it was closed. A hasp was similarly installed so the remaining portion of the gasket was compressed as the box was locked. The use of the gasket together with the glued joints produced a nearly airtight box. Hooks were added to the door of the test box to prevent warpage and excessive leakage of gas. The constructed boxes may be seen in Figure 6.

Three identical fittings were installed on each box. The first was a float valve water drain which consisted of a two inch length of two inch diameter tubing attached to the bottom of the box beneath a two inch hole drilled five inches away from the front right hand corner. The bottom end of the tubing was covered with a piece of plexiglas

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[5]These partitions are similar in construction to actual boat compartment partitions and may be seen in Figure 19.

Figure 6. Storage Boxes and Equipment Used in Test One

having a $\frac{1}{4}$ inch hole in its center. A ping-pong ball was placed inside the tubing to act as a valve which would open to allow drainage of water as it collected. The second fitting was a thermometer well, located in the center of the top of the box. It was made from $\frac{1}{4}$ inch pipe fittings and allowed the end of a thermometer to extend three inches into the box. The thermometer well was sealed to prevent leakage of gas. The third fitting was a $\frac{1}{2}$ inch vent hole drilled three inches to the right of the thermometer well. In the control box this allowed the free flow of air into the box as ice melted and drained. In the test box this hole was used for the introduction of the carbon dioxide atmosphere.

As may be seen in Figure 6, 50 pound cylinders of carbon dioxide were used as a source of gas. These and a pressure regulator were obtained from commercial sources. They are of the same type found and used in drug store soda fountains for the carbonation of water. Also seen in Figure 6 is a manometer attached to the right hand side of the test box. This was connected through a "T" fitting to the carbon dioxide line as it entered the test box. Pressure readings of gas entering the test box were taken with this manometer. The coil of $\frac{1}{4}$ inch copper tubing seen in Figure 6 was used in order that carbon dioxide entering the test box would be the same temperature as air entering the control box. In the original installation gas was allowed to flow

from the cylinder into the regulator, where the pressure was reduced to approximately $\frac{1}{2}$ inch of water. From the regulator, gas flowed through the coil and into the test box. On the sixth day of testing this installation was changed to that shown in Figure 6. There the gas flowed from the cylinder to the regulator where it absorbed heat from the light bulb and was reduced in pressure to 20 pounds per square inch. Next the gas flowed through the coil and a needle valve used to stop the flow when samples were taken; then through a second needle valve, where the pressure was reduced to $\frac{1}{4}$ inch of water, and into the test box. These changes were made in order that the flow of gas into the test box would be constant at a very low rate. Pressure was reduced in an attempt to conserve gas, as would have been the case in commercial practice.

With the use of this equipment it is believed that the desired conditions were obtained. Conditions within the control box closely simulated those found commercially. Conditions within the test box were identical to those of the control box except for the change of atmosphere. Moreover, the equipment used in the change of atmosphere within the test box could be used in commercial practice.

Storage Procedure and Data Obtained.--On July 5 the boat, Little Man, returned to the port of Brownsville, Texas. Arrangements had been made for the first phases of the test to be made on this boat. All equipment had been checked

through a simulation of the daily test procedure. During the morning of July 6 the storage boxes and other equipment were put aboard the Little Man. The test box was located on deck at the rear starboard corner of the cabin. A cylinder of carbon dioxide was tied to the starboard pin rail a few feet from the test box. The copper tubing was installed between the cylinder and the test box and the flow of gas checked. The control box was placed on deck on the starboard side of the cabin just forward of the test box. The water bath incubator was fastened to a shelf in the captain's cabin. An inverter was connected to the boat electrical system to provide power for the incubator. Operation of the incubator proved satisfactory. Water jars, packages of sterile pipettes and tubes of medium sufficient for ten days of testing were placed near the incubator. By about 3:00 p.m. that afternoon fuel, ice, water and food had been stored and the voyage begun.

At 6:30 p.m. July 6 the Little Man was located several miles north of the mouth of the Rio Grande River in about 16½ fathoms of water. The main net was put overboard and trawling continued at approximately the same depth for six hours. At 12:30 a.m. the net was emptied and trawling resumed. Trash was cleared from the shrimp on deck and heading begun. Midway in this operation six shrimp were picked at random from those that had been headed. These were placed in a jar of sterile water and shaken for two minutes. Three

tubes of medium were inoculated and placed in the incubator according to the testing procedure. This constituted Sample 1.

After heading was completed the entire lot of freshly caught shrimp was divided evenly into two baskets and washed with sea water. Each of these baskets was estimated to contain 45 pounds of headless shrimp. Crushed ice was brought up from the hold and placed in the test box along with the shrimp in one of the baskets. This was accomplished in the manner of commercial practice for storage of shrimp in the hold. Once the test box was filled, an additional supply of ice was brought up from the hold and the shrimp in the remaining basket placed in the control box in a similar manner. Filling of the boxes was completed at about 2:00 a.m. July 7. The regulator was then adjusted to allow carbon dioxide to flow into the test box at an approximate pressure of $\frac{1}{2}$ inch of water.

Because sufficient shrimp for the test were caught in the first drag of the net and so close to port, it was possible to return to port that morning and continue the testing on shore. The Little Man arrived at the dock at 10:30 a.m. By 11:30 a.m. the storage boxes and equipment had been removed from the boat and placed in the fish house of the J. R. Hardee Shrimp Company where they are shown in Figure 6 and remained throughout the testing period of 21 days.

On the morning of July 8 Samples 2 were taken according

to the daily test procedure. Every morning thereafter, for the remainder of the test, this procedure was followed. The results of these daily tests are given in Table 2. Plots of these data are shown in Figure 7 for comparison. Conversions of the times are listed in Table 3. Data from Table 3 are plotted in Figure 8 for another comparison. Testing was discontinued after the third week when both lots of shrimp stored had no commercial value.

Discussion of Results and Observations.--In Table 2 the days on which re-icing occurred have been noted. It was necessary to add ice to each box when they had first been placed in the fish house because of exposure to the sun and the sea wind. On the other days indicated in Table 2, the re-icing procedure found in the appendix was followed.

Also indicated in Table 2 are the days on which a new cylinder of gas was connected. On July 10, as Samples 4 were being taken, it was noted that the first cylinder of gas used was empty. A new cylinder was connected as a matter of routine because this first cylinder had been used to check for leaks during the construction of the storage boxes. On the next morning, however, the second cylinder was found to be exhausted. The third cylinder was connected with some question because the flow of gas had been observed until 5:00 p.m. the preceding day and found satisfactory. Al connections in the copper tubing were checked with soapy water for leaks. It was thought that the second cylinder was only partly filled

Table 2. Sample Record -- Test One

Sample Number	Minutes Required for Color Change		Boxes Re-iced after Sampling	Gas Cylinder Changed
	Control	Test		
1	496	-	P*	
2	405	482		
3	463	589	C**	
4	374	396		X
5	398	381		X
6	375	373	P	X
7	305	358		
8	340	388		
9	325	424	C	
10	347	501		
11	325	428		X
12	308	445	P	
13	295	385		
14	295	355		
15	265	425	C	X
16	312	440		
17	280	440		
18	295	390	P	
19	270	405		
20	265	395		Empty
21	250	365	Necessary	none added

* "P" denotes partial re-icing, see Appendix

** "C" denotes complete re-icing, see Appendix

Table 3. Bacteria Count of Samples -- Test One

Sample Number	Coli Conversion Bacteria Count in Thousands per Milliliter		Fieger Conversion Bacteria Count in Thousands per Milliliter	
	Control	Test	Control	Test
1	1.1	-	6.5	-
2	20	1.8	37	8.8
3	3.0	0.045	12	1.0
4	55	60	65	70
5	25	33	40	50
6	55	60	65	70
7	520	90	250	90
8	180	26	130	42
9	280	11	180	25
10	140	1.0	120	6.0
11	280	9.0	180	23
12	450	5.6	230	17
13	720	40	300	52
14	720	4.0	300	14
15	2,000	11	530	25
16	430	6.8	220	19
17	1,200	6.8	400	19
18	720	35	300	50
19	1,700	20	500	37
20	2,000	28	530	45
21	3,200	78	720	80

Note: To convert figures to approximate bacteria count per shrimp; multiply by 200 milliliters (the volume of wash water used) and divide by 6 (the number of shrimp in each sample).

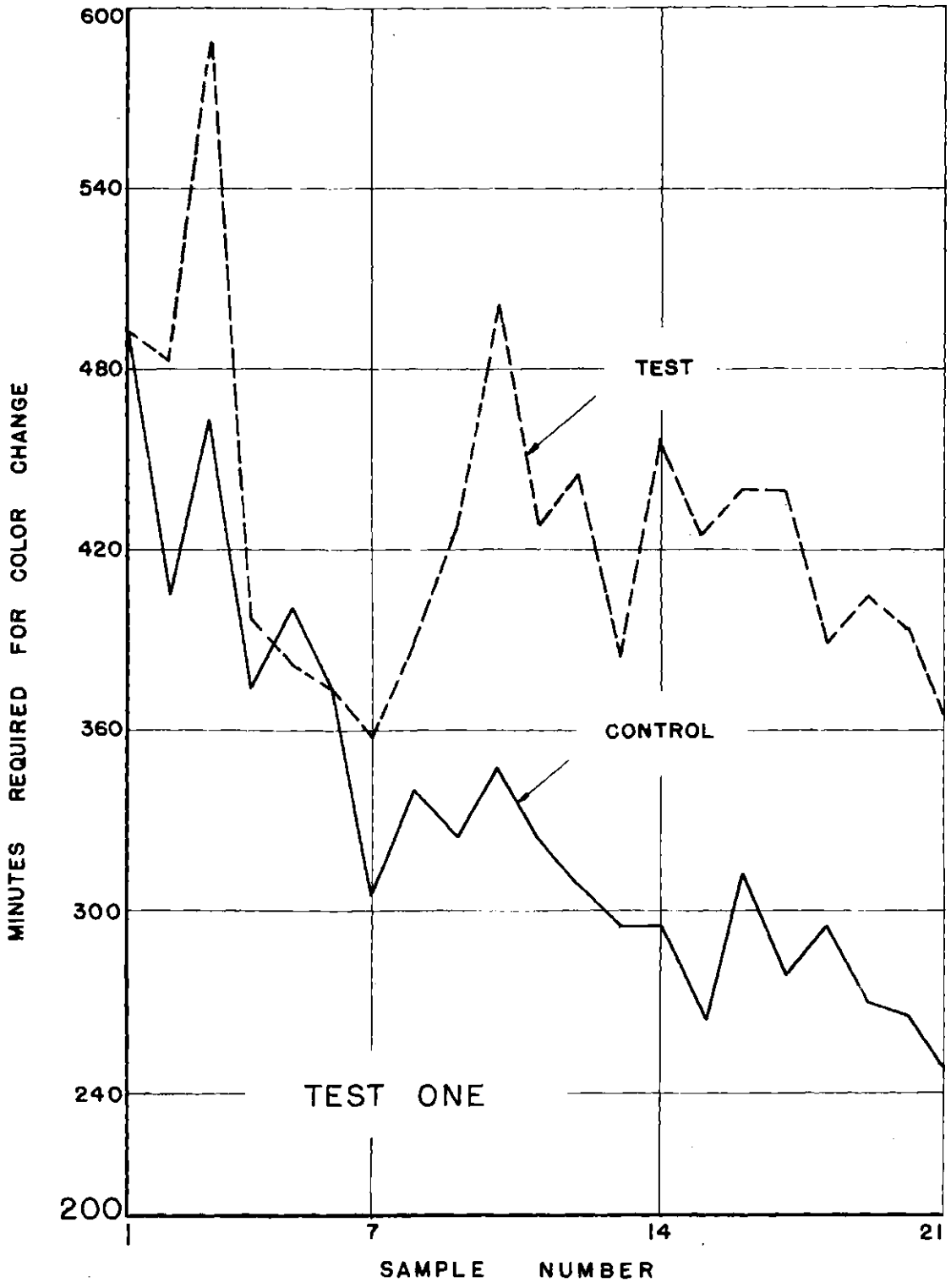

Figure 7. Comparison of Times for Sample Color Change -- Test One

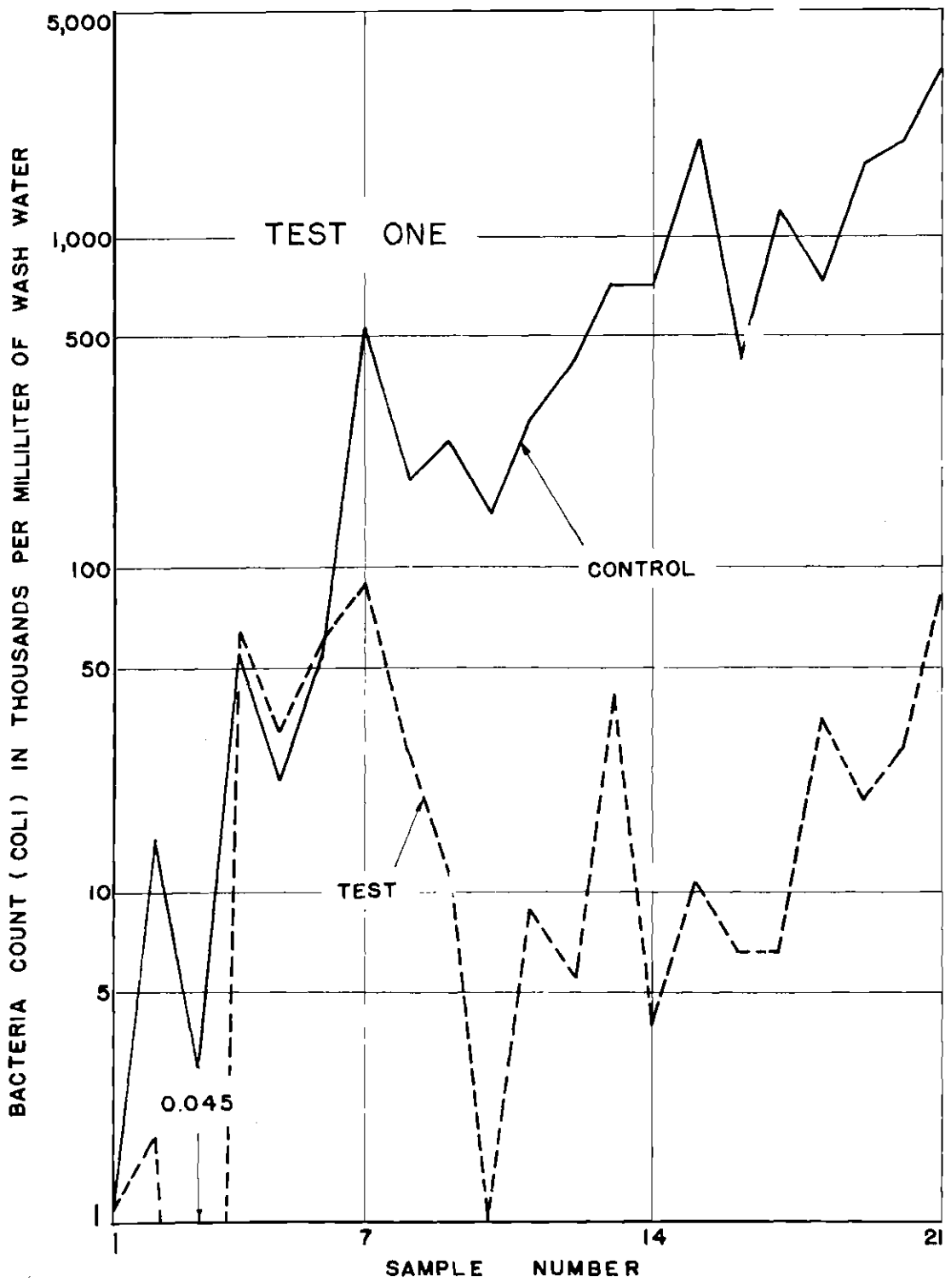

Figure 8. Comparison of Bacteria Counts -- Test One

when connected until the third cylinder was also found empty on the morning of July 12. On that morning it was observed that there was foam beneath the drain of the test box and there were drops of moisture on the stem of the third cylinder. The wet and dry bulb air temperatures recorded on the mornings when a cylinder was found empty were all between 78 and 80 degrees Fahrenheit. It was therefore reasoned: the regulator was acting as a refrigeration expansion valve; during the night when a condition of high humidity existed, moisture would condense on and in the regulator and freeze; ice forming inside the regulator would push upon its diaphram causing a greater flow of gas, which would cause more rapid formation of ice and exhaustion of the gas. Based upon this theory, a 100 watt light bulb was placed beneath the regulator to supply heat for the expansion of the gas and a needle valve was placed in the gas line to limit the maximum flow of gas into the test box. No further difficulty was experienced with the regulation of the flow of gas.

Beginning with control Sample 3 and every control sample thereafter, black spots were observed to be on the shrimp removed from the box. These spots were never observed to penetrate into the meat of the shrimp. The spots on the control Sample 19 may be seen in Figures 9 and 10. No spots were observed on any of the shrimp which had been exposed to the carbon dioxide atmosphere.

On July 19, the day of Samples 13, the odor of spoilage

Figure 9. Shrimp from Samples Number 19 (Control on Left)

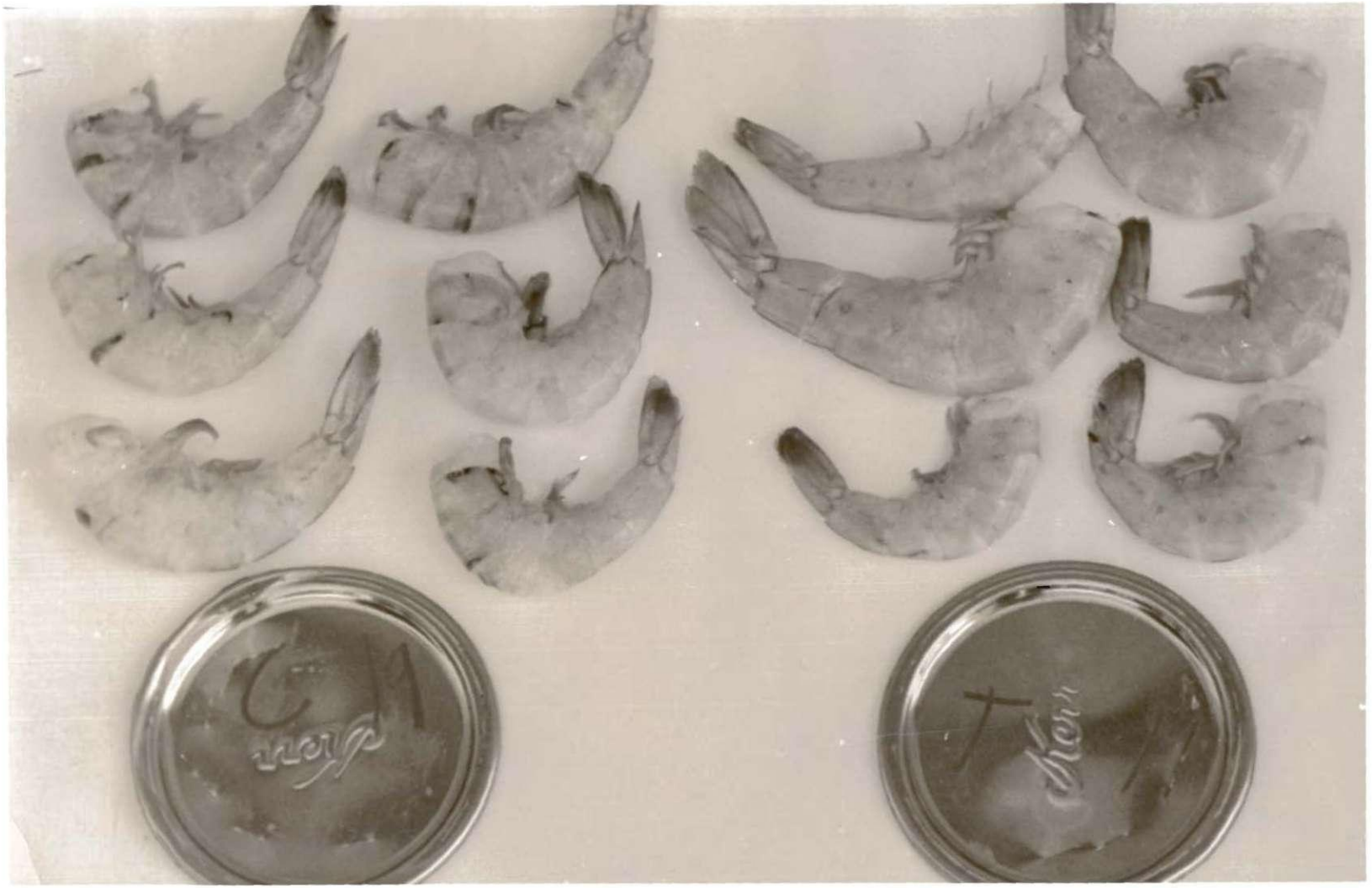

Figure 10. Reverse Side of Shrimp from Samples Number 19 (Control on Left)

was evident when the fish house was first opened. This odor was more in evidence on each succeeding morning. When Samples 14 were taken this odor was observed to be in the control box while there was no odor in the test box. Control Sample 15 was observed to have a strong odor which was decreased with washing, but the shrimp of this sample were still judged to be of poor commercial quality. Control Sample 18 was judged to be of questionable commercial quality. The shrimp of control Sample 20 had a coating of brown slime. When used as fishing bait control Sample 21 was of no value.

The routine comparison of Samples 13 revealed:

1. black spots on control shrimp, none on test.

2. characteristic shrimp odor on control shrimp, none on test.

3. shell of all six test shrimp soft and pliable very much like freshly molted shrimp, shell of all control shrimp tough.

4. meat of control shrimp had glossy opaque white color and texture of uncooked meat, all test shrimp meat had appearance of cotton.

In all later test samples the changes noted were observed to be progressively greater.

Once the difference in the shrimp from the two storage boxes was visually apparent, it was deemed necessary to gain more evidence of this difference through cooking and

tasting. The shrimp of Samples 14 were placed separately in two jars of boiling distilled water to which one level teaspoon of table salt had been added. They were allowed to cook for 20 minutes and then were drained, cooled and chilled. During the cooking foam was noted to form on the surface of the water in the jar containing the test shrimp. It was also noted that the test shrimp floated in the water while the control shrimp did not. For tasting, the shrimp of each sample were laid out separately on two paper towels with no identification. Three persons were separately asked to choose one shrimp from each towel, examine and taste them. All three identified the test shrimp and were unanimous in the items of difference noted. These were:

1. the test shrimp was all pink, it did not have the orange tint of the control shrimp.

2. the meat of the test shrimp was white and had a tough fibrous texture very much like cotton.

3. the test shrimp had less moisture than the control.

4. the test shrimp had very little flavor of any kind.

Samples 16 and 18 were also cooked and tasted with equal results.

The photograph which is Figure 11 was taken in an attempt to record the difference in texture found when the control and test shrimp were compared. This difference in the raw shrimp is almost too subtle for the camera. In Figure 11 it will be noted that the control shrimp stands out distinctly

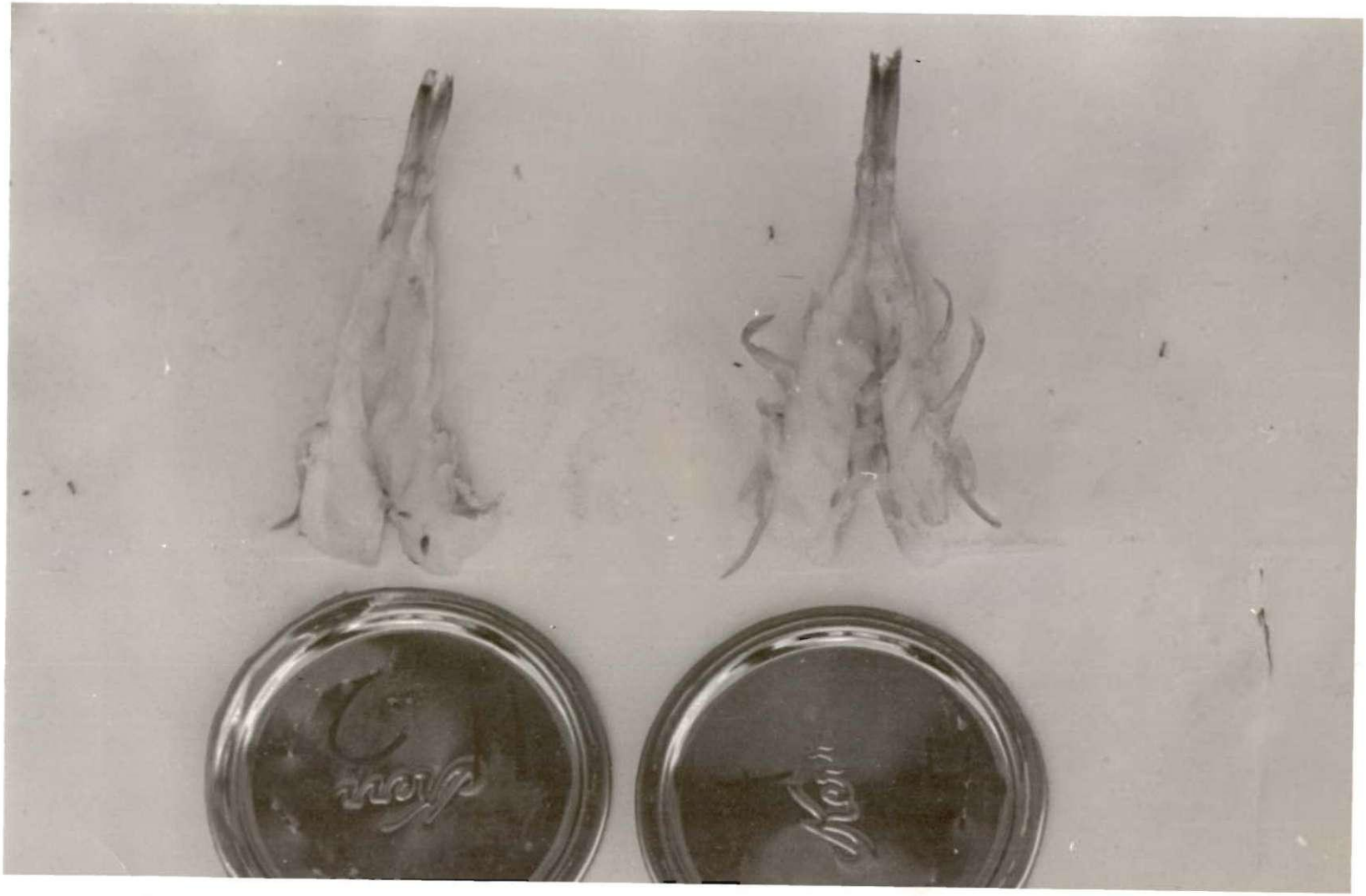

Figure 11. Meat from Shrimp from Samples Number 19 (Control on Left)

whereas the test shrimp tends to blend into the white background. The black area at the end of the test shrimp is part of its digestive tract, not the condition known as black spot.

Throughout the entire test period the daily test procedure developed in Chapter II produced the desired measures. After use, pipettes were rinsed in soapy water, rinsed with free flowing tap water, rinsed in commercially distilled water and wrapped in packages of two in kraft paper. As planned, pipettes were sterilized by placing them in the oven of a kitchen stove set at 300 degrees Fahrenheit for two hours.

Concurrent with the testing of Samples 4 and 11, duplicate tests were made using tubes of medium from the lot mixed and used in the calibration test. The times required for color change for these duplicate tests were the same as for the routine tests. In this manner the action of the lot of medium used for all daily tests was verified.

Only one additional fact was observed concerning the action of the inoculant in the medium. When the inoculant from test Samples 2 through 21 was first placed in the tubes of medium, a shade of yellow appeared immediately. After the tubes had been in the incubator for one hour most of this yellow color had disappeared. What yellow color did remain was concentrated in the lower half of the liquid. The top half of the liquid returned to the green color of the control

tubes and the green color standard. When such a tube was shaken to again mix the liquid, its color was that previously noted for the beginning of color change. However, this color would remain unchanged for several hours. When further change in such a tube did occur, it would occur concurrently with the two other tubes inoculated from the same sample.

The condition noted above made the beginning of the progressive color change difficult, if not impossible, to observe in so far as tubes inoculated from test samples were concerned. Since the complete color change indicates a change of pH from 7.0 to 6.0, the condition noted may be interpreted in terms of pH. The change to the yellow color, observed as the tubes were inoculated indicates a change of pH from 7.0 to about 6.5. The return of a portion of the green color after one hour indicates a rise in the pH. The fact that some of the yellow color remained indicates that the pH was higher than 6.5, but not 7.0. This means that growth conditions in the tubes containing test sample inoculant were slightly more acid than in the tubes containing control sample inoculant. Because of the magnitude of the bacterial action in the tubes of medium, it is felt that this condition did not affect the results of the testing to any great degree.

In Table 2 it will be noted that the times recorded for control and test Samples 4, 5 and 6 are nearly equal.

It will also be noted that a cylinder of gas exhausted into the test box during the night before each of these samples were taken. In Figure 7 it will be noted that, except for the first seven samples, the general slope of the two plots is approximately equal and the main difference is the spacing between them. This spacing indicates that the growth of bacteria on the test shrimp was delayed. The near equality of the times recorded for Samples 4, 5 and 6 indicates a sensitivity to the different rate of flow of gas into the test box. For this reason a second field test was made in order to determine whether or not this sensitivity could be reproduced.

CHAPTER IV

SECOND FIELD TEST OF SHRIMP

Storage Boxes and Equipment Used in Test.--In the test described in Chapter III there were three conditions of storage: air, intermittent flow of large quantities of gas, and continual flow of smaller quantities of gas. Three storage boxes were therefore necessary in the second test for the reproduction of these conditions.

In order to reduce the quantities of construction materials, shrimp, ice and gas necessary for the second test, smaller boxes were constructed. These boxes were similar in shape to the original boxes, but had approximate inside dimensions of 11 inches wide, 11 inches deep and 23 inches high. All surfaces of these three boxes were cut from a single 4 x 8 feet sheet of $\frac{1}{2}$ inch fir plywood. Waterproof glue and nails were used on all joints to insure against leakage. Construction of the inner partition was unnecessary in these boxes since they could be turned over and loaded face up. Two inches of styrofoam insulation was necessary on the outside surfaces to make up for the insulating effect of the large mass of ice in the original boxes. A comparison of the size and of the construction details of the two types of boxes is found in Figures 18 and 19 in the Appendix.

The boxes and equipment used in this second test are

shown in use in Figure 12. The boxes were labeled from left to right, Box C, Box B, Box A. Box C was the control box as may be seen by the fact that there is no tubing connecting it to the cylinder of gas. Box B had a continuous flow of a small quantity of gas. The manometer may be seen connected to the tubing leading to Box B. Conditions in Box B were intended to duplicate those existing in the test box during the last two weeks of the previous test. Box A had an intermittant flow of gas. Conditions in Box A were intended to duplicate those existing in the test box during the first few days of the previous test. As may also be seen in Figure 12, the ping-pong ball float valve drains were replaced by inserting a length of tubing in a jar of water. The regulator, cylinders of gas, coil of tubing and connections were the same as used previously.

The daily test procedure was altered in such a way that sampling of Box A was added to the procedure after sampling of the other two boxes. This addition of the third box meant that the water jar containing Sample A was shaken separately. A darkroom timer was used to insure consistent shaking of the jars. Pipettes were sterilized in packages of three. Wash water and tubes of medium used in the second test were from the same lot used previously.

Storage Procedure and Data Obtained.--Shrimp were obtained for this test on the afternoon of September 13. These shrimp were the first returned to port after tropical storm, Dora,

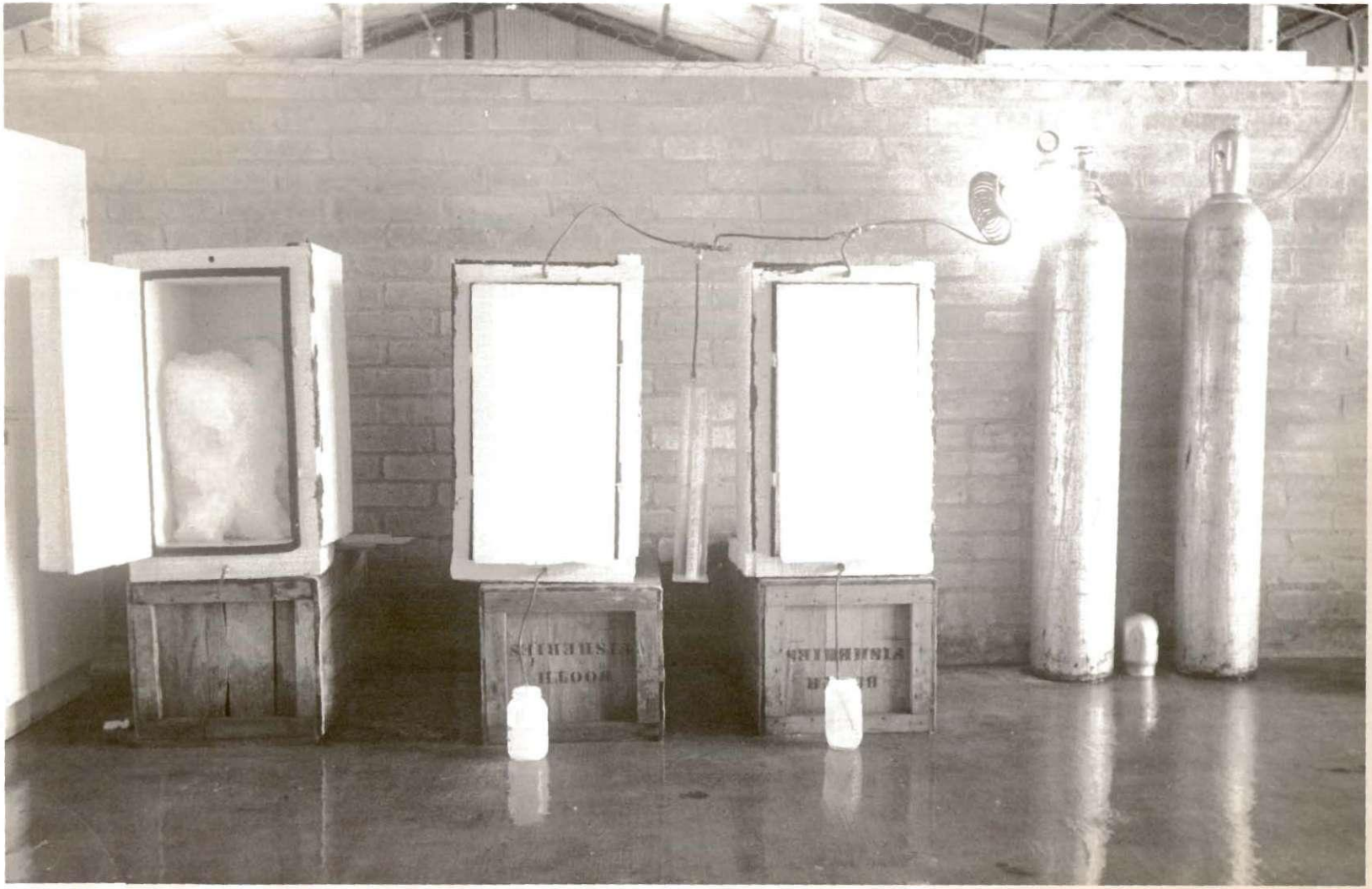

Figure 12. Storage Boxes and Equipment Used in Test Two

and were caught during that morning. They were stored on the boat in a normal commercial manner. With the storage boxes in place in the previous location in the fish house the shrimp were randomly separated into three ten pound lots and each lot placed in a box in the manner of the re-icing procedure found in the Appendix. Immediately after loading, at 5:00 p.m. the needle valves were opened slightly so that gas would flow into Boxes A and B. The pressure at Box B was adjusted to $\frac{1}{4}$ inch of water in order that it would receive a small steady flow of gas. After 15 minutes the flow of gas to Box A was stopped.

During the loading of the boxes Sample 1 was taken by choosing six shrimp at random before the lots were weighed. The next morning Sample 2 was taken from each box and the daily test procedure followed. Box B continued to receive a continuous flow of gas throughout this test. After the taking of Sample 2, Box A received a large amount of gas to an approximate pressure of three inches of water for one hour. Each morning after sampling and each afternoon thereafter Box A received this flow of gas. Data for this second test are presented in Table 4. Conversions of times to approximate bacteria counts are presented in Table 5. Plots of these data are found in Figures 13 and 14.

Discussion of Results and Observations.--The observations made for the previous test were found to apply equally in this second test. The condition of black spotting was noted

Table 4. Sample Record -- Test Two

Sample Number	Minutes Required for Color Change			Boxes Re-iced after Sampling	Gas Cylinder Changed
	Control	Test A	Test B		
1	525	-	-		
2	505	520	515		
3	480	530	530	C *	
4	400	445	475		
5	505	400	455		X
6	390	420	450	P **	
7	385	430	435		
8	365	440	440		

* "C" denotes complete re-icing, see Appendix

** "P" denotes partial re-icing, see Appendix

Table 5. Bacteria Count of Samples -- Test Two

Sample Number	Coli Conversion Bacteria Count in Thousands per Milliliter			Fieger Conversion Bacteria Count in Thousands per Milliliter		
	Control	Test A	Test B	Control	Test A	Test B
1	0.41	-	-	3.6	-	-
2	9.90	0.48	0.60	5.2	4.0	4.3
3	1.3	0.35	0.35	7.1	3.3	3.3
4	25	5.6	2.1	40	16	9.5
5	21	25	4.0	37	40	14
6	33	13	5.0	50	28	16
7	40	9.0	8.0	52	23	21
8	80	6.8	6.8	80	19	19

Note: To convert figures to approximate bacteria count per shrimp: multiply by 200 milliliters (the volume of wash water used) and divide by 6 (the number of shrimp in each sample).

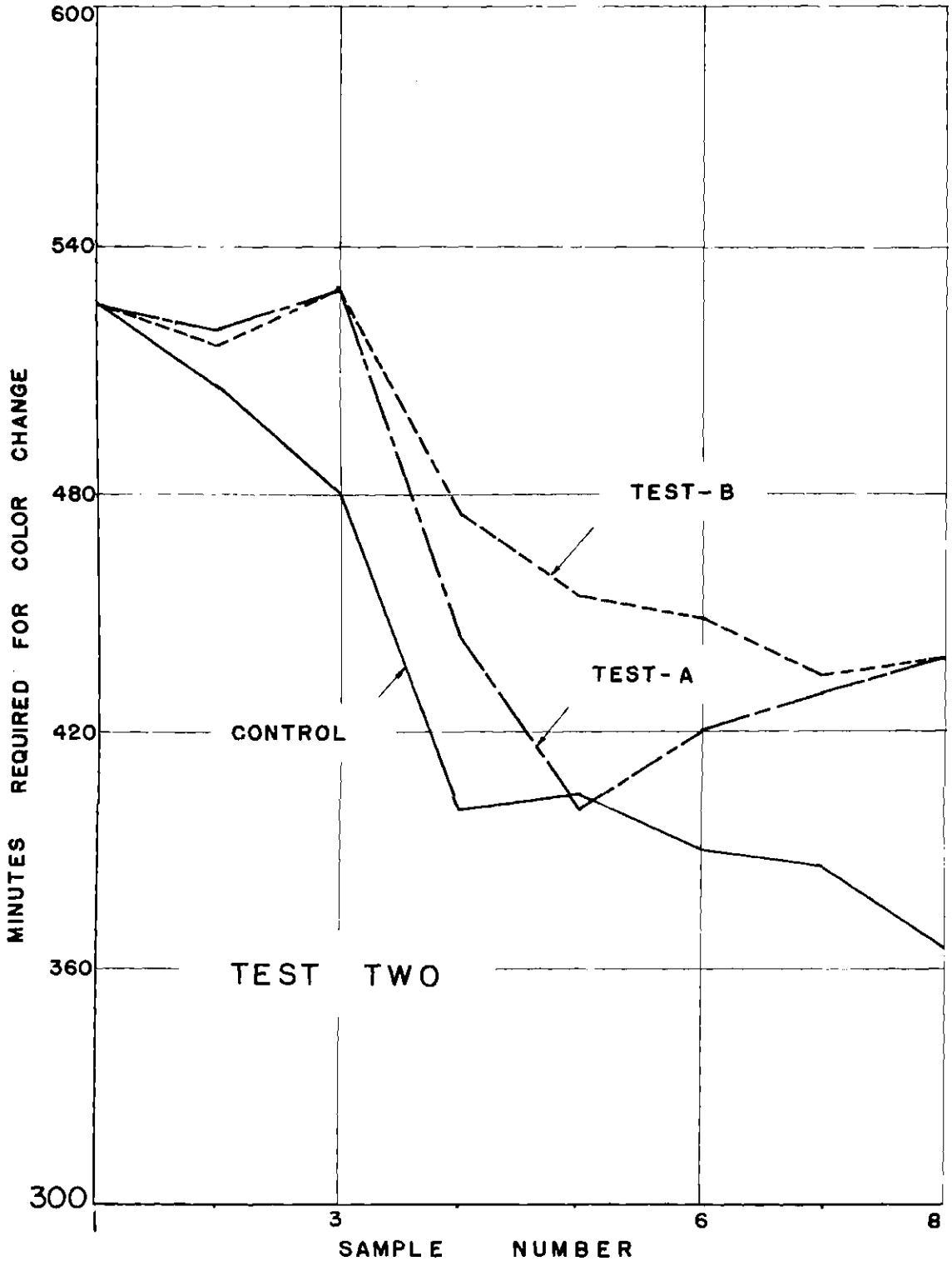

Figure 13. Comparison of Times for Sample Color Change -- Test Two

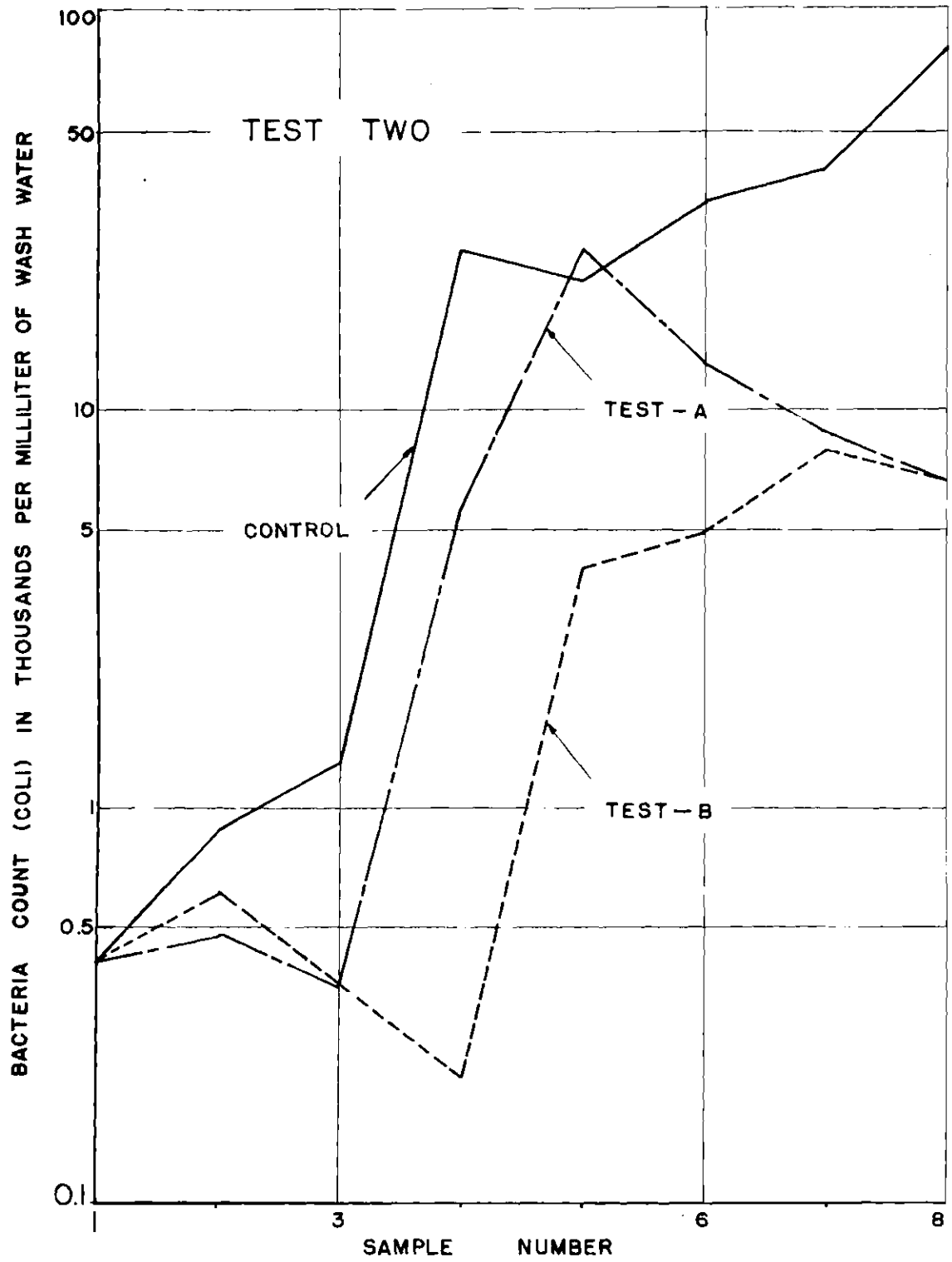

Figure 14. Comparison of Bacteria Counts -- Test Two

on the control samples. The initial change of color of the tubes of medium when inoculated from test samples was also noted as before, but was less intense for Box A samples than Box B samples. No softening of the shell or change of meat texture was noted in any of the uncooked samples.

After tubes of medium had been inoculated from Samples 2, the samples, still in their respective jars of wash water, were cooked by placing the jars in a pan of boiling water for approximately 15 minutes. Prior to cooking there was no apparent difference in the shrimp except for a few black spots on the control sample. After cooking the difference was visually apparent. The shrimp which had been exposed to carbon dioxide had none of the orange color of the control. Their shells were still hard, but the meat appeared to have the white texture of cotton.

A taste test was arranged by placing one shrimp from each sample unmarked on a paper towel. One of the persons who had tasted the shrimp in the previous test was asked to identify and taste the three shrimp. All three shrimp were correctly identified before tasting because of their color difference. Sample C, the control, was a shade of pink-orange. Sample B was a shade of almost pure pink. Sample A was a shade of lighter pink. Upon tasting the same change of texture and loss of flavor noted previously was observed in both samples of shrimp which had been exposed to carbon dioxide. Sample B had no taste or odor. Sample A, which

had been exposed to carbon dioxide for a total of only 15 minutes, had no taste or odor except that of iodoform. The other shrimp of Sample A lacked even the taste and odor of iodoform.

Throughout this second test samples of shrimp were cooked, compared and tasted as above and with similar results. After this second test had been discontinued a simple experiment was made. A fresh jar of wash water was opened. Carbon dioxide was allowed to bubble through this water for ten minutes. Six fresh shrimp were then placed in the jar. Gas was allowed to bubble through the shrimp and water for ten more minutes. Six additional shrimp were placed in another jar of water for comparison. No difference in the shrimp could be observed at this point. Both samples of shrimp were then cooked in the water in the jars. The shrimp which had been exposed to carbon dioxide for only ten minutes, when cooked, had changed in texture and color and had no flavor. Thus, mere exposure to carbon dioxide appears to be the cause of the changes noted.

CHAPTER V

COMPARISONS OF THE TWO FIELD TESTS

Comparison of Test Samples.--As has been stated previously, the second field test of shrimp storage was intended to be a reproduction of conditions existing during the simulated commercial storage of shrimp -- Test One. (For convenience the two tests will be hereafter called Test One and Test Two.) After Test One had been completed, but before Test Two was begun, there was some question as to whether or not the large quantities of gas exhausted into the test box during the first week of Test One had been the cause of the change in texture and flavor of the shrimp. The mere observance of this change made the commercial feasibility of use of a carbon dioxide atmosphere doubtful because of the difficulty of accurate regulation by operating personnel. Was the change caused by the amount of gas used or by the mere presence of the gas itself? It is this question that was to be answered by Test Two through replication.

There were two conditions of storage of the test shrimp during Test One: the first, an intermittent flow of large quantities of gas during the first week; and second, a continuous flow of small quantities of gas during the last two weeks. In Test Two an attempt was made to reproduce this first condition in Box A. Of special interest is the

fact that data obtained in Test One indicated a large rise in the number of bacteria on the shrimp while there was an intermittent flow of gas. (Samples 4, 5 and 6). A true reproduction of this condition in Test Two should have indicated a similar rise. In Figure 15 it will be noted that plotted data from the two tests do have similarities. In Figure 15 a rise in bacteria count is indicated by a shortening of the time required for color change since these two factors are inversely related. In both Test One and Test Two -- Box A the bacteria count reaches that of the respective control at Sample 5 (see Figures 7 and 13).

Data obtained during the last two weeks of Test One indicated a gradual rise in the number of bacteria on the shrimp over the entire period. In Test Two the reproduction of conditions during this period was intended to determine whether or not there would be a similar gradual rise in bacteria if the condition of continuous flow of gas had existed during the first week of storage in Test One. If such were the case a plot of data from Test Two - Box B and the last two weeks of Test One should define an average line of almost constant slope. In Figure 16 this comparison is made and found to be approximately correct.

Comparison of Control Samples.--Of all conditions that existed in Test One the conditions of the control box were more nearly reproduced in Test Two. In the case of the controls, the difference in the shrimp stored and the difference in

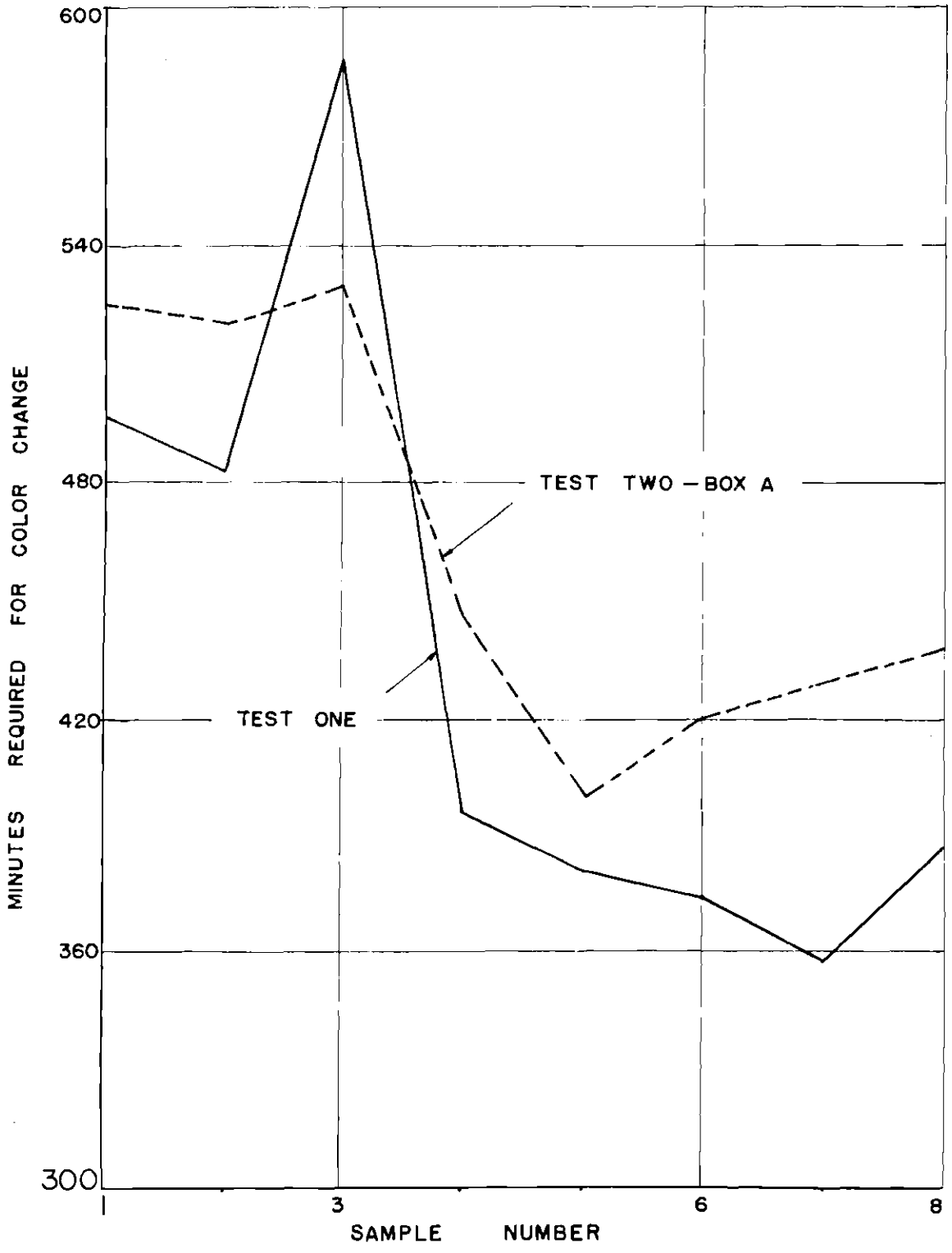

Figure 15. Comparison of Test One and Test Two -- Box A Samples

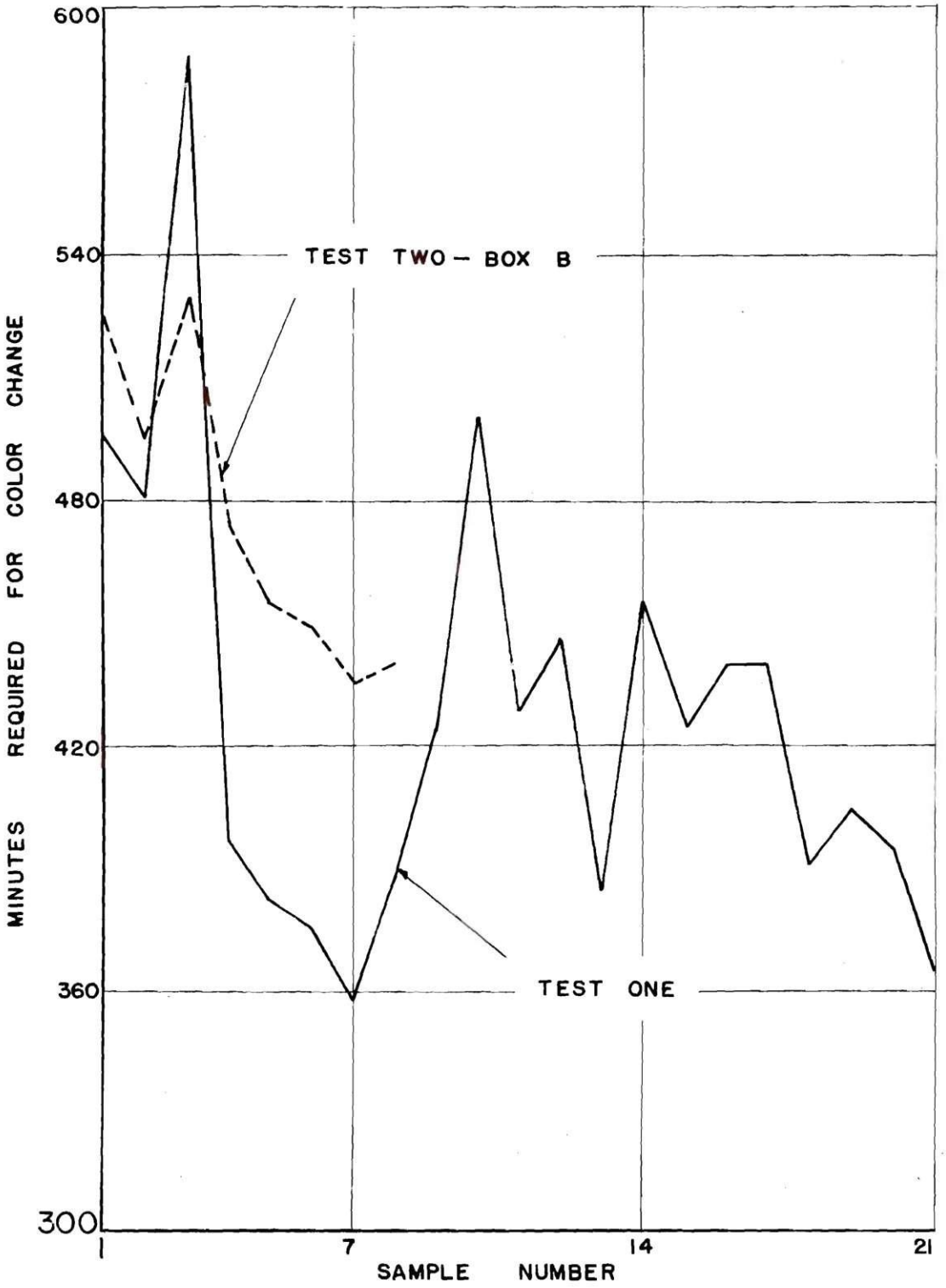

Figure 16. Comparison of Test One and Test Two -- Box B Samples

re-icing were the main factors which could cause variation in the data obtained for the two tests. In Figure 17 it will be noted that the plot of Test Two data begins above that for Test One. Since the shrimp used in Test Two were stored in ice for several hours and as a result were washed by the melting ice, the difference is an apparent true indication of condition of the shrimp.

In Figure 17 it will also be noted that the plot of the value for control Sample 2 - Test One is below average. Upon reference to Table 2 it will be noted that the storage boxes were partially re-iced on the day prior to the taking of Sample 2. Thus the storage box, prior to the taking of Sample 2, contained a large mass of ice which had a slow melting rate and allowed an increase in the number of bacteria on the shrimp because of decreased washing. This is correctly indicated in Figure 17 by a reduction in the time required for color change. Similarly there is a reduction in the time required for color change after each re-icing. By comparison, there was no re-icing in Test Two prior to Sample 2 because in Test Two the boxes had not been exposed to the sun and wind on the deck of a boat. Thus, there may be an accounting for differences in the plots shown in Figure 17.

Here may also be an accounting for similarities in the two plots. It will be noted that in both tests the storage boxes were re-iced prior to the taking of Samples 4.

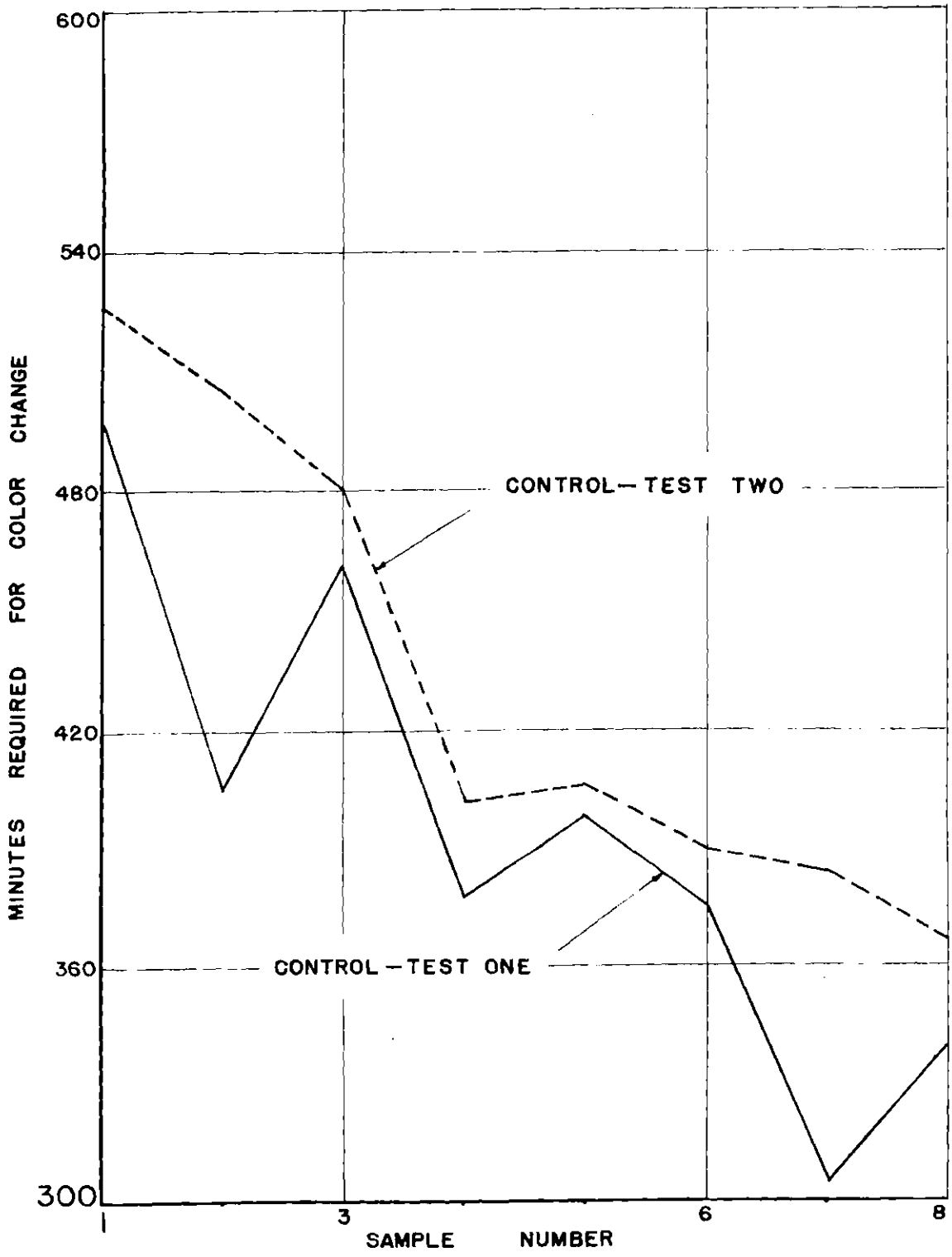

Figure 17. Comparison of Control Samples

Again the results of the treatment of the stored shrimp is reflected in Figure 17. More than anything else, these comparisons reflect the sensitivity to change of the method of measure.

Discussion of Reliability of Medium and Test Procedure.--It has been mentioned that a duplicate test was made on two separate occasions during Test One. A similar duplicate test was made during Test Two with control Sample 3. In these duplicate tests a different lot of medium was used. The fact that all of the duplicate tests reflected the same action as their respective routine tests indicates that the acid production medium is not materially affected by several months of age.

Throughout both periods of testing no problem was presented by contamination of the tubes of medium. On September 15 the single instance of contamination was observed. On that date a small spot of what appeared to be mould was found at the liquid surface on one side of a tube of medium which had been prepared on May 8 with those used in the calibration test. There had been no color change in this tube, nor was a change of color observed in any of the other tubes of medium stored.

In the daily test procedure used throughout both the periods of testing the step of agitation of the samples in the wash jars held the greatest possibility of producing an error in the results. For that reason extreme care was used

in the performance of this operation as had been specified. Throughout both of the testing periods the same person performed this operation. had it been necessary for different persons to shake the jars an additional error would have undoubtedly been introduced. Had such been the case, a mechanical shaking with a device such as used to mix cans of paint would have probably been recommended.

The acceptable sensitivity of the method of measure used in this experimentation has been mentioned. The fact that such sensitivity was observed and could be related to known causes justifies the assumption that the daily test procedure produced a reliable measure of bacterial activity on the shrimp stored during both the periods of testing.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

Conclusions.--The change of taste and texture of shrimp found to be caused by carbon dioxide precludes the commercial use of a carbon dioxide atmosphere for the preservation of stored wet shrimp. The presence of such an atmosphere around wet stored shrimp does, however, delay bacterial action for several days. It may be inferred that black spots on shrimp are caused, at least in part, by some component in air since shrimp stored in a carbon dioxide atmosphere developed no black spots.

The acid production medium developed by Novak, Fieger and Bailey (29) together with the testing procedure developed for the experimentation described here provide a reliable, satisfactory field measure of bacterial activity on shrimp.

Recommendations.--Any attempt at commercial use of a carbon dioxide atmosphere for wet shrimp storage should be seriously reconsidered because of the risk of loss of shrimp so stored. Other means of preservation of shrimp at sea should be emphasized in future research.

A standardized commercial testing procedure should be developed from the daily test procedure used in this experimentation. A commercial user of this test would require: an instruction booklet, a small incubator, a mechanical

vibrator for uniform agitation of samples, sealed tubes of laboratory prepared acid production medium, sealed jars of laboratory prepared water, and individually packaged sterile disposable pipettes. For extended use, tables of calibration could be published. Entry to these tables would be gained when the time required for color change and the degree of prior washing were known. From such a table an approximate degree of spoilage could be established. It is therefore recommended that this use of the acid production medium developed by Novak, Fieger and Bailey be called to the attention of the appropriate agencies for their consideration as a means of establishing quality control measures for shrimp and seafood products.

APPENDIX

DESCRIPTION OF WATER BATH INCUBATOR

Two items were purchased from a tropical fish supply store for the incubator. These were a $2\frac{1}{2}$ gallon rectangular aquarium and a 50 watt thermostatically operated heater. A cover for the aquarium was made from a sheet of plexiglas. At the front of this cover two rows of eight holes were drilled in such a way that 20 by 150 mm. test tubes placed in the holes would be 3/4 inches apart and at least one inch from the sides of the aquarium. A frame of aluminum sheet was bent and attached beneath the holes as a support for test tubes. This frame allowed the top of test tubes to extend about $\frac{1}{2}$ inch above the cover. Thus, the incubator was merely a tank of water maintained at the desired temperature with a simple test tube rack arranged in such a way that the test tubes could be viewed without removal.

When purchased the heater thermostat was set at 75 degrees Fahrenheit. This was reset at 95 degrees by filling the aquarium with 95 degree water and moving the adjustment screw until the heater turned off. At this setting the heater was turned on when the water temperature was about 94 degrees and was turned off when the water temperature was about 96 degrees. With the heater placed at the back of the aquarium behind a baffle, it was found that there was a ten degree difference in temperature in some locations in the aquarium.

To eliminate this temperature difference an agitator was made from a small electric motor and a plastic model boat propeller. This was placed near the heater so as to move water from the heater toward the test tubes. An aluminum baffle, painted white, was suspended between the heater and the test tubes to control the circulation of water and to provide a consistent background for the viewing of color changes.

The arrangement of the incubator proved entirely satisfactory throughout all the tests. Once the thermostat had been set no further adjustment was made or found necessary. Test tubes were easily placed in and removed from the incubator. Viewing of the test tubes during incubation was easy because of the glass sides of the aquarium.

FORMULA OF MEDIUM USED IN TESTS ONE AND TWO

Tryptone	5.5 grams
Proteose Peptone	11.0 gm.
Bacto-Beef Extract	2.2 gm.
Dextrose	3.3 gm.
Lactose	11.0 gm.
Maltose	11.0 gm.
Yeast Extract	1.1 gm.
Sodium Chloride	11.0 gm.
Bromthymol Blue	0.11 gm.
Distilled Water	2.2 liters

Note: The ingredients listed are those found in the formula for the acid production medium developed by Novak, Fieger and Bailey (29). The amounts of the ingredients shown are 22 times those published by the developers.

RE-ICING PROCEDURE

Test One, Complete Re-icing.--

1. When the height of the center of the mass of shrimp and ice in the boxes falls below 11 inches, open the control box and remove the inner partitions.

2. Remove the top half of the shrimp and ice contained in the box and place in a clean wash tub.

3. Remove the bottom half of the shrimp and ice and place in a second wash tub.

4. Carefully fill both wash tubs with water with a minimum of agitation to melt away the large pieces of ice from the shrimp.

5. Drain the wash tubs and remove small pieces of ice.

6. Place one of the boards in the partition and fill the bottom of the box with freshly crushed ice to a depth of about 12 inches.

7. With the hands, push ice to the sides of the box to make a bowl shaped depression about four inches deep and 16 inches in diameter.

8. Place two handfuls of shrimp from the second wash tub (the shrimp which were originally on the bottom of the box) in the depression.

9. Add one shovelful of ice on top of the shrimp and

mix so that ice is dispersed around the shrimp. Take care that the shrimp are kept away from the surfaces of the box.

10. Reform a bowl shaped depression and continue to place shrimp and ice in the box as above. Add additional partition boards as required. When all the shrimp are replaced in the box, the top shrimp should be about eight inches from the top of the box. Each shrimp should be replaced in about the same relative position in the box.

11. Fill the remainder of the top of the box with ice. About 600 pounds of ice are needed for this re-icing.

12. After the control box is re-iced, rinse the wash tubs and repeat the above for the test box.

Test One, Partial Re-icing.--Between each complete re-icing of the boxes it was possible to add ice to the top of the box. Each partial re-icing was made when the center of the mass of shrimp and ice was located about 18 inches above the bottom of the box. At that time ice had also melted away from the sides of the box for a distance of about three inches. Approximately 300 pounds of ice were required to fill the sides and top of each box. Had partial re-icing been repeated without complete re-icing, all of the shrimp would have eventually been located at the bottom of the boxes.

Test Two.--The same methods of re-icing described above were used in Test Two, except that the boxes were turned face up during loading and the pieces of ice removed from the boxes were broken away from the shrimp by hand instead of melting

in the wash tubs. Approximately 70 pounds of ice were required to completely re-ice each box in this test. Half that amount was required for partial re-icing.

Figure 18. Comparison of Size of Boxes Used in Tests

Figure 19. Comparison of Inner Construction of Storage Boxes

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