# THE DEVELOPMENT OF A PASTEURIZATION PROCESS FOR READY TO SERVE REFRIGERATED ROCK CRAB (Cancer irroratus) MEAT

CENTRE FOR NEWFOUNDLAND STUDIES

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# THE DEVELOPMENT OF A PASTEURIZATION PROCESS FOR READY TO SERVE REFRIGERATED ROCK CRAB (*Cancer irroratus*) MEAT

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A thesis submitted to the Faculty of Graduate Studies in partial fulfillment of the requirements of the degree of Masters of Science.

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## ABSTRACT

A pasteurization process for rock crab (*Cancer irroratus*) meat was developed for an 8 oz plastic container with an aluminum pull top lid. A refrigerated shelf life greater than 6 months was required maintaining good nutritional and microbial quality.

The optimal process for the ES308 plastic container, supplied by Kings Plastic, with a double seemed pull top aluminum lid, was found to be 130 minutes at 83°C. This process was developed for a fill weight of 180g of crab meat and 47 g of 3% brine. The slowest heating spot within the container was found to occur at a point 3.0 cm below the aluminum pull top lid. Thermal penetration studies revealed the following heating and cooling parameters for this container and meat: $j_h = 1.69$ ($\pm 0.04$), $f_h = 34.74$ ($\pm 1.04$), $j_c = 1.56$ ($\pm 0.03$), $f_c = 50.53$ ($\pm 1.73$)

The 83.0 °C upper temperature processing limit, for this plastic container, reduced the possible processing times. Reduced processing temperatures, from 83.0 °C, caused disproportionally great increases in processing times which further resulted in increases of energy expenditure per container produced. The process 130 minutes at 83.0 °C was found to be optimal from an energy expenditure point of view for this reason.

Nutritional quality did not show significant changes apart from some reductions in fatty acid content. The microbial quality from this process was shown to be more than adequate as the shelf life obtained was in excess of 12 months when held consistently at 2.2°C. The shelf life was dramatically lowered with increases in storage temperature (above 4.0°C) and with abusive handling or inadequate sealing.

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# 1.0 INTRODUCTION

Crab pasteurization follows a trend in the seafood industry toward the development of high priced, high quality secondary processed products utilizing some type of unique packaging style which aids in preservation. A great deal of product development and marketing effort have been accomplished in the United States with the sale of Pasteurized Blue Crab. Pasteurized Blue Crab has a refrigerated shelf life in excess of 6 months. Little has been done in Canada to create similar products for sale as a competitor.

Another trend in seafood processing prevalent today is the need to create marketable products from underutilized fish species. The term "underutilized" implies that the material is not traditionally profitable. Rock Crab is one such species which has a taste and texture similar to Blue Crab and is available in relative abundance in the Maritime area. It follows, then, that it is possible to create pasteurized products from this raw material which would be competitive and alternative to the pasteurized Blue Crab products.

Pasteurized Blue Crab has been packaged in many different container styles such as (Schellekens, 1996; Gates, *et al,* 1993; Ward, *et al,* 1984):

- two and three piece double seamed cans of aluminum and steel
- barrier and non-barrier pouches
- plastic containers with double seamed aluminum pull-top lids

This work involved the use of the plastic container with aluminum pull top lid as this form of packaging provides a product with a better sensory and microbial quality and longer shelf life than that obtainable in steel.

The purpose of this work was to prepare a commercial pasteurization process for Rock Crab (*Cancer irroratus*) in an 8 oz polypropylene container with a double seamed aluminum pull top lid. The product is to have at least a six month shelf life under refrigerated conditions (3°C or lower).

## 2.0 LITERATURE REVIEW

### 2.1 Rock Crab Processing and Handling Background:

In order to better understand the levels and types of bacteria most prevalent in pasteurizing rock crab, and other concerns, it is important to know where the rock crab is caught, how the live crab is stored until landing at the dockside, how the crab is processed and under what conditions any extracted meats are stored. The following is a discussion of the several important points about the rock crab (*Cancer irroratus*) production process as they relate to pasteurization or canning.

Figure 1: A typical rock crab found in the Maritime Gulf area (Atlantic Canada).

The rock crab, as with all crabs, is a decapod crustacean (has 10 legs and a shell). It is usually yellow-brown in color with a pale yellow underside. It can have an overlay of small purple or crimson spots on the carapace. The carapace is characterized by nine,

shallow, smooth edged marginal teeth at the widest part and is usually no larger than about 140 mm (Williams and Wahle, 1992). Rock crabs are usually no heavier than 0.25 kg (Underwater World, DFO Canada, 1985).

### 2.1.1 Habitat of Rock Crab:

Rock crabs are found from Labrador on the Canadian coast to the shores of South Carolina in the US. They are usually found in water of no greater depth than 600 meters. They are most commonly found in shallow waters, such as bays, and on open sand or sand-mud bottoms (Underwater World, DFO Canada, 1985; Williams and Wahle, 1992). Rock Crab for this work was obtained in the Gulf of St. Lawrence around the Miramashee area of New Brunswick, Canada.

### 2.1.2 Harvesting Rock Crab:

The rock crab has traditionally been a by-catch of the lobster fishery. New crab traps which specifically work on crab, and not lobster, have now been developed that allow a more directed rock crab fishery (Underwater World, DFO Canada, 1985).

Small fishing boats, usually less than 65 feet in length, take traps to the various fishing areas and lower the modified traps to the sea floor. A rope and buoy marker is attached to each trap. Each trap is baited with a cheap fish source, such as mackerel or waste fish. After an appropriate time is given for the crabs to react to the bait and enter the trap, each trap is retrieved using the rope and marker. The trap is opened and the crabs are removed and stored in plastic fish pans. Ice is layered on the crab to reduce their metabolism and prolong their life out of water. Crab can not be further processed after

death and must be discarded, therefore every effort is made to ensure the crabs are alive at the dock.

### 2.1.3 Processing Rock Crab:

Live rock crab are brought to a plant and are processed according to the following steps:

1. Liveliness testing and grading.
2. Butchering.
3. Cooking and cooling.
4. Meat Separation
5. Packing
6. Storing, including preservation means.

Figure 2 shows a graphical representation of these steps.

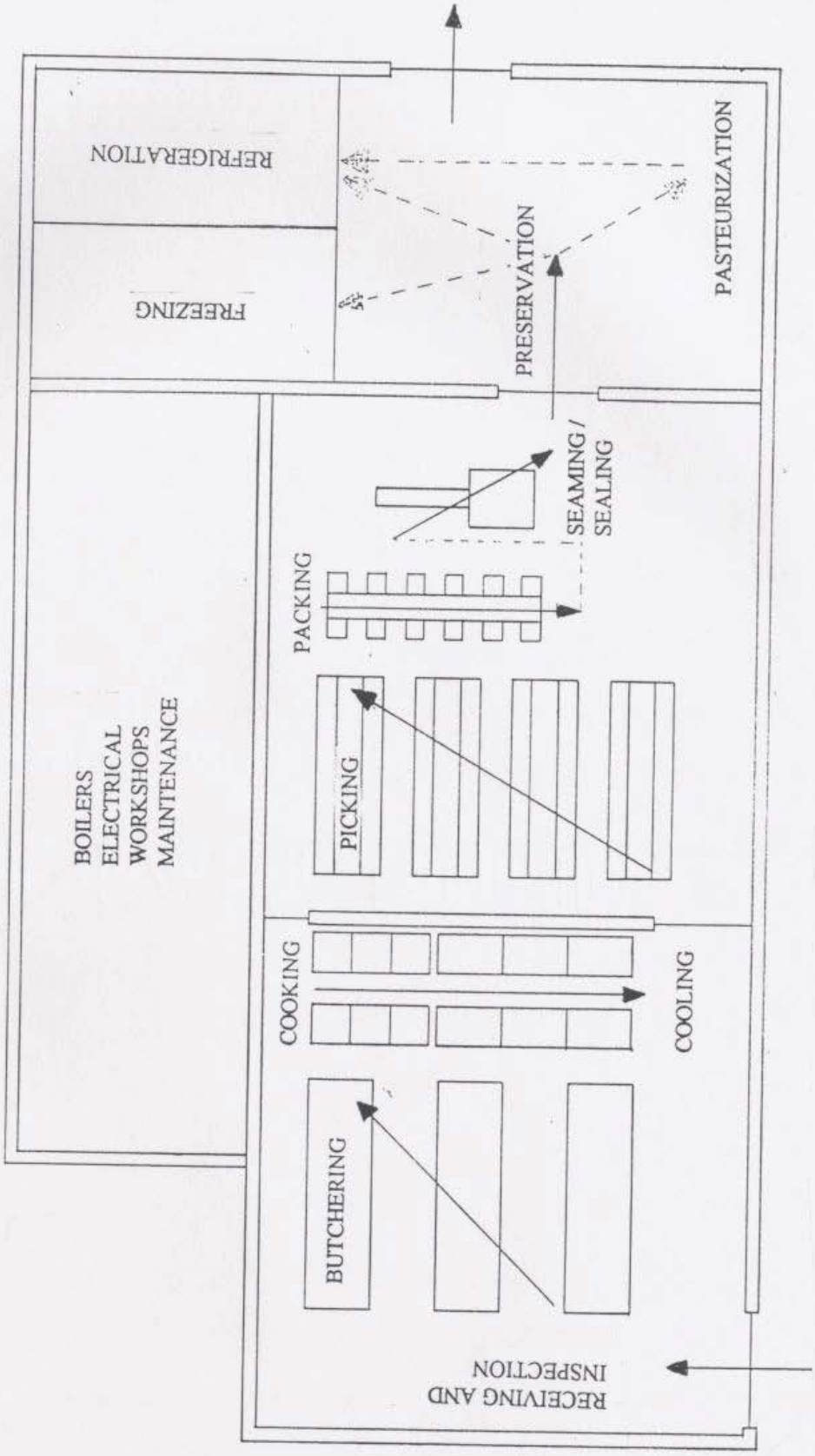


Figure 2: Sample plant layout showing the direction of raw material flow and potential location of unit operations.

#### 2.1.3.1 Liveliness Testing of Crab:

Crab must be live just before it is processed to avoid spoiled meat and extremes of bacteria load (Botta, 1994). Most complex living organisms have a natural immune system which reduces the likelyhood of pathogens growing within the flesh. Faghri (1983) showed that indicator bacteria was not detected in the blood of rock crab nor the muscle tissue after exposure but was detected after the death of the crab. If the crab is live just before it is processed, some insurance against the presence of large numbers of pathogens, and subsequently toxogenic compounds created by those pathogens, will be prevalent.

Most liveliness testing is done by examining responses to external stimuli such as by prodding the crab with a finger, by observing the height at which the main claws are held as the crab is elevated, or by electric shock. (Botta, 1994). Crabs are classified into dead, critically weak, weak, and lively categories (Botta *et al*, 1993). A statistical sample of the crab load is usually taken to determine the liveliness of the whole load. Tolerances for the number of dead crab are very low.

### 2.1.3.2 Butchering Crab:

Figure 3 shows a crab butchering line typically used for rock crab processing. The essential components of the butchering machine are the butchering pin and the roller brushes.

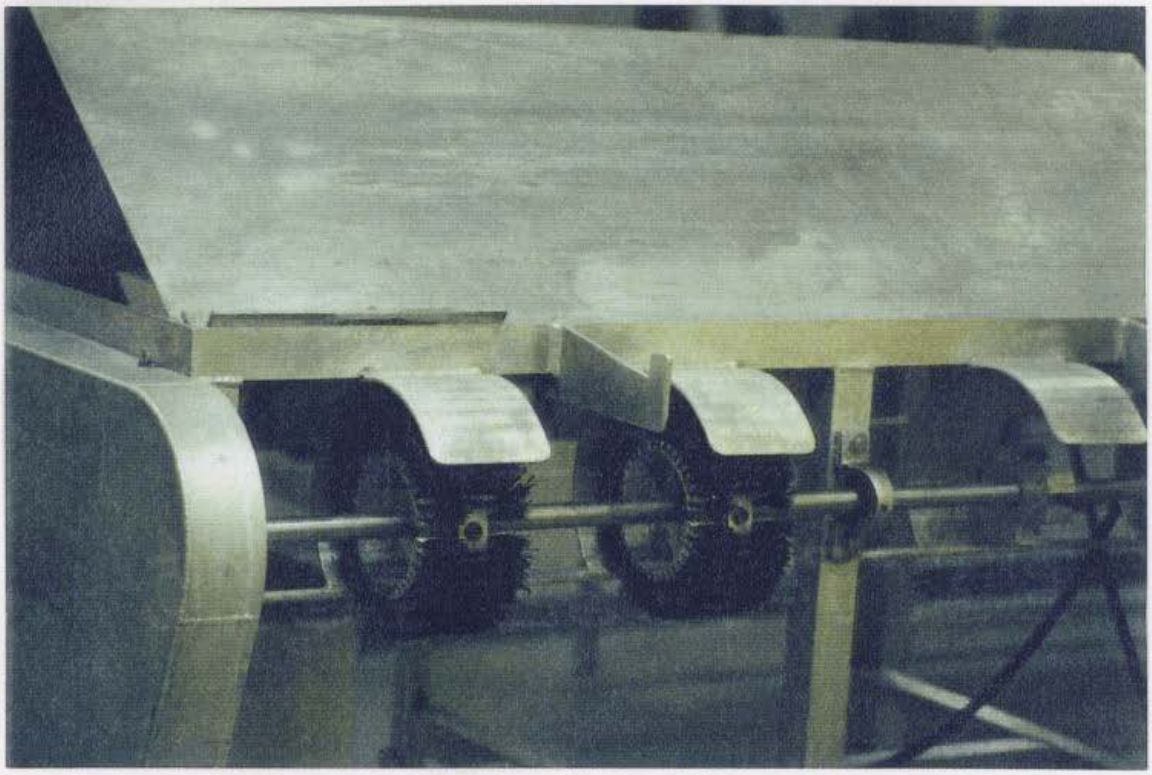

Figure 3: A butchering station typical of the industry

Butchering is a manual process which is accomplished as follows:

- The crab is grasped firmly by the legs with its head away from the butcher's body and the crab's bottom toward the ground. The butcher's left hand grasps the left hand side legs of the crab and the butcher's right hand grasps the right hand side legs of the crab.
- The crab is quickly smashed down over the butchering pin at the location of the crab's head (dead center), thereby popping off the crab's carapace (top shell) and separating the crab into a left and right hand "section".

- The left and right sections of the crab are then quickly pressed into the rotating roller brushes to clean off any gut, liver and gill which may be present.
- The crab sections are then thrown into cooking baskets, or onto a continuous cooking system conveyor, and are immediately cooked leaving little time for enzyme or bacteria activity.

The above methodology shows that, for the most part, the internal parts of the crab section (1/2 crab with gut removed after the butchering process) are not contacted during the butchering process. The external parts of the crab section, however, are coated with microorganisms residing in the digestive tract of the crab and on the cleaning rollers. Most of these microorganisms would be marine based.

### 2.1.3.3 Cooking Crab Sections:

Figures 4 and 5 show a batch cooker and batch cooler that are typically used for cooking and cooling rock crab. The cooker is comprised of a stainless steel vessel holding boiling water which is maintained at a boil through direct saturated steam injection at the vessels base. Steam injection is accomplished through a steam spreader bar to protect crab from direct steam impingement. The cooler is a large fiberglass container with circulated pre-chilled cooling water. Circulation is accomplished by maintaining a steady flow of cooling water to the tank via submerged pipes.

Figure 4: Stainless steel cookers used for cooking rock crab. Heating is accomplished through direct saturated steam injection at the cooker's base through a steam spreader.

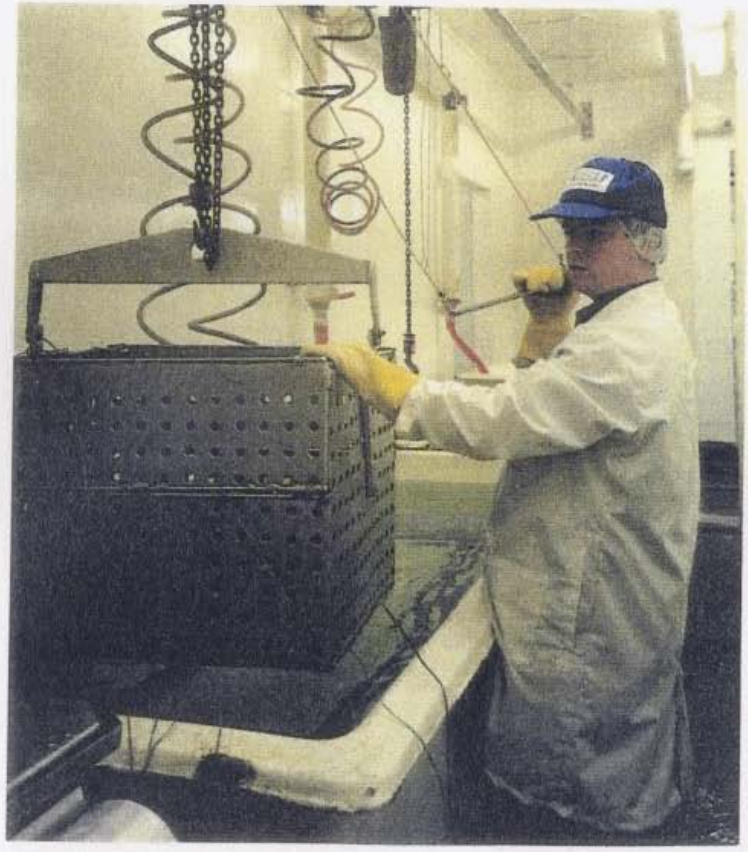

Figure 5: The cooling unit used for rock crab processing. Note the circulation pipes at the bottom of the tank.

Butchered crab shoulders are placed into cooking baskets, see Figure 5, and are lowered into the boiling water. When the water boils again, after a period of "come up", a timer is started. After approximately 10 minutes, a time which varies from plant to plant and cooking system, the cooked crab is removed from the boiling water and is placed directly into ice water. The internal temperature of the crab shoulder will have approached 85°C at the end of the cooking time and the crab shell will have turned to a reddish color.

Cooling takes place in the cooling tank which is constantly overflowing with chilled water at about 0°C. The temperature of the crab shoulder is reduced as rapidly as possible to the chilled water temperature. The rapidity of internal temperature reduction helps to prevent sticking of meat to the shell.

This cooking process subjects the bacteria placed on the surface by the butchering process, to 100°C water for 10 minutes. This cooking process is adequate to destroy most vegetative forms of pathogen pertinent to pasteurization. That the internal temperature reaches 85°C further indicates that any microorganism allowed into the center of the crab section will be controlled as well.

#### 2.1.3.4 Meat Separation from Cooked Sections:

At this point in time all of the meat separation of rock crab is done manually. Meat is usually separated into two distinct components: the meat coming from legs (leg meat) and the meat coming from the rest of the crab (salad meat). Leg meat usually has the higher value because it occurs in larger chunks with better color and flavor. The salad meat, although it is also flavorful when prepared manually, occurs in smaller pieces with a white or off white color. Salad meat is also more difficult to remove and, as such, has a higher incidence of cartilage or shell.

Both leg meat and salad meat is removed by hand so is therefore in contact with the human operator giving the potential for microbial contamination. The largest probability of contamination to the pre-pasteurized meat comes at this stage, depending on the sanitation and hygiene systems of the plant. Any bacteria present on extraction equipment and plant personnel can be transferred to the meat which provides the potential for contamination from many sources. Land based bacteria are most likely transferred at this point.

#### 2.1.3.5 Packing and/or Storing the Extracted Meat:

After the meat has been extracted it is either immediately packed into containers for pasteurization or is vacuum packed into 4 mil polypropylene bags, blast frozen, and mastered for later pasteurization or other use. Storage conditions for frozen meats are usually -20 to -30°C. Bacteria present in the frozen packages will not grow under these conditions.

Packages which have been immediately packed into pasteurizing containers are to be pasteurized within one hour of lid seaming to prevent the buildup of microorganisms within the container.

#### 2.1.3.6 Effect of Pre-Pasteurization Crab Handling on Pasteurization Effectiveness:

The overall effect of crab handling is crucial when considering the method by which pathogenic bacteria are introduced to the pasteurized crab food system. The initial cooking of the crab and the initial antiseptic nature of the live crab suggests that contamination will occur sometime between the butchering stage and the packaging stage of the process, just before pasteurization. If nonsanitary contact after cooking is allowed to occur, any number and type of bacteria may be introduced. Strict sanitation and hygiene will ensure a reduced level of bacterial activity. This work assumes that the worst case has occurred where control is necessary over psychrophillic and psychrotrophic bacteria which have been introduced by nonsanitary contact that are resistant to pasteurization heat treatments.

## 2.2 Thermobacteriology:

Pasteurization is a process which destroys certain microorganisms through the use of moist lethal heat. In order to analyze the effect of this heat on microorganisms, it is important to understand some basic concepts.

### 2.2.1 Factors Effecting Microbial Growth in Foods:

There are several factors which effect the growth of microorganisms in foods (Stumbo, 1973, Pelkzar et al, 1986) , in general:

- the substrate;
- the temperature;
- the pH;
- the available water or water activity;
- the presence or absence of oxygen;
- chemicals (ie. antibiotics)
- the oxidation/reduction potential.

#### 2.2.1.1 The Substrate:

The substrate, or food product itself, will permit growth of microorganisms accustomed to it as food source. Microorganisms having no food cannot function and will therefore not grow. Crab is high in protein with relatively low amounts of fat and little, if any carbohydrates (Krzeczkowski and Stone, 1974). Microorganisms must be largely proteolytic, capable of utilizing proteins for metabolism, to grow in crab meat. Fat, which can protect microorganisms from heat, is not present in great quantities.

The following process usually occurs with protein foods (Pelczar et al. 1986).

Protein Foods + proteolytic microorganisms → amino acids + amines + ammonia + hydrogen sulfide.

### 2.2.1.2 Temperature

Microorganisms are accustomed to living in certain temperature ranges above and below which they are inhibited from growing and even destroyed (Pelczar et al. 1986). Psychrophiles and psychrotrophs are those microorganisms which will grow at refrigeration temperatures. Psychrophiles grow best in the range of 0 to 15°C with a maximum growth temperature of 20°C. Psychrotrophs can grow at 0°C but have an optimal growth range around 20 - 30°C. Mesophiles are medium temperature growing microorganisms (25 - 40°C) and thermophiles are high temperature growing microorganisms (>45°C). Since our intention in this work is to store the pasteurized products in a refrigerator, the classification of microorganism of most concern would be the psychrophile or psychrotroph. Mesophiles are also of concern when considering abusive temperatures greater than 10°C.

### 2.2.1.3 The pH:

The pH of the food impacts the way microorganisms grow in foods. Generally speaking, the lower the pH the more inhibited are the microorganisms. Foods are generally broken into two different food categories: low acid and high acid foods (Stumbo, 1973; Lopez, 1987). High acid foods have a pH less than 4.6 and have more mild heat processing standards due to the inhibitory effect of the acidic conditions. The

crab product investigated in this work is a low acid food with little inhibitory effect due to the pH.

#### 2.2.1.4 The Water Activity:

The available water refers to that water in the food which is not bound by cellular membranes or hydroscopic forces. It is that water which is available for microbial use. A measure of this available water is water activity, the ratio of the water vapor pressure of the product to that of pure water. Water activities below 0.88 will inhibit most bacteria (Stumbo, 1973, Lopez, 1987). The water activity of the crab product investigated in this work is above any inhibition point, 0.85 for most bacterial microorganisms.

#### 2.2.1.5 Oxygen Availability:

The presence or absence of oxygen defines which type of microorganism will grow in the packaged food. Microorganisms are classed into three main groups of oxygen need (Pelczar et al, 1986):

1. aerobic, requiring oxygen for growth;
2. anaerobic, requiring the absence of oxygen for growth;
3. facultatively anaerobic, can live in both conditions.

The presence of oxygen in the container will inhibit the growth of all anaerobic microorganisms, conversely the absence of oxygen will inhibit the growth of aerobic microorganisms. In this work, the product container is packed with air in the head space. This will inhibit the growth of anaerobic microorganisms for a time, until the oxygen is used up by the metabolic processes of aerobic bacteria. The types of bacteria most likely

to be problematic in this work are initially the aerobic and the facultatively anaerobic bacteria, and finally the obligate anaerobes such as *Clostridium botulinum*.

#### 2.2.1.6 Chemicals:

Certain chemicals, such as antibiotics, can directly affect the microorganisms ability to survive in a food stuff. Chemicals may interfere with the metabolic processes of microorganism cell function or may directly cause microorganism death.

#### 2.2.1.7 The Oxidation/Reduction Potential:

Certain compounds, like peroxide, increase the oxidation/reduction potential of the food which make metabolic processes more difficult for microorganisms. The loss, (oxidation) or gain (reduction), of electrons can change the chemical conditions required for microbial growth and function. The crab product investigated in this work is not expected to have a significantly destructive or inhibitive oxidation/reduction potential. None of the literature referenced in this work, see literature review, has cited the oxidation/reduction potential as being inhibitive to bacterial growth in crab.

#### 2.2.1.8 Growth Factor Summary:

The rock crab meat product investigated in this work is a low acid food, low in fat and high in protein with a high water activity. The microorganism of concern with this product, then, would most likely be an aerobic or facultatively anaerobic proteolytic psychrophile or psychrotroph.

### 2.2.2 The Decimal Reduction Time:

The thermal death of specific microorganisms does not follow a linear path with a finite time to complete death, rather, it follows a logarithmic relationship (Stumbo, 1973). If a semi-logarithmic plot was made between the numbers of one type of bacteria surviving a single lethal heat and time, an approximately linear relationship would be observed with a negative sloping line. The length of time required to reduce the numbers of microorganisms by one log scale, by 90%, is referred to as the decimal reduction time and is given the symbol D. This is equal to the negative inverse of this semi-logarithmic or "survivor" curve slope.

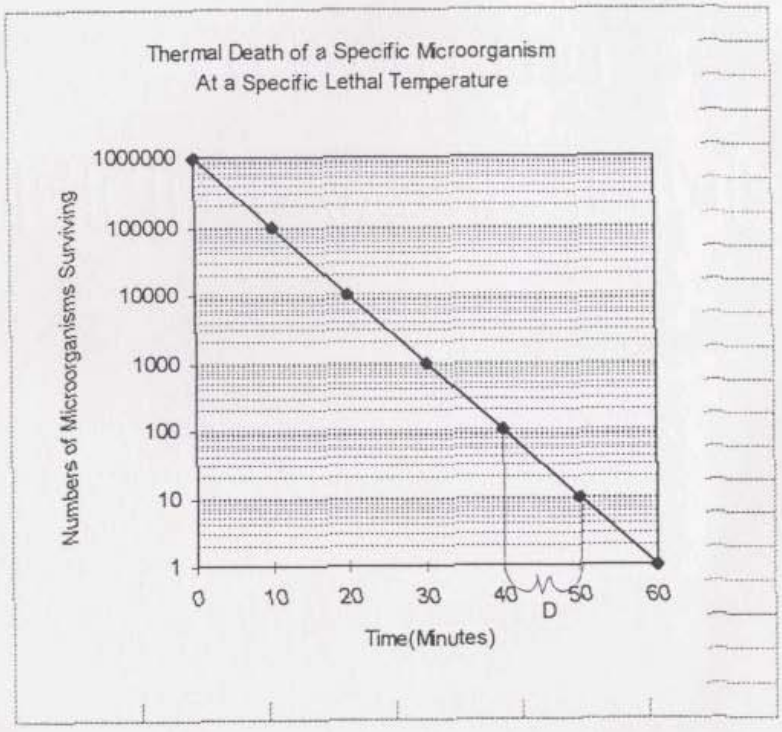


Figure 6: Decimal reduction time (D) determination.

### 2.2.3 The z-value:

If one were to subject microorganisms in a specific media to a wide range of lethal temperatures and determine the decimal reduction times over these temperatures a trend would be observed. It could be expected that as the lethal temperature increased the decimal reduction time would decrease, indicating that it takes less time to kill the microorganisms with greater heat. If a semi-logarithmic plot is made between the decimal reduction time observed, D, on the logarithmic scale, and the lethal temperature used, a linear relationship would be shown with a negatively sloping line. The temperature increase required to reduce D by 90% is referred to as z. This is equivalent to the negative inverse of the slope of this line (Stumbo, 1973).

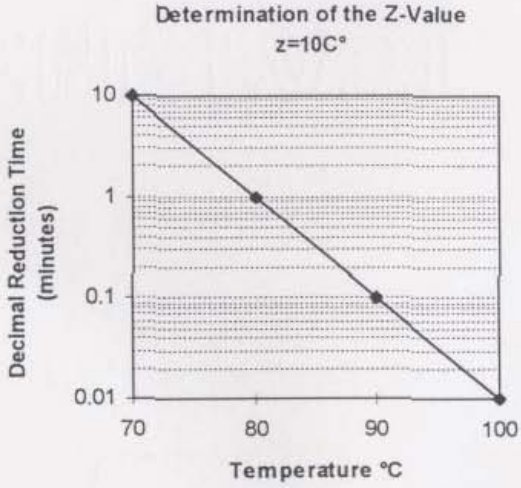


Figure 7: z-value determination.

### 2.2.4 Application of Thermobacteriology:

The decimal reduction time and the z-value describe how a specific microorganism dies in a specific medium with a specific kind of heat. In applying thermobacteriology to the pasteurization process development problem, one first needs to select a microorganism to study which will grow in and spoil the food in the storage conditions. The specific microorganism, usually a pathogen, is referred to as the target microorganism. The target microorganism is always the most heat resistant of all of the spoilage microflora which will grow in the post process storage conditions. Thermobacteriological analysis on various spoilage microorganisms in various media have been performed and is available in literature. Section 2.3.2 discusses the determination of the target microorganisms D and z values for crab meat.

Once the D value and z value is found we must determine the time which we are to subject the product to the lethal heat to kill the target microorganism. One D time increment at the lethal temperature reduces the population of microorganisms by 90%, the next D will reduce the remaining population by 90% and so on. Sterilization processes usually hold the product at the lethal heat for the equivalent of 12 D time increments while pasteurization processes usually involve 4 - 6 D time increments.

If D has a value of 0.20 minutes then 12D, a sterilization process, would be 2.4 minutes, reducing $10^6$ bacteria to $10^{-6}$, read as 1 in a million chance of 1 bacteria surviving. This would be read as a 12 D reduction in the target microorganism if it was held at the sterilization temperature for 2.4 minutes.

The above example implies that the whole food product was instantaneously heated to the lethal temperature and held for 12 D time increments, 2.4 minutes, for commercial sterility. The food product actually heats up gradually over time at a rate governed by the mode of heat transfer into the product, conduction or convection or both. Conductive product heat transfer is controlled by the thermal conductivity of the foodstuff while convective product heat transfer is controlled by both the thermal conductivity of the foodstuff and the level of physical internal mixing due to the convective behavior of the food stuff or agitation (Holman, 1976).

Gradually increasing internal temperatures complicate the lethal effect of the heat applied as lower lethal temperatures give higher D values. The z-value relates the temperature dependence of the D values for the target microorganism and can be used to calculate an equivalent effect on microbial population reduction as the internal temperatures rise. Microorganisms located at the slowest heating spot within the container will survive the longest as they will be subjected to far less heat that those on the surface of the products. The rate and pattern of heat rise at this spot must therefore be determined before application of z is possible. This is accomplished through a heat penetration analysis where the temperature at the slowest heating spot of the product is logged or plotted against time.

The effect of lethal heat on the microorganism is then combined with the actual lethal heat observed in the product due to the heating process to define the extent of thermal death obtained by the process. Two methods of calculation have been used in this

work to perform this calculation: The improved general method and the formula method according to Stumbo, 1973.

### 2.2.5 Heat Penetration Analysis:

The temperature at the slowest heating spot in the container is measured through the use of thermocouples. The temperature sensing part of the thermocouple is placed at the slowest heating spot of the container and secured there (Section 3.1.4 discusses the thermocouple). The product and thermocouple are then heated as they would be during the process. A data logger is used to log points of temperature against points of time. The following figure shows a typical plot resulting from this analysis:

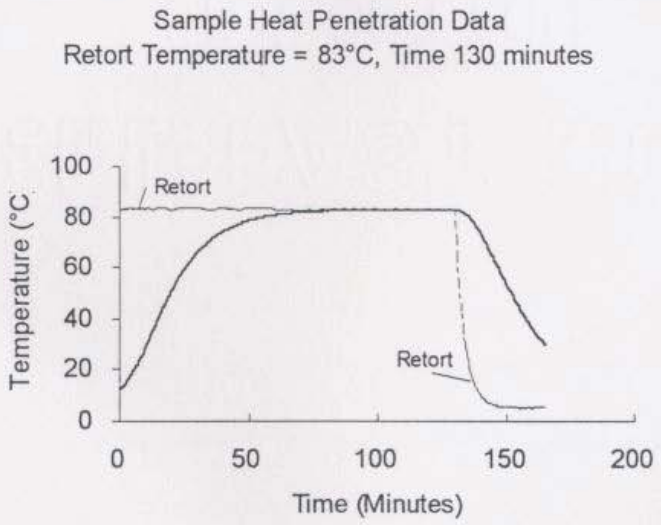


Figure 8: Example of a heat penetration plot (solid line) of an 8 oz plastic container filled with crab meat. Thermocouple is placed at the slowest heating spot of a product.

As can be seen from the example the slowest heating spot temperature lags the retort temperature in heating and in cooling. The plot shows the relationship between

retort temperature, heating media temperature, and the actual lethal temperature observed at the slowest heating spot. This heat penetration analysis is performed to obtain a statistical understanding of the heat penetration into and within the container in question and so is repeated at least three times.. This heat penetration information is usually logged as numerical data on a computer or data file with points of temperature against time at even intervals such as every minute.

### 2.2.6 The Improved General Method:

The improved general method is described by Stumbo (1973). The logged numerical data may be used to determine what sort of lethal effect the temperatures have had on the perceived microbial population. The z value can be used to define the relative lethal effect of each temperature point in the data compared to a reference temperature using Equation 1:

$$L = 10^{\frac{(Tx-Tr)}{z}} \qquad (1)$$

Where: L = Incremental lethality to a specific target microorganism.

$T_r$ = Reference temperature, in this case 85°C.

$T_x$ = Temperature at the slowest heating spot within the container.

z = the temperature increase required to reduce the D value by 90%. The z value in this case is 8.89 C°.

Incremental lethality is the fraction of lethal time compared to that which would be seen at the reference temperature. For instance if $T_x$ was equal to $T_r$, the internal temperature of the product was equivalent to the reference temperature, the incremental lethality would be 1.0, indicating that the fraction of lethal heat observed relative to the

reference temperature was 100%. Smaller $T_x$ values give proportionally smaller values of lethal heat fraction. At $T_x$ = 83°C, using the above referenced values for the other variables ($T_r$ = 85°C, z = 8.89C°), the L would be 0.60 indicating that the lethal heat observed at that temperature was only 60% of that obtainable at the reference temperature. The improved general method takes each data point at a uniform time increment and calculates the fraction of lethal heat for each point compared to the reference temperature and then adds these fractions to obtain the total fraction of lethal heat over the whole heat penetration process. This total fraction is then multiplied by the time increment to obtain an estimate of the amount of heating time observed relative to the reference temperature. Equation 2 is used in this calculation:

$$F = \sum L\Delta t \text{, (minutes)} \qquad (2)$$

Where:

$\Sigma L$ = sum of all incremental lethalities over the process time at the reference temperature.

$\Delta t$ = the time increment between data logging points.

F = Lethality, the equivalent, in minutes at some given reference temperature, of all heat considered in the product with respect to its capacity to destroy the target microorganism.

The F value, or lethality, is what is used to define the effect of the overall process and is compared to the 12 D calculation at the reference temperature. In our previous example, the 12 D value was 2.4 minutes. We would ensure that the heat penetration observed would yield at least an F of 2.4 minutes by holding the product at the retort temperature until the F calculation yielded 2.4 minutes.

The value F is also expressed as a pasteurizing lethality or pasteurization value P. The normal nomenclature for these values are as follows:

$$P^{Z}_{Tr} \text{ or } F^{Z}_{Tr} = X, \text{ (minutes)}$$

### 2.2.7 The Method According to Ball and Stumbo:

The "formula method", as referred to by Lopez (1987) involves a somewhat more complex procedure than the improved general method. Two authors have established a method for mathematically describing the heat penetration profiles of thermally processed products, Ball (1928) and Stumbo (1973).

Consider the following example plot:

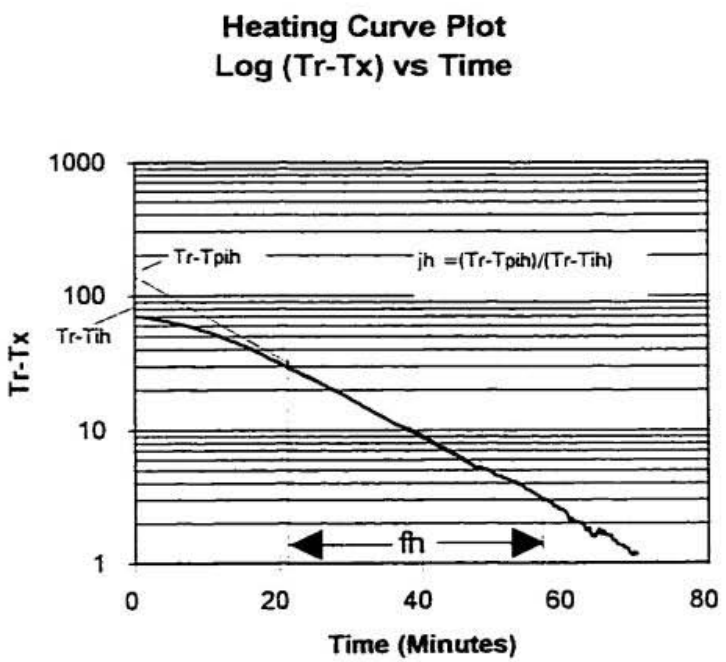


Figure 9: Example heat penetration plots of $T_r$-$T_x$ vs Time. Note calculation of $j_h$ and $f_h$.

The values $j_c$ and $f_c$ are found using a similar plot of $T_x$-$T_c$ vs time. The parameter $f_c$ is the slope of the cooling curve line and $j_c = (T_{pic}-T_w)/(T_{ic}-T_w)$.

The heat penetration parameters shown above are defined as follows:

$j_h$ = the heating lag factor;

$f_h$ = the time, in minutes, to traverse one log cycle of the straight line portion of the heating, (Tr-Tx) vs Time, curve plotted on semi-logarithmic paper;

$j_c$ = the cooling lag factor;

$f_c$ = the time, in minutes, to traverse one log cycle of the straight line portion of the cooling curve, (Tx-Tc) vs Time, plotted on semi-logarithmic paper.

These parameters define the heating and cooling behavior of the product measured, coupled with the following basic information:

$T_i$ = initial temperature of the food product;

$T_r$ = the retort temperature;

$T_c$ = the cooling water temperature;

$T_x$ = the slowest heating spot temperature inside the food;

CUT = the come up time; the time required to bring the retort up to the sterilizing temperature;

$t_p$ = the retort operators process time; time from the end of the CUT to the end of heating;

$t_b$ = the Ball's process time;

$$tb = tp + 0.42(CUT) \quad (3)$$

$$m = Tx - Tc \quad (4)$$

m = the difference between the center can temperature and the cooling water temperature.

$$g = Tr - Tx \qquad (5)$$

U = process equivalent (minutes).

Ball (1928) was the first author to mathematically define the heat penetration profile. They made the following assumptions:

1. The cooling water temperature was 21.1°C;
2. $j_c = 1.41$;
3. $f_h = f_c$;
4. z = 10 C°;
5. Heat transfer is by simple conduction;
6. Initial cooling behavior is hyperbolic.

Ball's assumptions were found to be quite limiting. Stumbo (1973) used much of the same method described by Ball (1928) but expanded it and made it more flexible reducing the assumptions to:

1. $f_h = f_c$;
2. m+g = 100 C° (180 F°).

Stumbo's method provided much more flexibility for process determinations and permitted the use of the cooling curve portion of the cook. Stumbo (1973) developed tables describing fh/U vs g for varying z values and $j_c$ values which could be used to relate the required lethality of the process to the actual heat penetration obtained. (Ghazala, 1989; Stumbo, 1973) The following equations were used:

$$U = \frac{Fo}{Fi} \qquad (6)$$

where: Fo = the lethality at the reference temperature

Fi = the relationship between lethality at the retort temperature and the lethality at the reference temperature.

$$tb = fh(\log(jhg\max) - \log(g)) \qquad (7)$$

where: $g_{max}$ = the initial difference between the retort temperature and the center temperature of the product, $T_r$-$T_i$

These equations are used to calculate the process time required to give a certain lethality or are used to determine the lethality obtained from a process time.

## 2.3 Pasteurization

### 2.3.1 What is Pasteurization?:

Pasteurization is the treatment of packaged foods to temperatures below 100°C over a given time to eliminate pathogenic microorganisms which may grow under certain storage conditions (Hackney *et al*, 1991). It is a heat treatment which kills part of the vegetative microorganisms present in the food and relies heavily on handling and storage conditions to further minimize bacteria growth (Karel *et al*, 1975). In this situation the pasteurization heat treatment will destroy pathogens in crab in a sealed plastic container with aluminum pull top lid which would otherwise grow in this container under refrigerated storage conditions (under 3°C).

### 2.3.2 Target Microorganism for Pasteurized Rock Crab:

As discussed in section 2.1.3.4, cooking of butchered rock crab is usually done in boiling water for approximately 10 minutes. This would imply a temperature treatment of 100°C for 10 minutes to any surficial bacteria and somewhat less than that for any bacteria which may have found its way to the protected inside of the butchered crab, the maximum internal temperature reached by a large crab is about 85°C. Governmental regulations require that the crab be live just before it is butchered and that it be cooked to minimize the activity of degradative enzymes present in the crab (Botta, 1994). The natural immune system of the live crab will maintain it essentially free of microorganisms until death as well (Fahgri, 1983).

The bacteria of major concern in pasteurisation are pathogens such as *Listeria monocytogenes*, *Clostridium botulinum* E, Non-proteolytic *Clostridium botulinum* B, and Non-proteolytic *Clostridium botulinum* F. These bacteria have the potential to grow under refrigeration conditions, 3°C, and will not be destroyed by cooking heat treatments. Wentz *et al* (1985) showed that the bacteria of most importance in post-processed Blue Crab meat were *Escherichia coli* and *Staphylococcus aureus*. This was in agreement with observation reported by Phillips and Peeler (1972), and Hackney *et al* (1991). Later, Betts (1992) indicated that the decimal reduction times for *E. coli* and *S. aureus* were, respectively, 0.47 min at 62.8°C and 1.36 min at 62°C in beef, which were not as hardy as the *Clostridium* species.

Although no mention has been made of any of the *Clostridia* or *Listeria* species in any post processed crab meat microbial tests in the literature, it is quite logical to assume that the potential for such contamination still exists through unsanitary human contact (Kautter *et al*,

1974; Hackney *et al*, 1991). Ray *et al* (1976) showed increased numbers of psychrotrophs in food pilot plants not observing proper sanitary practices. The same author showed reduced psychrotroph numbers in plants observing sanitary practices indicating that contamination occurred during processing. Ward *et al* (1977), compared the microflora of unpasteurized and pasteurised crab meat. They found that the surviving bacteria from the process were largely lactic acid producing, non-spore forming, gram positive rods. They determined the decimal reduction time of these rods to be 2.5 min at 55°C in a phosphate buffer. Harrison and Huang (1990), showed that the decimal reduction time of *Listeria monocytogenes* at 60°C in crab meat was 2.61 minutes which is far less than that of the *Clostridium* species. Solomon *et al* (1982) showed that non-proteolytic *Clostridium botulinum* types B and F, and proteolytic type B do not grow in blue crab meat at reduced temperatures after heat treatment. Harrison *et al* (1995), supported this study by showing that obvious spoilage had occurred in blue crab packed in containers, identical to those studied in this work, well before the potential for *Clostridium botulinum* toxins formation. Cockey and Tatro (1974), also indicate a reluctance of *Clostridium* E to grow in autoclaved crab meat and demonstrated negative toxin production in blue crab heated to 85°C and held for 1 minute. In a study on the meat of blue crab, Lynt *et al* (1977) showed that the decimal reduction times for *Clostridium botulinum* type E spores were 0.74 min in Beluga blue crab and 0.51 min in Alaska blue crab, at a temperature of 82.2°C. The greatest z-value for the Beluga was 8.33°C. Lynt *et al*, 1979, showed that the decimal reduction times for *Clostridium botulinum* type F are similar to those for *Clostridium botulinum* type E. Strain 202 demonstrated a decimal reduction time of 0.53 minutes at 85°C in crab meat with a z value of 6.4 C°. Scott and Bernard (1982) compared the heat resistance

of spores of non-proteolytic *Clostridium botulinum* type B with type E and proteolytic type B spores. They found that non-proteolytic type B strains had a greater thermal resistance than type E. The non-proteolytic type B strains were found to be far less heat resistant than the proteolytic strains. Non-proteolytic *Clostridium botulinum* type B strains 17B, 2129B and ATCC 17844 were identified as being the most heat resistant (Scott and Bernard, 1982). Decimal reduction times at 82.2°C were 16.7 min for 17B, 32.3 min for 2129B and 4.17 min for ATCC 17844. The z values for 17B, 2129B and ATCC 17844 were found to be 6.5, 9.7 and 16.5C°, respectively (Scott and Bernard, 1982). However, the question still remains as to the importance of these bacteria in the crab medium (Solomon *et al*, 1982; Harrison *et al*, 1995; Cockey and Tatro, 1974).

### 2.3.2 Target Lethality of the Intended Pasteurization Process:

Ward *et al* (1984), suggested a pasteurization lethality of 31 minutes be used on blue crab meat products at a reference temperature ($R_T$) of 85°C and a z value of 8.89°C in order to maintain assurance of control over strains of *Clostridium botulinum* type E. Gates and Parker (1992), adequately reduced plate counts in blue crab by pasteurizing at 83.3°C giving a lethality of 44 minutes at a reference temperature of 85°C and a z value of 8.9C°. Gates *et al* (1993) used a pasteurization lethality of 40 min with a z value of 8.89°C at a temperature of 85°C to reduce the potential for microbial spoilage. They cited work done by Webster *et al* (1991) which showed that crab meat spoiled at a lethality of 31 min with z value and reference temperature similar to those reported by Ward *et al* (1984) and Gates *et al* (1993). Rippen and Hackney (1992), indicate the same concern because of the isolation of a new non-proteolytic strain of *Clostridium botulinum* with a

decimal reduction time of 31 minutes at 85°C. Segner (1992), describes a psychrotrophic microorganism, similar to *Clostridium arcticum*, with a D of 35 minutes at 85°C requiring a higher pasteurization lethality than F=31 minutes at a reference temperature of 85°C and z value of 8.89C°. This work will pursue a target lethality similar to that suggested by Gates *et al* ($F^{8.89}_{85}$ = 40 minutes), which will provide approximately a 3D reduction (three times the decimal reduction time, D) of the strongest strain of *Clostridium* B (2129B) and over 100D to strong strains of *Clostridium* E (Beluga) (Hackney *et al*, 1991).

# 3.0 MATERIALS AND METHODS

## 3.1 Sample Preparation Procedure:

### 3.1.1 Rock Crab Meat Preparation:

Rock crab was received vacuum packed and frozen on Styrofoam trays. A light brine (3%) had been added prior to freezing. Each tray held about 1 lb of meat and brine. Meat portions were made up of leg meat portions and de-shelled body meat portions known as "salad". The approximate ratio of leg meat to salad on each tray was 40:60. The following picture, Figure 10, is of the raw material as received:

Figure 10: The rock crab (*Cancer irroratus*) raw material as received from the supplier. The top layer of each tray was leg meat with the "salad" portions underneath.

### 3.1.2 The Container:

The container used was a #308 polypropylene container supplied by King Plastics, Orange, CA, USA. The lid used for the container was an aluminum pull top lid designed for consumer convenience. The container held 8 oz of crab and brine when full. Figure 11 shows the container used.

Figure 11: The 8 oz container used in this work (King Plastic, Orange, CA, USA). Note the presence of the thermocouple entrance hardware.

The container was guaranteed heat tolerant to only 83°C. Temperatures above this, with some tolerance, caused the plastic material to liquefy, ruining the hermetic seal of the container. This posed a serious limitation to the temperatures useable in this work.

### 3.1.3 Seaming the Aluminum Lid on the Plastic Container:

The containers were closed with a Dixie Seamer model 25D, Figure 12 (Dixie Canner, Athens, Georgia), using normal double seaming techniques. The seamer was fitted with first and second operation rolls specific to the plastic container and aluminum pull top lid used.

Figure 12: The Dixie Seamer used to close the containers. Supplied by Dixie Canner, Athens, Georgia.

### 3.1.4 Thermocouples, Data Logger and Leads Used to Collect Thermal Penetration Information:

The data logger used was a Molytek data logging system, supplied by MMIS, Inc., Aurora, ON, Canada, Figure 13. The unit was set to log 20 points of data simultaneously at 20 second intervals.

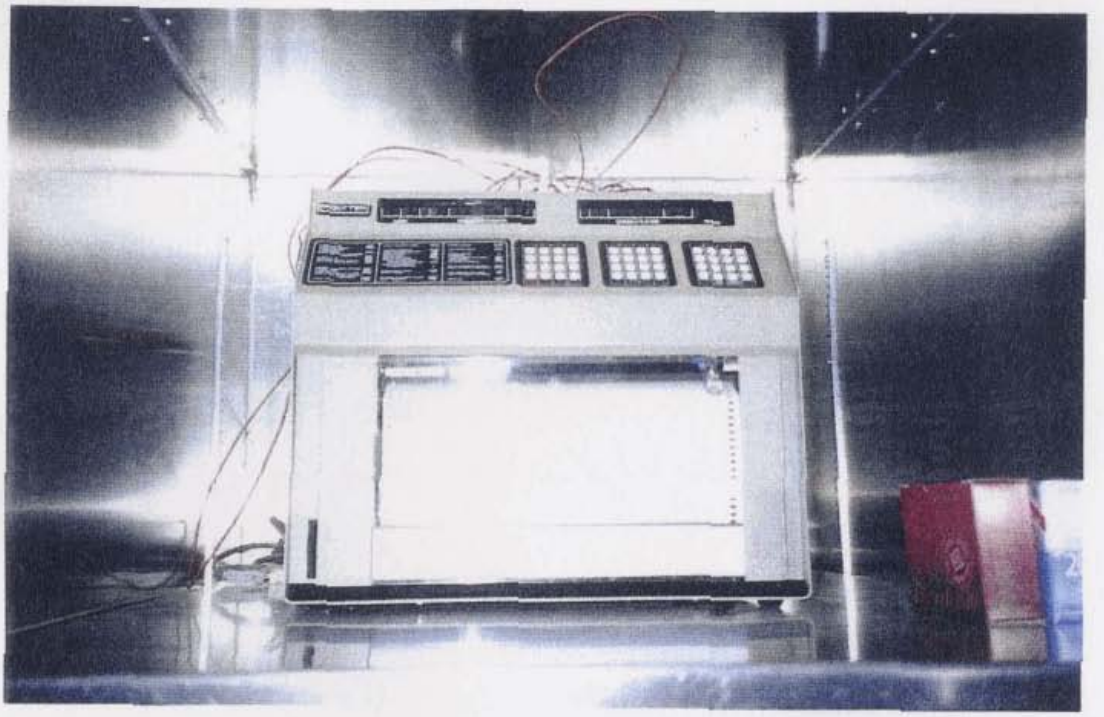

Figure 13: The multi-point data logging system used.

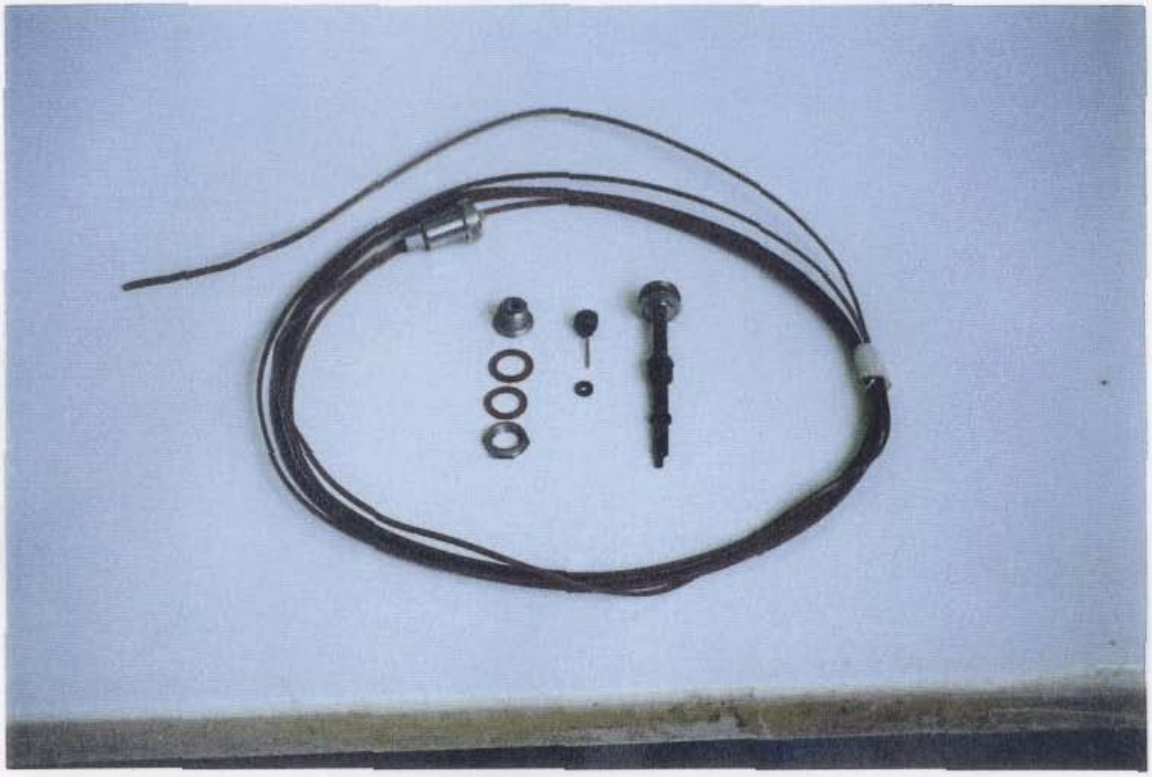

Figure 14: The thermocouples, leads and equipment used. *Supplied by Ecklund-Harrison* Technologies.

The leads and thermocouples used were supplied by Ecklund-Harrison, Fort Meyers Florida, USA. Please refer to Figure 14. *Leads (Item TEF-20) were composed of* a 20 gauge copper - constantan wire pair (Type T). Two types of thermocouples were utilized during this work. Adjustable thermocouples, to the right in the above figure, were used to obtain heat penetration information and the slowest heating spot in the container. Needle type thermocouples, in the center of the above figure, were obtained for more precise measurements of lethality.

### 3.1.4.1 Thermocouple Installation and Use:

The container used for this work was plastic and had a tendency to crack when stressed. For this reason the only stable point of entry into the container was through the aluminum pull top lid. A special hole punch, supplied by Ecklund Harrison Technologies,

was used to punch a hole of appropriate size at the geometric center of the lid. A special effort was taken to determine the precise location of the geometric center of each lid. This hole was to house the thermocouple assembly.

Each thermocouple assembly was made up of 6 main parts as follows:

- 1 hollow bolt with threaded interior and exterior (Item SC9);
- 1 nut (Item C-9);
- 2 rubber washers (Item C-16);
- the needle or adjustable thermocouple and a rubber ring washer (Items CNS, C17).

Prior to seaming the lid on the container, the hollow bolt was passed through 1 rubber washer and then through the top side of the lid. Another rubber washer was placed on the hollow bolt end protruding from the bottom of the lid and the nut was started on the outside of the hollow bolt. The nut was turned with sufficient torque to press the rubber washers together on the inside and outside of the lid, creating an air tight seal. The lid with equipment attached was then seamed onto the filled container. See the assembled unit in Figure 11. After seaming, the thermocouple was passed through a rubber ring washer and then through the center of the bolt, sensing end first, into the food. The threaded end of the thermocouple was turned into the internal bolt threads of the hollow bolt and was tightened using a thermocouple wrench. Once all bolts, nuts and thermocouples were securely installed and tightened, the product was ready for testing.

The adjustable thermocouple apparatus was similar to the needle type except for a sliding threaded brass plug which was fitted to the thermocouple shaft. The rubber ring washer discussed previously was placed onto the shaft of the adjustable thermocouple

after the brass threaded plug. The desired length of thermocouple was passed through the hollow bolt center and then the brass threaded plug was turned into the hollow bolt internal threads against the rubber ring washer. The pressure of the rubber ring washer against the shaft of the adjustable thermocouple, provided by the brass plug, caused an air tight seal.

### 3.1.5 Filling the Container:

The vacuum packaged crab meat was thawed in cold water for approximately 3 hours. The packages were removed from the thawing water once thawed and were opened. Excess brine in each package was drained off into a container for later re-use. Leg meat and salad meat were separated and placed in individual bowls. Salad meat was lightly squeezed to remove most of the absorbed brine. Brine squeezed out of the salad meat was collected for later re-use.

Three possible heat penetration conditions can occur in solids and liquids, excluding radiation: conduction, convection and partial convection (Holman, 1976). Of these three conditions, the slowest and most predictable is conduction. This therefore is deemed the worst case situation and the safest to study. The product fill weights desired by the marketplace provided a partially convective product. In order to enhance conduction, and hence predictability, an artificially conductive recipe was required.

A salad meat weight of 108 g and 72 g of leg meat was mixed together and placed into each container. The brine from the original sample, supplemented by additional 3% brine, was poured into each container until 227g (8 ounces) was obtained. All heat

penetration work was done using this conductive sample giving a "safety factor" to all results.

### 3.1.6 The Pasteurizing Retort:

The Stock Pilot Rotor 900 retort (Stock America, Milwaukee, WI) was used in full water immersion mode to process the containers. Figure 15 shows the retort used.

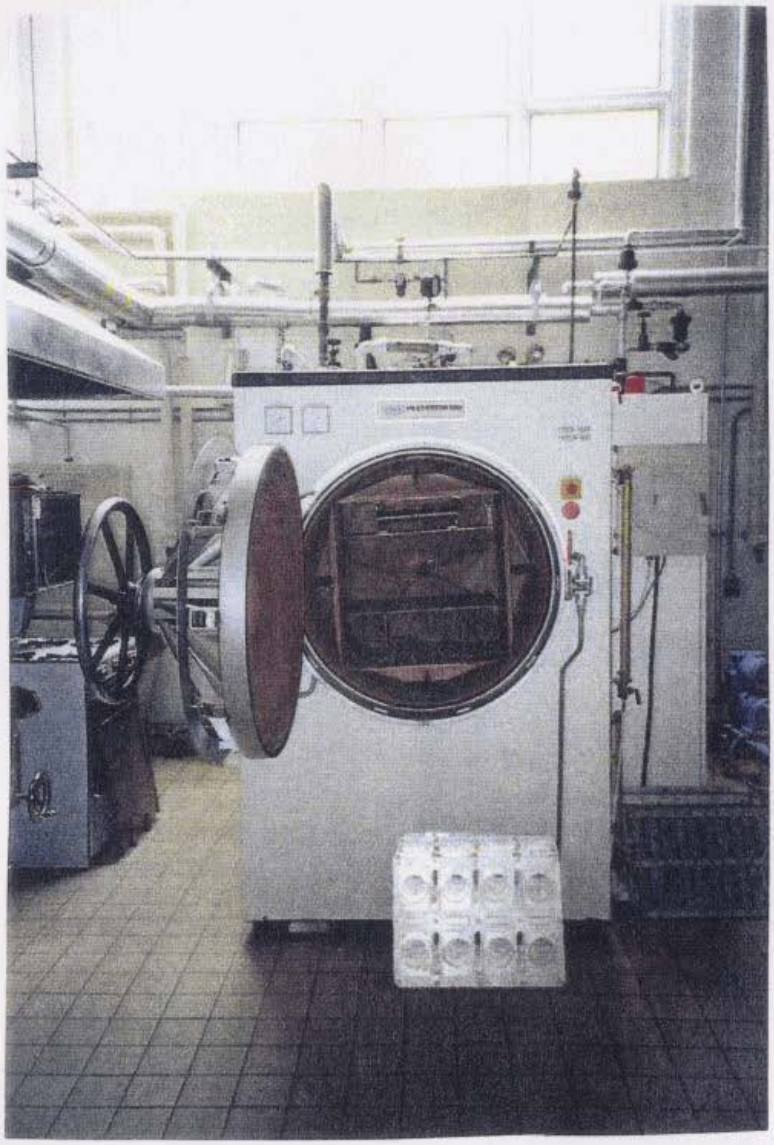

Figure 15: The Stock Rotomat Retort PR 900 with leads in processing basket.

A rack and jack down holding system was used to keep the containers submerged in the heating medium. Please see Figure 16.

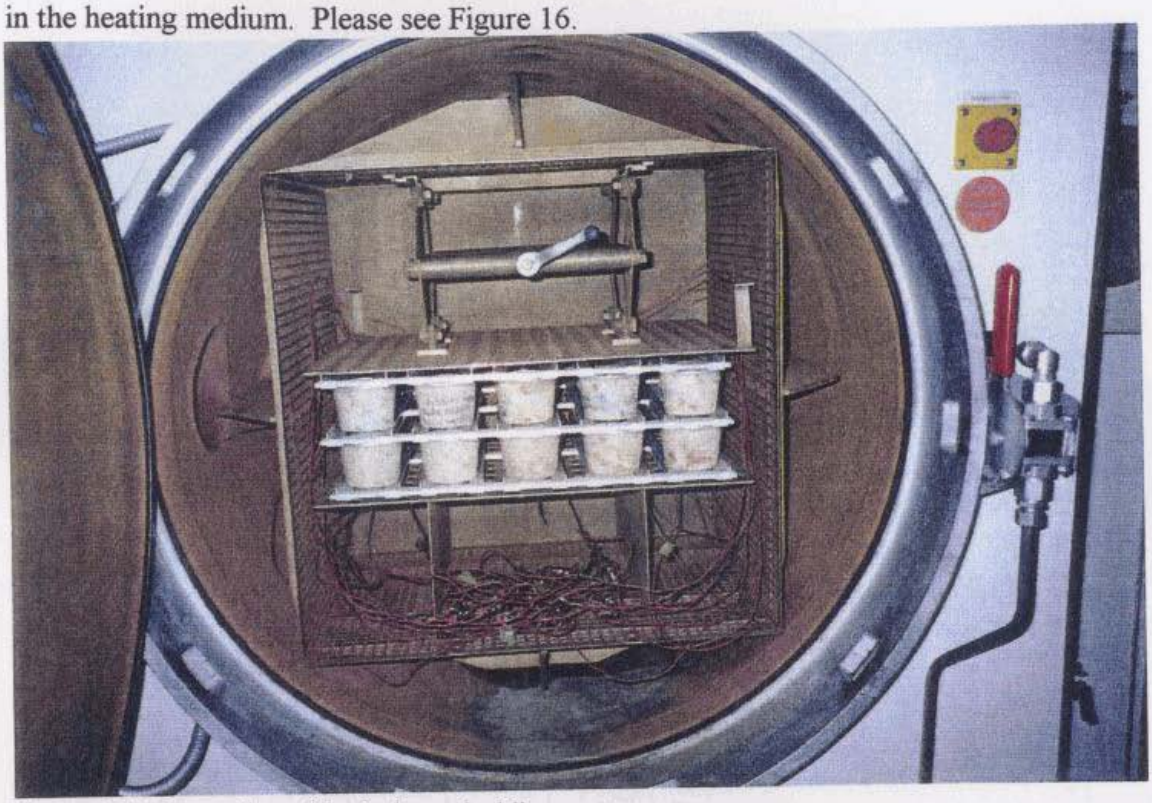

Figure 16: The rack and jack down holding system.

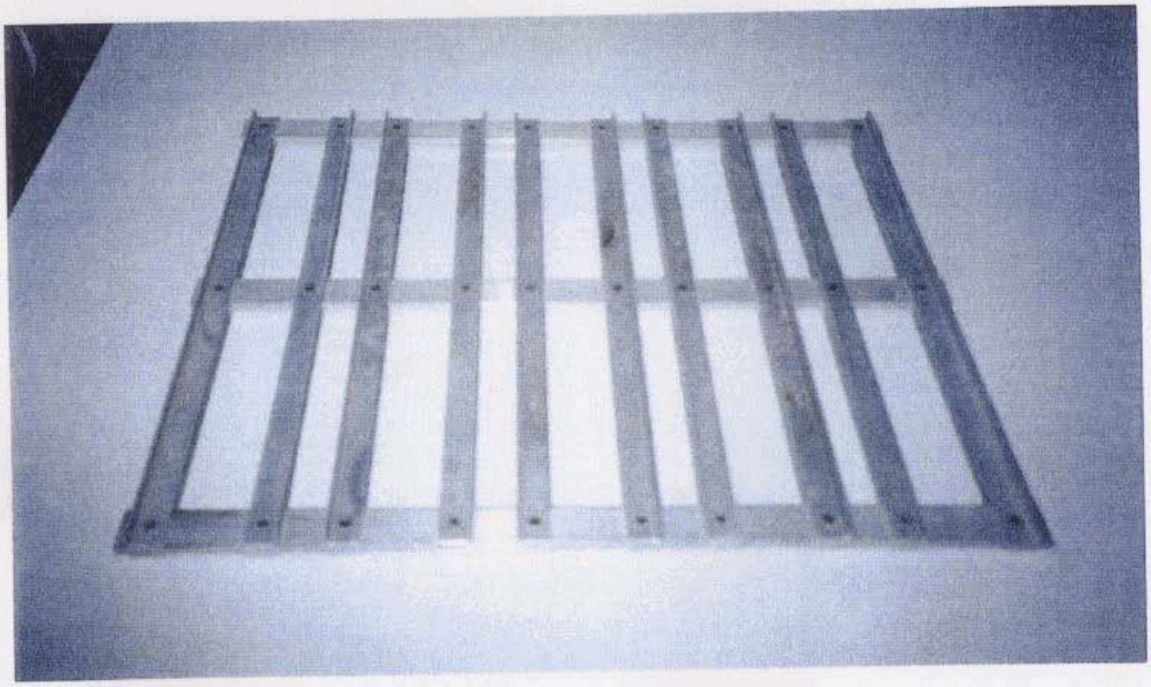

Figure 17: The rack invented to hold the containers in the retort.

In order to hold the containers in a predictable fashion within the retort and to allow space for thermocouple hook-up, the following rack was invented (Figure 17). Figure 16 shows how this rack was utilized.

### 3.1.7 Stacking the Containers in the Retort:

Containers stacked in the retort were placed upright to better facilitate thermocouple placement (See Figure 16). A rack and jack down holding system was developed to hold approximately 48 containers firmly. Pressure was exerted on the top and base of the container to prevent inverting. The concave base on the container inverts to convex when unchecked by increases in internal pressure during heating and cooling.

#### 3.1.7.1 Connecting the Data Logger Leads Within the Load:

Each retort load for heat penetration was stacked gradually interspersing the thermocoupled containers evenly throughout the load. Thermocouple lead wires for each layer were connected at one time through the over-lying tray before the next layer of containers were added. The lead wires exited the load at the shortest possible path and were attached to the load basket to prevent movement, see Figure 16.

### 3.1.8 Pasteurizing Methodology:

Heating and cooling was performed in the same vessel within the Stock Rotomat system. Water, heated to 10°C over the process temperature, flooded the retort process vessel, and containers, and was maintained at the set point temperature by a steam injected circulation temperature control system. The temperature of the heating water loses about 10°C in moving from the storage vessel into the cool process vessel and piping. Timing

10°C in moving from the storage vessel into the cool process vessel and piping. Timing was started when the water temperature within the retort was being controlled at the set point temperature by the temperature control system. Cooling within the retort occurred after the set amount of pasteurizing process time elapsed. Cooling water was introduced into the hot water in the process vessel filling the process vessel to overflowing. Overflowed water was directed back to a storage vessel until filled and then to the drain thereafter until cooling was complete. The cooling water temperature did not attain its lowest value for a number of minutes because of the overflow cooling methodology. This may have had some effect on the cooling parameters.

### 3.1.9 Storage of Pasteurized Containers:

Pasteurized crab containers were stored in a controlled refrigerated storage set at 2.2°C.

## 3.2 Slowest Heating Spot Evaluation and Pasteurization Heat Penetration Studies:

### 3.2.1 Slowest Heating Spot Evaluation:

The slowest heating spot within the container was determined using adjustable thermocouples along with Cadkey$^{R}$, a computer drafting program. The cross sectional profile of the container was drawn to scale. Lines parallel to the exterior of the container, lines of uniform heat penetration, were drawn at even intervals toward the center of the scale drawing. These evenly spaced lines represented the even heat penetration rate expected through a uniform conductively heating sample (Jackson and Olson, 1940). Smaller line spacings represented slower heat penetration rates, such as was evident at the

top of the container where an air layer decreases the heat penetration rate. Side and bottom spacings were identical and were considered full scale or 100%. Spacings from 100% to 65% of that from the sides and bottom were used to represent heat penetration from the top. The distance, in each case, to the convergence point was measured graphically. The adjustable thermocouples were then placed through the lid of the container to varying depths (2.6, 2.8, 3.0, 3.1, and 3.2 cm) around the range of graphically measured cold spots and the heat penetration data was obtained. Lethalities for each thermocouple data set, see section 2.3.2, were compared to determine the slowest heating point ($z$=8.89 C°, $T_r$ = 85°C, where $T_r$ is the reference process temperature). Only the heating lethalities were used as industrial cooling methods will vary from those used in this work. The lowest lethality corresponded to the slowest heating spot.

### 3.2.2 Pasteurization Heat Penetration Studies:

Needle thermocouples were obtained from Ecklund Harrison Technologies of a length which placed the temperature sensing junction of the thermocouple at the cold spot of the container. Containers fitted with thermocouples were pasteurized, in the Stock Rotomat Retort, at three different temperatures (83.0, 82.0 and 81.0 °C) to a heating lethality of 40 minutes based on a reference temperature of 85°C. Several pasteurization runs at each temperature were performed to define the process time required to reach a 40 minute lethality at that temperature. Further heat penetration runs were performed at each temperature to obtain required heat penetration parameters.

### 3.2.3 Computerized Calculation of Heating/Cooling Parameters:

This work used computerized calculation techniques to determine $j_h$, $f_h$, $j_c$, $f_c$ from data file spreadsheets accumulated with a computer and a data logger. (Ghazala, 1989)

The plot of $\log(T_r-T_x)$ vs Time and $\log(T_x-T_c)$ vs Time, necessary for the determination of $j_h$, $f_h$, $j_c$, $f_c$ can be easily determined from data logger data files. Since the computer data files contain matching data points for $T_x$ and time for the whole process, it is a relatively simple matter to calculate matching points of $\log(T_r-T_x)$ and time and $\log(T_x-T_c)$ and time for the whole process. Regression analysis can be applied to the linear portions of this data to obtain the slope and y intercept of the best fit line. Data yielding $r^2$ values for fit of 0.999 or better are used. The $f_h$ and $f_c$ values can be calculated as follows using the slope and intercept from the heating and cooling curves respectively:

$$fh, fc = \frac{-1}{slope} \qquad (8)$$

The jh and jc values can be calculated the following way:

$$jh = \frac{Intercept}{(Tr - Tih)} \qquad (9)$$

$$jc = \frac{(Intercept + Tc)}{(Tic - Tc)} \qquad (10)$$

where: $T_{ih}$ = initial center temperature.

$T_{ic}$ = initial center temperature at start of cool.

### 3.3 Quality Analysis:

#### 3.3.1 Proximate Analysis:

The components of the proximate analysis determined in this work were:

- fat content;
- crude protein;
- moisture content;
- ash;
- carbohydrates (by difference).

##### 3.3.1.1 Fat Content:

The fat content of the crab was extracted by the method of Bligh and Dyer (1959). A weight of 25 g of blended rock crab sample, 25 ml of chloroform and 50 ml of methanol was placed in a Waring blender jar. The blender was capped with the blender lid wrapped in a double layer of aluminum foil. The mixture was blended for 2 minutes then another 25 ml of chloroform was added and blended for another 30 seconds. A weight of 25 g of water was added and the mixture was blended for a further 30 seconds. The solution was then filtered through a vacuumized buchner funnel fitted with Whatman #41 filter paper into a 250 ml filtering flask. Vacuum was obtained through a GAST vacuum pump (GAST Mfg. Co., Benton Harbor, MI) set to 20 inches of mercury vacuum pressure. The filter paper and residue was then transferred to a blender with 25 ml of 1:1 chloroform/methanol, then blended for 15 seconds. The ensuing solution was filtered through the buchner funnel. The filter cake was pressed to ensure all excess fluids were

clear from the system then the filtrate was poured into a 250 ml separatory funnel. The filtering flask was rinsed with 10 ml of 1:1 chloroform/methanol and the resulting solution added to the separatory funnel. The lid was placed on the separatory funnel and vented. The separatory funnel was covered with aluminum foil to protect the contents from light exposure. The solution was allowed to separate over night. The separated fat/chloroform layer was placed in a pre-weighed round bottom flask. The chloroform was then evaporated from each sample using the rotary evaporator. Once all of the chloroform was evaporated, the round bottom flask was re-weighed to determine the amount of fat present in grams. The following formula was used to determine the fat content of the food.

The following formula was employed to determine the fat content of the food:

$$F = \frac{(W_2 - W_0) x V_1 x 100}{V_2 x W_3}, \quad (11)$$

Where: F = percent (g/100g) fat in the sample;

$V_1$ = total volume (ml) chloroform layer in graduated cylinder;

$V_2$ = volume (ml) chloroform aliquot removed to weigh dish;

$W_0$ = weight (g) of empty aluminum weigh dish;

$W_2$ = weight (g) of aluminum dish with dried lipid residue;

$W_3$ = weight (g) of tissue sample blended.

### 3.3.1.2 Kjeldahl Method to Determine Crude Protein Content:

The Kjeldahl method was used to determine the crude protein levels in the crab. A Buchi 426 Kjeldahl analysis system was used. Well blended and mixed freeze dried crab meat (300mg) was placed on K.F. weigh paper #109967 (Nitrogen free). The weigh paper and sample was folded and transferred to a 250 ml digestion tube. A blank sample containing only nitrogen free weigh paper was also prepared. Kjeldahl catalyst tablets, 2 (Kjeltabs) were placed in each container followed by 20 ml of concentrated sulfuric acid.

The tubes were then connected to the Buchi digestion unit. The Kjeldahl tubes were vacuumized and the heating elements turned on. Varying levels of heat, denoted by a knob scale between 1 and 10 on the heat controlling potentiometer, were used. The system was warmed up for 10 minutes on a setting of #10, to warm the system rapidly. The vacuumized Kjeldahl tubes were then placed into the heating receptacles and the heating potentiometer turned back to #3 for 20 minutes. The potentiometer was turned to #9 for 40 minutes and then finally to #10 for 10 minutes giving a total digestion time of 70 minutes. The final heating temperature was in the order of 400°F (205°C). The digestion proceeded until the mixture in the tubes was clear to slight yellow. The tubes were then removed and allowed to cool about 15 minutes, no precipitate was allowed to form. The Buchi distillation equipment (Brinkman Instruments (Canada) Ltd., Rexdale, ON) was warmed up and prepared with a preliminary charge of water for 15 minutes. The Buchi distillation system was outfitted with auto filling systems for water and sodium hydroxide (NaOH), a tank containing a large quantity of 40% sodium hydroxide was located at the top of the distillation system. Six (6) flasks each containing 50 ml of 4% boric acid

($H_3BO_3$)with 15 drops of N-Point Indicator were prepared. Aliquots of 60 ml of de-ionized water was placed into each cooled digestion tube with digested material. Each digestion tube was then placed onto the digestion system in turn with a $H_3BO_3$ containing flask on the receiver end of the distillation unit. A measured quantity, 100 ml, of NaOH was added to the digestion tube, using the automatic feeding system, and the ensuing reaction allowed to subside. The steam distillation process was started and was allowed to proceed until 150 ml of solution was distilled. All of the distilled gasses were channeled through the $H_3BO_3$ solution. The indicators, normally red (acidic) will eventually turn green (basic) as the distillation proceeds, ammonia is evolved by the distillation process and taken up by the $H_3BO_3$ solution. Each of the six digestion flasks, including the blank sample, with their own $H_3BO_3$ indicator solution was run through the digestion sample in the same way.

The resulting green $H_3BO_3$ solution was then titrated to an end point, pink color, with 0.1 N $H_2SO_4$. The blank was also titrated similarly. The calculation of % crude protein was performed as follows:

$$\%\,\mathrm{Pr}otein = (Va - Vb)x1.4007xNx6.25, \qquad (12)$$

Where: Va = Volume of acid titrated for sample.

Vb = Volume of acid titrated for blank.

N = normality of titrating acid.

6.25 = protein factor for meat products.

### 3.3.1.3 Moisture Content:

The moisture content of the crab sample was determined by gravimetric means (AOAC 1990). Pre-marked aluminum dishes were placed in a drying oven (Precision Scientific Group, Chicago, Illinois, U.S.A.) set between 103 and 105°C for 1 hour. The heated dishes were then placed in a desiccator for about 20 minutes to cool and were then weighed to 0.0001 g. Approximately 5 grams of well mixed crab sample was weighed onto each marked aluminum dish, and the weight recorded to 0.0001g. The tray and wet sample were then placed into a drying oven set between 103°C and 105°C and were left until a constant weight was obtained, approximately 24 hours. The tray and dry sample were then cooled in a desiccator and weighed to 0.0001g. The following calculation was employed to determine the moisture content on a wet weight basis:

$$\%\,Moisture = \frac{(M_{ds-t} - M_t)}{(M_{ws-t} - M_t)} x100\%\,, \qquad (13)$$

Where: $M_{(ds-t)}$ = Mass of dry sample plus tray.

$M_t$ = Mass of tray.

$M_{(ws-t)}$ = Mass of wet sample plus tray

### 3.3.1.4 Ash Content:

The ash content was determined using a dry ashing technique (AOAC,1990). Three (3) crucibles with lids were heated in an oven at 103 - 105°C for 1 hour and then cooled in a desiccator. Each crucible and lid was weighed to 0.0001 g. Approximately 4 g of the crab sample was placed in each pre-weighed crucible and weighed to 0.0001 g. The materials were charred on a hot plate until black in color and no smoking occured. The covered crucibles were then placed in a muffle furnace (Blue M. Electric Company, Blue Island, Illinois, U.S.A.) set at 550°C and left overnight. The crucibles were removed from the furnace and cooled in a desiccator for 20 - 30 minutes and then weighed with cover to 0.0001 g. The material was pulverized with a glass stirring rod. If black particles were observed the materials were again placed in the muffle furnace overnight and then re-weighed again.

The ash content was determined according to the following formula:

$$A = \frac{W_3 - W_1}{W_2 - W_1} x100\% , \qquad (14)$$

Where: A = percent ash;

$W_1$ = weight of empty crucible with cover;

$W_2$ = weight of crucible, cover and sample added;

$W_3$ = weight of crucible, cover, and ash.

#### 3.3.1.5 pH Analysis:

The pH was determined using a pH meter (Orion Research Inc., U.S.A.). Each time the pH meter was used it was calibrated using buffers of pH 4 and 7. Containers, including quantities of crab and brine, were well blended and transferred into a 50 ml beaker. The pH meter electrode was placed into the stirred mixture and then let to stand until the pH meter reading stabilized. Once the reading stabilized the pH of the crab was recorded.

### 3.3.2 Free Amino Acid Analysis:

The crab samples were prepared in the following way and then sent to the amino acid analysis lab at the Biochemistry Department of Memorial University for free amino acid determination. The samples were analyzed on a Beckman Model 121 MB amino Acid Analyzer. A single column three buffer sodium citrate elution method was used at a flow rate of 8 ml/hr with buffers and column temperature as per Beckman 118/119 CI AN-001 application notes. Quantitative determination of the results was achieved using a Hewlet Packard Computing Integrator Model 3395A.

In duplicate, 15 ml centrifuge tubes were weighed to 0.0001g. Approximately 0.5 g of homogenized freeze dried crab product, moisture content known, was placed in each tube and re-weighed to 0.0001g. A measured quantity, 5 ml, of 14% sulfosalicylic acid was added to the tubes and the weight and volume measured again. The samples were then vortexed for 15 seconds and placed in the refrigerator for 30 minutes. The cold samples were then vortexed again before pouring into 1.5 - 3.0 ml labeled eppendorf tubes. The tubes and contents were then centrifuged for 5 to 7 minutes then the

supernatants were transferred to another two 1.5 ml tubes and centrifuged again. The supernatants were combined and the volume was recorded with a graduated cylinder. The supernatant was divided into 1 ml portions and was standardized to a pH of between 1.9 to 2.4 with quantities of either 2N HCl or 2N LiOH. The quantities of acid or base used was recorded. A 1 ml quantity of supernatant and 1 ml lithium citrate buffer with internal standard [(s)-2-aminoethyl-L-cysteine hydrochloride], supplied by the amino acid lab, was combined in a s13x100 mm tube. Each subsequent 1 ml sample of supernatant was placed in 13 x 100 mm test tubes with the quantity of acid or base as previously determined and topped up to 2 ml with lithium citrate buffer with internal standard. The samples were then ready for the amino acid testing lab.

### 3.3.3 Fatty Acid Analysis:

#### 3.3.3.1 Fat Extraction:

Fat samples were extracted from the crab using a modified Bligh and Dyer (1959), procedure. The samples were prepared to the separatory funnel stage as described in Section 3.3.1.1 and were allowed to separate into the chloroform methanol layers. Round bottom flasks (125 ml) were weighed to 0.0001g. The fat/chloroform layer was drained into the round bottom flask from the separatory funnel. The round bottomed flask was immediately purged of oxygen using gaseous nitrogen and attached to a Caframo VV 2001 Rotary Evaporation system (Heidolph-Elecktro GmbH and Co. KG, Kelheim, Germany). The heating water of the rotary evaporator was set at 55°C and 90 rpm. The rotary evaporation system was also purged of oxygen through a stop cock in the top of the condenser using gaseous nitrogen. The rotary evaporation system was vacuumized to

20 inches of mercury vacuum and was set to operate with the round bottom flask rotating in the heating water. Cold water was circulated through the condenser to facilitate the condensation process. Chloroform was thereby evaporated from the fat extracted. Once evaporation was complete the system was shut down and the round bottom flask was capped and cooled. The round bottom flask and the contained fat were then weighed to 0.0001g. The mass of fat extracted from the sample, obtained by difference, represented as the fat content of the sample. Fat obtained was resuspended in 500 µl of Chloroform and was pipetted into vials with a small amount (approx. 0.05g) of anti-oxidant (hydroquinone), blown down with nitrogen and stored at -60°C until further needed.

**3.3.3.2 Transmethylation:**

Transmethylation was performed according to the method of Keough and Kariel (1987). A fat sample, 0.05 g, was placed into a conical reaction vessel. A measured mixture, 2 ml, of methanol/hydrochloric acid solution (94:6 v/v well mixed) was added to the fat. A small amount of anti-oxidant (hydroquinone) was added and vortexed as well and a disposable screw on cap was applied. The sample was placed inside a metal tube with screw on lid and was incubated at 70°C for 16 hours. The tubes and samples were cooled and opened carefully. The contents of the reaction vessel was poured into a small test tube (#1). An aliquot of hexane, 1.5 ml, was added to the sample and vortexed with the top layer formed removed to another test tube (#2). This procedure was repeated removing the top layer to the same test tube (#1). Distilled water, 1.5 ml, was placed in the removed fluid and vortexed. The top layer formed was removed to another test tube (#3). Another aliquot, 1.5 ml, of distilled water was placed in the subsequent fluid (test

tube 3) and top layer removed to a different test tube (#4). This test tube was covered in parafilm and placed in the freezer at -20°C for 3 - 4 hours to remove any remaining water. The resulting fluids were then immediately poured into a clean test tube and evaporated to dryness with nitrogen, approximately 30 - 40 minutes. Carbon disulfide, 1 ml, was placed into the evaporation test tube and was subsequently pipetted into a labeled autosampler vial. Each vial was topped up with carbon disulphide and then stored in the ultrafreezer (-60°C) until GC analysis.

#### 3.3.3.3 Gas Chromatography:

The methyl esters formed from this procedure were analyzed via gas chromatography with a flame ionization detector. A Hewlett Packard 5890 Series 2 Gas Chromatograph (Hewlett packard, Atlanta, GA) equipped with 30 m x 0.53 mm I.D. and 1 mm film thickness Supelcowax 10 (Supelco Canada Ltd., Mississauga , ON) fused silica capillary column was used. The oven temperature was set at 220°C and the injection port and detector were maintained at 230°C. Identification of fatty acids was based on two standards; a mixture of PUFA-1 (Supelco Cat. No. 4-7033), and lipid standards (181-1 and 181-15, Sigma).

### 3.4 Shelf Life Analysis:

#### 3.4.1 Sample Preparation:

A number of containers were prepared using the finished commercial product formulation, giving a drained weight of 185 g. The samples were to be representative of those made commercially for sale giving a good estimate of the potential shelf life of the product.

#### 3.4.2 Sampling Plan:

The quantity of samples prepared were based upon the following sampling plan. Note that during the shelf life study the containers were exposed to three temperatures: 2.2°C, 4°C and 10°C. The 4°C test was performed after the 10°C set for shelf life confirmation at a mediocre challenge temperature.

Table 1: Sampling schedule for the 12 month microbial shelf life study.

| Sampling Time | Quantity at 2.2°C (Containers) | Quantity at 4°C (Containers) | Quantity at 10°C (Containers) |
|---|---|---|---|
| 0 Time | Unprocessed 4<br>4* | 2** | 2 |
| Week 1 | 4 | 2 | 2 |
| Week 2 | 4 | 2 | 2 |
| Week 3 | 4 | 2 | 2 |
| Week 4 | 4 | 2 | 2 |
| Week 6 | 4 | 2 | 2 |
| Week 8 | 4 | 2 | 2 |
| Week 10 | 2 | | |
| Week 12 | 2 | | |

| | | | |
|---|---|---|---|
| Week 16 | 2 | | |
| Week 20 | 2 | | |
| Week 24 | 2 | | |
| Week 36 | 2 | | |
| Week 48 | 2 | | |
| Week 52 | 2 | | |
| Totals: 12 months | 48 | 14 | 14 |

* All samples except the first are processed.

** The 4°C set was only followed for 2 months with a small set at 2.2°C for comparison.

The above sampling scheme shows that 76 samples were prepared and stored for analysis. An additional 20 samples were prepared for contingencies.

### 3.4.3 Plating Media and Method:

Tryptic soy agar (TSA) (DIFCO) was used as the main plating medium for both the anaerobic and aerobic plate counts. It was prepared and autoclaved.

A 7 step serial dilution was prepared from each crab sample as follows: Dilution blanks, 9 ml, made with 0.1% peptone water, were prepared in test tubes, plugged with cotton, capped with aluminum foil and autoclaved. Serieal dilutions were labeled from $10^{-2}$ to $10^{-8}$. A weighed quantity, 50 g, of crab sample was placed, using aseptic techniques, into a sterile Waring blender with 450 ml of sterile 0.1% peptone water and blended thoroughly at high speed (2 minutes). This created a $10^{-1}$ dilution. From this initial blended mixture, 1 ml solution was taken with a sterile pipette and placed in the first test tube resulting in a $10^{-2}$ dilution. This test tube was vortexed for two minutes and then 1

ml of it was placed, with a sterile pipette, in the second test tube resulting in a $10^{-3}$ dilution. This procedure was repeated until a $10^{-8}$ dilution was made.

Pour plates were made in duplicate for each of the dilutions. Sterile petri dishes were labeled appropriately. Previously autoclaved TSA agar was melted and held in a water bath at 45°C until needed. A 1 ml aliquot of each dilution was placed in each appropriately labeled petri dish using aseptic techniques. Each petri dish was then filled to about half full with melted sterile agar and allowed to stand (covered) until the agar solidified, roughly 45 minutes. The plates were then inverted and placed in a 37°C incubator for 72 hours. Poured and solidified plates for anaerobic analysis were inverted and placed in anaerobic jars and oxygen was evacuated using a vacuuming system and maintained with a BBL Gas Pack Anaerobic System (Becton Dickinson). The anaerobic jars were then placed in the incubator (Model 307, Fisher Scientific) held at 37°C for 72 hours.

Plates were counted using a standard back-lighted colony counting system and a hand held counter.

## 4.0 RESULTS AND DISCUSSION

### 4.1 Slowest heating Spot:

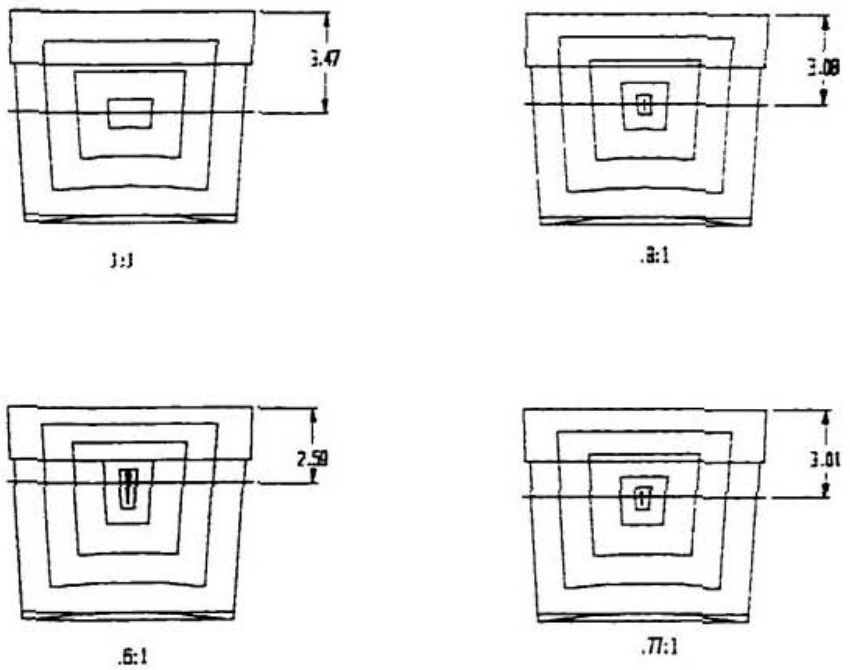


Figure 18: Graphical analysis of heat penetration rates into an 8 oz polypropylene container with an aluminum pull top lid filled with rock crab meat and brine. Heat penetration ratios from top to side are varied from 1:1 to 0.6:1.

A number of Cadkey [R] graphs were drawn, Figure 18. In a transient, conductive heating condition, isothermal lines, lines along which the same temperature exists in the container at any time, can be drawn at even intervals toward the center of the container based upon the rate of heat transfer from each side of the container. Each line represents a constant temperature at uniform time intervals. The slower heat transfer rates will result in lines closer together for each uniform time interval. This will approximate the pattern of heat transfer toward the center of the product, (Holman, 1976). Jackson and Olson

(1940) describe the conductive heating of food stuffs as being conducted symetrically from the outside of the can toward its center For the conductively heated container used in the present study, heat transfer rates are uniform on the side and bottom of the container, as the heat is transferred through plastic of constant width, and meat/brine in close contact in both situations. Heat transfer through the top occurs through an aluminum lid in close contact with loosely packed meat above the level of brine in the container, containing air pockets. A lower rate of heat transfer can be expected through the top as these air pockets entrained within the loosely packed meat in contact with the aluminum lid will act as an insulator (Holman, 1976). Since heat transfer rates through the lid are expected to be less, the distance between the isothermal lines are expected to be smaller. Stumbo (1973) also demonstrated the use of a similar method where the lines of constant temperature were actually representaed as lines of constant F or iso-F and also as iso-j. The iso-j concept was also utilized in work by Yawger (1978). Figure 18 shows isothermal lines from four different lid heat transfer rates utilizing top to side width ratios from 1:1 to 0.6:1. From these scale drawings it was anticipated that the slowest heating spot should occur between 2.6 and 3.5 cm below the lid

The actual slowest heating center was determined experimentally around those values obtained by the above graphical analysis, through the use of adjustable thermocouples fitted to the lids of the conductively heating containers. Table 2 shows the sample heating lethalities obtained from thermocouples placed at depths between 2.6 and 3.2 cm below the lid. Tukey's multiple comparison test, $\omega = 1.1$, showed significant effect of depth on lethality between 3.1 and 3.2 cm and also between 2.6 and 3.0 cm. Mean

values for depths of 2.8, 3.0 and 3.1 cm were found to be statistically the same. Using this methodology one would expect to find gradually decreasing values of lethality until the slowest heating spot was reached. After this point, increasing levels of lethality would be observed. The lethality at 3.0 cm and at 3.1 cm depth equally correspond to the slowest rate of change and lowest lethality. The 3.0 cm depth was selected as the slowest heating point.

Table 2: Mean lethality value vs. Vertical depth from lid in containers of rock crab meat processed at reference temperature of 85°C.

| Depth (cm) | Lethality (minutes) $\omega = 1.1$* | n |
|---|---|---|
| 2.6 | 44.7 (±0.21)** | 2 |
| 2.8 | 43.7 (±0.63) | 3 |
| 3.0 | 43.1 (±1.05) | 4 |
| 3.1 | 43.1 (±0.72) | 3 |
| 3.2 | 44.6 (±0.64) | 3 |

* From Tukey's multiple comparison test (Mendenhall and Sincich, 1984). Means separated by this value are statistically significant.

** Values in parentheses refer to the standard deviation of the mean.

## 4.2 Heat Penetration:

### 4.2.1 Parameters and Process Times:

The effect of processing temperature on heat penetration parameters obtained using needle thermocouples are shown in Table 3. No significant differences were observed between processing temperatures apart from $j_c$.

Table 3: Mean value of heat penetration parameters for rock crab meat samples processed at three temperatures to $P^{85}_{8.89} = 40$ minutes.

| Treat-ment No. | Temper-ature (°C) | $j_h$ | $f_h$(min.) | $j_c$ | $f_c$(min.) | n |
|---|---|---|---|---|---|---|
| 1 | 81 | 1.71(±0.03) | 34.87(±0.43) | 1.53(±0.04) | 51.78(±1.73) | 12 |
| 2 | 82 | 1.72(±0.03) | 34.58(±0.66) | 1.58(±0.02) | 50.06(±0.45) | 6 |
| 3 | 83 | 1.69(±0.04) | 34.74(±1.04) | 1.56(±0.03) | 50.53(±1.73) | 9 |
| $F_{0.05} = 3.39^*$ | | 1.24 | 0.33 | 3.47 | 3.00 | |

* F test statistic from 1 way ANOVA (F=MST/MSE, Reject when $F > F_{0.05}$). The analysis is 1 way based on temperature because each processing time yields an identical lethality, 40 minutes.

The value of $f_h$ in a conductively heating product should be in close agreement with the $f_c$ value provided that the product does not change its thermal conductivity over the cook. It was expected that this would be the case for the product investigated here. However, pronounced differences were found between the $f_h$ and the $f_c$ values. Although the specific reason for this is not clear, one can hypothesize two potential reasons. The first is that the method of cooling was not instantaneous. In this regard, the Stock Rotomat retort did not instantly introduce the containers into the cooling medium at the

required temperature, such as would be the case if the containers were plunged into cooling water. Rather, the cooling water was added into the hot process water until the cooling water temperature was attained. This lag in temperature drop in the cooling water is believed to cause an increase in the $f_c$.

The second possibility is that a reaction (ie. physical bonding or complexing) between added brine and the meat sample may have caused a change in the thermal conductivity values during heating and cooling. The container may have been convectively heated at first on a micro scale. Free pockets of water within the meat would assist heat transfer when heating through micro-convection. After heating, these pockets of water would have been absorbed by the meat preventing the micro-convection and decreasing the thermal conductivity. This decrease in thermal conductivity would decrease the rate of cooling and increase the $f_c$.

The analysis of variance of the heat penetration parameters shows that there are at least two values different in the $j_c$ data. Since $j_c$ is defined by the initial cooling characteristics of the cooling segment of the process, it follows that the variations in the $j_c$ could also be due to the cooling method of the retort as explained previously. This could also be an individual characteristic of the processing temperature but is unlikely because the $f_c$ values among the temperatures were found to be statistically the same. The cooling curve portion of this cook can be considered unreliable in processes using the Stock Rotomat Retort when comparing to processes involving a separate cooling system like that observed in commercial operations. Guaranteed lethalities can only be based on the

heating portion of the cook as lower lethalities would be expected from the cooling curve portion in an industrial setting.

The heating curve parameters at each process temperature were found to be the same which indicated that, from temperature to temperature, there was no difference in the heating characteristics of the product. This may have been due to the small differences in temperatures used but could have reflected a uniformity in physical heat transfer characteristic over a range of temperatures.

Times for processing to a lethality of 40 minutes, referenced at 85°C and z = 8.89C°, for each of 81°C, 82°C and 83°C were found to be 175, 155, and 130 minutes, respectively (Table 4).

Table 4 : Process times corresponding to process temperature giving a lethality of $P^{85}_{8.89}$ = 40 minutes.

| Treatment No. | Temperature (°C) | Processing Time (minutes) | n |
|---|---|---|---|
| 1 | 81 | 175 | 12 |
| 2 | 82 | 155 | 6 |
| 3 | 83 | 130 | 9 |

A 1°C shift in the temperature caused an increase of 20 - 25 minutes in the processing time. This demonstrates two very important points:

1. The pasteurization lethality in this temperature range is very temperature sensitive;
2. Energy expenditures rise very quickly as the temperature is slightly reduced.

The first point indicates that the control system used to maintain the temperature of the heating medium must be very accurate and precise. A small drop in processing temperature can cause severe process deficiencies. As a result, the processor should be cautious and hold the temperature slightly higher than required (+0.5°C) to account for any temperature fluctuations in the control system.

The second point infers that the process temperature must be maintained as high as possible to prevent excessive energy expenditures which will increase the cost to produce the product. Process time effects the product flow rate through the processing plant for a given size of equipment as well. It is in the processor's, and subsequently the product process developer's, best interest to see the process proceed in the shortest possible time with the most cost effective energy expenditures giving the highest consistent finished product quality. The following analysis demonstrates the level of effect of the lower process temperatures on energy expenditures.

### 4.2.2 Energy Efficiency Relationship to Processing Temperature:

In this case, the container has an upper process temperature limit of about 83°C. This limits the shortest process time to that attainable at 83°C which is 130 minutes. A 1°C drop in the process temperature results in an increase of 25 minutes to the process time. A 2°C drop increases the time by 45 minutes. The amount of energy expended over an extra 25 to 45 minutes processing time outweighs the additional energy required to maintain the temperature 1 - 2°C higher for shorter periods as is evidenced by the following example:

In commercial systems, pasteurizing water is usually pre-heated to just above the process temperature so that when the product is introduced, the drop in temperature will not cause a large system response time or come up time. The same heated water is used for many pasteurization runs. The heating control system then must maintain a constant temperature against heat losses to the cold product and to the surroundings.

Assume:

- a lidded stainless steel pasteurization vessel with dimensions 91.44 cm x 91.44 cm x 91.44 cm.
- The plant temperature is 12°C.
- 286 kg of crab in the pasteurizer
- Cp (specific heat) of crab = fish = 3.6 kJ/kg.°C (Lund, 1992)
- $h_{nat}$ (natural convection coefficient) = 11.4 $W/m^2.°C$ (Lund, 1992)
- the stainless steel pasteurization vessel is at the same temperature as the contained cook water.
- the come up time is 0 minutes.

An energy balance using the pasteurizer just after the crab is introduced suggests the following:

$$Qin = Qproduct + Qout \qquad (15)$$

Where: Qin = the amount of heat put into the system over the process time;

Qproduct = the amount of heat taken up by the cold product over the process;

Qout = heat losses to the plant over the process time.

Singh (1977) uses a similar energy balance method for making energy consumption evaluations on sterilizing retorts. The formulas used in this analysis correspond closely to those used by Singh.

We can use the formula:

$$Qproduct = mCp\Delta T \text{ (Holman, 1976)} \qquad (16)$$

to calculate the amount of heat taken up by the product.

The change in temperature will be over three possible ranges as shown in Table 5:

Table 5: Product temperature change during process.

| Temperature Range # | Process Temperature (°C) | Plant temperature (°C) | ΔT (°C) |
|---|---|---|---|
| 1 | 83 | 10 | 73 |
| 2 | 82 | 10 | 72 |
| 3 | 81 | 10 | 71 |

Heat lost to the product then is as shown in Table 6 for the three ranges:

Table 6: Heat required to increase the product temperature to the process temperature from plant ambient conditions.

| Temperature Range # | Mass (kg) | Cp (kJ/kg.°C) | ΔT (°C) | Qproduct (kJ) |
|---|---|---|---|---|
| 1 | 59.1 | 3.6 | 73 | 15,498 |
| 2 | 59.1 | 3.6 | 72 | 15,286 |
| 3 | 59.1 | 3.6 | 71 | 15,074 |

The heat convection equation:

$$Qout = hnatA\Delta T \qquad \text{(Holman, 1976)} \qquad (17)$$

can be used to determine the rate of heat lost to natural convection over the process time.

Where: hnat = the natural convection coefficient = 11.4 $W/m^2.°C$;

A = surface area of the pasteurizer, 5 sides exposed to air;

$\Delta T$ = difference in temperature between the air of the plant and the stainless steel vessel.

The heat lost to the surroundings then is as follows for the three temperature ranges:

Table 7: Heat lost from the hot tank due to natural convection.

| Temp. Range # | hnat ($W/m^2.°C$) | A ($m^2$) | $\Delta T$ (°C) | qout (W) | Time (min.) | Qout (kJ) |
|---|---|---|---|---|---|---|
| 1 | 11.4 | 4.16 | 73 | 3370 | 130 | 26,292 |
| 2 | 11.4 | 4.16 | 72 | 3323 | 155 | 30,906 |
| 3 | 11.4 | 4.16 | 71 | 3275 | 175 | 34,395 |

The total heat to be supplied for the three ranges then is:

Table 8: Total heat required during the process, Qin.

| Temperature Range # | Qproduct (kJ) | Qout (kJ) | Qin (kJ) |
|---|---|---|---|
| 1 | 15,498 | 26,292 | 41,790 |
| 2 | 15,286 | 30,906 | 46,192 |
| 3 | 15,074 | 34,395 | 49,469 |

As can be seen by this conservative example, the amount of heat used (Qin) rises rapidly based on holding time, more so than the increase in heat required due to the temperature rise. The 83°C and 130 minutes process time (Range #1) is greater than 15% more efficient than the 81°C, 175 minute process (Range #3). It is greater than 9% more efficient than the 82°C and 155 minute process (Range #2).

In addition, some pasteurizing systems use cold compressed air as a mixing medium to ensure adequate mixing among the containers. This introduces another heat loss which must be overcome on a continuous basis increasing the differences noted here.

### 4.2.3 The Optimal Process:

From an energy consumption point of view the product process will be optimal at the highest temperature and shortest time or 83°C for 130 minutes. Product quality, however, must be considered before the product process is deemed acceptable. If the product quality coming from the above process is deemed unacceptable then the optimal

process must be reselected at a lower temperature and longer process time sacrificing energy.

### 4.3 Quality Assessment:

The product quality coming from the energy optimal process selected above will be evaluated through the following means:

- Nutritional Quality
  - ⇒ proximate analysis and pH;
  - ⇒ fatty acid analysis;
  - ⇒ essential amino acid analysis.
- Microbiological Quality
  - ⇒ Shelf Life Analysis

#### 4.3.1 Nutritional Quality:

Nutritional quality will be examined through the following factors:

- proximate analysis and pH;
- fatty acid analysis;
- essential amino acid analysis.

Comparisons will be made with analyses for other crab species from other published authors.

#### 4.3.1.1 Proximate Analysis and pH:

Proximate analysis involves the determination of five main attributes namely protein content, fat content, water content, ash content and carbohydrate content. Table 9 describes the proximate analysis of the pasteurized rock crab as it relates to other species of crab from various areas:

Table 9: Table of proximate analysis values for rock crab, snow crab and dungeness crab.

| Attribute | Processed Rock Crab (*Cancer irroratus*) | Soviet Snow Crab [a] (*Chionecites opilio*) | Snow Crab, Canned [a] (*Chionecetes opilio*) | Dungeness Crab [b] (*Cancer magister*) Average Males and Females |
|---|---|---|---|---|
| % Protein | 14.09 (±0.38)<br>14.33 (±0.17)* | 16.8 | 19.5 | 15.1 |
| % Water | 82.33 (±0.36) | 79.5 | 78.2 | 78.4 |
| % Lipid | 0.78 (±0.03) | 0.1 | 1.0 | 1.2 |
| % Ash | 2.13 (±0.08) | 2.4 | 1.6 | - |
| % Carbohy-drates | 0.43<br>(By difference) | 0 | 0 | - |

* Unprocessed value. No significant difference was found between processed and unprocessed values of crude protein ($p<0.05$), $n=6$. (a) Krzeczkowski and Stone, 1974. (b) Allen, 1971

The proximate analysis of the processed rock crab was similar to the analysis for Dungeness (*Cancer magister*) and Snow crab (*Chionecetes opilio*). The relative ratios were somewhat consistent with variations being explained by different species and various processing methodology. Krzeczkowski and Stone (1974), described proximate analysis differences determined between snow crab located in different areas of the world as being related to processing differences. The high ash content of the Soviet Snow crab was attributed possibly to the presence of tendon in the merus (leg meat) section of the

extracted meat. Similarly, the relatively high ash content in the Rock crab could be attributed to the presence of shell or cartilage in the meat.

There was no significant change observed between the unprocessed and processed values of crude protein in the sample ($p<0.05$). This seemed reasonable given the relatively mild heat treatment undergone by the food (83°C) during pasteurization as compared to the cooking process at 100°C.

Carbohydrate was calculated by difference and as such is subject to experimental error. Since none of the other crab species show the presence of carbohydrate, this value was questionable.

The marginally higher moisture content could be attributed to the added brine in the sample. The proximate analysis was performed on the blended crab and brine not crab meat only. This difference will also explain the relatively reduced quantities of protein and fat observed.

The pH of the pasteurized product was found to be 8.17 ($\pm$0.22), n=4. This product was found to be a low acid food with a pH ideal for the growth of many microorganisms.

#### 4.3.1.2 Fatty Acid Analysis:

Table 10 describes the differences between the processed and unprocessed crab fatty acids:

Table 10: Fatty acid analysis for unprocessed (n=3) and processed rock crab (n=12).

| | Name | Unprocessed Value mg/g sample | Processed Value mg/g sample | t, Significance (Stndrd t-test) for df = 13, $t_{0.025}$=2.160 |
|---|---|---|---|---|
| 14:0 | Myristic | 0.039 (±0.002) | 0.035 (±0.001) | 5.13, sig |
| 16:0 | Palmitic | 1.097 (±0.059) | 0.958 (±0.004) | 9.18, sig |
| 16:1 | Palmitoleic | 0.343 (±0.009) | 0.300 (±0.001) | 18.26, sig |
| 17:0 | Heptadecanoic | (3*) | (3) | |
| 18:0 | Stearic | 0.415 (±0.031) | 0.379 (±0.002 | 4.54, sig |
| 18:1 | Oleic | 1.175 (±0.063) | 0.934 (±0.009) | 14.32, sig |
| 18:2 | Linoleic | 0.153 (±0.028) | 0.105 (±0.001) | 6.75, sig |
| 18:3 | Linolenic | 0.046 (±0.002) | 0.034 (±0.001) | 15.38, sig |
| 18:4 | Octadecatetraenoic | 0.017 (±0.002) | 0.016 (±0.001) | 1.28, ns |
| 20:4 | Arachidonic | 0.711 (±.002) | 0.661 (±0.007) | 11.94, sig |
| 20:5 | Eicosapentaenoic | 3.376 (±0.023) | 3.069 (±0.031) | 15.90, sig |
| 22:5 | Docosapentaenoic | 0.138 (±0.001) | 0.123 (±0.002) | 12.35, sig |
| 22:6 | Docosahexaenoic | 1.291 (±0.012) | 1.128 (±0.009) | 26.52, sig |
| | Total | 8.801 | 7.742 | |

* = Internal standard, sig = significantly different ($p<0.025$), ns = Not significantly different ($p<0.025$).

The data in Table 10 shows that the pasteurization heat treatment significantly effected the levels of fatty acid in the product. This was expected because the low levels

of fat in the product increased the sensitivity of any measurements made. The most notable reductions were those associated with oleic, linoleic, and linolenic acids which showed greater than a 20% drop. Eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) reduced 9.1% and 12.6% but comprised over 38% and 14% of the total available fatty acids. Nesheim (1974) indicated that carbohydrates and fats are generally not adversely affected by heat processing except at extremes. The above noted reduction may not have had that great an importance given this observation. Giddings and Hill (1975), however, indicated that specific slight changes in the lipid of certain seafoods can conceivably effect eating quality. The determination of the true effect of any changes must be made with a sensory evaluation. A structured sensory evaluation was beyond the scope of this work and should be completed in further efforts.

Table 11 describes the processed and unprocessed fatty acid percent of total as compared to different species of crab. It shows that the relative percentages of fatty acid are close between those measured in this study and those of other studies except for the relative levels of eicosapentaenoic acid and oleic acid, apart from dungeness crab. The larger relative quantities of eicosapentaenoic acid may be due to variations in the crab habitat, processing treatment and sample preparation method as samples were prepared with brine intact.

The relative percentages of unsaturated, monunsaturated and polyunsaturated fatty acids are similar for both the processed and unprocessed pasteurized rock crab. Comparisons with other crab types show a variation in the percentages of mono- and polyunsaturated fatty acids, the percent of unsaturates seems to remain constant. This is

also attributed to variations in habitat, processing method and sample preparation. In all cases, the largest group of fatty acids were the polyunsaturated fatty acids. The polyunsaturated fatty acids made up greater than 60% of the fatty acids present in the pasteurized rock crab.

This analysis has shown that the quantity of fatty acid has been reduced by 1.1 mg/g sample or 12% but the relative percentages of fatty acid have remained approximately the same. Further work should identify the impact of this reduction given the low levels of fat present in the rock crab through a sensory analysis.

Table 11: Processed and unprocessed percentages of fatty acid for different species of crab as compared to Rock Crab.

| | Name | Unpro-cessed | Pro-cessed | Rock Crab [a] | Jonah Crab[a] | Red Crab [a] | Snow Crab [b] | Dun-geness Crab [c] Male Uncooked |
|---|---|---|---|---|---|---|---|---|
| 14:0 | Myristic | 0.4 | 0.5 | 1.2 | 0.6 | 0.4 | 0.4 | 0.1 |
| 16:0 | Palmitic | 12.5 | 12.4 | 12.4 | 11.6 | 11.4 | 12.5 | 14.4 |
| 16:1 | Palmitoleic | 3.9 | 3.9 | 3.2 | 4.2 | 3.4 | 3.3 | 5.6 |
| 17:0 | Heptadecanoic | - | - | 1.0 | 0.6 | 0.8 | 1.3 | 1.2 |
| 18:0 | Stearic | 4.7 | 4.9 | 4.3 | 5.1 | 4.1 | 3.4 | 7.0 |
| 18:1 | Oleic | 13.4 | 12.1 | 7.1 | 5.3 | 4.0 | 16.0 | 14.0 |
| 18:2 | Linoleic | 1.7 | 1.4 | 1.0 | 1.1 | 0.9 | 3.5 | - |
| 18:3 | Linolenic | 0.5 | 0.4 | - | - | - | 0.5 | - |
| 18:4 | Octadecatetraenoic | 0.2 | 0.2 | - | - | - | 0.6 | - |
| 20:4 | Arachidonic | 8.1 | 8.5 | 3.1 | 7.0 | 4.8 | 4.8 | 0.0 |
| 20:5 | Eicosapentaenoic | 38.4 | 39.6 | 24.8 | 27.9 | 23.7 | 28.0 | 34.8 |
| 22:5 | Docosapent | 1.6 | 1.6 | - | - | - | 1.4 | 1.6 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | aenoic | | | | | | | |
| 22:6 | Docosahexa enoic | 14.7 | 14.6 | 13.4 | 9.7 | 15.9 | 15.0 | 13.7 |
| | Saturates | 17.6 | 17.8 | 18.9 | 17.9 | 16.7 | 17.6 | 22.7 |
| | Mono-unsaturates | 17.3 | 16.0 | 10.3 | 9.5 | 7.4 | 19.3 | 19.6 |
| | Poly-unsaturates | 65.2 | 66.3 | 42.3 | 45.7 | 45.3 | 53.8 | 50.1 |

(a) Krzynowek et al (1982), (b) Krzeczkowski and Stone (1974).

(c) Allen (1971), "-" = unavailable.

#### 4.3.1.3 Amino Acid Analysis:

Table 12 shows the comparison between free amino acids found in unprocessed and processed crab:

Table 12: Comparison of free amino acids profile in unpasteurized and pasteurized Rock Crab. Essential amino acids are bolded.

| | Unpasteurized (n=3) | | Pasteurized (n=7) | | | |
|---|---|---|---|---|---|---|
| Compound | Average (mg/g dry sample) | Standard Deviation (±) | Average (mg/g dry sample) | Standard Deviation (±) | t | $t_{0.025}$ =2.306 |
| Aspartic Acid | 0.0566 | 0.0132 | 0.0686 | 0.0049 | -2.214 | ns 5%* |
| **Threonine** | 0.0430 | 0.0078 | 0.0513 | 0.0053 | -1.996 | ns 5% |
| Serine | 0.0641 | 0.0034 | 0.0705 | 0.0167 | -0.635 | ns 5% |
| Asparagine | 0.0364 | 0.0234 | 0.0467 | 0.0119 | -0.959 | ns 5% |
| Glutamic Acid | 0.0823 | 0.0266 | 0.1097 | 0.0136 | -2.230 | ns 5% |
| Glutamine | 0.1726 | 0.1153 | 0.1416 | 0.0656 | 0.554 | ns 5% |
| Proline | 0.2920 | 0.2030 | 0.4041 | 0.0448 | -1.495 | ns 5% |
| Glycine | 1.1445 | 0.3408 | 1.1940 | 0.1565 | -0.330 | ns 5% |
| Alanine | 0.4345 | 0.0054 | 0.5362 | 0.1459 | -1.165 | ns 5% |
| **Valine** | 0.0558 | 0.0104 | 0.0676 | 0.0064 | -2.242 | ns 5% |
| Cystine | Not Available | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Methionine** | 0.0534 | 0.0192 | 0.0639 | 0.0034 | -1.528 | ns 5% |
| **Isoleucine** | 0.0439 | 0.0112 | 0.0501 | 0.0025 | -1.503 | ns 5% |
| **Leucine** | 0.0596 | 0.0120 | 0.0720 | 0.0076 | -2.008 | ns 5% |
| Tyrosine | 0.0554 | 0.0416 | 0.0717 | 0.0211 | -0.853 | ns 5% |
| **Phenylalanine** | 0.0301 | 0.0169 | 0.0405 | 0.0055 | -1.555 | ns 5% |
| **Tryptophan** | 0.0109 | 0.0081 | 0.0115 | 0.0064 | -0.127 | ns 5% |
| **Lysine** | 0.0701 | 0.0053 | 0.0776 | 0.0135 | -0.894 | ns 5% |
| ***Histidine*** | 0.0367 | 0.0111 | 0.0436 | 0.0033 | -1.614 | ns 5% |
| ***Arginine*** | 2.4488 | 0.8730 | 2.6611 | 0.2441 | -0.634 | ns 5% |

* ns = Not Significant at the 5% Level. Sig = Significant at the 5% Level.

The free amino acids were not significantly affected by the heat treatment ($p<0.05$). Nesheim (1974) indicated that the most heat labile of the essential amino acids were lysine and threonine. In this study, these two amino acids were not significantly reduced at the 5% level of significance. One would not expect a change in the amino acid content because of the fact that the crab meat, through pasteurization, was being cooked for a second time at a reduced temperature.

#### 4.3.1.4 Nutritional Quality Results Summary:

The proximate analysis and the free amino acid analysis did not show significant changes both from the processed - unprocessed condition and as compared to other species. Most of the fatty acids were found to have been significantly reduced by the pasteurization giving a total average reduction of approximately 12%. The impact of this reduction is not fully known but is expected to be minimal. A structured taste panel should be performed in further work to define the significance of any reduction in flavour noticed. Overall, these results show that the effect of the process on the nutritional quality

of the prepared rock crab meat was minimal, apart from the impact of a 12% reduction in fatty acids. This seems reasonable given the cooking procedure, prior to pasteurization, resulted in internal temperatures exceeding 85°C at the slowest heating points of the crab, 2°C higher than the process temperature, see section 2.1.3.3. Most of the crab meat, in the legs and surface components, would be treated to temperatures around 100°C (boiling).

### 4.3.2 Microbiological Quality:

Microbiological quality was evaluated through two shelf life tests. The first test extended for 12 months and the second for 8 weeks. Results from the second test were intended to examine the first.

#### 4.3.2.1 Shelf Life Analysis:

Figures 19 and 20 describe the aerobic and anaerobic plate count results with associated pH of rock crab samples held at 2.2°C. The results showed that no growth occurred over 40 weeks apart from one abnormally high count around week 20 which can be attributed to sampling error.

Figure 20 shows the anaerobic plate count results for the samples held at 2.2°C. All counts, apart from week 20, showed zero anaerobic growth during the test.

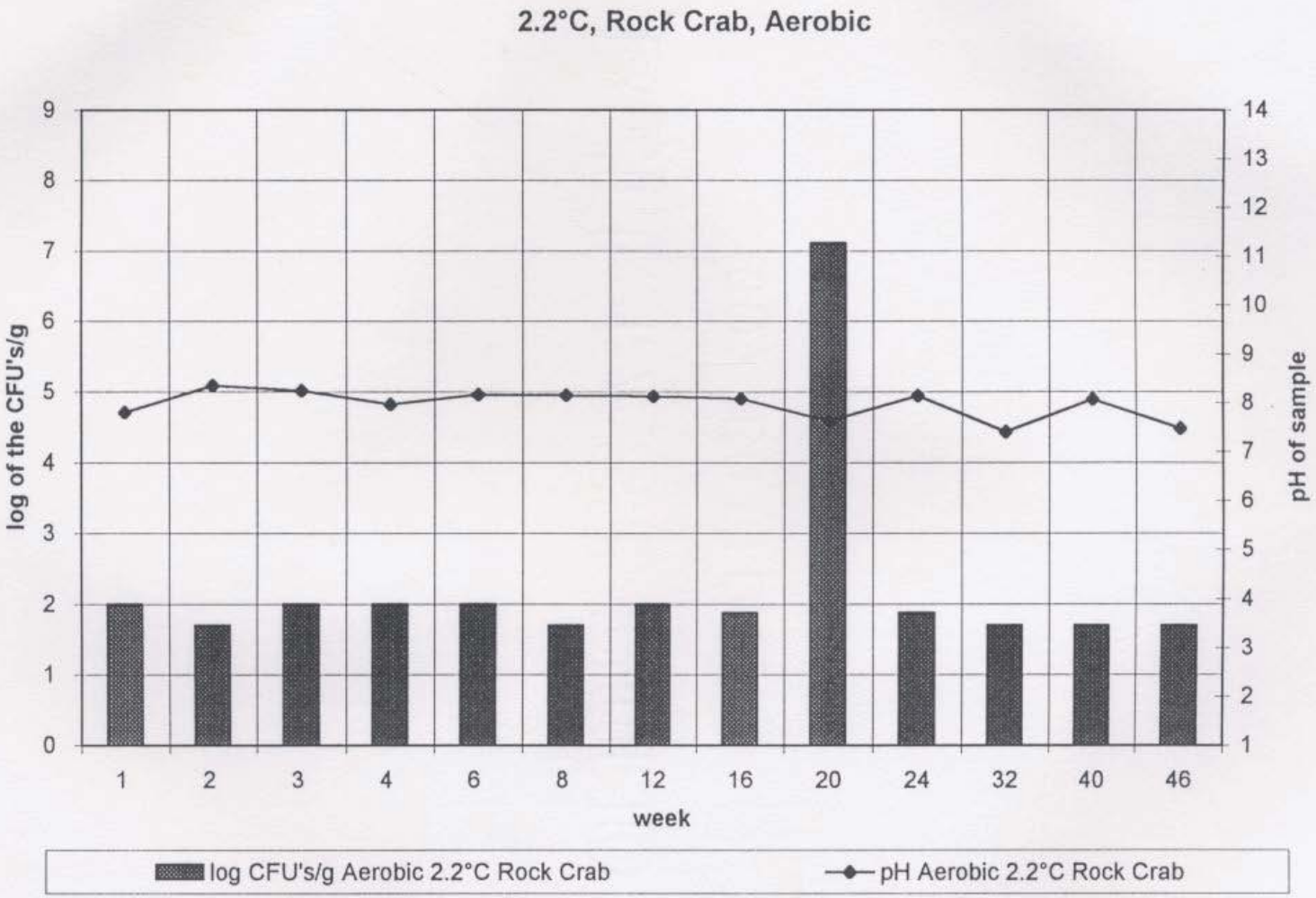


Figure 19: Aerobic shelf life results from rock crab sealed and pasteurized in 8 oz plastic containers with aluminum pull top lid stored at 2.2°C. (n=2) Recorded as pH and logarithm of aerobic colony forming units per gram (CFU/g).

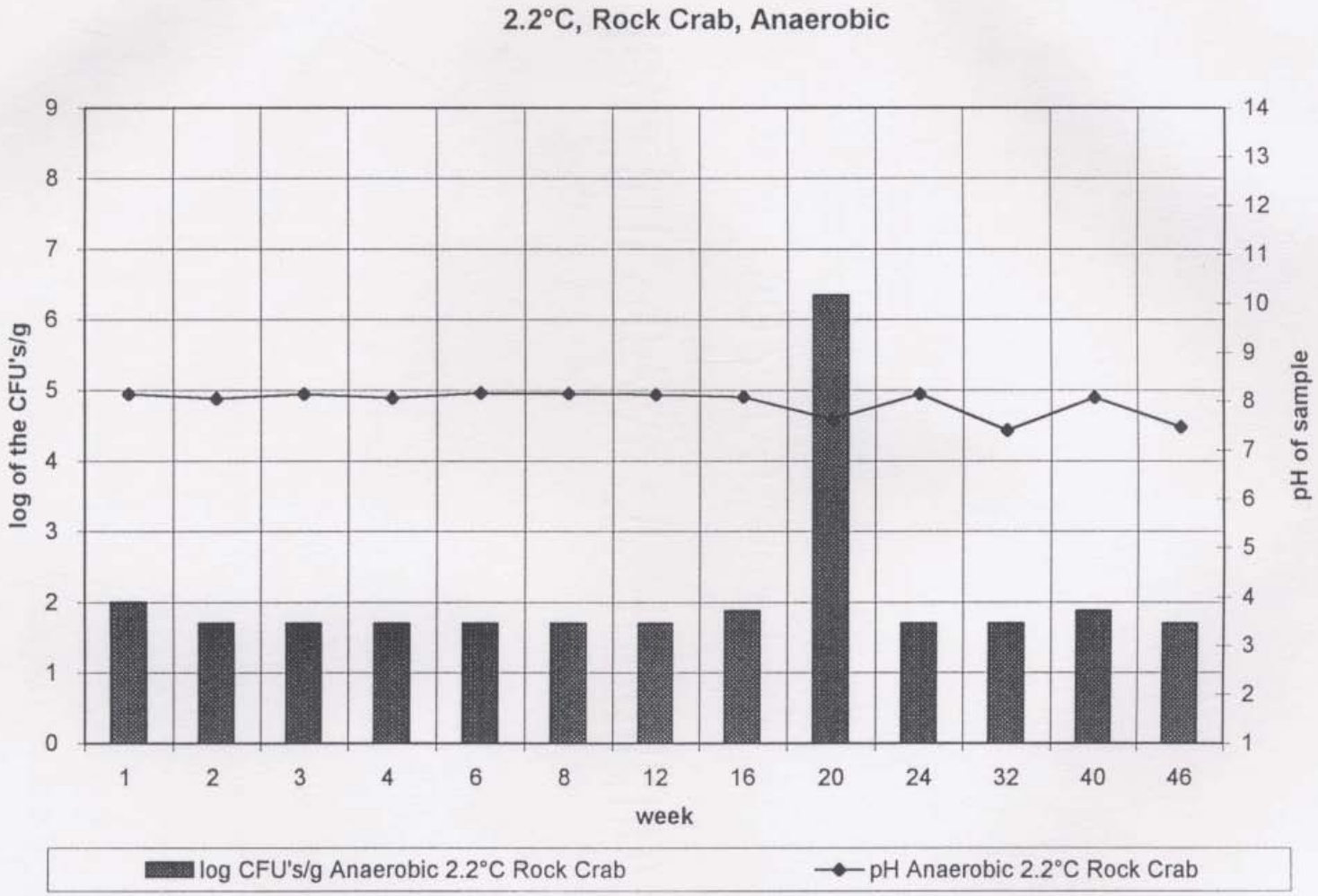


Figure 20: Anaerobic shelf life results from rock crab sealed and pasteurized in 8 oz plastic containers with aluminum pull top lid stored at 2.2°C. (n=2) Recorded as pH and logarithm of anaerobic colony forming units per gram (CFU/g).

Figure 21 and 22 shows the aerobic and anaerobic growth which occurred at the 10°C challenge temperature. Significant aerobic growth occurred by week 2 and significant anaerobic growth at week 3. Associated drops in pH occurred at weeks 4 and 6 respectively which may indicate the presence of some lactic acid producing bacteria in the sample. Ward et al (1977) identified and isolated lactobacilli as the predominant facultatively anaerobic microflora in pasteurized blue crab meat which supports this theory. It is also interesting to note that the samples spoiled aerobically first and subsequently anaerobically indicating the presence of free oxygen in the container, reducing the possibility of *Clostridium botulinum* growth, a strict anaerobe, and toxin production prior to spoilage.

Figures 23 and 24 show the 4°C results of the follow up work which examined product held at 2.2°C and 4.0°C. The later temperature, 4.0°C, was deemed a mediocre challenge temperature and was hoped to indicate the potential for growth during mild abuse or home style refrigeration conditions. All bacterial counts, both aerobic and anaerobic, showed zero growth during the test apart from one occurrence in the anaerobic results at week 8 in the 4°C set. This occurance was likely due to experimental error as it is unlikely that the microflora would have grown to this level at a 4°C temperature. No change was observed in the pH. These results indicated that if pasteurized rock crab meat, in good microbial condition, was placed in a consumers refrigerator, set at 4.0°C, the product would have over a 6 week shelf life. This is very important when considering product versatility. Consumers need the ability to purchase a food one day and consume it

some days later. Further studies should be performed on transportation systems, for products such as these, and the associated temperature abuse conditions prevalent.

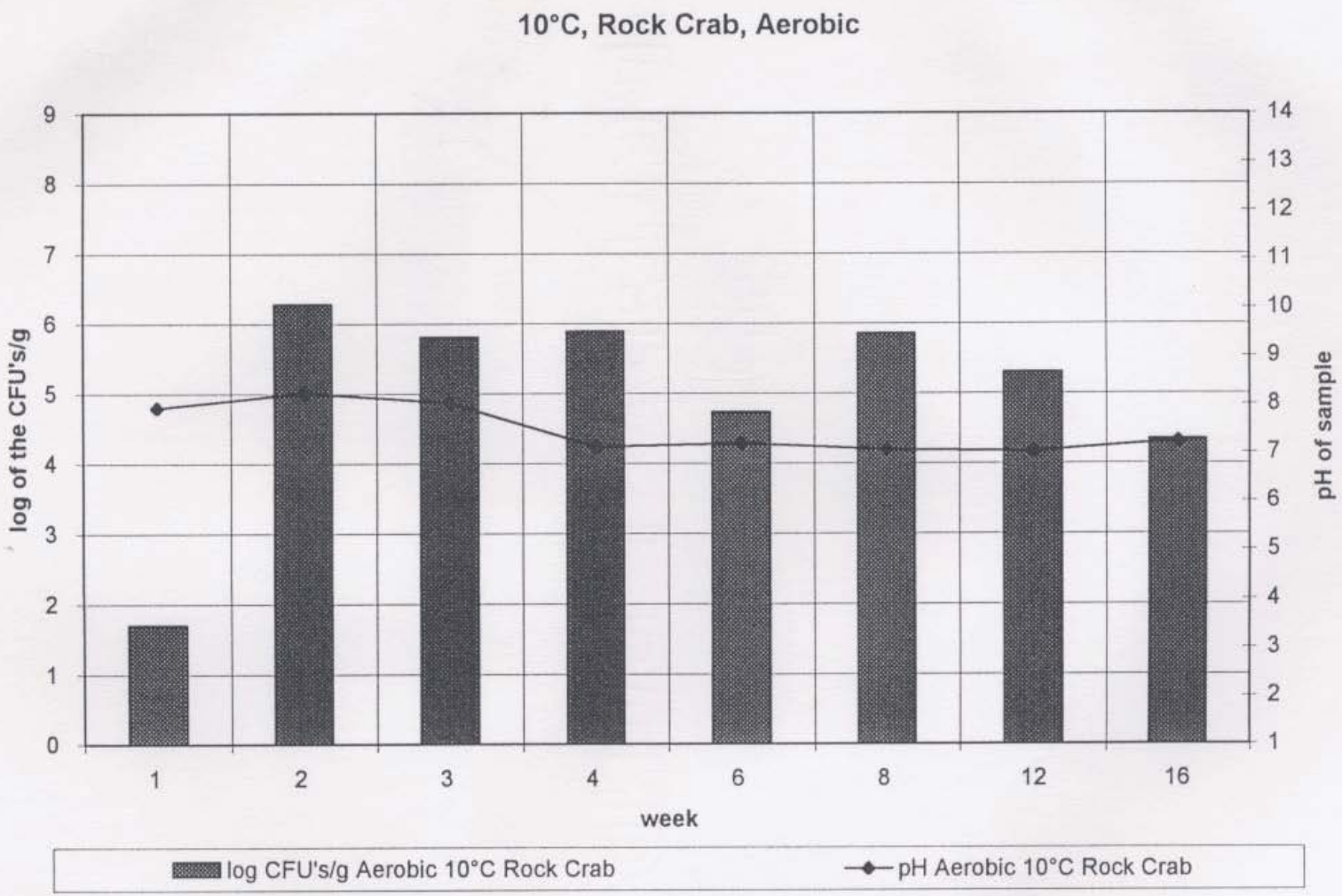


Figure 21: Aerobic shelf life results from rock crab sealed and pasteurized in 8 oz plastic containers with aluminum pull top lid stored at 10.0°C. (n=2) Recorded as pH and logarithm of aerobic colony forming units per gram (CFU/g).

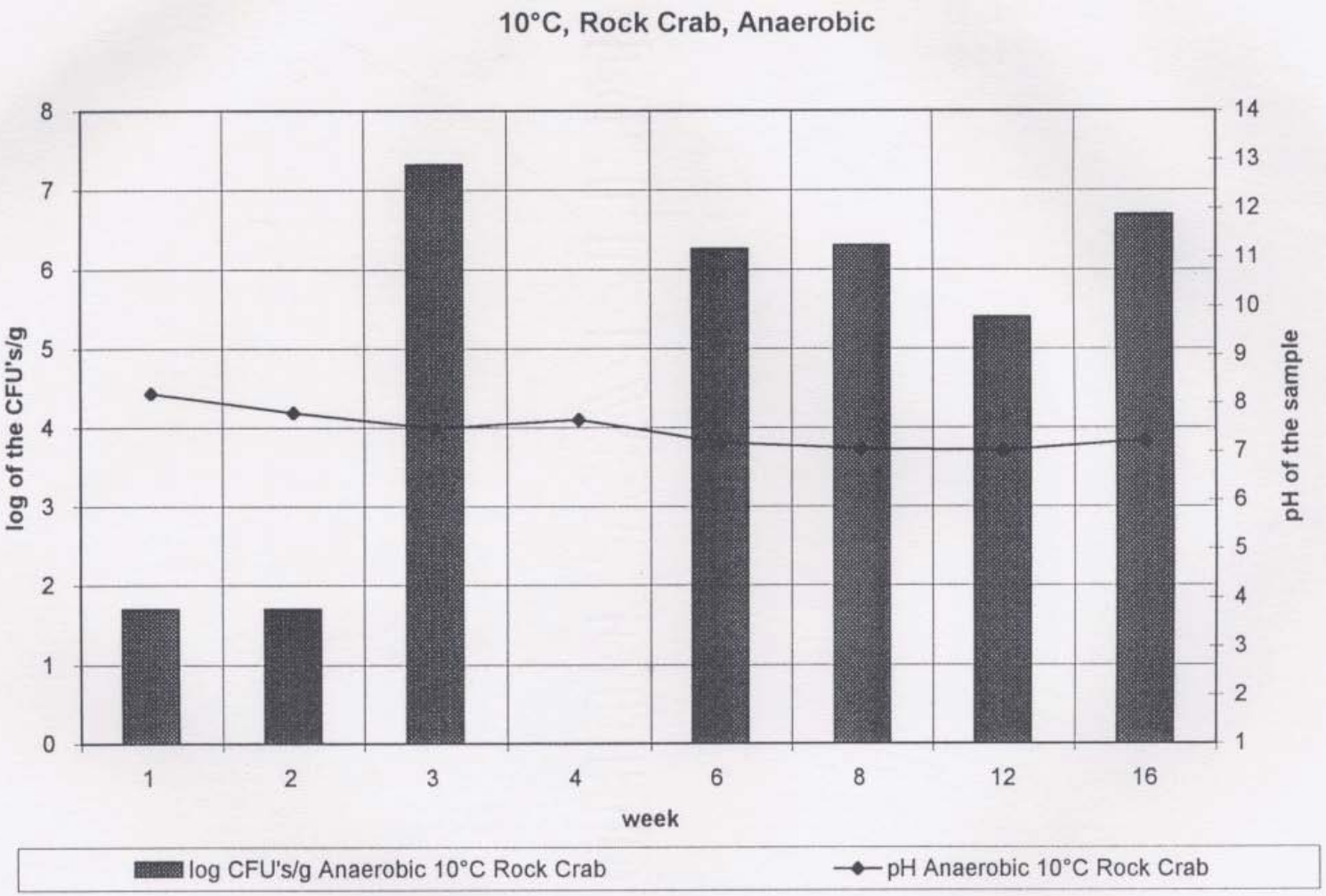


Figure 22: Anaerobic shelf life results from rock crab sealed and pasteurized in 8 oz plastic containers with aluminum pull top lid stored at 10.0°C. (n=2) Recorded as pH and logarithm of anaerobic colony forming units per gram (CFU/g).

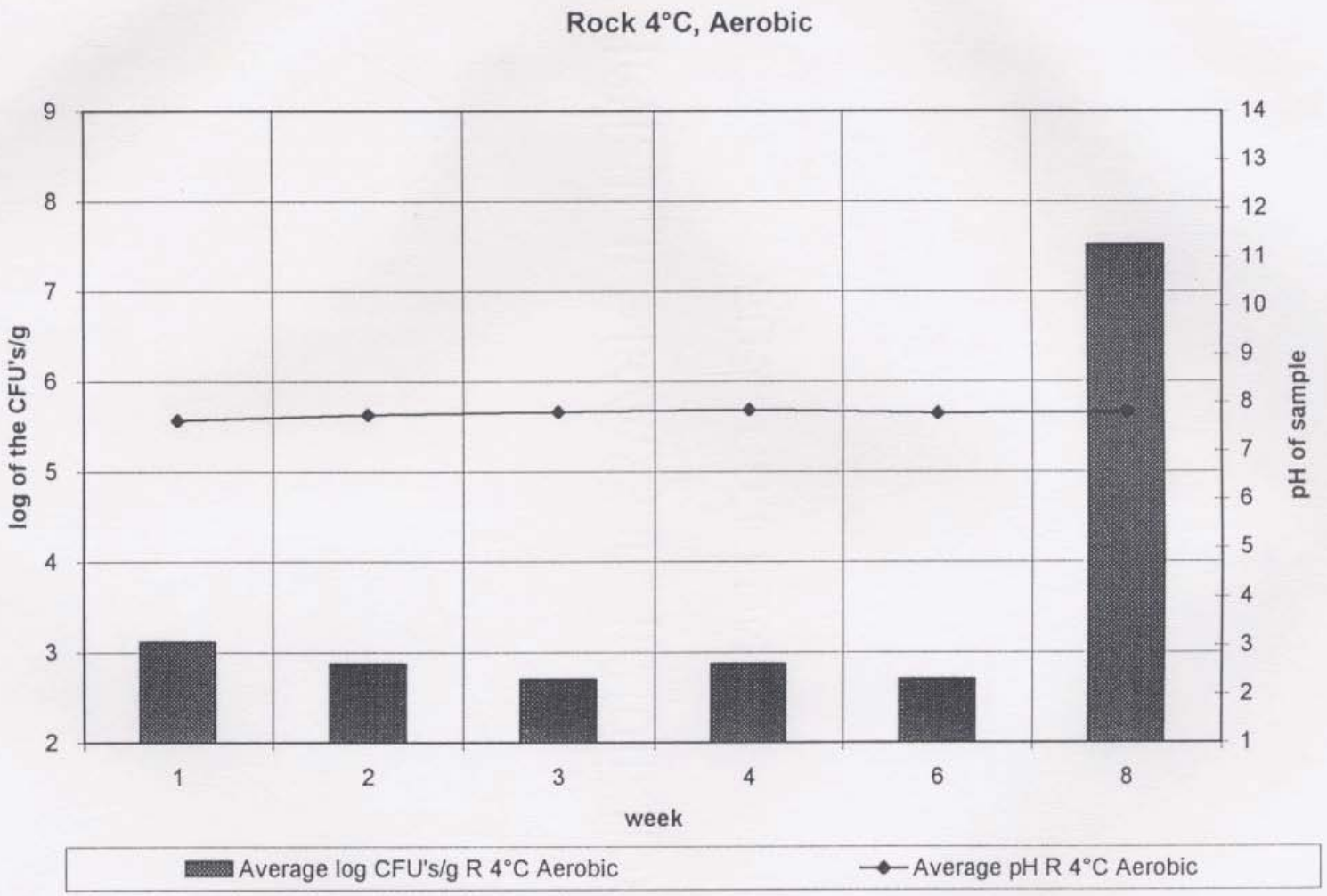


Figure 23: Aerobic repeated shelf life results from rock crab sealed and pasteurized in 8 oz plastic containers with aluminum pull top lid stored at 4.0°C. (n=2) Recorded as pH and logarithm of aerobic colony forming units per gram (CFU/g).

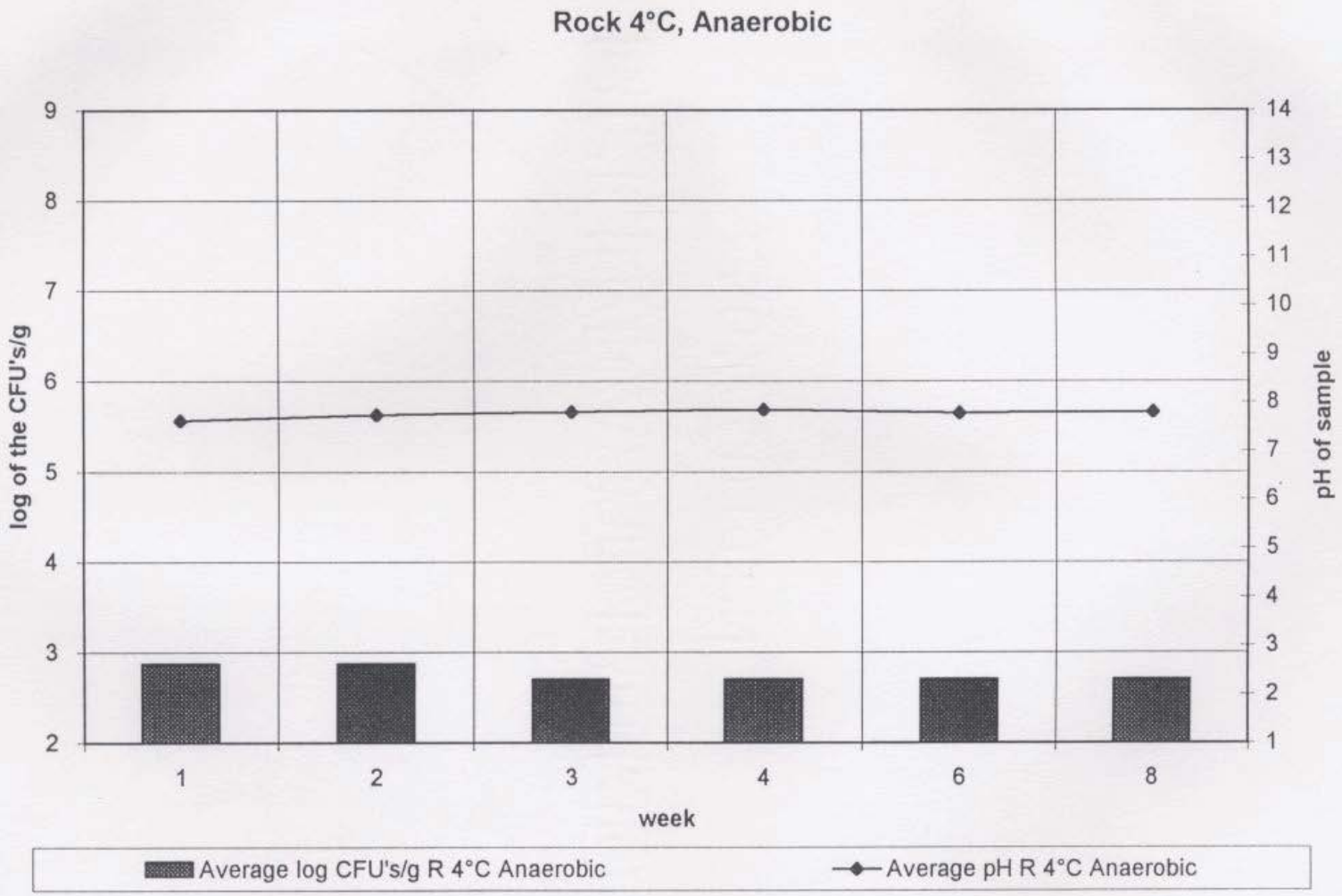


Figure 24: Anaerobic repeated shelf life results from rock crab sealed and pasteurized in 8 oz plastic containers with aluminum pull top lid stored at 4.0°C. (n=2) Recorded as pH and logarithm of anaerobic colony forming units per gram (CFU/g).

#### 4.3.2.2 Spoilage Characteristics:

Product which had spoiled during storage displayed the following characteristics. (Please see Figure 25):

- The product color darkened to varying degrees of gray to black;
- The contained product had a "rotten egg smell" associated with sulfurous compounds;
- The container was typically swollen due to internal pressure increase from gas production;
- The product pH reduced;
- A yellow slime was produced which accumulated in the bottom of the container.

Hard (1992), indicated that heat denatured hemocyanin may be the cause of discoloration in crab flesh. The copper based crab blood protein, hemocyanin or oxyhemocyanin, turns white after processing and, in the presence of hydrogen sulfide, forms a blue green color. It is possible that the micro flora surviving the process may have been hydrogen sulfide producing and so cause this darkening provided metal ions were present. Sun Pan and Kuo (1994) indicated that hydrogen sulphide would be produced by the interaction of heat and sulpher containing compounds in shellfish flesh such as the sulphur containing amino acids. Hydrogen sulfide gas formation would also explain the increase in internal pressure or swelling. The presence of hydrogen sulfide may explain the sulfurous smells associated with spoiled product. In addition, hydrogen sulfide is usually black in color which may account for some discoloration within the product.

Figure 25: Spoiled pasteurized rock crab product in top tray of 10.0°C incubator.

The darkening of the product may form a basis for determining the condition of stored products identifying spoilage before consumption. The reliability of this discoloration as an indicator is yet to be proven; however, most spoiled containers from this work displayed this discolored result. The potential contribution of these discoloration reactions should be investigated in detail in further work.

Other potential causes of this discoloration have also been outlined by Hard (1992), and include: enzymatic browning by phenolases, the Maillard browning reaction, protein-lipid browning and bacterial browning.

The reduction in pH of the product may be due to the presence of lactic acid producing bacteria.

The accumulation of a yellow slimy substance at the base of the container may be due to the accumulation of bacterial metabolites which may be protein or lipid based.

## 5.0 SUMMARY AND CONCLUSIONS

The optimal process for pasteurizing Rock Crab in ES308 plastic containers supplied by Kings Plastic with double seemed pull top aluminum lids was found to be 130 minutes at 83°C. The slowest heating spot within the container was found to occur at a point 3.0 cm below the lid at the center of the container. The heating and cooling parameters which defines the heat penetration behaviour for this product were found to be as follows:

- $j_h = 1.69\ (\pm 0.04)$
- $f_h = 34.74\ (\pm 1.04)$
- $j_c = 1.56\ (\pm 0.03)$
- $f_c = 50.53\ (\pm 1.73)$

Pasteurization processes utilizing the above process temperature and time for the container specified will give a refrigerated shelf life of 1 year or more if held at a temperature of 2.2°C. Increases in temperature appreciably above this value, greater than a 2°C rise, can cause a significant reduction in the expected shelf life. This work has shown that a storage temperature of 4°C, similar to the temperature of a consumer refrigerator, will provide a shelf life greater than 6 weeks.

Energy consumption calculations, for the equipment and condition assumptions outlined, showed that the highest temperature process possible, 83.0°C, was the most energy efficient. Process time reduction based on a unit temperature increase gave a greater energy savings than the energy consumed to maintain the higher temperature.

The quality assessment of the crab showed that there was no measurable effect on the proximate analysis and on the amino acids of the crab due to the established pasteurization process. The overall quantities of fatty acid were reduced by approximately 12%, however, with a questionable impact. The impact of this on the flavour profile of the crab meat is anticipated to be minimal due to the presence of an approximately equivalent percentage of each of the fatty acids in the post processed state.

The information contained in this thesis can be used by thermal processors to establish useful pasteurization processes for rock crab meat in brine. The process identified in this thesis has been successfully implemented in a commercial setting.

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