ELIMINATION OF PARASITES USING NON-THERMAL AND LOW TEMPERATURE TECHNIQUES AND EFFECT ON CHEMICAL AND SENSORY QUALITY OF RAW OYSTERS

by

BRIANA HAYES

(Under the direction of Dr. Yao-wen Huang)

ABSTRACT

The effects of non-thermal and low temperature treatments on parasite elimination and chemical and sensory quality of raw oysters were evaluated using: low temperature pasteurization, cold pasteurization, individual quick freezing, ultra-low freezing, and blast freezing. *C. parvum* was inactivated by all experiments. The results on the effect of treatments on *C. cayetanensis* were inconclusive because no sporulation was observed. Oysters ultra-low frozen for 7 days showed no significant difference from the control in the 2-thiobarbituric acid reactive substances (TBARS). Sensory testing based on appearance, aroma, and texture, showed that raw oysters treated with low temperature pasteurization were preferred to ultra low freezing, individual quick freezing, and the control, but were not significantly different from oysters which were blast frozen. Thus all treatments have the potential to be used to eliminate *C. parvum*, but with low temperature pasteurization having little effect on chemical and sensory quality of raw oysters.

INDEX WORDS: Oysters, *Cyclospora cayetanensis*, *Cryptosporidium parvum*, 2-thiobarbituric acid reactive substances test, low temperature pasteurization, individual quick freeze, blast freeze, cold pasteurization, ultra-low freeze
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DEDICATION

First and foremost, I would like to dedicate this to God without which none of this would be possible. This thesis is dedicated to my family, Veta Hayes, Wayne Hayes, and Wayne Hayes II, who have helped me immensely throughout this process with their support and encouragement. Next, I would like to dedicate this to my friends Devin Raines and Jennifer Davis who were always there to lend their support. Also, I would like to dedicate this to Reshaun Finkley who has inspired me in every way possible, and has given me his encouragement and support day after day.
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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW
**Purpose of the Study**

Molluscan shellfish are responsible for a large number of disease outbreaks caused by pathogenic bacteria, although they only constitute about 0.1% of the seafood consumed in the U.S. During the filter-feeding process shellfish absorb particles from the surrounding water, which may include pathogenic microorganisms. This mechanism is a major reason why eating raw shellfish is not advised and why it has resulted in an extremely poor safety record (Huss, et al., 2000). In developed nations like the United States, a large percentage of food borne illnesses are attributed to such diseases as campylobacteriosis and salmonellosis. However, developing countries have a wider range of food borne illnesses which also include parasitic diseases (Molins, et al., 2001). Parasites are responsible for a large number of seafood-associated infections, mostly from the consumption of raw or undercooked seafood (Butt, et al., 2004).

Although there are many post-harvest treatments which have been developed to inactivate harmful pathogenic bacteria, in most cases, their effectiveness against parasites has not been evaluated. Because of the emergence of pathogenic protozoa as harmful pathogens that can be found in raw oysters, it is necessary to evaluate current post-harvest treatments to determine their effect on these pathogens. It is evidenced that there is a need for parasite elimination techniques which maintain the characteristic flavor and consistency of raw oysters, but do not promote chemical changes in the oyster tissue. This research was directed towards the potential use of non-thermal and low temperature techniques as a method to eliminate parasites from oysters, which are to be consumed raw.
The purpose of this study was:

1. To evaluate five non-thermal and low temperature sanitation methods, blast freezing, ultra-low freezing, low temperature pasteurization, cold pasteurization, and individual quick freezing, to determine their effect on inactivating parasites in raw oysters
2. To study the effects of these sanitation methods on lipid oxidation of oyster tissue
3. To study the effects of these sanitation methods on the sensory characteristics of raw oysters

**Oyster Characteristics**

Shellfish are mollusks which live in one shell or in two shells close together, either in water or on land. The oyster is a bivalvular shellfish with a rough shell, where the lower half is waved and the top half lies on top like a lid and the two shells move by means of a strong contractor muscle. The oyster is a hermaphrodite in which the larvae develop in the shell until three months when the young oysters are released from the shell. The oysters then attach themselves to places which they deem as suitable, where the shell formation begins. Oysters are ready for consumption after about six years (Catsberg et al., 1990). Shellfish, such as oysters, filter large quantities of water, removing small particles for food, which is referred to as their filter-feeding mechanism (Fayer, 2004). This is the reason why it is imperative that oysters which are to be eaten raw live in water which is free of pathogenic microorganisms (Catsberg et al., 1990).

Three oyster species of economic importance are the eastern oyster (Crassostrea virginica), the Pacific oyster (Crassostrea gigas), and the Olympia oyster (Ostrea lurida) (Noomhorm and Vongsawasdi, 2004). The eastern oyster (Crassostrea virginica), which was used in this study, is an economically and ecologically important bivalve species which inhabits
the coastal region of the United States from Maine to the Gulf of Mexico (Chu, et al., 2003).

**Oyster Harvesting**

Oysters can be harvested by picking, tonging, and dredging, where picking is generally confined to the area of oysters which are exposed at low tide, and tonging and dredging captures oysters in larger numbers. Tongs consist of two poles crossed like scissors that have toothed iron baskets at the ends of the poles. These tongs are lowered to the sea floor, used to scoop up the animals, and are closed before being raised from the bottom. Similarly, a dredge is a metal rake that is dragged across the bottom of the ocean, scraping up oysters in its path. After harvesting the oysters are gathered and held in a chain mesh bag, then carried up from the bottom either by a conveyor belt or by flowing water through a large diameter hose (Noomhorm and Vongsawasdi, 2004).

**Market Preparation**

Oysters are marketed as either in shell (unshucked), which requires washing, packing, and chilling, or in the more popular shucked form, which requires more laborious steps for market preparation. After shucking, oysters are rinsed as pieces of shell and torn or discolored oysters are removed, then aerated in blowing tanks to remove sand, silt, and shell fragments. The washed meats are then graded by size and placed in suitable packaging, dependent on the freezing method. Oyster meat may remain in good condition for more than nine months when stored at -29°C, however, storing oyster meat for more than six months may degrade raw material quality if the storage conditions are not consistent (Noomhorm and Vongsawasdi, 2004). Traditionally, shellfish, such as crab, crawfish, and oyster deteriorate in quality immediately upon death. For this reason, they are usually shipped live, in refrigerated containers, or fresh frozen. When the shellfish arrive at a processing plant they are placed in a cooler, where
they are kept under carefully controlled temperature of 32-38°F, where higher temperatures may lead to product spoilage (Voisin, 2003).

**Raw Oysters**

A live oyster has a well-closed shell, and can live out of the water for about 14 days without oxygen if stored under refrigeration. For raw oysters it is necessary to cut the contractor muscle with a special knife before opening and consumption. Sometimes raw oysters are delivered ready for the table, which means the shell is opened and the oyster meat is loosened from the shell. Shellfish spoil quickly because of the high water and protein content and quality deterioration is obvious because of an unpleasant smell, discoloration as a result of drying out, and the meat becoming tough (Catsberg, et al., 1990). It is recognized that a good raw oyster has a mild salty taste. An ideal oyster has about twelve parts per thousand of salt in the juice (Voisin, 2003).

**Freezing Effect on Shellfish**

As a result of chemical, physical, bacteriological, and histological changes which quickly degrade the quality, shellfish are perishable and spoil easily after harvesting. These quality changes are greatly affected by many factors, although temperature is the most important factor (Jiang and Lee, 2004). Heating is most effective in eliminating pathogenic parasites from seafood. For heating methods, the internal temperature of the thickest part of the meat must reach a minimum of 63°C (145°F) for 15 seconds or longer (Butt, et al., 2004). High heat treatment as a means of controlling microorganisms in food products results in diminished taste and reduced nutritional content. Substantially elevated temperatures are considered unsatisfactory for processing of raw oysters where the purpose of the process is to retain sensory qualities of oysters and sell them on the half shell (Voisin, 2003). Freezing is also effective in eliminating
parasites from seafood with a minimum of 15 hours at -35°C (-31°F) or 7 days at -20°C (-4°F) (Butt, et al., 2004). Freezing inhibits the activity of food spoilage and food poisoning organisms, and the low storage temperature greatly slows down the enzymatic and biochemical reactions that normally occur in unfrozen foods (Jiang and Lee, 2004).

Conversion of water into ice initiates complex physical and physicochemical changes that can cause generally deteriorative quality changes not ordinarily occurring in fresh foods (Jiang and Lee, 2004). During refrigeration of shellfish, some microbiological, physical, chemical, biochemical and sensory changes may occur depending on the duration and conditions of storage and the initial quality of the product (Khan, et al., 2005). Principally, shellfish spoils because of self digestion and as a result of the action of bacteria. Both self digestion and the action of bacteria are encouraged by enzymes, which remain active in the shellfish after it dies, however, enzyme activity can be reduced by lowering the temperature (Jiang and Lee, 2004).

**Post-Harvest Treatments Not Evaluated in This Study**

The post-harvesting process for oysters, which is not evaluated in this study, is high hydrostatic pressure treatment.

**High Hydrostatic Pressure**

Increasing attention has been directed in recent years to the application of high hydrostatic pressure (up to 800 Mpa) to inactivate microorganisms (Murchie et al., 2005; Erickson, 2002). High pressure foods were first commercialized in Japan in 1992 (Murchie et al., 2005). High pressure is preferable to heat treatment because it does not destroy many of the substances found in fresh foods, such as vitamins, chlorophyll, and aroma substances. It is believed to increase food safety by reducing bacteria in the processed products, while retaining the products nutritional value, color, flavor, and texture (Voisin, 2003). This treatment is also
believed to retain sensory qualities in seafood (Murchie et al., 2005; Voisin, 2003). The principle of cold isostatic pressure processing is as follows: food is placed in a container and is surrounded by a pressure medium. Pressure is fed into the pressure vessel and is distributed evenly through all parts of the product, preventing mechanical damage of delicate food products. This process may be conducted with no or minimal heat treatment. Minimal heat is used to decrease the pressure and/or time required for processing of the food items. The higher the temperature, the less time and pressure is required to eliminate pathogens in raw seafood products. The cost of high pressure equipment is of some concern because it approaches one million dollars (U.S.) (Voisin, 2003).

As a result of the high pressure treatment, pathogenic organisms are destroyed without substantially adversely affecting the sensory qualities and shelf life of the product. Also, the connective tissues of the adductor muscle holding the two shell halves are separated from the shells and the shells open without any manual cutting of the muscle (Murchie et al., 2005; Voisin, 2003). Products processed with this method may be sold to oyster bars and restaurants, where oysters are sold on the half shell (Voisin, 2003).

**Post Harvest Treatments Evaluated in This Study**

The post-harvest treatments evaluated in this study are those using minimal or no heat to treat raw oysters: blast freezing, ultra-low freezing, individual quick freezing, cold pasteurization, and low temperature pasteurization.

**Blast Freezing**

Blast freezing is suitable for many aquatic food products, and utilizes very cold air (0 to -40°F) to remove heat from the products and transport this heat to the refrigeration coils. There are several types of blast freezers which have the same general operating principles, but different
airflow, loading method, and capacities. Most blast freezers use average air velocities of 2.5 to 7.5 m/s, while 2.5 to 5.0 m/s are reported as the most economical velocities (Noomhorm and Vongsawasdi, 2004).

Two important blast freezing modes are tunnel freezing and fluidized bed freezing. In tunnel freezing, products are placed on trays which are on a moving mesh belt passing through a tunnel or enclosure where the cold air is blown from the opposite end. The main advantage of this mode is its versatility, because it is suitable for irregular shaped, different-sized, and non-deformable foods. Tunnel freezers have a slightly slower freezing rate than immersion freezers, and dehydration may occur, which causes equipment frosting. With the high demand for individual quick frozen products, fluidizing belt freezers are extensively used. This procedure requires a sufficiently powerful stream of cold air to keep the products in suspension. The great advantage of fluidized bed freezing is short freezing times, since each piece of food is kept loose and free flowing by the air pressure, resulting in a higher yield. The retention time for this freezing operation depends upon the size of the products (Noomhorm and Vongsawasdi, 2004).

**Ultra-Low Freezing**

Ultra low freezing is a method in which the food products are placed in an Ultra low freezer (So-Low Environmental Equipment, Cincinnati, OH), with a temperature range of -40°C to -85°C, which is controlled digitally, for a specified period of time. A study by Fayer and Nerad (1996) showed that *Cryptosporidium parvum* oocysts frozen at -70°C for 1 hour or more failed to infect mice, which corresponds to the ultra-low freezing techniques used in this study.

**Individual Quick Freezing**

Cryogenic freezing is the ultra-fast freezing process that results in excellent product quality. In this method, the products, either unpacked or thinly packed, are exposed to an
extremely cold refrigerant, where heat removal occurs as a result of a change of state by the refrigerant. The advantages of cryogenic freezing are rapid freezing rate, simplicity, flexibility, and inexpensive equipment design. Commonly used refrigerants are liquid nitrogen and carbon dioxide. Liquid nitrogen, the refrigerant used in this study, is a by product obtained during the production of oxygen from air, it is nontoxic, and relatively cheap (Noomhorm and Vongsawasdi, 2004).

Liquid nitrogen freezing systems are divided into three types: the immersion type, the spraying of liquid nitrogen type, and the circulation of very cold liquid nitrogen vapor type. In the method commonly applied in the food industry, which uses a spray of liquid nitrogen, the products are placed on a conveyor belt in a single layer. The conveyor carries the products through the freezer in the direction opposite to the flow of nitrogen. During a pre-cooling stage, warm products entering the freezer are first subjected to a blast of cold nitrogen gas, which prevents stress cracking in the products as a result of too rapid cooling. Shrimp and oysters are successfully frozen by this procedure and it is found that the frozen product obtained from liquid nitrogen freezing provides lower indole and trimethylamine content than those obtained from conventional methods. In addition, smaller ice crystal are formed and less protein changes occur, which results in less drip loss during thawing (Noomhorm and Vongsawasdi, 2004).

**Cold Pasteurization**

Cold pasteurization is a non-thermal treatment which can be used effectively to eliminate or reduce pathogenic bacterial numbers to acceptable levels in raw or minimally processed foods (Molins et al., 2001). It is the process of subjecting materials to electromagnetic radiation or electronic beams of sufficient energy levels to sever chemical bonds (Erickson, 2002). Currently 40 countries allow cold pasteurization of one or more food products and 29 are applying it
commercially (Molins et al., 2001). Food cold pasteurization is used sparingly because it has come under scrutiny from some anti-irradiation and anti-technology groups who challenge the evidence of food safety and effectiveness of the process (Crawford and Ruff, 1996).

The most important element in food cold pasteurization is the dose used on the product because it must be adequate to produce desired results, yet low enough to maintain the quality of foods. High doses kill all microorganisms except for viruses, while irradiation at low doses produces many of the same effects of pasteurization, achieved by conventional heating products (Crawford and Ruff, 1996).

Cold pasteurization is used to inactivate parasites and vegetative forms of bacteria from poultry, meat, and meat products, fish and other seafood as well as fruits and vegetables. It can eliminate pathogens in food without substantial increases in product temperature and without causing any significant physical or chemical changes in food (Robinson, 1996; Molins et al., 2001; Crawford and Ruff, 1996). Cold pasteurization, as opposed to thermal pasteurization, is a more efficient pasteurization method for solid foods, especially those of animal origin, without causing significant changes in taste and quality of the products. This method is unique because it ensures the hygienic quality of foods of animal origin, especially those consumed raw or undercooked (Loaharanu, 1996).

Oysters and clams can be radiation-treated without killing the mollusks, therefore allowing live storage under refrigerated conditions. Cold pasteurization of fish and seafood is approved in ten countries and is being considered for approval in several more (Molins et al., 2001). Parasites require cold pasteurization of lower doses (<1.0 kGy) than most bacteria (2-5 kGy), since the objective of the treatment is usually not to destroy the organism but to render it uninfective (Loaharanu, 1996; Molins et al., 2001). This process is endorsed by national and
international bodies such as the World Health Organization, the Food and Agriculture Organization of the United Nations, and others (Molins et al., 2001; Crawford and Ruff, 1996).

**Low Temperature Pasteurization**

According to Tesvich et al. (1999), low temperature pasteurization is a process for the heat treatment of oysters where the oysters remain in their natural shell during the heat treatment step of the process, and are then quick chilled in cold water. In this treatment, live, raw oysters in their natural shell, are placed in warm water at a temperature of about 49-55°C, for about 10-45 minutes, depending on oyster size. The meat within the oyster must reach 49°C, but not exceed 53°C. It has been determined that the internal temperature must exceed 49°C to kill bacteria, but if the temperature exceeds 53°C the edges of the meat begins to curl and shrink due to protein degradation. Internal meat temperatures may be ascertained by random sampling during the actual processing to ensure quality. The temperature and time period are sufficient enough to destroy harmful pathogenic bacteria while leaving the oysters in a raw state. Different temperature and time durations have been found to give good results depending on the size of the oyster. After the oysters reach the correct internal temperature, the oysters are further treated with either a medium or high treatment process, which is an increase in the amount of time the oysters are heated. This medium or high treatment is dependent upon the bacterial populations in harvesting areas and the time of year the oysters are harvested. After mild heating, the oysters are cooled in cold water, which chills the meat to less than 7°C, and are then ready for shucking (Tesvich et al., 1999).

During the mild heat treatment step the live oyster is killed, which causes the abductor muscle of the oyster body to relax, making the oyster easier to shuck. It has been found that oysters treated by this process have increased shucking yields, due to the fact that most of the
muscle remains with the shucked oyster meats, as opposed to non-treated oysters where a large portion of the muscle remains with the shell to be disposed of. Another factor is that the treated oysters appear to absorb some of the water, which in turn, increases their weight (Tesvich et al., 1999). A disadvantage to this method is that it is difficult to control the high temperature of commercial size batches when the size and thickness of oysters differ from batch to batch (Voisin, 2003). Oysters treated by this process are safe and able to be served at a raw bar in a raw state (Tesvich et al., 1999).

**Lipid Oxidation**

Lipid oxidation is a leading cause of meat deterioration which affects fatty acids, especially highly vulnerable polyunsaturated fatty acids, which are prevalent in shellfish (Fernandez, et al., 1997). During refrigerated and frozen storage, many muscle foods are susceptible to degradation by lipid oxidative mechanisms (Erickson, 2002). Lipid auto oxidative degradation results in products which change food quality, such as color, aroma, flavor, texture, and also nutritive value (Fernandez, et al., 1997). Lipid oxidation of seafood reflects flavor qualities of stored products and results in the development of a condition called oxidative fat rancidity (Khan, et al., 2005; Santos-Yap, 1996). The extent of oxidation in fish lipids depends on the quantity and type of lipids in the fish muscle, for example, fatty species are more prone to oxidation than lean species, and species with more highly unsaturated fatty acids are less stable than other species (Santos-Yap, 1996). When oxidative rancidity progresses sufficiently, it leads to the development of obvious off-taste and odor, resulting in reduced shelf life (Santos-Yap, 1996).

The two major components involved in lipid oxidation are unsaturated fatty acids and oxygen. In this process, oxygen from the atmosphere is added to certain fatty acids creating
unstable intermediates that eventually break down to form unpleasant flavor and aroma compounds. The most common and important process by which unsaturated fatty acids and oxygen interact is a free radical mechanism characterized by three main phases: initiation, propagation and termination.

1. Initiation:
   (a) RH + O₂ → R⁺ + OOH

2. Propagation:
   (b) R⁺ + O₂ → ROO⁻
   (c) RH + ROO⁻ → ROOH + R.
   (d) ROOH → RO⁻ + OH

3. Termination:
   (e) R⁺ + R⁻ → R-R
   (f) R⁺ + ROO⁻ → ROOR
   (g) ROO⁻ + ROO⁻ → ROOR + O₂

Initiation occurs as hydrogen is abstracted from an unsaturated fatty acid, resulting in a lipid free radical, which in turn reacts with molecular oxygen to form a lipid proxy radical. In the propagation step of lipid oxidation the lipid peroxyl radical abstracts hydrogen from an adjacent molecule, resulting in a lipid hydroperoxide and a new lipid free radical. The termination step occurs when two free radicals combine to terminate the process (Erickson, 2002).

The products of lipid oxidation decrease either directly or indirectly the sensory quality of the fish and fish products. Lipid oxidation produces unstable intermediate compounds, like free radicals and hydroperoxides, and also leads to the formation of volatile compounds which are involved in the development of off-flavors (Eymard, et al., 2005). Lipid hydroperoxides are not considered harmful to food quality by themselves; however, it is their degradation products that cause quality changes (Erickson, 2002). Oxidized lipids also interact with proteins inducing modification of textural properties of food products (Eymard, et al., 2005).
**Methods to Evaluate Lipid Oxidation**

Changes in physical, chemical, or sensory properties of fats and oils during oxidation may be monitored to assess the extent of lipid oxidation. The available methods to monitor lipid oxidation in foods and biological systems may be divided into two groups: one group measuring primary oxidative changes and the second group determining secondary changes that occur in each system (Shahidi and Wanasundara, 2002). The most common techniques developed to assess the extent of lipid oxidation in fish and shellfish are the peroxide value tests, which measure the amount of peroxides or hydro peroxides formed during oxidation, a measurement of primary changes, and the TBA (2-thiobarbituric acid) test, which measures the amount of malonaldehyde formed after the decomposition of hydroperoxides during the second stage of oxidative rancidity, a determination of secondary changes (Jiang and Lee, 2004).

**TBA Test**

One of the oldest and most frequently used tests for assessing lipid oxidation in foods and other biological systems is the 2-thiobarbituric acid (TBA) test. The extent of lipid oxidation is reported as the TBA value and is expressed as milligrams of malonaldehyde (MA) equivalents per kilogram sample or as micromoles MA equivalents per gram sample (Shahidi and Wanasundara, 2002). As shown in figure 1.1, one molecule of malonaldehyde, a secondary product of oxidation, reacts with two molecules of TBA to form a pink-colored complex which absorbs at 532 nm. TBA may also react with other lipid oxidation products such as 2-alkenals, and 2, 4-alkadienals, and therefore reflects the total content of reactive aldehydes in the TBA test. TBA values correlate well with sensory data (Khan, et al., 2005).
The TBA test may be conducted directly on the sample, its extracts, or distillate. In the distillation method, volatile substances are distilled off using steam and allowed to react with the TBA reagent in an aqueous medium. The advantage of this method is the absence of interfering substances. In the extraction method, TBA-reactive substances (TBARS) are extracted from the food material into an aqueous medium prior to color development with the TBA reagent. The main disadvantages of both of these methods are long assay time and possibility of artifact formation. In the direct assay method, lipid sample (oil) reacts with the TBA reagent and the absorbance of the prepared colored complex is observed. This method is simple and requires less preparation time. It has been suggested that TBARS values produce an excellent means for evaluating the relative oxidative state of a system as affected by storage condition or process variables. However, it is preferable to quantify the extent of lipid oxidation by a complimentary analytical procedure in order to verify the results (Shahidi and Wanasundara, 2002).
Raw Oyster Consumption Risks

Some of the largest food poisoning outbreaks have been associated with seafood, with one major concern being raw oysters. In recent years, considerable attention has been paid in the media to tragic results of consumption of raw oysters where the individuals become infected with life-threatening pathogenic microorganisms (Sumner and Ross, 2002; Voisin, 2003). The predominance of oysters and clams as vehicles of seafood-borne disease is most likely a result of their filter feeding mechanism, and the fact that they are normally eaten whole, including the intestinal tract, and raw or following a very mild heat treatment (Murchie et al., 2005).

Bacteria which live in marine environments, especially warm waters like that of the Gulf area, are: *Vibrio*, *Listeria monocytogenes*, *Salmonella* (nontyphoidal), and *Salmonella typhi*, *Campylobacter jejuni*, *Escherichia coli*, *Yersinia enterocolitica*, *Clostridium botulinum*, *Clostridia perfringens*, *Shigella*, *Staphylococcus aureus*. Some other organisms that can cause disease in normal, healthy adults which are proven pathogens in seafood are: Helminths; Viruses: poliovirus, other picomaviruses; Hepatitis A and E and non-B Hepatitis, and *Bacillus cereus*. Additionally, there is a series of organisms that can cause disease most often in special population groups; rotavirus and Listeria. Public fear of the potential dangers associated with bacterial poisoning through raw oyster consumption adversely affected the oyster harvesting industry (Voisin, 2003).

Even with the negative publicity, consumption of raw shellfish is widespread, especially in the South, and many restaurants continue to carry raw oysters as part of their menu. However, many restaurants post warning signs which display the possible danger to individuals with liver or immune system disorders (Voisin, 2003).
Protozoan Parasites as Food borne Pathogens

A large number (nearly 40) of parasitic protozoans are known to be infectious to humans, with the most important being transmitted primarily through water (Cryptosporidium, Entamoeba histolytica, Giardia, and Cyclospora) (Huss and Ben Embarek, 2004). Since the 1970s interest in parasitic protozoa as water and food borne pathogens has grown globally. A primary reason stems from the worldwide increase in immuno-compromised (mainly human immunodeficiency virus) populations, which readily contract a number of infectious and non-infectious microbial mediated diseases (Shields and Olsen, 2003). The two parasites which were examined in this study are Cryptosporidium parvum and Cyclospora cayetanensis.

Cryptosporidium parvum

Cryptosporidium parvum is a cause of gastrointestinal disease in humans and farm animals (Gomez-Bautista et al., 2000). Cryptosporidium was first described in 1907 in asymptomatic mice. It is the least host specific species and has been identified in mice, cattle, humans, horses, and many other mammalian hosts (Fayer, 2004; Jay, 1996). It is a major cause of diarrhea in children and those with compromised immune systems and one of the most common protozoan parasites to cause disease in humans. This protozoan parasite is transmitted mainly by the ingestion of oocysts by fecal-oral contact or through contaminated food or drinking water (Gomez-Bautista et al., 2000). Cryptosporidium has been labeled a waterborne pathogen because it is most often associated with surface and drinking water and has been implicated in many outbreaks involving contaminated drinking water (Macrae, et al., 2004). Approximately 49 drinking water related outbreaks were reported between 1984 and 1999, mostly in North America, the UK, and Japan, with the largest recorded outbreak for any pathogen being in Milwaukee, Wisconsin. Oocysts are transmitted from an infected host to
susceptible hosts in various ways: human-to-human, animal-to-animal, human-to-animal, animal-to-human, water-borne, food borne, and possibly airborne (Fayer, 2004).

Fig. 1.2 Diagram displaying the most important cycles of transmission for maintaining Cryptosporidium (Hunter and Thompson, 2005)

Data has shown that the eastern oyster (Crassostrea virginica) can remove C. parvum oocysts from the water through filter feeding, and then accumulates the parasites on the gills and in the hemolymph (Gomez-Bautista et al., 2000; Fayer et al., 1997). Several reports have indicated the presence of oocysts, mostly C. parvum, in oysters in estuarine tributaries of the Chesapeake Bay (Fayer, 2004). Oocysts of C. parvum have also been recovered from oysters,
clams, mussels, and cockles in coastal waters of Ireland, Spain, and the United States (Fayer, 2004). The *C. parvum* oocysts are capable of surviving in seawater and in freshwater shellfish (Gomez-Bautista et al., 2000). According to Freire-Santos et al. (2002), oocysts of *C. parvum* recovered from shellfish in experimentally contaminated saltwater aquaria were infectious for mice over 30 days after exposure. There is a potential for *C. parvum* to contaminate inshore shellfish by run off from agricultural practices or waste disposal systems (Macrae, et al., 2004). The highest percentage of contaminated shellfish identified in a three year study were found shortly after the greatest rainfall during that time, suggesting that runoff was the most likely source (Fayer et al., 2002; Fayer, 2004).

**Cyclospora cayetanensis**

*Cyclospora cayetanensis* has gained the attention of public health agencies as human pathogens from many food and waterborne outbreaks in North America and Canada during 1996-1999 (Mansfield and Gajadhar, 2004; Shields and Olsen, 2003). Previously, this parasite had been thought to be in developing countries or associated with traveler's diarrhea; however it can rapidly become a problem in developed countries due to food importation (Shields and Olsen, 2003). Although cases of cyclosporiasis were recognized in the late 1970s by Ashford (1979), it was not classified as a protozoan until Ynes Ortega (Ortega et al., 1993, 1994) described it in Peruvian children (Shields and Olsen, 2003).

In the case of *Cyclospora cayetanensis*, humans seem to be the only hosts for the parasite (Mansfield and Gajadhar, 2004). Similar to other coccidian pathogens, *C. cayetanensis* is an obligate intracellular parasite. Figure 1.2 displays the life cycle of *C. cayetanensis*. *Cyclospora* infects the cells of the upper portion of the small intestine causing reoccurring diarrhea disease (Shields and Olsen, 2003). *C. cayetanensis* infects humans after ingestion of
oocysts and requires a minimum amount of time, moisture and moderate temperature to sporulate and become infective (Mansfield and Gajadhar, 2004). The symptoms of this infection are very similar to those of *C. parvum*, which include diarrhea and nausea, and the detection methods are based on those for other parasitic protozoa such as *Cryptosporidium* and *Giardia* (Shields and Olsen, 2003). Mansfield and Gajadhar (2004) found that freezing and heat treatment appeared to kill the parasite and that treatments used to remove or destroy *Cryptosporidium* oocysts would also be effective for *C. cayetanensis*.

In the case of food products, detection is difficult. The limited availability of the suspected food of transmission and inconsistent distributions of oocysts present sampling problems. Foods associated with past outbreaks, mostly fresh fruits and vegetables are perishable, which is an issue given the 7 day incubation period of cyclosporiasis. This may leave little or no food material available for testing. In most reported methods, the food is washed in sterile distilled or de-ionized water in a container and agitated. The wash is decanted, further purified and then examined by microscopy or molecular methods (Shields and Olsen, 2003).
Fig. 1.3 Proposed life cycle of *Cyclospora cayetanensis* in humans (Adams, et.al, 2003).
References


CHAPTER 2

ELIMINATION OF PARASITES USING NON-THERMAL AND LOW TEMPERATURE TECHNIQUES AND EFFECT ON CHEMICAL AND SENSORY QUALITY OF RAW OYSTERS

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1 Hayes, B., Huang, Y.W., and Ortega, Y.R. To be submitted to J. of Food Protection
ABSTRACT

The effect of non-thermal and low temperature treatments on parasite elimination and effects on chemical and sensory quality of raw oysters were evaluated using: low temperature pasteurization, cold pasteurization, individual quick freezing, ultra-low freezing, and blast freezing. Samples were subject to microbial, chemical, and sensory evaluation. *C. parvum* was inactivated by all treatments on the half shell and whole shell. The effects of the temperature treatments on *C. cayetanensis* were inconclusive. Raw oysters stored in an ultra-low freezer for 7 days showed no significant difference from the control in the 2-thiobarbituric acid reactive substances (TBARS), but were not significantly different from oysters treated with the low temperature pasteurization medium treatment. According to sensory testing based on appearance, aroma, and texture, raw oysters treated with low temperature pasteurization were preferred to ultra low freezing, individual quick freezing, and the control, but were not significantly different from oysters which were blast frozen. Thus all treatments have the potential to eliminate *C. parvum*, but with low temperature pasteurization having the little effect on chemical and sensory quality of raw oysters.
INTRODUCTION

*Cryptosporidium parvum* and *Cyclospora cayetanensis* are parasites which can cause gastrointestinal disease in humans. *Cryptosporidium* is a major cause of diarrhea in children and those with compromised immune systems and one of the most common protozoan parasites to cause disease in humans (8). It has been labeled a waterborne pathogen because it is most often associated with surface and drinking water and has been implicated in many outbreaks involving contaminated drinking water (16). Data has shown that the eastern oyster (*Crassostrea virginica*) can remove *C. parvum* oocysts from the water through filter feeding, and then accumulates the parasites on the gills and in the hemolymph. *C. parvum* oocysts are capable of surviving in seawater and in freshwater shellfish (8). There is a potential for *C. parvum* to contaminate inshore shellfish by run off from agricultural practices or waste disposal systems (16). Parasites of the genera *Cyclospora* have recently gained attention as human pathogens from many food and waterborne outbreaks in North America and elsewhere. In the case of *Cyclospora cayetanensis*, humans seem to be the only hosts for the parasite (17). The symptoms of this infection are very similar to those of *C. parvum*, which include diarrhea and nausea. Mansfield and Gajadhar (17) found that freezing and heat treatment appeared to kill the parasite and that treatments used to remove or destroy *Cryptosporidium* oocysts would also be effective for *C. cayetanensis*.

Molluscan shellfish are responsible for a large number of disease outbreaks caused by pathogenic bacteria, although they only constitute about 0.1% of the seafood consumed in the U.S. During the filter-feeding process shellfish absorb particles from the surrounding water, which may include pathogenic microorganisms. This mechanism is a major reason why eating...
raw shellfish is not advised and why it has resulted in an extremely poor safety record (9). In developed nations like the United States, a large percentage of food borne illnesses are attributed to such diseases as campylobacteriosis and salmonellosis. However, developing countries have a wider range of food borne illnesses which also include parasitic diseases (19). Parasites are responsible for a large number of seafood-associated infections, mostly from the consumption of raw or undercooked seafood (4).

Oysters can become susceptible to lipid oxidation during storage. Lipid oxidation is a leading cause of meat deterioration which affects fatty acids, especially the highly vulnerable polyunsaturated fatty acids prevalent in shellfish. Lipid autooxidative degradation results in products which change food quality, such as color, aroma, flavor, texture, and also nutritive value (7). The most common techniques developed to assess the extent of lipid oxidation in fish and shellfish are the peroxide value tests, which measure the amount of peroxides or hydroperoxides formed during oxidation, and the thiobarbituric acid test, which measures the amount of malonaldehyde formed after the decomposition of hydroperoxides during the second stage of oxidative rancidity (10).

Heating is most effective in eliminating pathogenic parasites from seafood. However, in the case of raw oysters treatments which use lower temperatures are necessary, so that cooking of the oyster meat does not occur. It has been proposed that freezing is also effective in eliminating parasites from seafood with a minimum of 15 hours at -35°C (-31°F) or 7 days at -20°C (-4°F) (4). It is evidenced that there is a need for parasite elimination techniques which maintain the characteristic flavor and consistency of raw oysters, but do not promote chemical
changes in the oyster tissue. The objective of this study was to evaluate five techniques: blast freezing, ultra-low freezing, low temperature pasteurization, individual quick freezing, and cold pasteurization, to determine their effect on inactivating parasites, lipid oxidation, and sensory characteristics of raw oysters.

MATERIALS AND METHODS

Oocysts. *C. parvum* oocysts, Iowa isolate, were purified from experimentally infected calves and were obtained from the Parasitology Laboratory at the University of Arizona, Tucson, AZ. *C. parvum* oocysts were purified with a discontinuous sucrose gradient and then a cesium chloride discontinuous gradient (3). Oocysts were stored at 4°C in an antibiotic solution (100 U of penicillin, 0.1 mg of streptomycin, and 0.1 mg of gentamycin per 10 mL) until used (within two months). *C. cayetanensis* oocysts were prepared as described in Sathyanarayanan and Ortega, (22). Fecal samples from contaminated individuals from Peru containing *C. cayetanensis* oocysts were sieved and stored in 2.5% potassium dichromate (Sigma, St. Louis, MO). Only samples containing unsporulated oocysts were used in this study. An initial concentration of oocysts was prepared using a modified ethyl acetate method. Briefly, fecal samples were resuspended in distilled water. Twenty milliliters of this suspension were mixed thoroughly with 5 ml of ethyl acetate (Fisher Scientific, Pittsburgh, Pennsylvania) and centrifuged at 1,500 g for 5 min. The supernatant and organic layers were discarded and the pellet resuspended in saline solution and centrifuged at 1,500 g for 10 min. This process was repeated 3 times. Pellets were diluted in distilled water and layered over a primary discontinuous sucrose gradient and centrifuged at 2,000 g for 20 min. Briefly, the discontinuous sucrose gradient was prepared using a Sheather’s solution (sucrose, 0.025 M phosphate-buffered saline, and Tween 80) to make a 1:2 solution (specific gravity 1.103) and a 1:4 solution (specific gravity 1.064). In 50-ml
polypropylene tubes, 10 ml of 1:4 was layered over 10 ml of the 1:2 solution. Five milliliters of 3-layer gauze-filtered fecal samples were carefully layered over the sugar layers and centrifuged at 1,500 g for 25 min. The sugar layers and interface were then collected from each sample and washed by centrifugation with saline solution. Pellets containing oocysts were stored in 2.5% potassium dichromate solution at 4 C until use (20).

**Oysters.** Oysters, (75-125 g) *Crassostrea virginica*, harvested from Apalachicola Bay, FL, were purchased from commercial retailers in Atlanta, GA and washed with tap water before use. Oysters were stored at 4°C up to 4 days or until experimental inoculation.

**Half-shell Inoculation.** Inoculates were prepared from stock solutions of *Cryptosporidium parvum* and *Cyclospora cayetanensis* and diluted to produce 1 x 10^4 oocysts/oyster for *Cryptosporidium* and 1 x 10^3 oocysts/oyster for *Cyclospora*. Oysters were opened using oyster knife and were. Positive and negative controls were also maintained. Positive control samples were inoculated with both parasites but were not temperature treated. The negative control samples, which were not inoculated, were maintained for examination of the effect of the temperature treatments on oyster tissue.

**Whole-shell Inoculation.** Oysters were placed in autoclaveable pans containing saltwater for 24 hrs before inoculation. The oysters were then placed in autoclaveable pans with fresh saltwater and plastic tubing which allowed for air flow. Inoculates were prepared from stock solutions of *Cryptosporidium parvum* and *Cyclospora cayetanensis* and diluted to produce 1 x 10^4 oocysts/50uL for *Cryptosporidium* and 1 x 10^3 oocysts/50uL for *Cyclospora*. Inocula was
pipetted into saltwater containing 24 oysters per 2L saltwater. The oysters were left for 30 min with the air flow turned off. The air in the tubing was then turned on and the oysters were left for 24 hrs for self-inoculation of these pathogens by filtration and concentration. After the 24 hrs the oysters were removed from the water and temperature treated. Positive control samples were inoculated with both parasites but were not temperature treated. The negative control samples, which were not inoculated, were maintained in saltwater for 24 hrs, and temperature treated to examine the effect of the temperature treatments on oyster tissue.

**Saltwater Filtration.** After the oysters were removed from the saltwater, it was collected and filtered using a 1.2 µm polycarbonate filter (Fisher Scientific, Pittsburgh, PA). The filter paper was washed with distilled water in 50mL tubes on a rocker at speed 6. The filters were then discarded and the tubes were centrifuged at 3000 rpm for 15 min. The supernatant was aspirated down to the pellet, and the pellet was then transferred to an eppendorf tube. The amount of oocysts in 3µL was counted and then used to calculate the amount of oocysts retained by the oysters after inoculation.

**Temperature Treatments.** For ultra low freezing, oysters were placed in -70°C freezer (So-Low Environmental Equipment, Cincinnati, Ohio) for 24 hrs and 7days. To simulate blast freezing oysters were placed in -31°C for 15 hrs. For the individual quick freezing treatment, liquid nitrogen was applied to each individual oyster. The oysters were placed on a tray and the liquid nitrogen was poured directly onto each oyster. After the excess liquid nitrogen evaporated, approximately one minute after pouring, the oysters were placed in -23°C for 12 hrs, 24 hrs, 36 hrs, and 48 hrs. For low-temperature pasteurization, the oysters were placed in a 50°C water bath
for 18.5 min (medium treatment) and 22 min (high treatment). The oysters were then immediately placed in an ice water bath for 15 min (23). After exposure to these treatments oysters were placed in a 4°C refrigerator.

For the cold pasteurization treatment, inoculated oysters were shipped to Food Technology Services Inc. (Mulberry, FL) to be irradiated at 0.5, and 1.5 kGy. Parasites require cold pasteurization at doses of <1.0 kGy (Loaharanu, 1996; Molins et al., 2001). Oysters treated by this process were not included in the lipid oxidation or sensory evaluation portions of the experiment.

**Half-shell Oyster Processing.** The gills and hemolymph were removed from the oysters using scissors and forceps. The gills were cut into small pieces, and put into 50mL tubes. Forty-five milliliters of elution buffer were added to each tube and the tubes were placed on a rocker for one hour at speed 8. Para-pak tubes (Fisher Scientific, Pittsburgh, PA) were used to filter the samples. The samples were centrifuged at 3000 g for 15 min, and then the supernatant was aspirated. Approximately 5mL of elution buffer (laureth 12, 1M Tris, EDTA, distilled water, and antifoam A) was left in the tubes and the tubes were vortexed. The oyster tissue was then rinsed twice with distilled water and centrifuged at 3000 g for 15 min, then aspirated carefully to leave the pellet. The tubes was rinsed with phosphate-buffered saline [PBS] and centrifuged at 3000 g for 15 min. The tubes were then aspirated carefully to leave the pellet. The pellet was the transferred to a 1.5mL eppendorf tube. *Cryptosporidium* oocysts were stored at 4°C until detection and determination of viability. *Cyclospora* oocysts were suspended in 2.5% potassium dichromate and stored at room temperature for 15 days.
Whole-shell Oyster Processing. Oysters were opened and the gills were dissected. Both the oyster and the gills were placed in a 50mL tube. A 0.85% saline solution (0.85 g of NaCl in 100 mL of distilled water) was added to the tube at an equal volume to the oyster in the tube. The tube was placed on a rocker for 15 min at speed 4. The liquid was then transferred to a clean tube and centrifuged for 15 min at 3000 g. The liquid was aspirated, the tube was filled with saline, and centrifuged for 15 min at 3000 g. The liquid was then aspirated off, leaving approximately 1.5mL in the tube. The sample was then transferred to an eppendorf tube and refrigerated at 4°C. Cyclospora oocysts were suspended in 2.5% potassium dichromate at room temperature for 15 days.

Cryptosporidium detection. Oocysts were detected in the samples by an indirect fluorescent-antibody test (IFAT) (Meridian Diagnostics, Inc., Cincinnati, OH). Smears of oyster pellet were made by placing 10µL of solution onto blank three well slides. The slides were allowed to dry, then heat fixed by placing on a slide warmer set to 2.75 for 10 min. The IFA reagent was prepared from 20 µL detection reagent, 20 µL counter stain, and 40 µL phosphate buffer solution. Ten microliters of IFA reagent were added to each slide well and the slides were incubated in a humid chamber for 20 min. Ten microliters of mount media were then added to the slides, a small cover slip was placed on the drop and the slides were refrigerated at 4°C. Oocysts were identified as fluorescent green cells after examination by epifluorescence microscopy (Olympus BX60, Olympus America Inc., Melville, NY) under the NB filter.

Viability and Infectivity. Viability was determined by the neonate animal infectivity assay. Term-pregnant CD1 mice were obtained from Harlan Sprague Dawley Inc. (Madison,
Upon delivery, each mother was housed separately with 12 neonate mice. After 5 days each mouse pup was orally inoculated with a 50 µL aqueous suspension containing the temperature-treated and processed oyster tissue containing *C. parvum* oocysts. Each treatment was evaluated in 10 to 12 mice. Twelve neonate mice were used as negative controls (inoculated only with water). Five days post-inoculation, all pups were euthanized by CO₂ inhalation. A segment of the ileum was removed and smears of the fecal material were made on poly-L-lysine coated three well slides. These slides were stained using an acid fast staining kit (Remel, Lenexa, KS). The slides were heat fixed, covered with carbolfuscin stain for 30 min, destained with acid alcohol for 10 sec, then covered with methylene blue for 5 min and rinsed with tap water, allowed to dry and observed under a microscope at 40x magnification using immersion oil. The stains of fecal material from each mouse were scored positive (infected) or negative (uninfected) by microscopic observation, and the amount of animals infected at each treatment were recorded.

A 1-cm segment of terminal ileum was removed, fixed with 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The 4 µm histological sections were examined by light microscopy at 40x magnification. Evidence of infection was defined as the observation of *C. parvum* parasite developmental stages in the microvilli of prepared ileal tissue sections. Tissues from each mouse were scored plus or minus by microscopic observation, and the proportion of animals infected at each treatment were calculated and recorded.

**Cyclospora detection and viability.** Sporulation was used as an indicator of viability, since there is no current *in vivo* viability or infectivity assay available for *C. cayetanensis*. Potassium dichromate (2.5%) was used to allow oocysts to sporulate and to prevent the growth of bacteria, fungi, or yeast. After all treatments *Cyclospora* oocysts were incubated at 23°C in potassium dichromate for 15 days. Ten microliters of the suspension were placed on a glass
Samples were examined by bright field microscopy at 20x and 40x magnification under the WU filter and observed by autofluorescence. The number of sporulated and unsporulated oocysts were recorded.

**Lipid Extraction.** Lipid extraction was performed according to the conventional Soxhlet extraction of Luque-Garcia (15) with certain modifications. Before extraction, the temperature treated oysters were cut up and dried in an oven at 100°C for 16 hrs, with one sample consisting of 6 oysters. The dried sample was placed in a cellulose thimble (43 x 123mm, Fisher Scientific, Pittsburgh, Pa). The distillation flask was filled with 300-mL of hexane (Sigma, St. Louis, MO).

**TBARS Test.** 2-thiobarbituric acid reactive substances (TBARS) values were determined according to the AOCS method (2). All observations were compared to the control sample. Three observations for the control (Two half shell and one whole shell) and each of the treatments (blast freezing, ultra-low freezing, individual quick freezing, and low temperature pasteurization) were performed.

**Sensory Evaluation.** Sensory evaluation of temperature treated oysters was carried out by 20 consumer panelists, which according to Meilgard, Civille, and Carr (18), improves discrimination among samples. Panelists were asked to evaluate five oyster samples, oysters which were blast frozen, individual quick frozen, low-temperature pasteurized, and ultra low frozen, and a control, which was not temperature treated. The participants were asked to rank the samples based on appearance, texture, and aroma, without tasting the samples. The most preferred sample corresponded to 1 and the least preferred sample corresponded to 5. Each
sample consisted of one oyster, and the five samples were assigned random three digit codes. The sensory analysis was performed immediately following treatment and storage at 4°C.

**Statistical Analysis.** Data was analyzed using Statistical Analysis Software (SAS®, Duncan, SC), with treatment being the comparative factor. Analysis of variance (ANOVA) with the general linear model (PROC GLM) was conducted to determine the means and standard deviations among samples for all treatments and the control.

**RESULTS AND DISCUSSION**

**Oocyst recovery in positive samples.** Oocyst recovery in the positive controls varied dependent upon the experiment, half shell or whole shell (Tables 2.1 and 2.2). Previous studies (16) found that whole tissue homogenates gave the best recoveries because oocysts were not concentrated on the gills or in the hemolymph in sufficient numbers to enhance detection. However, for the half shell experiment whole oysters were not homogenized for oocyst detection, just the gills and hemolymph were removed, which may account for the low recovery in these trials. Previous studies (16) also found the recovery rate for *C. parvum* in seeded oyster tissues to be 69.5% which corresponds with 75.6% recovered in this study. Another issue that affects oocyst recovery is the health of the oyster upon experimental inoculation. Unhealthy or dying oysters will not concentrate and filtrate oocysts.

**Effect of treatments on *C. parvum* and *C. cayetanensis.** No sporulated cells of *Cyclospora* were found in positive controls, or temperature treated oysters. The results of this
study on the inactivation of *C. cayetanensis* are inconclusive, because there was no sporulation observed in the positive control samples. It is possible that there was an adverse reaction between the *C. cayetanensis* oocysts and the oyster tissue. Sathyanarayanan and Ortega (22), found that *Cyclospora* sporulation can be inactivated when oocysts are exposed to extreme hot or cold temperatures -70°C, and 70°C to 100°C. *Cryptosporidium* oocyst viability was also affected by the experiments. After temperature treatments, there were no viable cells detected by the acid fast stained slides, or by the tissue histology. The results of this study can be compared with the results of Fayer and Nerad (6) wherein they found that *C. parvum* oocysts that were frozen at -70°C for 1 hr or more, and -20°C for 24 hrs or more had no infectivity in mice. According to the acid fast stain, infectivity of the positive control mice (n = 12) varied (Tables 2.1 and 2.2), but none of the negative control mice were infected (n =12). However, tissue histology showed no positive control mice infected. *C. parvum* infectivity in the positive control samples of the acid fast stained fecal material shows conclusive evidence that the mouse intestine was infected. It is possible that the terminal ileum tissue used for tissue histology was infected but the sections made visible by the cutting of the tissue did not display this infection.

**Effect of treatments on lipid oxidation.** TBARS results showed that there was no significant difference between the values of the control and the ultra low frozen oysters stored for 7 days (Table 2.3). There was also no significant difference between the ultra low frozen oysters and the low temperature pasteurization medium treatment. Khan, Parrish, and Shahidi (13) found that TBARS values of mussels increased gradually with storage on ice. However, in this study the TBARS values did not increase with storage time because of the quick freezing, and low temperature methods employed. A limitation of the TBARS test is that it measures secondary lipid oxidation products and is not very effective with very short or very long storage
times (7, 21). Although cold pasteurization was not evaluated for lipid oxidation in this experiment, Kanatt, Chander, and Sharma (12) found that lipid oxidation, as measured by the TBARS test, increased with radiation processing of meat. This goes along with the findings of Jo and Ahn (11).

**Effect of treatments on sensory quality.** Sensory evaluation results showed that the low temperature pasteurized oysters had the lowest rank sum, followed by the blast frozen oysters (Table 2.4). These two values were not significantly different from one another, but the low temperature pasteurization rank sum was significantly different from the individual quick frozen, ultra low frozen, and control oysters. It can be concluded that the low temperature pasteurized oysters were preferred over the other temperature treatments. Although cold pasteurization was not evaluated for sensory quality in this experiment, other investigators found that cold pasteurized raw fish meat was indistinguishable from non-cold pasteurized raw fish meat (5).

**CONCLUSIONS**

In conclusion, contamination of oysters with pathogenic protozoa has become a major concern of the seafood industry in recent years. Oysters can concentrate and filtrate the oocysts of *C. cayetanensis* and *C. parvum* from the water in which they are harvested due to run-off or other fecal contamination. Post-harvest treatments are the key to ensuring that oysters which are to be consumed raw are free of harmful pathogens. The present study has confirmed the effectiveness of current post-harvest treatments, low temperature pasteurization, cold pasteurization, individual quick freezing, and blast freezing on the inactivation of *C. parvum*, but has also presented a new treatment, ultra low freezing, which can also be used to inactivate this parasite. The effects of the five treatments on *C. cayetanensis* are inconclusive because there was no sporulation observed in the positive controls after 15 days. Although all five treatments were
shown to be effective against *Cryptosporidium*, low temperature pasteurization emerged as the recommended treatment because it also had little effect on the chemical and sensory properties of the oyster tissue. Further studies are needed to evaluate oocyst recovery of both parasites in positive control samples. Further examination of the effect of oyster tissue on sporulation of *C. cayetanensis* is also needed.
Table 2.1 – Oocyst viability and infectivity of *C. parvum* and *C. cayetanensis* in positive control oysters after half shell experimental inoculation

<table>
<thead>
<tr>
<th>Parasite</th>
<th>No. of oocysts inoculated (per oyster)</th>
<th>No. of positive controls containing oocysts*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. parvum</em></td>
<td>1x 10^4</td>
<td>4/12</td>
</tr>
<tr>
<td><em>C. cayetanensis</em></td>
<td>1x 10^3</td>
<td>4/13</td>
</tr>
</tbody>
</table>

Positive control = oysters experimentally inoculated but not temperature treated

* Positive controls containing oocysts are presented as number of positive samples/total number of positive control oysters

Table 2.2 – Oocyst recovery and infectivity of *C. parvum* and *C. cayetanensis* in raw oysters after whole shell experimental inoculation

<table>
<thead>
<tr>
<th>Parasite</th>
<th>No. of oocysts inoculated (per pan)</th>
<th>No. of oocysts after inoculation (saltwater)</th>
<th>No. of oocysts recovered by positive controls</th>
<th>No. of positive controls containing oocysts *</th>
<th>Approximate recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. parvum</em></td>
<td>240000</td>
<td>58672</td>
<td>181328</td>
<td>4/12</td>
<td>75.6</td>
</tr>
<tr>
<td><em>C. cayetanensis</em></td>
<td>240000</td>
<td>2034</td>
<td>21966</td>
<td>11/12</td>
<td>91.5</td>
</tr>
</tbody>
</table>

Positive control = oysters experimentally inoculated but not temperature treated

* Positive controls containing oocysts are presented as number of samples displaying infection/total number of positive control oysters
Table 2.3 - TBARS values (expressed as mg malonaldehyde/kg sample) of temperature treated oysters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of treatment</th>
<th>Mean TBARS value</th>
<th>Level of significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>A 0.1110</td>
<td>-</td>
</tr>
<tr>
<td>Individual quick freeze</td>
<td>12 hour</td>
<td>CD 0.2613</td>
<td>0.0087</td>
</tr>
<tr>
<td></td>
<td>24 hour</td>
<td>CD 0.3127</td>
<td>0.0009</td>
</tr>
<tr>
<td></td>
<td>36 hour</td>
<td>D 0.3330</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>48 hour</td>
<td>D 0.3373</td>
<td>0.0003</td>
</tr>
<tr>
<td>Blast freeze</td>
<td>15 hour</td>
<td>CD 0.2693</td>
<td>0.0061</td>
</tr>
<tr>
<td>Low temperature pasteurization-medium</td>
<td>8.5 minutes</td>
<td>BC 0.2227</td>
<td>0.0430</td>
</tr>
<tr>
<td>Low temperature pasteurization-high</td>
<td>12 minutes</td>
<td>CD 0.2443</td>
<td>0.0179</td>
</tr>
<tr>
<td>Ultra low freeze</td>
<td>24 hours</td>
<td>CD 0.2320</td>
<td>0.0296</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>AB 0.1230</td>
<td>0.8187</td>
</tr>
</tbody>
</table>

ABCD: Mean values in a column not followed by the same uppercase letter are significantly different (P < 0.05).
Each mean value represents the average of three samples (n = 3).
Control = storage at 4°C

Table 2.4 - Rank sums from sensory evaluation of temperature treated oysters (ranking preference test)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rank Sums</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual quick freeze</td>
<td>64 bc</td>
</tr>
<tr>
<td>Blast Freeze</td>
<td>53 ab</td>
</tr>
<tr>
<td>Low temperature pasteurization-medium</td>
<td>43 a</td>
</tr>
<tr>
<td>Ultra Low Freeze</td>
<td>66 bc</td>
</tr>
<tr>
<td>Control</td>
<td>74 c</td>
</tr>
</tbody>
</table>

abc: Mean values in the same column not followed by the same lowercase letter are significantly different.
Control = storage at 4°C
REFERENCES

CHAPTER 3
SUMMARY AND CONCLUSIONS
In this study, five temperature treatments were evaluated to determine their effect on the parasites *Cyclospora cayetanensis* and *Cryptosporidium parvum*. Certain post-harvest treatments already in use, and some new treatments, can now be used to inactivate *C. parvum*. All treatments evaluated in this study inactivated *C. parvum*. *C. parvum* was inactivated when whole, shell-stock, oysters and half shell oysters were temperature treated. The results for the parasite, *C. cayetanensis* were inconclusive because there was no sporulation observed in the positive control samples.

Infectivity of the positive control mice varied greatly, showing that oocyst recovery in the positive controls oysters also varied. This low oocyst recovery rate possibly affected the results for the temperature treatments evaluated in this study.

The effects of four temperature treatments, blast freezing, ultra-low freezing, individual quick freezing, and low temperature pasteurization, on lipid oxidation of raw oyster tissue were also examined using the TBARS test. The study showed that the TBA values of the ultra low frozen oysters, which had been stored for 7 days were not significantly different from the control. However, the TBA values of the low temperature pasteurized oysters were not significantly different from the ultra low oysters frozen for 7 days. From this study, it can be concluded that the ultra low freezing technique, with 7 day storage, had the least effect on lipid oxidation in oyster tissue.

Sensory evaluation of the raw oysters, using the ranking preference test, evaluated the temperature treated samples based on the appearance, aroma, and texture of the oyster tissue. The ranking preference test showed that the low temperature pasteurized oysters were preferred over the other three treatments and the control. With the second most preferred treatment being blast freezing.
Although the results of this study displayed that all post harvest treatments evaluated could be used to eliminate the parasite *C. parvum*, it can be recommended that low temperature pasteurization be used as a post harvest treatment of raw oysters to eliminate this parasite, because it was the most preferred in sensory testing, and it had little effect on lipid oxidation of the oyster tissue. However, oocyst recovery by raw oysters should be investigated to further evaluate the results of this study. Also, further examination of the effect of oyster tissue on *C. cayetanensis* sporulation is also needed.
Ranking Preference Test Score Sheet

**Instructions:**

On the given tray, there are five samples which you will examine and compare based on aroma, appearance, and texture. **Do not taste the samples!** Write the sample codes in the space provided, and rank the samples according to preference, with 1 being the most preferred sample, and 5 being the least preferred sample.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>528</td>
<td>_____</td>
</tr>
<tr>
<td>396</td>
<td>_____</td>
</tr>
<tr>
<td>875</td>
<td>_____</td>
</tr>
<tr>
<td>264</td>
<td>_____</td>
</tr>
<tr>
<td>759</td>
<td>_____</td>
</tr>
</tbody>
</table>

**Comments:**