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Development of safe and ready to eat frozen oyster products using microwave steam-venting technology

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DEVELOPMENT OF SAFE AND READY TO EAT FROZEN OYSTER PRODUCTS USING
MICROWAVE STEAM-VENTING TECHNOLOGY

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ABSTRACT

The Gulf Coast region, principally Louisiana, leads the nation in oyster production. The National Marine Fisheries Service reported that 19.7 million pounds of oyster meat, valued at $62.3 million dollars were harvested from the Gulf Coast region in 2010. One of the main concerns for this industry is the health risk associated with the consumption of oysters, particularly for at-risk populations. Oysters are filter feeders. They tend to concentrate microbes present in surrounding waters, some of which can cause severe illness in susceptible humans. Among pathogens that contaminate the gastrointestinal system of oysters, *Vibrio* spp (especially *V. parahaemolyticus* and *V. vulnificus*) are of greatest concern.

The overall goal of the proposed study was to develop a protocol for the production of high quality and safe frozen oyster products in steam venting packages that are microwavable. Based on this study, a combination of 100 g of frozen oyster meat with 200 g of frozen mixed vegetables provided retention of almost 95% of the meat's moisture after 300 s of microwave cooking. In addition, the achieving of an internal temperature of 90°C after 258 s of microwave cooking assured inactivation of naturally occurring pathogenic bacteria in oyster meat. The study demonstrated that steam venting technology could be used to inactivate pathogenic bacteria in frozen oysters and oyster products cooked in the steam packages retained the texture and the desirable flavor composition traditionally associated with oysters.

CHAPTER 1   INTRODUCTION

The Gulf Coast region, principally Louisiana, leads the nation in oyster production. The National Marine Fisheries Service reported that 19.7 million pounds of oyster meat, valued at $62.3 million dollars was harvested from the Gulf Coast region in 2010 (NOAA, 2011). Today, one of the main concerns for this industry is the health risk associated with consumption of oysters.

Oysters are filter feeders. They concentrate microbes present in surrounding waters, some of which can cause severe illness in humans (Cook, 2003; DePaola and others, 1997; Koo and others, 2006; Richards and others, 2010). Since most oysters are eaten alive, raw, or poorly cooked, they can act as vectors for pathogenic microbes. Among pathogens that contaminate the gastrointestinal system of oysters, *Vibrio spp* (especially *V. parahaemolyticus* (*Vp*) and *V. vulnificus* (*Vv*)) are of greater concern (CDC, 2013; Richards and others, 2010; Scallan and others, 2011). *V. vulnificus* strains have been strongly associated with severe and life-threatening conditions in immunocompromised patients, especially those with chronic liver disease. *V. vulnificus* is the second leading cause of seafood-related fatality in the U.S. (Andrews and others, 2003; Haq and others, 2005).

There is always a high consumer demand for oysters that are safe while retaining their original flavor, nutrient content, texture, and appearance. In addition, these oysters are expected to be additive-free as well as presenting a longer shelf life (Horst and others, 2011). A combination of rapid freezing and appropriate packaging can accomplish the later statement. The available freezing techniques, such as cryogenic freezing, provide a multitude of advantages. Cryogenic freezing is fast and thus reduces the weight loss during the freezing process while maintaining the overall quality closer to the freshest product. Combining cryogenic freezing

with high barrier packaging and modified atmosphere packaging may increase the shelf life of a product compared to similar ones frozen with traditional freezing techniques.

It is believed that with the emerging category of value added meal solutions the development of an oyster based, safe, frozen and ready-to-eat meal product using steam-venting microwavable packaging will create new opportunities for oyster processors to expand their market. Ready–to-eat (RTE)-meals are available in a wide variety of recipes and they are consumed with increasing market share. At the same time, consumer acceptance of these foods has been hindered by the perception that they lack freshness. Additionally, consumers have gained knowledge regarding the content of the products they purchase, with the expectation that the meal would be nutritious, palatable and safe to eat (Peck and others, 2008). To counter count the perception that the foods are lacking of freshness, the development of steamed meals have given a new dimension to RTE meals. Steaming is a gentle, fat-free cooking method that maintains the natural moisture in foods. This feature makes it an excellent choice for preparing delicate meals, especially those having seafood as a main ingredient. Steaming protects against drying, keeping flavorful juices and nutrients inside the seafood, rather than letting them escape into the surrounding liquid.

The overall goal from this study is to develop high quality and safe frozen oyster products in steam venting packages that are microwavable. An integral part of this research will be the measurement of *V. parahaemolyticus* and *V. vulnificus* along the processing chain. Population levels of these bacteria will be carefully assessed at freezing, during frozen storage, and, more importantly, to assess the conditions required to achieve non-detectable levels through the process of microwaving.

CHAPTER 2   LITERATURE REVIEW

Vibrio vulnificus and *Vibrio parahaemolyticus*, are free living, naturally occurring bacteria, not associated with pollution, which are changing the way raw oysters from the Gulf States are eaten. These *Vibrio sp*. are common in the Gulf waters during the warmer months, and filter feeding shellfish such as oysters can accumulate them as they feed (USGAO, 2011). Especially *V. vulnificus* is of important interest for people with pre-existing medical conditions, which are considered "at risk" for severe blood infection (primary septicemia) that could cause death.

Because oysters are usually eaten raw, special efforts are made to protect public health. The U.S. Food and Drug Administration (FDA) and coastal state governments oversee the National Shellfish Sanitation Program (NSSP), which sets standards for waters in which oysters are grown and requires those waters to be tested regularly.

2.1   Foodborne diseases background

Infectious diseases spread through food or beverages are a common, distressing, and sometimes life-threatening problem for millions of people in the United States and around the world. Data acquired by active and passive surveillance as well as other sources by the Centers for Disease Control and Prevention (CDC) estimates that 31 identified pathogens cause 48 million episodes of foodborne illnesses, 128,000 hospitalizations, and 3,000 deaths each year (Scharff, 2012). The average cost per case of foodborne illness is $1,343 calculated from two different economic models, resulting in an aggregated annual cost of illness of $ 64.5 billion (Scharff, 2012). Epidemics of foodborne diseases are not only a threat to public health but also erode consumer confidence in the causal food product and thus, impact the economic viability of

the industry. Consequently, the added cost for this impact is conservatively estimated in economic models and does not accurately represent its true extension.

Infectious agents associated with food-borne illnesses include bacteria, viruses, and parasites, and the illnesses caused by these agents range from mild gastroenteritis to life-threatening syndromes. The CDC through the Foodborne Disease Outbreak Surveillance System collects data on foodborne disease incidents submitted from all states and territories. The most recent year for which data are finalized was for 2010 and a total of 9,024 cases were listed as foodborne disease outbreaks with 4,540 (50.3%) suspected or confirmed cases due to bacteria (CDC, 2011). Foodborne illness cases due to bacteria have exceeded viral, parasitic, and chemical sources since 2007 as shown in Figure 2.1. Previous to this year, reported data shows that viruses caused more foodborne illnesses, however, bacterial infections associated with foodborne illnesses have always required hospitalization or resulted in death (Figure 2.2).
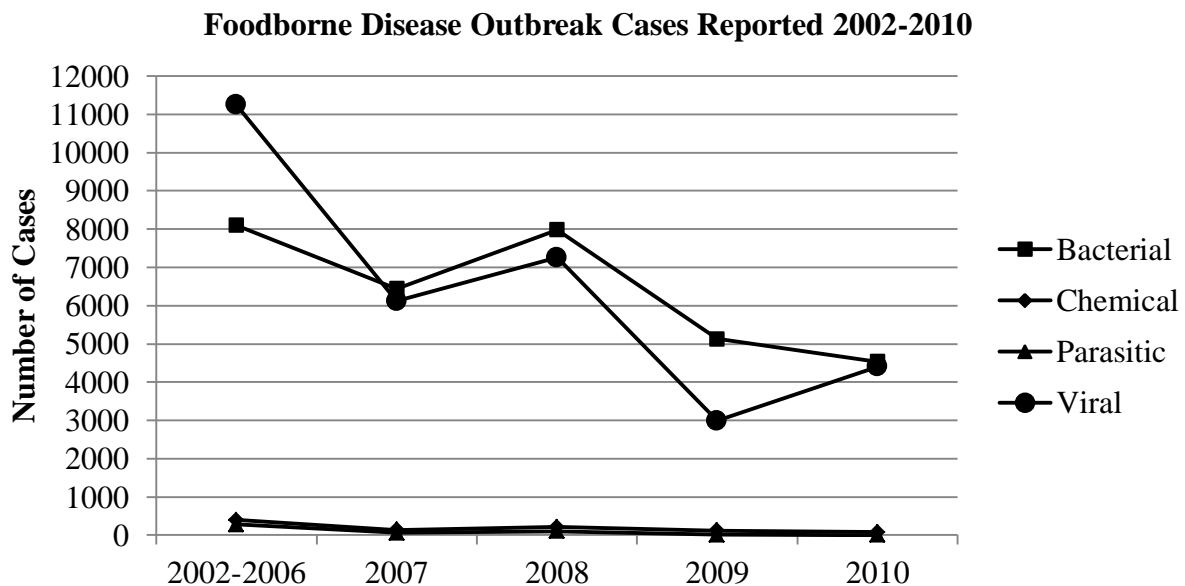
Figure 2.1: Graphical representation of foodborne disease outbreak cases sorted by etiological causes as reported by the CDC (CDC, 2006, 2011, 2010b, 2013)

Fatalities Associated with Foodborne Disease Outbreaks from 2002-2010
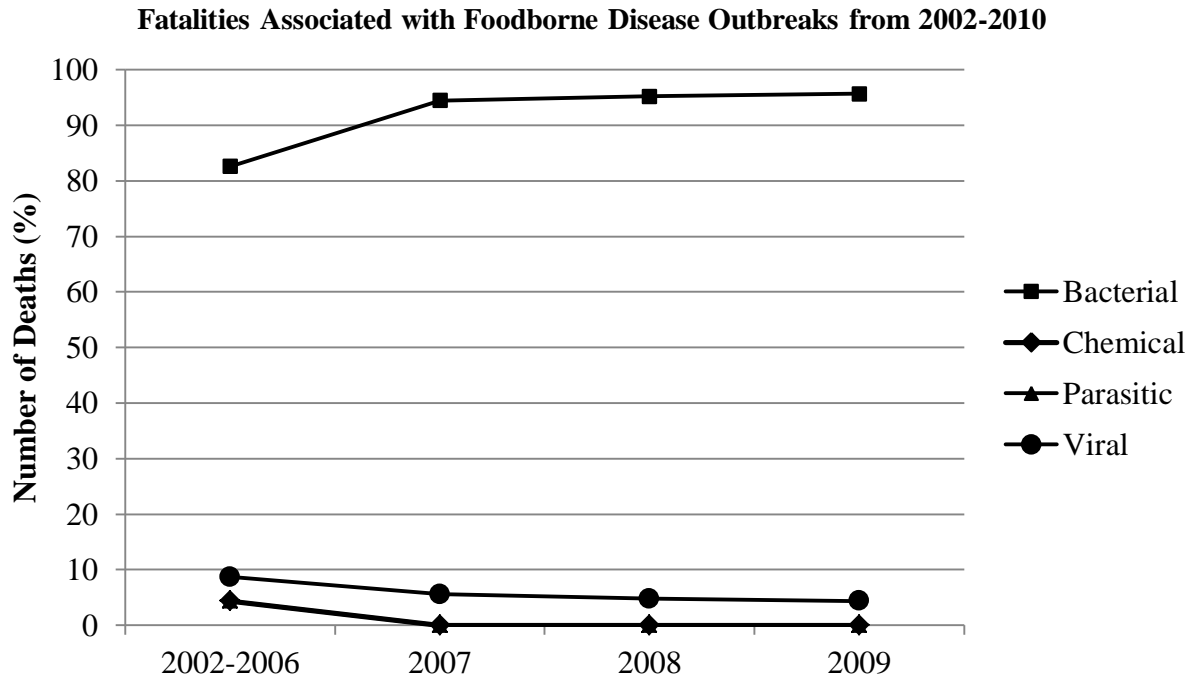
Figure 2.2: Graphical representation of fatalities associated with foodborne disease outbreaks sorted by etiological causes as reported by the CDC (CDC, 2006, 2011, 2010b, 2013)

Seafood is responsible for an important proportion of food-borne illness and outbreaks both in the United States and worldwide. Seafood includes mollusks (e.g., oysters, clams, and mussels), finfish (e.g., salmon and tuna), marine mammals (e.g., seal and whale), fish eggs (roe), and crustaceans (e.g., shrimp, crab, and lobster). Many of the detailed investigations of bacterial-related foodborne illnesses have focused on oysters, since they are often consumed uncooked (Iwamoto and others, 2010). Raw oysters may contain a number of different harmful bacteria, and have been linked to serious illness and death. As such, food safety experts and public health agencies have consistently warned of the serious potential risk created by these mollusks, when consumed uncooked.

2.2   Risks associated with consumption of oysters

In general, seafood-associated infections are caused by a variety of bacteria, viruses, and parasites. From these, *Vibrio* spp. are widespread and naturally present in marine and estuarine

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environments. *Vibrio* species are characterized as Gram-negative, rod-shaped or curved rod-shaped, halophilic bacteria. In 2009, according to the Cholera and Other *Vibrio* Illness Surveillance (COVIS) system, of the 825 cases of vibriosis (excluding toxigenic *V. cholerae*) 217 (26%) were from Pacific Coast states, 256 (31%) from Atlantic Coast states, and 101 (12%) from non-coastal states (Figure 2.3) (COVIS, 2011). In those states sharing waters from the Gulf of Mexico, the *Vibrio* species most frequently reported were *V. vulnificus* (27%) and *V. parahaemolyticus* (21%), followed by *V. alginolyticus* (19%), and non-toxigenic *V. cholerae* (13%).

On the other hand, in non-Gulf Coast States *V. parahaemolyticus* (58%) exceeded *V. alginolyticus* (14%), *V. vulnificus* (7%), and non-toxigenic *V. cholerae* (7%). Detailed investigation of these cases showed that improperly prepared or mishandled shellfish was the primarily cause of infection. More specifically, in cases reporting eating a single seafood item, consumption of oysters was the principal vector in 48% of the cases, of which 94% consumed them raw.

Oysters are more inherently risky than other seafood commodities owing to many factors, including the nature of the environment from which they come, their mode of feeding, the season during which they are harvested, and how they are prepared and served (Horst and others, 2011). The pathogens most commonly associated with their raw consumption are *Vibrio parahaemolyticus* and *Vibrio vulnificus*. Related infections are acquired through ingestion or through exposure of an open wound to seawater. *V. parahaemolyticus* has been associated with sporadic infections and outbreaks of gastroenteritis while *V. vulnificus* infections occur almost exclusively as sporadic cases in the United States (COVIS, 2011).

Clinical features most often associated with *V. parahaemolyticus* infection include watery diarrhea, abdominal cramps, nausea, and vomiting. Wound infections and septicemia occur less commonly (Painter and others, 2013; Scallan and others, 2011). Although *V. parahaemolyticus* is recognized as a major cause of seafood-borne gastroenteritis, most strains of this species are not pathogenic to humans (Iwamoto and others, 2010). *V. vulnificus* is particularly virulent, especially among patients with liver disease and iron storage disorders, who are at increased risk of invasive disease. *V. vulnificus* infections can lead to sepsis and severe wound infections. Severe infections, such as bloodstream and wound infections, require prompt antimicrobial therapy. The case fatality rate is about 50% for bloodstream infections and 25% for wound infections (Iwamoto and others, 2010).

The Food and Drug Administration (FDA) is the governmental agency responsible for ensuring oyster safety and works with the Interstate Shellfish Sanitation Conference (ISSC), which includes representatives from the FDA, states, and the shellfish industry to establish guidelines for sanitary control of the shellfish industry. The FDA and the ISSC have the common goal of reducing *Vibrio* related illnesses, especially from *V. vulnificus*. Joint forces of both entities concentrate to produce a significant reduction during the warmer months of the year. Cases of vibriosis (excluding toxigenic *V. cholerae*), have a definite peak time during the summer months (Figure 4). Most cases occur from May to September, with the greatest number during July and August (COVIS, 2011, 2007, 2008, 2009). Accordingly, the same trend observed in Figure 2.4 agrees with the numbers of reported illnesses caused by the consumption of oysters.
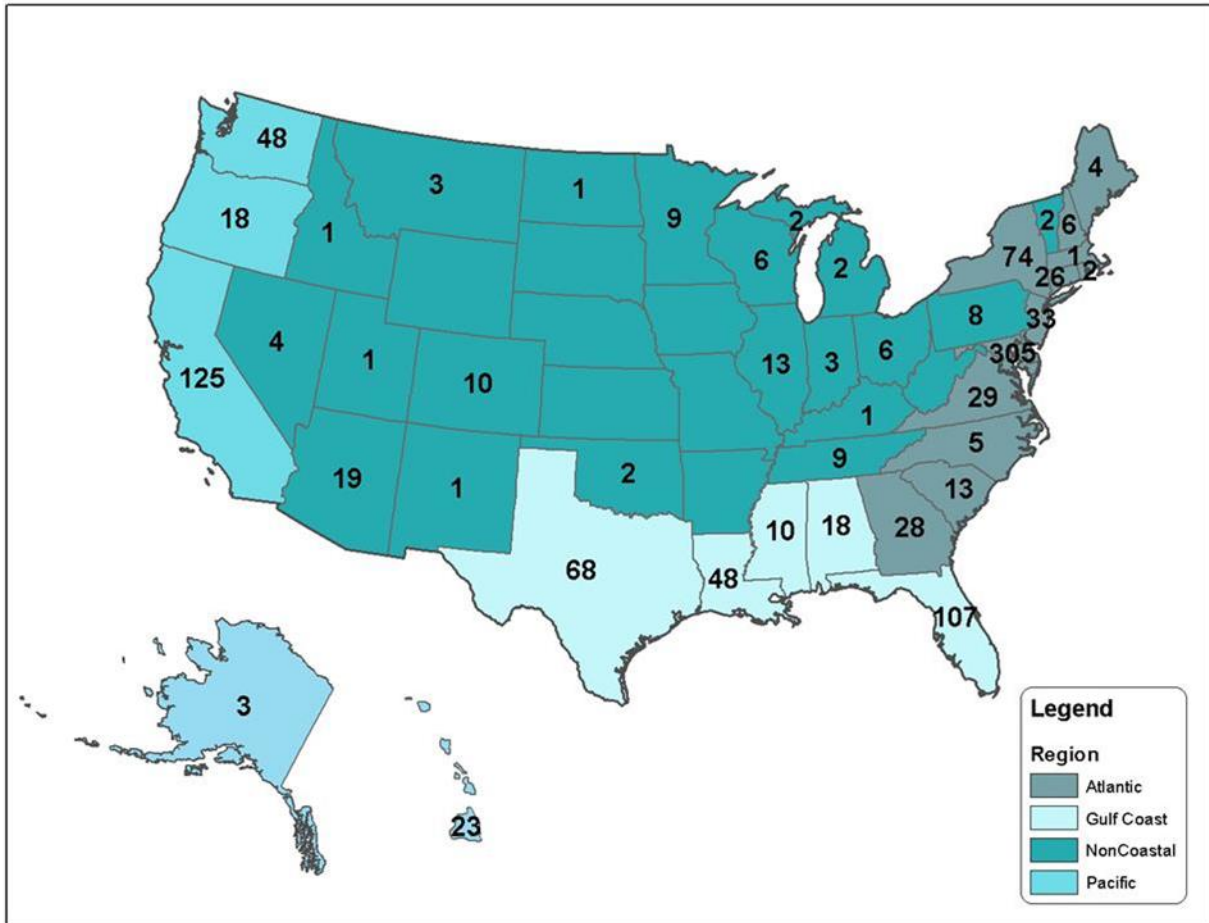
Figure 2.3: Number of cases of *Vibrio* infections (excluding toxigenic V. cholerae), by state, 2009 (n=825 in 42 states) (COVIS, 2011)

Since 2004, the FDA's and the ISSC's efforts have been primarily aimed at consumer education, however, tangible success on reducing the number of illnesses related to consumption of raw oysters has not been achieved. In 2010, the FDA called oyster harvesters to implement time and temperature controls to ensure that oysters are cooled to specific temperatures within a specified time frame to reduce *V. vulnificus* growth and thus decrease the incidence of foodborne illnesses originated by the consumption of raw oysters. However, direct evaluation of the effectiveness of these measures has not being assessed either by the FDA or ISSC (USGAO, 2011).
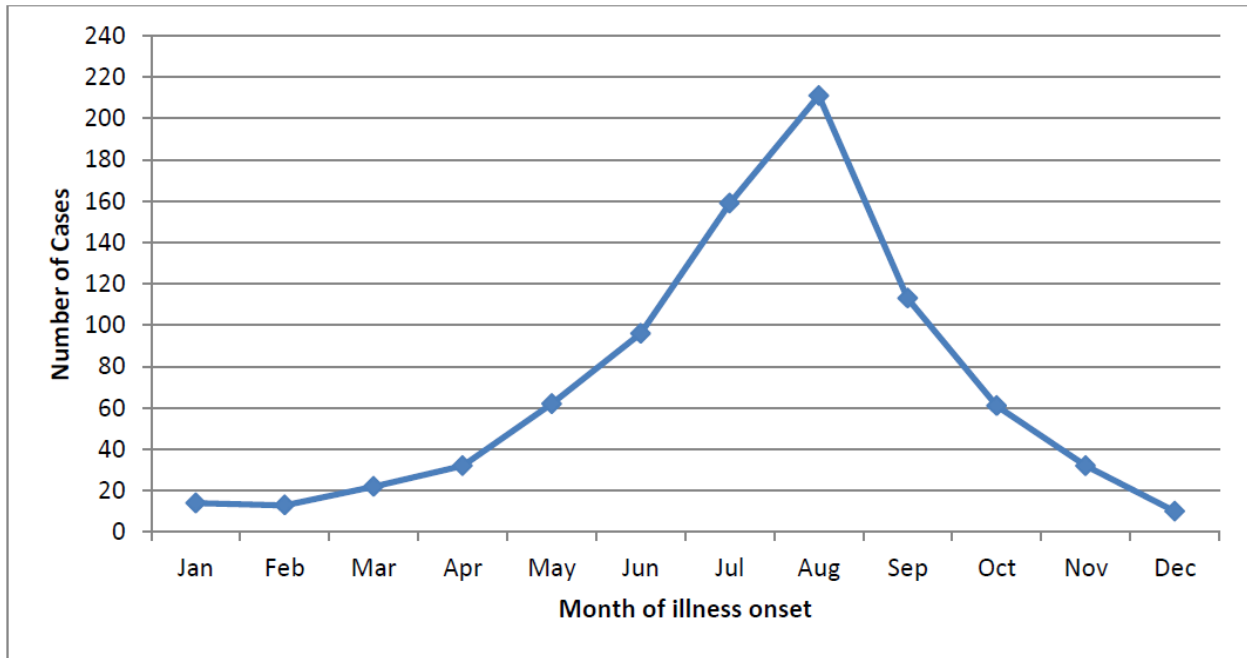
Figure 2.4: Number of cases of vibriosis by month of illness onset, 2009 (n=825 in 42 states) (COVIS, 2011)

It has been suggested by state governments and oyster industry officials that 100 percent compliance with the controls is highly unlikely and thus the FDA has proposed the mandatory requirement to use postharvest processing technologies and methods as the only alternative to eliminate the risk of foodborne illnesses due to raw oyster consumption as observed in California. California, unlike Gulf Coast states, requires that all raw Gulf Coast oysters harvested during the summer and sold in the state be processed to reduce *V. vulnificus* to nondetectable levels. This requirement has reduced *V. vulnificus* illnesses in California to nearly zero.

2.3 Existing Post-harvest Process Technologies for Controlling *Vibrio vulnificus* and *Vibrio parahaemolyticus* in oysters

Post-harvest processing (PHP) technologies for oysters are continually being developed to provide safer oysters to consumers. Alternatives offered by modern technology are focus to promote quality, food safety, and extension of the shelf life of oysters. Some of the post-harvest

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processing technologies currently being investigated are irradiation (electric beam irradiation, X-ray, cobalt and microwave technology) (Jakabi and others, 2003; Mahmoud, 2009), high salinity treatment (Larsen and others, 2013), and value added product (smoked, char-grilled, steamed, pickled, marinated or pre-cooked convenience meals) (Horst and others, 2011). However, applicable PHP methods are those that have been determined to reduce *Vibrio vulnificus* and *Vibrio parahaemolyticus*, by >3.52 log and by > 4 log respectively, to nondetectable levels (<30 MPN/gram) (FDA, 2009). These PHP methods are, to a certain degree, economically feasible for summer-harvested Gulf oysters intended for the raw half-shell market. FDA-approved applicable PHP methods include mild-heat pasteurization, high hydrostatic pressure (HHP) processing, and cryogenic individual quick freezing (IQF) with extended frozen storage.

2.3.1 Mild-Heat Pasteurization

This PHP process was developed and patented in 1995 by AmeriPure in Franklin, Louisiana (Horst and others, 2011). In general, this process comprises a mild heating of oysters in the shell, followed by a rapid cooling. The internal temperature of the oysters rises high enough to reduce the loads of *Vibrio sp.* bacteria to nondetectable levels without cooking them during the time of exposure. First, oysters are washed, individually banded in order to avoid extreme loss of internal juices, arranged in racks, and then submerged in water at 52°C (126°F) for 24 minutes. Temperature and time have been validated through several studies (Andrews and others, 2000) showing reduction of bacterial loads with minimal cooking of the meat. The process continues with a cold shock by dipping the trays into iced water at 4.44°C for 15 min, finalizing with packaging the oysters either for half shell or shucked to extract the meat.

Pasteurization of oysters gives several advantages to the processors. Oysters processed by this method are claimed to have high moisture content with shucking yields increased by 15

to 30 percent relative to untreated oysters (Andrews and others, 2000; Muth and others, 2000). The shelf life is extended from one week for untreated oysters to three weeks under refrigeration. Another advantage of this type of PHP process is its versatility. The process can be adjusted to a small- or large- scale to fit the needs of an individual processing plant. However, the processing temperature and time alter the desirable organoleptic characteristics that make oysters so unique and appreciated.

2.3.2   High Hydrostatic Pressure

Pressurization processing applied to oysters was initiated in 1999 in Houma, LA. The process was developed and patented by Motivatit Seafoods. The process inactivates vibrios and spoilage bacteria in shellfish and facilitates the shucking of oysters (Kural and others, 2008; Li and others, 2009; Murchie and others, 2005). *Vibrio sp.* are much more sensitive to pressure than most of other bacteria due to the complexity of Gram-negative's cell membrane which can be inactivated by pressure levels ranging from 200 to 350 MPa. Bio-membranes are the main sites affected by pressure. High pressure disrupts membrane function due to phase transition causing leakage through the inner and outer membranes as well as inactivation or disruption of key enzymes (Cook, 2003; Koo and others, 2006). During the pressurization and de-pressurization cycles the adductor muscle is detached from the inner walls of the shell which opens the oysters and facilitates the extraction of the meat (He and others, 2002).

The process starts with cleaning, washing, sorting or grading the oysters, then banding and containerizing, usually by placing in a stainless steel cylinder, undergoing pressurization for 4-6 minutes. Following treatment, oysters intended for the raw half-shell market are boxed and iced with their bands on and oysters intended for shucking are shucked and packed in containers.

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High Hydrostatic Pressure technology offers food processors several advantages over other processing methods. Oysters are treated evenly throughout, regardless of their shape and size in a relatively short time in comparison to mild-heat pasteurization (Murchie and others, 2005). The system is considered to be energy efficient (Muth and others, 2011) and produces safe-to-eat oysters that retain the appearance, flavor, texture and nutritional qualities similar to untreated fresh oysters (Murchie and others, 2005) with a shelf-life of almost 30 days (He and others, 2002) under refrigerated conditions. The extracted oyster meat possesses good shape and appearance, looking slightly more voluminous and juicy. These characteristics are derived because of the preservation of the adductor muscle. There is a higher retention of moisture when the adductor muscle of the oyster is not cut from the shell, providing shucking yields of 25–50% (Murchie and others, 2005). The main drawback from the high hydrostatic process is not related to safety or quality of the oysters but to economic factors. The initial capital investment to acquire the equipment alone is considerable, making it affordable to only consolidated large-scale processors (Muth and others, 2011).

2.3.3   Cryogenic Individual Quick Freezing

Freezing is a common preservation technique for foods in general and oysters in particular. Freezing oysters to extend shelf life was first applied in 1989 and rapidly popularized in Australia, Canada, New Zealand, and especially in the United States. Aside from the resulting decrease of microorganisms including *Vibrio sp*. bacteria to nondetectable levels, extended shelf life is a major selling point of the process (Muth and others, 2011). Gram-negative bacteria are more susceptible to freezing compared to Gram-positive. Freezing temperatures drastically disrupt membrane transport mechanisms, which are intricate due to complexity of the membrane's structure, altering functional metabolic and enzymatic processes (Archer, 2004).

Preparatory steps to cryogenically freeze oysters include cleaning, rinsing, and shucking of incoming shellstock. Then oysters on the half shell are placed on specially designed trays and loaded onto a freezer tunnel where they are rapidly frozen using liquid carbon dioxide or liquid nitrogen. Processing temperature ranges between - 40°C to - 30°C with retention times of 8 to 12 minutes (Motivatit Seafoods, 2013). A common practice after freezing is the application of a mist of water on top of the oysters which freezes into a glaze of ice on contact. Glazing protects the product against quality deterioration that can occur during frozen storage (Sundararajan and others, 2011). After glazing, the oysters are stored in wax-coated corrugated boxes and placed in a freezer for a period of time sufficient to achieve non-detectable levels of *Vibrio spp* (Muth and others, 2011).

One of the main advantages of this process is the convenience it provides to costumers. Establishments which do not have trained staff who are able to properly shuck oysters may benefit from this process which permits the serving of raw oysters on the half-shell. For this purpose, the oysters are simply removed from their packaging and raised to the desired serving temperature before they are served to the consumer. Also, cryogenically frozen oysters have longer shelf-life than any other post-harvest process technology keeping most of the flavor and appeal of non-processed oysters between six and twelve months under frozen storage (Berne, 1996; Songsaeng and others, 2010), and thus, it is a common practice among oyster processors to freeze oysters from the winter harvest, which yields higher quality oysters (particularly in the Gulf of Mexico), and then offered for sale during other times of the year.

Adoption of this technology has also disadvantages. The cryogenic freezing of oysters is not recommended during the warmer months of the year, especially in the Gulf Coast area. Gulf operations that have cryogenic freezing equipment very rarely freeze summer-harvested oysters

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because of quality issues (Muth and others, 2000; Mary K. Muth and others, 2011; Motivatit Seafoods, 2013). During the summer, oysters present lower weights because of spawning as well as possessing a grainy or poor texture (Gullian and others, 2009) making them not a less desirable product to freeze. They also have a dark color, deviating from their normal creamy appearance, making them unacceptable for half shell presentation which has been verified by consumer acceptance studies (Posadas and others, 2011). Capital investment in equipment is not as expensive as in High Pressure Processing (Muth and others, 2011); however, oyster processors that implemented this technology have had to augment the size of their facilities to accommodate frozen storage warehouses. In addition, an efficient supply of liquid carbon dioxide or liquid nitrogen may become a burden when the physical location of the plant is away from distribution centers or they are not easily accessible for delivery. In such cases, where there is not a guaranteed supply of these gases, this technology should not be considered.

2.4   Effect of freezing on *V. vulnificus* and *V. parahaemolyticus*

2.4.1   Survival of Microorganisms during Freezing

Laboratory studies on freezing microorganisms in simple aqueous solutions has allowed investigators to study the direct effects of freezing without the interventions of undesired variables and has simplified the isolation and enumeration processes following freezing treatments (Koo and others, 2006; Mahmoud, 2009). However, the actual mechanism of freeze damage to the cells has not been defined yet, and scientists have different theories as to what is affecting the survival of microorganisms during freezing, frozen storage, and thawing.

Factors expected to cause damage to cells during freezing are: low temperatures, formation of intracellular ice, formation of extracellular ice, intracellular solute concentration, and extracellular solute concentration. All of these factors will be affected by the rate of

freezing. It must be noted that damage can also occur during thawing. The rate of thawing will affect the extent of damage as well (Lund and others, 2000).

Cellular damage during freezing has been widely explained under the 'two-factor' hypothesis of freezing injury due to the osmotic behavior of cells during freezing. Hypertonic damage at slow rates of cooling and lethal intracellular ice formation at high rates of cooling describe the freezing response of many cell types. In a review about the freezing of biological systems (Ken and others, 2004), it is explained that cells generally undergo supercooling, by remaining unfrozen at -10 or -15º C, even when the medium around them has ice. Since the vapor pressure of supercooled water is higher than that of ice, cells need equilibrium. For slowly cooled cells or cells with high water permeability, equilibrium will be reached by transferring internal water to external ice, resulting in dehydration. However, if the cell membrane has low water permeability or the cells are cooled rapidly, equilibrium will be achieved when a substance (colloids or dissolved substances) acts as a nucleus for intracellular ice formation. The critical rate that defines slow and fast cooling is specific for each cell and depends on its permeability to water and on the ratio of the cell volume and its surface area (Ken and others, 2004).

Regardless of how the equilibrium is achieved, cells will be subjected to "solute effects", which refers to intracellular and extracellular solute concentration that may cause a precipitation of the solutes, if their solubilities are exceeded, and result in a change in pH, that can be detrimental to cells. Slowly cooled cells will be exposed to this effect for a longer time (Mazur, 1970).

Formation of intracellular ice produces small crystals that have high surface energies, which will be reduced by growing or by fusing with other small ice crystals. The rate of this process will be higher in smaller crystals and at higher temperatures, so this process is very

important during warming (Goswami, 2010). In fact, it was found that the size of the crystals is a function of initial crystal size, storage temperature and time, and that crystal growth is detectable in temperatures as low as -45ºC (Goswami, 2010). The formation of large ice crystals is lethal to cells in most cases and death seems to occur as a result of the extraction of bound water from vital structures and from proteins (Damodaran and others, 2008). It has been suggested that the optimum freezing rate range for cell survival is one that will be slow enough to prevent intracellular ice formation, but fast enough to prevent the cell from being affected by prolonged exposure to concentrated solutes (minerals, metals, etc). However, for some cells, there is no optimum range (Mazur, 1970).

Responses to freezing stress have been extensively studied in a large diversity of organisms such as prokaryotes, plants or animals (Lalaymia and others, 2012; Panoff and others, 1998; Rivals and others, 2007). Response to freezing stress is often passive and leads to a decrease in viability and metabolic activity that is associated with cryoinjury (Panoff and others, 1998; Thieringer and others, 1998). DNA denaturation is also listed as a possible cause of death after freezing and thawing. DNA damage and fragmentation occurs due to the presence of reactive oxygen species (ROS) (Riesco and others, 2012). ROS include free radicals, which are active oxidizing agents, and these peroxidation products are highly deleterious and can produce both DNA strand breaks and base modification which includes single- and double-strand breaks, abasic sites, and base damage. At low temperatures, repair mechanisms will probably be slow and the extent of the damage may impair various vital functions.

2.4.2 Cold Adaptation of *V. vulnificus* and *V. parahaemolyticus*

Numerous studies have investigated the response of *V. vulnificus* to low temperatures (Bryan and others, 1999; Johnston and others, 2002; McGovern and others, 1995; Quevedo and

others, 2005) focusing on the viable but nonculturable (VBNC) state induced in *V. vulnificus* when subjected to the unfavourable conditions of low temperatures. The VBNC state is a dormancy state in which the bacteria fail to form colonies on routine bacteriological media growth, but will remain alive and capable of renewing metabolic activity (Oliver, 2005). It was believed that the general inability to isolate estuarine vibrios during the winter months or from colder waters was due to the sensitivity of these bacteria to cold temperatures. However, studies have presented evidence that the bacteria, although unable to be cultured, have entered into VBNC state in response to the reduced temperatures (Johnston and others, 2002).

Results from these investigations (Bryan and others, 1999; Johnston and others, 2002; Oliver, 2005; Panoff and others, 1998; Thieringer and others, 1998) suggest that *V. vulnificus* can have improved tolerance to colder temperatures by keeping the cultures at the intermediate temperature of 15°C before final storage at 6°C. This adaptation was possible by the development of cold-adaptive protein(s), which differ from those found in *E. coli.* In addition, the protein that was not present before the temperature downshift and appeared increased by a factor of 35 one hour after the temperature change. These largely differ in apparent molecular mass and isoelectric point from cold shock proteins found in other bacteria (McGovern and others, 1995).

Finally, the authors investigated the effect of the intermediate 15°C temperature before freezing, and showed that the "pre-cooled" bacteria had an overall 2.2 log higher viability than the one frozen directly from room temperature. This result suggests that submitting the bacteria to refrigeration temperatures may be counter-productive for freezing as a postharvest process to reduce *V. vulnificus* in oysters.

Vibrio parahaemolyticus also shows tolerance to colder temperatures as *Vibrio vulnificus* with previous exposure to low temperatures. The effect of cold shock on the survival of *V. parahaemolyticus* subjected to subsequent lower temperatures (5 and -18ºC) showed that regardless of the cold shock treatment, survival of *V. parahaemolyticus* increased when stored at these low temperatures (Johnston and others, 2002; Lin and others, 2004). In fact, studies on the morphological changes of *V. parahaemolyticus* under cold and starvation stresses attribute this tolerance to the rapid and drastic changes on the shape and structure of the cell (Chen and others, 2009). Under concomitant cold and carbon starvation, *V. parahaemolyticus'* cells entered the viable but nonculturable state and their shape changed from rod-like to coccoid. Electron microscopy revealed characteristic features in the cells such as a densely stained peripheral part and lightly stained central part of cytoplasm along with a thick peptidoglycan cell wall.

2.4.3 Previous freezing studies with *Vibrio vulnificus* and *Vibrio parahaemolyticus*

Several studies have been conducted to evaluate the effect of freezing on *Vibrio vulnificus* and *Vibrio parahaemolyticus* in oysters as well as in suspension media. Freezing studies using *Vibrio parahaemolyticus* has been less extensive when compared to those of *Vibrio vulnificus* due to the ability of this pathogen to cause primary sepsis in certain high-risk populations since it is fatal in 50% of cases (Iwamoto and others, 2010). In general, freezing studies report a reduction of these bacteria in oysters and oyster meat which vary depending on the freezing method employed.

Parker and others (Parker and others, 1994) reported significant reductions of 3 to 4 logs in *Vibrio vulnificus* in oysters injected with 10^6 CFU/g and then frozen at -20ºC in an air blast freezer. Greater reduction was observed in sets of oyster samples that were vacuum packed. Most reductions occurred within the first 7 days and continued to decline during storage.

However, after 30 days, 2 logs of the bacteria were still detected and, after 70 days, some samples still contained 1 log.

The effects of cryogenic freezing on *Vibrio vulnificus* was studied by Mestey and Rodrick (Mestey and others, 2003). Whole and half-shell oysters were frozen using CO_2 (-67ºC) and liquid nitrogen (-91ºC) followed by frozen storage. The study demonstrated that there was a lower number of recoverable *V. vulnificus* when CO_2 was used for freezing of half shell oysters. For whole oysters, bacterial levels were undetectable after 14 days for carbon dioxide and after 21 days, in most cases, for liquid nitrogen. Recoverable numbers for half-shell oysters were lower at all storage intervals, were undetectable after 14 days for both methods, and a few times only after 7 days. Muntada-Garriga and others (Muntadagarriga and others, 1995) reported that viable cells of *V. parahaemolyticus* (10^{5-7}CFU/g) in oyster homogenates were completely inactivated by freezing at -18 and -24°C for 15 to 28 weeks depending on initial populations of the microorganism and freezing temperatures.

Su and others (Liu and others, 2009) investigated the effects of cryogenic freezing using liquid nitrogen (-95.5ºC) followed by frozen storage, on reducing *V. parahaemolyticus* in half shell Pacific oysters. Oysters were frozen in a cryogenic tunnel with a retention time of 12 minutes. The population of bacteria in the oyster declined slightly by 0.22 log MPN/g after the freezing process. Frozen storage studies at -10, -23 and -30°C found that the population of the bacterium decreased faster in oysters stored at -10 than at -23 or -30°C. Holding half-shell Pacific oysters at -10°C for three months or at -23°C for four months was capable of achieving a greater than 3-log (MPN/g) reduction of *V. parahaemolyticus* in the Pacific oyster from starting at 10^5 MPN/g inoculum.

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2.4.4   Assessment of air blast and cryogenic freezing of oyster meat

Oyster purists would claim that oysters are intended to be eaten raw and freshness is mandatory when the briny flavors of the sea are mostly desired. Under this premise, oysters would not be considered as a seafood item suitable for freezing. However, frozen oysters are commercially available as cryogenically individually quick frozen half-shell oysters and frozen sucked oyster meats. Oyster meats frozen either individually or in blocks yield a product which are used in cooked dishes.

The freezing of oyster meats using conventional freezers is a well-established process in the United States and Japan (Horst and others, 2011). The shucked meats are thoroughly washed with water to remove sand, grit and other shell debris, drained to remove excess water, and frozen in an air blast freezer before packing. However, the use of cryogenic freezing for oyster meat is a rare practice between oyster processors. The production cost of this process is considered to be high in terms of the end use of the final product where pursuing of oyster flavor is more important than appearance. However, the emerging consumer tendency of ready-to-eat (RTE) food items might change this perception and more attention should be paid to improve the overall quality of frozen oyster meat.

There is always a high consumer demand for oysters that are safe while retaining their original flavor, nutrient content, texture, and appearance. In addition, these oysters are expected to be additive-free as well as possessing a longer shelf life. Since the oyster industry is so important to Louisiana's economy, it is necessary to protect the consumers' confidence in the product. A combination of cryogenic freezing and appropriate packaging can accomplish the later statement as well as helping to penetrate into the ready-to-eat market. A scientific approach designed to evaluate the quality and safety concerns during the freezing (air blast and cryogenic)

of oyster meat is necessary to establish the foundations for the development of RTE oyster meat products. Specifically, these studies were customized to target *V. vulnificus* and *V. parahaemolyticus*; the pathogens which are a threat to oyster consumers.

2.5 Modified atmosphere packaging in food applications

Benefits of extending the shelf life of food by modifying the atmosphere of the storage unit have been extensively studied since first demonstrated by the work of Kidd and West (Kidd and others, 1933) in 1936. The prolonged shelf life extension was accomplished by the substitution of air with other gases for the storage of fresh produce in a controllable atmosphere. Headspace gas modification started to appear 30 years later at the packaging level, and it was branded as modified atmosphere packaging (MAP) (Del Nobile and others, 2012).

Process-wise, MAP is defined as placing a perishable product in a package, removal of atmospheric air by vacuum or flushing, and replacing it by a pre-determined gas or mixture of gases with a composition different than air, followed by sealing the package (Kropf, 2004). After closing the packaging there is no additional manipulation of the internal environment. The headspace composition may change during storage. This is the critical difference when compared to controlled atmosphere systems (CA), where continuous monitoring of the environment is necessary to maintain a stable gas atmosphere and other conditions (Del Nobile and others, 2012).

Extensive studies have been performed using MAP in a variety of commodities, i.e. produce, poultry and meat products. Most of these studies have the common factor of tailoring mixtures of gases, either to enhance or avoid the development of organoleptic characteristics. The MAP conditions protect products against deteriorative effects, which may include discoloration, off-flavor and off- odor development, nutrient loss, texture changes, and other

measurable factors. However, the predominant concern is related to bacterial growth, which is most often the limiting factor of shelf life. Thus, an important objective of MAP systems is to minimize the changes that those attributes experience throughout the storage of the product.

The utilization of MAP in seafood has also been studied as a tool to extend shelf life during cold or chilled storage. In recent years, customers' interest in fresh, mildly preserved, and conveniently packed seafood products has increased. As a result of this demand, application of MAP in seafood has been customized to account for the origin of the raw material, temperature and type of cold storage, gas mixtures, and packaging materials (Gunsen and others, 2010). However, despite the numerous studies on this topic, there are few published studies about the effects of MAP on shellfish, and no data are available, to the best of my knowledge, on oyster meat packed in modified atmospheres.

The present study was mainly initiated to evaluate the effects of MAP on the quality retention of oyster meat in a ready-to-cook form, frozen cryogenically with liquid nitrogen and stored at -20ºC for six months. The effect of MAP on the shelf life was assessed primarily by comparing the volatile flavor compounds of fresh oyster meat with the different MAP treatments.

2.5.1   Principal gases used in modified atmosphere packaging of foods

The three main gases used in MAP are nitrogen (N_2), oxygen (O_2), and carbon dioxide (CO_2). Additionally, carbon monoxide (CO) has become of special interest to the meat industry which has required more research. Each gas has a role and importance which are related to the gas' specific properties.

Nitrogen is an inert gas that is tasteless, colorless and odorless. Chemical characteristics of this gas include a lower density than air, low solubility in water and fat and it is nonflammable. Nitrogen is used to replace oxygen and prevent package collapse, to retard

oxidative rancidity and inhibit growth of aerobic microorganisms. However, no direct effect of N_2 on microbial growth has been observed and, as a result, it has no impact on anaerobic bacteria (Arvanitoyannis and others, 2012a).

Oxygen, is a colorless, odorless gas that has a relatively low solubility in water, supports combustion (is explosive) and is very reactive with a wide variety of biological compounds (Grebitus and others, 2013). Oxygen inhibits the growth of anaerobic microorganisms, but promotes the growth of aerobic microbes. Additionally, oxygen is responsible for several undesirable reactions in foods, including oxidation and rancidity of fats and oils, rapid ripening and senescence of fruits and vegetables, staling of bakery products, and color changes (Del Nobile and others, 2012).

In addition to O_2 and N_2, CO_2 is the other gas used in significant amounts in MAP systems. Carbon dioxide is a colorless gas with a slightly pungent odor and greater solubility in water than nitrogen and oxygen. CO_2 has been utilized as a preservative for fresh produce, meat and poultry for over 100 years and, consequently, its use in MAP systems has been studied extensively.

Carbon dioxide is considered as the centerpiece of MAP systems due to its ability to inhibit a wide range of microorganisms (Arvanitoyannis and others, 2012a). (Garcia-Gonzalez and others, 2009) determined that CO_2 has a greater inhibitory effect on Gram negative bacteria, which grow rapidly on seafood, than it does on Gram positive. The mechanism of action depends on the gas dissolution in the food that reduces the pH, thus inhibiting microbial growth.

Although the pathway through which CO_2 exerts its inhibitory effect on bacteria is not yet fully understood, it is known that the gas dissolves readily in water and will produce carbonic acid (H_2CO_3) in solution (Kropf, 2004). Carbonic acid will then cause a drop in meat pH and, in

turn, negatively affect microbial growth. Carbon dioxide can also penetrate into microbial cells, disrupting the cell membrane (Del Nobile and others, 2012) where the bicarbonate ion, a dissociation product, changes cell permeability, and affects metabolic processes.

In MAP studies utilizing CO_2, it has been shown that its effects are usually related to the concentration of CO_2 in the food and not to its concentration in the package headspace. Thus, the gas that affects the food and the microorganisms is the one that dissolves in the food and not the one in the headspace (Arvanitoyannis and others, 2012a; Caleb and others, 2013; Chae and others, 2011; Grebitus and others, 2013; Kim and others, 2012).

2.5.2   General considerations using MAP to extend shelf life of food products

Application of MAP to extend shelf life depends on the mixture of the gases employed. The optimum level of each gas depends on different aspects, such as product characteristics, respiring surface area, storage conditions, and package barrier properties (Rodriguez-Aguilera and others, 2011, 2009a, 2009b). Furthermore, different considerations need to be taken in account when working with respiring and nonrespiring food to optimize the selection of gas headspace.

Antimicrobial effects in nonrespiring food can be achieved by making carbon dioxide the predominant gas in the headspace composition (Arvanitoyannis and others, 2012a). Addition of nitrogen is commonly practiced to avoid the package collapsing effect due to the high solubility of carbon dioxide (Conte and others, 2013). Pure nitrogen is usually employed in food with low water activity, especially in those with relatively high lipids content like breakfast cereals. Although, these products are less susceptible to microbial spoilage, enzymatic and chemical reactions can occur and results in deterioration (Klensporf-Pawlik and others, 2009).

For respiring food, such as horticulture produce, more than one aspect should be kept in mind before selecting the proper gas combination. Storage under reduced oxygen and elevated carbon dioxide partial pressures are commonly used to reduce respiration rate, metabolic activity, and ethylene sensitivity, thus increasing shelf life (Wang and others, 2011). On the other hand, extremely low levels of oxygen and/or high levels of carbon dioxide have to be avoided because they induce anaerobic metabolism with the possibility of off-flavor generation, and/or the risk of anaerobic microorganism proliferation (Del Nobile and others, 2012). Therefore, the most relevant applications of the usage of MAP in respiring foods are those where the development of the desired headspace is created as a result of the product's respiration (passive MAP) (Costa and others, 2011; Kudachikar and others, 2011). Under passive MAP conditions, the respiration of the product and the gas permeability of the film are the two factors that influence the change in gas composition of the environment surrounding the product (Conte and others, 2013).

MAP techniques have expanded and are now used on a wide range of fresh or chilled foods, including raw and cooked meats and poultry, fish, fresh pasta, fruit and vegetables and more recently coffee, tea and bakery products. The major advantages and disadvantages on the adoption of these techniques at retail and processing levels are listed in Table 2.1.

2.5.3   Previous studies of MAP applied to seafood

Several methods have been used by the food processor to slow down or inhibit deteriorative changes in seafood, including chilled storage, freezing, heat processing, drying, and the use of chemical additives and preservatives. However, the increasing consumer concern regarding preservatives as well as the demand for fresh products has forced the food industry to seek alternative methods of food preservation. Seafood is a highly perishable food which has a relative short shelf life.

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Table 2.1 Advantages and disadvantages of MAP as they apply to fresh products (Conte and others, 2013)

Advantages of MAP for the consumer	Disadvantages of MAP
Increased shelf life allowing less frequent loading of retail display shelves	Capital cost of gas packaging machinery
Reduction in retail waste	Cost of gases and packaging materials
Improved presentation-clear view of product and all round visibility	Cost of analytical equipment to ensure that correct gas mixture are used
Hygienic stackable pack, sealed and free from product drip and odor	Cost of quality assurance systems to prevent distribution of leakers
Reduction in production and storage costs due to better utilization of labor, space and equipment	Increase of pack volume which will adversely affect transport costs and retail display space
Increased distribution area and reduced transport costs due less frequent deliveries	Benefits of MAP are lost once the pack is opened or leaks
Little or no need of chemical preservatives	
Centralized packaging and portion control	

Modified atmosphere packaging is a system that offers a way of extending the shelf life of seafood products, maintaining quality and inhibiting bacterial growth. Studies on the application of MAP in seafood can be grouped in two main areas. The first one is related to the extension of shelf life, while the second one targets the improvement of quality aspects in products that underwent processes such as salting, smoking, and curing. In general terms, MAP has been utilized as a supplement to ice or refrigeration to extend the storage shelf-life of fresh seafood products which has led to a greater variety of products. Fresh fish and other fresh seafood products are highly susceptible to spoilage from postmortem microbial growth, biochemical endproducts (e.g., enzymes) resulting from the microbial growth, or combinations of both (Arvanitoyannis and others, 2011).

The higher water and free amino acid content along with the lower content of connective tissue as compared to other flesh foods lead to the more rapid spoilage of fish (Masniyom, 2011). Moreover, shellfish flesh containing higher carbohydrate and lower nitrogen content can be used as a nutrient source for microbial growth. Immediately after death, several biochemical and enzymatic changes are triggered in seafood muscles, especially with improper handling. Therefore, spoilage in fish and shellfish depends on species and chemical components (Masniyom, 2011).

It has been widely reported that the use of high barrier films along with modified atmosphere packaging containing CO_2 effectively inhibits bacterial growth during refrigerated storage of packaged fresh fishery products. The first extensive research on seafood stored in CO_2 was reported in the early 1930s in the United Kingdom, the United States of America and Russia (Stansby and others, 1935). In a 100% CO_2 atmosphere, fish were kept fresh 2-3 times longer than the control fish in air at the same temperature. Elevated CO_2 levels between 40% and 100%, have been shown to inhibit normal Gram negative spoilage bacteria (ie *Pseudomonas, Listeria, Salmonella, Alteromonas, Shewanella, Moraxella* and *Acinetobacter*) in fish from cold and temperate waters (salmon, atlantic cod, swordfish, european sea bass, carp, rainbow trout) (Hansen and others, 2009; Hovda and others, 2007; Hudecova and others, 2010; Kykkidou and others, 2009; Lauzon and others, 2009; Noseda and others, 2012; Oguzhan and others, 2012; Pantazi and others, 2008; Poli and others, 2006; Provincial and others, 2013; Tryfinopoulou and others, 2002; Yilmaz and others, 2009) doubling or tripling shelf life at refrigerated temperatures.

The effects of mixtures of oxygen, carbon dioxide, and nitrogen have been also studied in ready-to-cook seafood products (hake fillets, yellow gurnard fillets, chub mackerel fillets,

shrimp, mussels, and entire eviscerated cuttlefish) (Arvanitoyannis and others, 2011; Kamadia and others, 2013; Qian and others, 2013; Speranza and others, 2009; Ulusoy and others, 2011) showing an increase in the sensorial shelf life ranging from about 95% to 250%. Results have shown that sensorial quality was the subindex that limited their shelf life. In fact, based primarily on microbiological results, samples under MAP remained acceptable up to the end of storage (that is, 14 days), regardless of seafood type. In these studies, lower concentrations of CO_2 were desired to avoid unpleasant effects on some sensorial parameters of the packed product such as excessive exudate, softening of texture, and discoloration.

The synergistic effect between MAP and storage temperatures has been addressed by several researchers to maximize the effect of MAP in shelf life of seafood products (Table 2.2). Based on sensory evaluation, super chilled storage alone (-2ºC to 0ºC) compared with traditional chilled storage (4ºC - 6ºC) increased the shelf life of Cod loins from 9 to 17 days. Chilled MAP increased the shelf life from 9 to 14 days, and when MAP and super chilled storage were combined, the shelf life was further extended to 21 days (Wang and others, 2008).

In addition, this study and several other ones (Bøknæs and others, 2000; Fernandez and others, 2010; Li and others, 2011; Penney and others, 1994; Torrieri and others, 2011) clearly demonstrates the need for strict temperature control during storage. Temperature abuse (above 8ºC) causes the shelf life to shorten due to microbial activity, especially from psychotropic microorganisms, but also opens the door for possible growth of food poisoning microbes such as *Clostridium bolulinum* (Garcia de Fernando and others, 1995). *C. botulinum* produces toxin in many different atmospheres, but is unable to grow at temperatures below 3.3ºC.

In previously processed seafood (boiled/cooked, salted, smoked, etc.), MAP also significantly increases the shelf life of the final product. Furthermore, in such cases, MAP is

reported to improve other quality aspects such as color stability, texture, and reduction of fatty acids oxidation. Concentrations of CO_2 between 50% and 70% coupled with nitrogen, increased the shelf life of seafood salad from 4 to 7 months at a storage temperature of 2 ± 2ºC (Gunsen and others, 2010).

Table 2.2 Shelf life extension of fish and fishery products under MAP (Masniyom, 2011)

Fish and fishery products	Storage temperature (ºC)	MAP condition $CO_2:O_2:N_2$	Shelf life (days)	References
Mediterranean Swordfish	4	40:30:30	12	(Pantazi and others, 2008)
Pearlspot	2	60:40:0	10	(Sankar and others, 2008)
Cod	-0.9	50:5:45	21	(Wang and others, 2008)
Sea Bass	4	60:10:30	13	(Kostaki and others, 2009)
Atlantic Salmon	2	90:0:10	22	(Fernandez and others, 2010)
Mediterranean Swordfish	4	50:5:45	13	(Kykkidou and others, 2009)
Atlantic Salmon	1.2	60:0:40	15	(Hansen and others, 2009)
Sea Bass	4	60:0:40	18	(Provincial and others, 2010)

Determination of thiobarbituric acid reactive substances (TBARS), which measure the level of fatty acids oxidation, and sensory evaluation, determined that MAP seafood salad was edible until the 7th month of storage as compared to air-packed. MAP with combined 80% CO_2:10% O_2:10% N_2 gases prevented a pH rise, purge loss, and texture toughening while

minimizing oxidative changes in precooked, shell-less red claw crayfish tails stored at refrigerator temperature.(Chen and others, 2008). Stability in color, texture and considerable extension of shelf life was also observed in MAP smoked blue cod (Penney and others, 1994). Product in carbon dioxide filled packs remained acceptable when stored at 3ºC and -1.5ºC after 49 and 113 days respectively (3.5 and 4.0 times larger than in control samples packed aerobically). Extension of shelf life in CO_2 samples was attributed to a significant extension of the lag phase before spoilage micro flora proliferation commenced and to the selection of low-spoilage-potential lactic-acid-bacteria-dominated flora.

Although elevated concentrations of carbon dioxide extend shelf life of diverse seafood products by its effects on spoilage bacteria, negative effects on quality and sensory attributes have been reported in pearl spot (*Etroplus suratensis* Bloch) (Lalitha and others, 2005). CO_2 flush packed products had less desirable odor, texture and flavor with an increase in exudates which lead to a less acceptability. This may be due to a greater loss of the water holding capacity (WHC) of the muscle protein at lower pH values (Masniyom, 2011). The combination of other treatments helped to overcome the exudate losses in fish kept under MAP such as addition of sodium chloride (Fuentes and others, 2012), which increased the WHC, in salmon when compared to air packed samples. Also, to improve the quality of desalted cod, phosphate brining was used as a pretreatment before MAP (Rotabakk and others, 2009) which significantly increased the weight of the portions as compared to the control group. Polyphosphates and polyanion might interact with the positive charges of the protein molecules to increase the net negative charge, resulting in the increased water uptake ability. As a result, the repulsive forces between protein molecules may increase, leading to the increased water retention (Masniyom, 2011).

There is also a general concern related to the safety of MAP in fish products in relation to the potential for growth and toxin production by *C. botulinum* (Sankar and others, 2008). Contamination of seafood with *C. botulinum* may arise from marine and fresh water environments, and thus the facultative anaerobic conditions often encountered in MAP low acid foods (pH > 4.6) can be favorable to growth and toxin production by the bacteria if the suitable storage temperatures prevail. *C. botulinum* type E, nonproteolytic and psychrotrophic strain, can grow and produce toxin at low temperatures (Sankar and others, 2008). Trout and salmon samples kept in MAP at 10, 15, and 20ºC and inoculated with spores of *C. botulinum* spoiled before they became toxic (Cann, 1984). However, toxin has been found in MAP or vacuum packed flounder fillets prior to the fish being spoiled (Arritt and others, 2007). As discussed before, the storage temperature plays a major role in the extension of shelf life, but it also sets the margin of safety between sensory spoilage and onset of *C. botulinum* growth as reported in prolonged storage of MAP salmon at abusive temperatures (above 8ºC) that may present a public health hazard because toxin formation preceded sensory spoilage (Peck and others, 2008). Temperature control of MAP seafood has become one of the major challenges for retail stores, to the extent of acquisition of sophisticated temperature sensor devices attached to packaging to monitor temperature abuse (Masniyom, 2011).

2.5.4　MAP and frozen seafood

Temperature abuse of seafood packed under modified atmosphere conditions and stored at super chilled or chilled temperatures is probably one of the main causes of complaints from customers. MAP fish products are described as being spoiled and exhibit a typical amine-like odor developed from microbial activity (Bøknæs and others, 2000). Most recent reviews on the shelf life extension of fish and fishery products by modified atmosphere packaging (Conte and

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others, 2013; Del Nobile and others, 2012; Masniyom, 2011; Sivertsvik and others, 2002) compile in great detail the effects of MAP on the microbial reduction, retention of fresh quality characteristics, synergistic effect with storage temperature, and safety issues, however, the effect of MAP in seafood during frozen storage for extended periods was not addressed.

The few available studies of MAP and frozen storage have been developed to explore consumer demands for conveniently packaged seafood products (Bak and others, 1999; Bøknæs and others, 2000; Bono and others, 2012). Overall better quality in relation to color stability, lipid oxidation and shrimp meat toughening was achieved in boiled shrimp packed in nitrogen atmosphere and stored at -17°C for 12 months (Bak and others, 1999) in containers resistant to the puncture of the shrimp horns. The effects of photooxidation in astaxanthin (red color in shrimp) and lipid oxidation were minimal in samples stored under MAP conditions. The distinct red color in shrimp remained in MAP samples even with prolonged exposure to fluorescent light. Packaging in atmospheric air resulted in pronounced lipid oxidation during frozen storage as compared to packaging in modified air, almost 4 times greater based on TBARS values. In both cases, the exclusion of oxygen in the package did not trigger chemical reactions for degradation of astaxanthin and fatty acid oxidation by photooxidation extending the shelf life of the product by 50%.

An interesting study authored by Bøknæs et al (Bøknæs and others, 2000) was performed to explore the effect of freshness and frozen storage on sensory characteristics, trimethylamine (TMA) development, water holding capacity (WHC), and microbial content of fresh cod fillets packed in trays under a mixture of 60% CO_2:40% N_2. The authors suggest that the degree of freshness coupled with MAP extends shelf life by improving sensory attributes and considerably reducing spoilage microorganisms in thawed MAP cod fillet production. This conclusion was

obtained after comparing fresh cod samples that were MAP packed and frozen by air blast freezing with samples stored aerobically at 0ºC for 7 days before MAP packing followed by freezing. Both sets of samples were stored at -20 and -30ºC for 6 weeks. Cod fillets were then thawed and transferred for chill storage at 2ºC for 17 days. In general, significantly lower values of TMA and high sensory scores were measured in samples stored at -20ºC without exposure to aerobic environment prior MAP, but no significant difference in WHC was found between any sets of samples. Inactivation of a specific spoilage microorganism (*P. phosphoreum*) occurred during frozen storage because it was not detected during chill storage of cod samples. Development of frozen storage odor and taste was most pronounced for thawed chilled MAP fillets with 7 days of aerobic chill storage prior to MAP and freezing which was related to the production of compounds associated with lipid oxidation.

The application of combined preservation techniques is considered as a future alternative in the stability of foods in general and seafood in particular (Kerry, 2012). The study and application of combined techniques has already shown promising results in shellfish. Techniques such as the application of bactericides, irradiation, and ozonation (Arvanitoyannis and others, 2012a; FDA, 2005; Kerry, 2012) continue to be explored due the tremendous interest expressed in this area. Alternative decontamination/disinfection have demonstrated a great potential to decrease pathogenic bacteria, yet the effects against viruses is questionable (Kerry, 2012). There is a need to acquire knowledge on the effect of MAP methods towards viruses due to a global concern of increasing infections and outbreaks attributed to viruses transmitted by food.

Although an increase in shelf life and improvement in quality are concluded from studies of MAP in frozen storage, this alternative is still not included, and not even mentioned, as a

viable option in any of the recent publications dedicated to advances in MAP (Arvanitoyannis and others, 2012b; Conte and others, 2013; Del Nobile and others, 2012; Kerry, 2012). Probably the main cause may reside in the considerable extra cost and amount of effort manufacturers would have to go through when dealing with MAP products. According to US regulations, all manufacturers producing products with reduced oxygen content or protective atmosphere have to set up critical control points regarding both the gas content and the seal integrity (FDA, 2005). Usually MAP is carried out at the level of retail packs, and food processors and manufacturers are free of this responsibility.

2.6   Microwave cooking/heating

The major uses of the microwave oven in the United States (U.S.) beginning in the 1990s was mainly for reheating or defrosting foods, cooking vegetables, preparing snacks, preparing meals for one person, or quickly preparing traditionally long-cooking foods (Happel, 1992). Microwave heating is not considered by most as a cooking method. However, the microwave oven has become a very important appliance in the modern kitchen. In fact, data from the 2009 US Department of Energy, Residential Energy Consumption survey, indicate that 95.9% of consumers own a microwave oven and 39% use it to prepare at least one meal every day (USEI, 2009).

2.6.1   Fundamentals of microwave heating

2.6.1.1 The microwave oven

The invention of the microwave oven has an interesting history. In 1946, Dr. Percy LeBaron Spencer, an American engineer working for the Raytheon company was exploring the use of a magnetron to produce microwaves in a radar system to locate Nazi airplanes during World War II (Bih, 2003). During the manufacturing of the magnetrons, at some stage, many

tubes simultaneously were allowed to radiate into space creating a "hot environment" which led to perform a series of experiments using different food items inside a metal box (Osepchuk, 2009). He found that the energy entering the metal box was unable to escape, thereby creating a higher density electromagnetic field, rising the temperature of the food very rapidly.

In 1947, Raytheon built the "Radarange®", the first commercially available microwave oven (Bih, 2003). It was 1.8 m (5ft 11in) tall, weighed 340 kilograms (750lb) with a cost of about US$5,000 ($51,408 in today's dollars) each. Between 1952 and 1955, Tappan introduced the first home model priced at $1295. The world first 115-volt countertop, domestic oven was introduced in 1967. The 100-volt microwave oven, was under $500 and smaller, safer and more reliable than previous models (Bih, 2003). However, the use of the microwave oven in the American household started to increase in the late 1970s by the introduction of models with electronic touch controls, automatic temperature settings, and the capability to defrost.

Since then, advances in microwave ovens include employing more sensors to regulate temperature and automatic features to decrease programing. The development of digital displays has allowed the delivery of more information to the consumer while microwaving (when to stir, uncover, add salt, etc.). Furthermore, microprocessors and packaging has reduced preparation steps on the part of the consumer. Bar code readers are one of these possible features where the oven is set up by scanning the product's packaging, which pre-program the heating instructions (Lodgher and others, 2012). State of the art microwave ovens are totally computerized and can cook, bake and roast almost any food by microwave-assisted convection hot-air capabilities; however, these types of ovens are niche products due to high prices and complex technology-dependent communication systems which deviate from the concept of practicability.

2.6.1.2 Microwaves

Microwaves are located between 300 MHz and 300 GHz on the electromagnetic spectrum (Fig. 5). Microwave heating is defined as the heating of a substance by electromagnetic energy operating in that frequency range (Dekruijf and others, 1994). The high frequency range, which also can be used for heating, is very large and it can be subdivided into kHz high frequency (10 kHz < f < 1 MHz) and MHz high frequency (1 MHz < f < 300 MHz). The latter range is used when referring to high frequency heating. Only restricted microwaves or high frequencies are allowed for heating in industrial, scientific, and medical applications which are the so-called ISM frequencies (Galema, 1997).

Of these, only 2450 MHz is commonly used for food processing in Europe, while 915 MHz dominates in the United States (but also 2450 MHz and 5800 MHz are permitted) and 896 MHz in the United Kingdom (Galema, 1997; Venkatesh and others, 2004). Although higher frequencies are not in active use, it has been suggested that by combining higher frequencies with lower frequencies (infrared) it would be possible to get surface browning when microwaving food (Decareau, 1992).
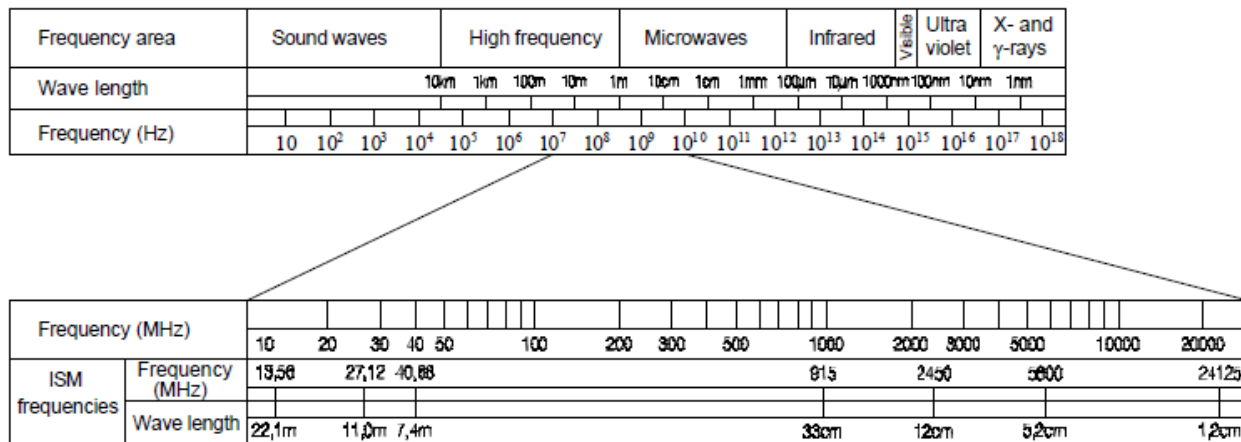
Figure 2.5: The electric spectrum and the ISM frequencies (Peräniitty, 1988)

2.6.1.3 Dielectric heating

The dielectric properties of a material describe the interaction with electromagnetic radiation (Bih, 2003) . Electromagnetic waves, such as microwaves, contain electric and magnetic field components. The electric field component applies a force on charged particles and as a result they migrate or rotate. Natural biological materials absorb only the electric part of the electromagnetic field. Food materials are practically non-magnetic, as they contain only trace amounts of magnetic material, such as iron and cobalt (Sosa-Morales and others, 2010).

Many molecules have a permanent dipole moment, and orientation (dipolar) polarization is due to the partial alignment of these dipoles. Water is a dipole and is usually a major component in biological materials. In a microwave or high frequency field, the dipoles try to follow the rapidly changing field. The concerted forces applied by the electric and magnetic components of microwaves change rapidly in direction (2.4×10^9 per second) causing warming because the assembly of molecules, *e.g.,* a liquid or a semi-solid, cannot respond instantaneously to the changing direction of the field. This creates friction which manifests itself as heat, which is known as dielectric heating (Galema, 1997).

Dielectric properties are the most important physical properties associated with microwave heating since the dielectric behavior of foods affect their heating characteristics. Knowledge of the dielectric properties of foods is essential in research, modeling and development of thermal treatments based on radio frequency (RF) and microwave (MW) energy. These properties provide information about the interaction between the foodstuff and electric fields. In food products, the dielectric properties are primarily determined by their chemical composition and, to a much lesser extent, by their physical structure. The influence of water and

salt (or ash) content depends to a large extent on the manner in which they are bound or restricted in their movement by the other food components.

Fortunately, many studies on the dielectric properties of agricultural and biological materials have been reported for different frequency ranges, temperatures, and moisture contents (Ryynanen and others, 1996; Shaheen and others, 2012; M. E. Sosa-Morales and others, 2010; Venkatesh and others, 2004). Literature data is mostly limited to food ingredients and their components. However, for complex multicomponent systems the dielectric properties must be measured or estimated, or from a practical approach, utilization of temperature sensors distributed uniformly in the food could guide the choosing of ingredients and their mixing based on temperature profiles (Decareau, 1992; Dekruijf and others, 1994).

2.6.2 Effects of microwave distribution in microwave ovens

Even heat distribution is very important in microwave cooking/heating. One useful property of microwaves is that they reflect on metal surfaces (Bih, 2003). After microwaves have been generated by the magnetron, they are directed towards a metal spinning propeller to be distributed into the oven chamber. The oven chamber is covered with metal as well and thus microwaves continue bouncing around in the chamber until randomly absorbed by the product (Bloomfield, 2013). To improve the distribution of microwaves around the food, the carousel, or turntable, is one of the earliest methods of increasing temperature uniformity inside a microwave oven. Furthermore, mathematical models have been developed and the exact role of the carousel in improving heating uniformity has been studied (Geedipalli and others, 2007).

Additionally, constructive and destructive interferences between microwaves also cause uneven heating in the food (Meier and others, 1998). Microwaves interfere with each other and the resulting electric field, at certain location in the microwave chamber, causing the sum of

individual electric fields to develop uneven thermal patterns. A constructive interference will impart more kinetic energy to polar molecules in the food due to positive interaction of electric fields resulting in points of higher temperature. In contrast, lower localized increase in temperature will occur when the resulting electric field is less (destructive interference) at the neighboring point (Bloomfield, 2013). These effects have been observed and have also been described as surface effects better known as 'hot' spots.

Several studies have established that other factors affect microwave heating, besides the oven (design and field distribution), and depend on uneven dielectric properties of foods, food geometry and thickness, packaging, and placement in the microwave oven (Fakhouri and others, 1993; Geedipalli and others, 2007; Manickavasagan and others, 2006; Ryynanen and others, 1996; Sosa-Morales and others, 2010; Vadivambal and others, 2010). Simulation of microwave heating by mathematical models and verified by thermal analysis have shown that hot spots or maximum temperature values occur in the center of spheres and at the surface, while center temperatures reached the highest values in cylinder-shaped food. In cubes and brick shaped products microwave energy concentrates in the corners, giving rise to hot spots (Campañone and others, 2005; Geedipalli and others, 2007). In addition, the uneven heat distribution in food, especially in multicomponent food, creates a common physical effect described as the pressure cooker effect (Galema, 1997). Localized 'hot spots' increase the temperature while the bulk temperature remains low creating pockets. Inside the pockets, the pressure increases by the phase change of water from liquid to gas due to heating. The pressure release is accompanied by a loud popping sound, displacing food particles many times outside of the packaging which is an undesired outcome.

2.6.3 Food safety in microwavable foods

The microwave oven has become a very important appliance in the modern kitchen. Microwave heating is not considered by most as a cooking method but a way to heat food products in a short period of time. Microwaveable foods are a group of ready-made, prepackaged, frozen, or prechilled products that can be consumed with minimal preparation.

The uneven heat distribution in microwave ovens is the main concern related to microbiological quality in food. Microbiological safety is often achieved by heating and thus if the food product is not adequately heated pathogenic microorganisms may survive. For precooked, ready-to-eat products, microwave cooking is a preferred method of food preparation because it can quickly warm the products prior to consumption; however, some products may contain raw meats, particularly raw pork, beef, and poultry meats that are potentially contaminated with foodborne pathogens (Huang and others, 2010).

Outbreaks of foodborne salmonellosis have been reported in Canada in connection to the consumption of undercooked microwavable products which contained raw or partially cooked poultry meat (MacDougall and others, 2004). In this case, consumer misconceptions of frozen product considered to be precooked contributed to the risk of infection, and clear labels identifying the product as raw poultry were needed. In the United States, four outbreaks of salmonellosis were reported from 1998 through 2006 associated with raw, frozen, microwavable, breaded, pre-browned, stuffed chicken products(Smith and others, 2008). As in the previous case, some affected individuals thought the product was precooked. In addition, most of them did not follow package cooking instructions, and none took the internal temperature after microwaving. Although modification of labels, verification of cooking instructions by the

manufacturer, and notification to alert the public were implemented after the first outbreaks in 1998, these measures did not prevent the following outbreaks.

The concerns regarding foodborne illnesses originated from consumption of products which have microwave heating as the last step before consumption. This has been the base for many studies aim at specific pathogens. Inactivation of *Salmonella sp* and *Listeria sp*. has been evaluated in a variety of products such as whole chickens, ready-to-eat products, popcorn, and fresh vegetables. In whole products, like chicken carcasses (1.8 kg), the inability of microwave ovens (seventeen units from various commercial suppliers) to uniformly heat the product was verified by temperature probes and the presence of viable *Listeria sp*. found in 5.74% of the chickens after cooking (Farber and others, 1998). Factors such as wattage, cavity size, and the presence or absence of a turntable were not significant in the survival of *Listeria sp*. However, in small sized chicken products, temperature distribution was improved and inactivation was successful (Jamshidi and others, 2009). Also, a 5-log reduction in Salmonella was achieved in popcorn after microwaving following the manufacturer's instructions (Anaya and others, 2008) as well as in jalapeno peppers and coriander foliage after 25 seconds and 10 seconds respectively reaching 63ºC in a 950 W microwave oven. In addition, inactivation of Salmonella was also achieved in sliced meat microwaved for 4 minutes followed by a standing time of 2 minutes (Levre and others, 1998). Samples were inoculated with 10^7 cells/g and no viable cells were recovered from both core and surface samples. On the other hand, some results showed that preset controls in microwave ovens are not safe enough when reheating contaminated foods. Survival of *Salmonella typhimurium* was evaluated in different types of baby food and it was found that 47.8% of food samples were positive for the contaminant using conventional settings while in a microwave with preset controls it was 93.3% (Tassinari and others, 1997).

Another recognized foodborne pathogen that has been the object of studies in microwavable food is *E. coli* O157:H7. Fecal contamination becomes a major route that contributes to foodborne infections associated with this pathogen and is commonly associated with raw or undercooked foods (Sargeant and others, 2003). In whole, and in 20-g portions of chicken breast both inoculated with $10^5 – 10^6$ cfu/g of this bacterium, elimination occurred at full power only after 35 seconds (73.7ºC) in the meat portions, however, viable cells were recovered from whole chickens after 22 minutes of cooking (variable temperature 60.2ºC -92ºC) (Apostolou and others, 2005). The correlation between time of exposure and microwave power intensities to eliminate *E. coli* O157:H7 was established in bovine minced meat inoculated with $10^7 – 10^9$ cfu/g (Quesada and others, 2003). The number of survival bacteria diminished as the time and temperature increased, however, a prolonged exposure was necessary for the complete inactivation, especially at lower power levels causing undesirable organoleptic characteristics.

Microwave heating in a smart microwave oven, which provides continuous and adjustable output, was evaluated in the survival of a cocktail of pathogens inoculated in catfish fillets as part of a microwavable meal (Sheen and others, 2012). *Listeria monocytogenes* (4-strain cocktail), *E. coli* O157:H7 (5-strain cocktail), and *Salmonella sp* (6-strain cocktail) were surface inoculated onto 110 g catfish fillets. After 2 minutes of 1250 W of microwave heating (80ºC -90ºC), 4 to 5 log CFU reductions of each of the pathogen were obtained. The results also showed that *E. coli* O157:H7 was more sensitive to microwave heating than *Listeria monocytogenes* and *Salmonella sp*.

Research on microwave heating shows that infection hazards linked to microwaved cooked food can be avoided by following adequate procedures concerning exposure time, temperature, and post heating hold time. However, general instructions cannot be applied to new

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products and the industry must responsibly perform testing and deep examination of food products to determine the extent to which microwave cooking is safe for these products.

2.6.4 Properties of microwave heated foods – sensory and nutritional aspects

Recent publications on microwave heating and sensory attributes are focused on the use of microwave-assisted steps in the processing of food rather than on household applications. However, sensory comparison between conventional and microwave heating or cooking is the principal focus of the majority of studies targeting household microwavable products. In addition, the majority of these studies have been on reheating or cooking vegetables, pasta dishes, and meats. While comparing these studies and their results, outcomes differ greatly even in similar types of products, which is dependant on the experimental design. Techniques used for sensory evaluation vary from simple difference tests with well-trained panels to large consumer tests. Nutritional quality in microwave heated and cooked food has also been studied. The same tests were performed as in sensory studies where researchers compared cooking methods and the retention or loss of specific components (nutrients).

Reviews on sensory and nutritional quality of microwave cooking indicated that early research reported inferior quality of microwave cooked products. Many later, better-controlled studies show that microwave cooking compares favorably to conventional cooking which is attributed to advances in microwave oven design and packaging (Ohlsson and others, 1982; Risch, 2009). Studies on vegetables showed that there were no differences in visual color, texture scores, and chroma in microwaved-blanched broccoli florets (Brewer and others, 1995) or carrots and green cabbage (Rennie and others, 2010) when compared to conventional blanching. Furthermore, it was found that microwaved blanched samples retained more nutrients (reduced ascorbic acid content) with similar results obtained in tomatoes (Begum and others,

2001), potatoes (phenolic constituents) (Barba and others, 2008), onions (antiplatelet agents) (Cavagnaro and others, 2012), and in beans, brinjal, knol-khol, and radish (different nutrients) (Kala and others, 2006). Retention of vitamins (retinol, beta-carotenes, thiamin, riboflavin, niacin, and ascorbic acid) was also higher after cooking chicken, lamb chops, fish, cassava, taro, and plusami in a microwave oven when compared to other cooking methods (Kumar and others, 2006).

The lack of browning during microwave cooking makes an obvious difference in meat when this parameter is part of sensory evaluation. However, when it is excluded, no differences are found in tenderness of beef between different cooking methods, if the final temperature is the same (Hoskins, 1986). This is also corroborated by similar studies in buffalo meat patties (Nisar and others, 2010) and chicken meat (Mendiratta and others, 1998). In fact, microwaved cooked samples scored high in juiciness irrelevant of their fat content. To obtain higher overall sensory scores in microwaved cooked meat products, improvement in color has been achieved by the use of additives and marinates (Perez-Juan and others, 2012). Furthermore, tenderness and juiciness were also enhanced by the use of the same.

Some authors have studied the changes on fatty acid composition and lipid oxidation to assess warmed-over flavor (WOF) in meat products. WOF, or reheated flavor, is caused by the oxidation of fatty acids during refrigerated storage (Cheng and others, 2007). The effect of microwave heating on the perception of WOF seems to vary depending on the nature of the meat and the way it was cooked. For example, no difference in WOF was found in roast beef slices reheated in microwave and conventional ovens (Johnston and others, 1980). In contrast, maximum WOF was produced in microwave cooking of pork compared to pan-frying, grilling, conventional cooking in water (Satyanarayan and others, 1992). However, addition of natural

and synthetic antioxidants have shown an ability to control WOF during refrigerated storage and minimal perception was observed during reheating regardless of the method utilized (Jayathilakan and others, 2007).

Most recent studies have been focused on the effect of cooking methods on the fat content and fatty acid composition of meat due to their importance for quality and nutritional values. Microwave cooking provided higher contents of fatty acids than boiling and grilling, which likely resulted from the higher moisture loss (Alfaia and others, 2010; Nisar and others, 2010). It was observed that samples increased the percentages of mono- and saturated- fatty acids related to raw meat. Interestingly, the results showed that heating decreased the polyunsaturated fatty acids to saturated fatty acids ratio but did not change its *n-6/n-3* index. In addition, the thermal procedures induced only slight oxidative changes in meat immediately after treatment but hardly affected the true retention values of its individual fatty acids. Similar results were observed in chicken and meat patties when cooked using a microwave oven (Echarte and others, 2003; Sharma and others, 2005).

Published studies of consumer acceptance of microwave cooked or reheated products are few and variations on the methods are large. Shrimp cooked at different microwave powers and times were evaluated for color, flavor, texture, juiciness, and overall acceptability using a nine-point hedonic scale (Gundavarapu and others, 1998). Nearly 80% of the consumers were willing to buy shrimp cooked at 240 W (24% power level) for 140 seconds and with a 120 seconds holding before serving based on tenderness and appearance. Consumer's willingness to purchase precooked microwavable-reheatable top round steak processed from different grades was higher in samples that were marinated and tenderized (McWatters and others, 1999). The marination/tenderization process increased the flavor ratings of steaks, regardless of grade.

On another note, researchers have also investigated the effect of cooking techniques, including microwave cooking, to assess the reduction and/or elimination of antibiotics, chemotherapeutics, and antimicrobial additives used in chicken farming (Javadi, 2011; Javadi and others, 2011a, 2011b; Lolo and others, 2006; Tarbin and others, 2005; Zhang and others, 2005). Most of these studies concluded that heat processes cannot annihilate the total amount of these chemicals in chicken meat samples, but they can only decrease their amounts. Also, the development of heterocyclic amine compounds (HCAs), which are potent mutagens and possible human carcinogens that are formed during heating of protein-rich foods, has been studied in fish, chicken, and beef products (Jung and others, 2009; Oz, 2010; Oz and others, 2010; Sugimura and others, 2004). While some of these studies favor and encourage the usage of microwave ovens for cooking due to low occurrence of HCAs (Jung and others, 2009; Sugimura and others, 2004), others reported higher contents of these compounds in microwaved samples of fish than in other meats suggesting to avoid microwave-cooking from a health perspective (Oz and others, 2010). A more careful review of these studies shows significant variability in sample preparation and handling protocols, and specifically in microwave cooking, when samples are cooked for the same time intervals in ovens differing in wattage, the resulting product is completely different which makes it difficult to draw a conclusive opinion.

2.6.5 Influence of packaging in microwavable foods

The three primary functions of packaging materials are to provide protection, utility, and communication in three different environments. The environments are physical, atmospheric, and human (Lockhart, 1997). Advances in packaging have evolved to provide for all three functions efficiently in all three environments. However, one of the critical aspects for the success of food packaging is to quickly adapt to the human's lifestyle. In the U.S., the

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consumer's experience of increased time constraints, nutritional awareness, and the desire for foods that taste and smell like they were cooked in a conventional oven have changed microwavable foods. The concept of microwave cooking has evolved from simply heating food in a shorter time to be an integral part of the way people live.

In microwave cooking, packaging composition and geometry affect heating. The incorporation of material components in the packaging (active packages), such as metal foil or strips, modify the microwave field and result in a more uniform heating or browning of a food product. Passive packages are transparent to microwaves and do not affect the heating pattern and thin materials do not either absorb or reflect microwaves (Guillard and others, 2010). There are a multitude of different microwave packaging formats which are adaptable to distribution channels, product type, pack size and point of consumption. However, round or oval shapes with vertical sides and round edges are the common characteristics shared in the majority of them, which minimizes corner and edge overheating during microwaving. These effects are one of the major problems in microwave heating due to the concentration of high intensity of electromagnetic fields in these areas (Knoerzer and others, 2009; Risman and others, 1987; Zhang and others, 2001). Even rectangular packages possess rounded corners and edges to decrease the influence of these effects (Ryynänen and others, 2001).

The most important package requirements for microwave heating are the following: legible and easy to follow instructions, thermal resistant, especially at temperatures between – 20ºC and 120ºC, be grease- and waterproof and protect the food during transport and storage (Robertson, 2012). In addition, packages should be user friendly (stable and easy to handle even after heating with minimal risk of injury), and the lid/film must be easy to remove (Datta and others, 2013). Furthermore, the package must be suitable with regard to health (material

migration) and sensory aspects (no off-taste imparted to the product) (Thambiraj and others, 2013). The material should be microwave transparent, except when it is used to shield the food or to modify the microwave field. The most commonly used microwave transparent materials are plastic packages (mono- or multi-layer polymers), plastic-coated cardboard or fiber trays, and glass packages (Risch, 2009).

2.6.6 Steam-venting technology in microwave cooking

Steam meals are ready-to-eat (RTE) meals composed of raw and semi-cooked ingredients, which get cooked during microwave heating and have become increasingly popular in the last two decades, particularly in metropolitan areas (Mejia and others, 2011). The development of steamed meals has given a new dimension to RTE meals. The products are packed in a sealed plastic container in a gas mixture and consumers cook the meals at home before consumption.

The proven challenge in RTE meals is food safety related to the inability of microwave ovens to heat evenly (Allan, 2009; Ramaswamy and others, 1992; Vadivambal and others, 2010). There is a particular concern when steamed meals are composed of raw (meat, seafood, and vegetables) and semi-cooked ingredients (rice, pasta or noodles). In commercially produced steam meals, microwave oven cooking prior to consumption is the only protective step against pathogenic microorganisms in raw meat, seafood, and vegetables, which may have a significant effect on food safety. It is known that inappropriate home cooking, particularly with the use of a microwave oven, has caused food borne illnesses (MacDougall and others, 2004; CDC, 2008; Smith and others, 2008).

Self-venting technology has rapidly developed via several different approaches to be applied in steamed meals for microwave cooking. The packaging is designed so that steam

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builds during the cooking process. This technology utilizes the concept of pressurized steam cooking inside sealed plastic pouches or containers that have a self-venting release adaptation (Mast, 2000). Steam-venting technology for microwave cooking has been engineered to regulate cooking quality through controlled package expansion in conjunction with proprietary self-venting mechanisms. Pouches, flowpacks and lidded trays are the most common presentations (Fowle and others, 2005).

During microwave cooking, food items absorb radio waves and are heated by dielectric heating. Rise in temperature, rapidly heats up the water content of the food, producing steam. Positive pressure is created in the package by the production of steam and microwaving simultaneously resulting in reduced cooking time and evenly distributed heat (no hot spots). The package distends with the increase of the internal pressure and provides a visual indication that cooking is occurring while maintaining a temperature between 100ºC to about 105ºC (Mast, 2000). The general description of microwave cooking using steam-venting technology is shown in figure 6.

Many of the self-venting and steaming systems are variations on the same theme. Confidence in the functional ability of the system rely on one (or a combination of) partial or complete de-lamination, rupturing, peeling or a strategically placed hole or series of holes (Fowle and others, 2005). In each case there is a build-up of pressure inside the pack to start the process. For instance, to simulate the 'pressure cooker' effect in a microwave oven, the package consists of a heat and pressure resistant deep polypropylene tray with a lidding film having a one-way steam release valve (Keller, 2003). During cooking, which takes between four and five minutes, the tray and lid expand until the pressure reaches 1.2 to 2.5 bar, while the one-way valve is activated at a predetermined internal pressure to vent off excess steam.

a)
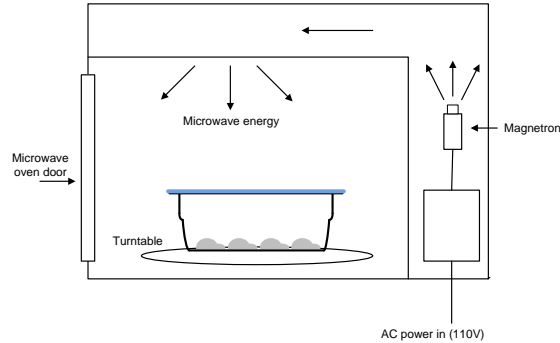
b)
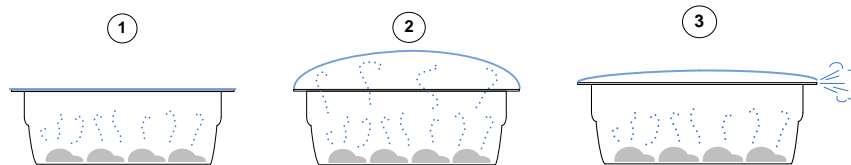
Figure 2.6: a) Overall view of a household microwave oven, b) Microwave cooking schematic showing the steam production (1) , built up (2), and release at constant pressure (3)

Self-venting is also achieved with co-extruded films like a 2-ply PET/PE laminates (Polymers, 2010). Steam-venting occurs as the laminating adhesive dissolves when exposed to steam, preventing the pack from bursting while maintaining a constant pressure during cooking. This mechanism can be incorporated into roll-stock, lidding or pre-made pouches. Both peel and non-peel versions are available with anti-fog and high-barrier properties suitable for chilled and frozen conditions. Another proprietary variant from the co-extruded laminate is the use of micro-perforation technology in the lidding film (Lin, 2009). The film is treated with a series of laser- perforated holes, which are covered by a peelable label that is removed prior to cooking. The perforation matrix is designed for optimal cooking for different products.

2.6.7 Product development of microwave ready oyster meat-based meals

There is not much literature dealing with the design of dinners for microwave heating but some general guidelines and workshops have been developed by packaging manufacturers

(Cryovac, 2013). In addition, investigations on placement, composition, and geometry of the food provide useful information for developing microwaveable food (Bows and others, 1990; Ryynanen and others, 1996). The results from these studies show that placement gives the most notable effect regarding heat distribution. The temperature distribution could be balanced partly by taking advantage of edge and corner heating intensification (Ryynanen and others, 1996). This could explain the distribution of food items in many of the available microwavable products in two or three compartments in multiple component meals.

From the food safety point of view, microwave cooking using steam venting technology could assure the inactivation of harmful organisms. In general, each thermal process must be considered in its own right and it is important to choose the organism most likely to carry the greatest risk. However, control and uniformity of temperature is crucial to achieve lethality. At a temperature of 70ºC for two minutes the outcome is roughly the same as 43 min at 60ºC and less than 10 seconds at 80ºC based on calculations for *Clostridium botulinum*, related to *F, Fo*, and *z*-values (Fowle and others, 2005). During microwave cooking using steam-venting technology, the temperature is maintained between 100ºC and 105ºC throughout the entire cooking period. This can be considered to be enough to produce a safe product based on section 3-401.12 of the 2009 edition of the FDA Food Code which requires that raw animal foods, including seafood, heated via microwave energy must attain an internal temperature of at least 73.8ºC (165ºF) (USFDA, 2009).

In seafood products, steaming is a process that usually provides desirable characteristics. Steaming is a gentle, fat-free cooking method that retains the natural moisture of foods. This feature makes it an excellent choice for preparing delicate meals, especially those having oysters as the main ingredient. It is believed that with the emerging category of value added meal

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solutions the development of oyster-based product will create new opportunities for oyster processors to expand their market.

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CHAPTER 3   EFFECTIVENESS OF CRYOGENIC AND AIR BLAST FREEZING ON REDUCING PATHOGENIC-OYSTER ASSOCIATED BACTERIA AND THEIR IMPACT ON QUALITY RELATED CHARACTERISTICS IN OYSTER MEAT

3.1   Introduction

The pathogens most commonly associated with the consumption of raw oysters are *Vibrio parahaemolyticus* (*Vp*) and *V. vulnificus* (*Vv*) (CDC, 2006, 2011, 2010a, 2010b, 2013). These *Vibrio spp*. are free living and naturally occurring in the Gulf waters during the warmer months. Filter feeding shellfish such as oysters can accumulate these bacteria and act as vectors for foodborne illnesses. *V. vulnificus* has been strongly associated with severe and life-threatening conditions in immunocompromised individuals, especially those with chronic liver disease, and is the second leading cause of seafood-related fatalities in the U.S. (Haq and others, 2005). However, infections due to *V. parahaemolyticus* have increased worldwide in recent years. In the U.S., improperly prepared or mishandled shellfish is the primary cause of *V. parahaemolyticus* infections in territories with no coastal areas, exceeding *V. vulnificus* cases (58% vs. 7%) (COVIS, 2011). Infections caused by *V. parahaemolyticus* result in diarrhea, abdominal cramps, nausea, and vomiting.

Vibrio parahaemolyticus* and *V. vulnificus* appeared to be sensitive to cold temperatures. These *Vibrio spp*. seem to enter viable but nonculturable (VBNC) state in response to low temperatures (Johnston and others, 2002), and thus freezing cannot be relied upon for immediate destruction of these bacteria. Furthermore, tolerance to freezing temperatures has been reported with previous exposure of these bacteria to intermediate temperatures (8-15ºC) before refrigeration and freezing (Bryan and others, 1999; Chen and others, 2009; Lin and others, 2004; McGovern and others, 1995). Parker and others (1994) reported significant reductions of 3 to 4 logs in *V. vulnificus* in oysters injected with 10^6 CFU/g and then frozen at -20ºC in an air blast

freezer. Most reductions occurred within the first 7 days and continued to decline during storage. However, after 30 days, 2 logs of the bacteria were still detected and, after 70 days, some samples still contained 1 log. Muntadagarriga and others (1995) reported that viable cells of *V. parahaemolyticus* (10^{5-7}CFU/g) in oyster homogenates were completely inactivated by freezing at -18 and -24°C for 15 to 28 weeks depending on initial populations of the microorganism and freezing temperatures.

Regulation for commercializing oysters requires that during transport, shell stock oysters must be cooled to an internal body temperature of 10ºC or less when transportation time exceeds 2 h. The regulation also requires that during processing and shipment, shucked oysters must be maintained at 7.2ºC or less (FDA, 2009). However, documented cases have shown that temperature abuse is often the main cause in the development of foodborne illnesses in oysters (USGAO, 2011). Commercialization of adequately packaged and frozen oyster meat could provide an alternative to reduce the incidence of foodborne illnesses related to consumption of raw oysters.

Freezing is a much preferred technique to preserve food for long periods of time. It permits the preservation of flavors and nutritional properties of foods more effectively than storage above the initial freezing temperature. It also provides the advantage of minimizing microbial or enzymatic activity. Extended shelf life is a major selling point of freezing which is convenient and practiced for oysters cryogenically frozen on the half shell. The freezing of oyster meat using conventional freezers is also a well-established process in the United States and Japan (Horst and others, 2011), however, the end use of the product is for preparation of stews and other dishes.

Safe oysters with maximum retention of original flavor, nutrients, texture, and appearance are difficult to obtain and thus they have high consumer demand. In addition, these oysters are expected to be additive-free as well as possessing a longer shelf life. Since the oyster industry is vital to Louisiana's economy, it is necessary to protect the consumers' confidence in the product. This study was therefore conducted to determine if differences exist in the inactivation of *V. vulnificus* and *V. parahaemolyticus* when inoculated oyster meat is frozen by conventional (air blast) and cryogenic freezing, followed by storage in a microwavable package with a continuous evaluation of quality. Evaluation of the quality and safety concerns during the freezing (air blast and cryogenic) of oyster meat could provide information to establish the foundations for the development of possible ready-to-eat oyster meat products.

3.2 Materials and methods

3.2.1 Wild caught oysters

Gulf oysters (*Crassostrea virginica*) were purchased from a supplier in Houma, Louisiana. The oysters were harvested off the coast, iced on board the vessel and then transported to Louisiana State University Agricultural Center Food Processing Pilot Plant. Oysters were scrubbed under running water to remove mud and debris adhering to the shell, allowed to drain and refrigerated at 4ºC for 2 h to obtain temperature uniformity. Shucking was conducted aseptically with an ethanol-sterilized shucking knife and the meat was collected in one large sterile bag. Then the oyster meat was divided into two groups. One of these groups was designated for inoculation with the *vibrio* species while the other one was analyzed for quality characterization. All experimental samples and controls were processed within 36 h of harvesting.

3.2.2 *Vibrio* strains and inoculation

Three clinical strains of *Vibrio vulnificus* (ATCC® 27562, 7184, and 1007) and one strain of *Vibrio parahaemolyticus* (ATCC® 17802) were used to assess the effect of air blast freezing and cryogenic freezing on these bacteria. Cultures were obtained as -70ºC frozen stocks from the Louisiana State University Department of Food Science culture collection. *Vibrio vulnificus* and *Vibrio parahaemolyticus* strains were grown separately and maintained at 37ºC for 12 h in sodium chloride solutions of Bacto nutrient broth (Difco Laboratories, Detroit, MI) at 2% and 3% (wt. /wt.) respectively. After 18 h, cultures produced approximately 10^6 CFU/mL for *Vibrio vulnificus* and 10^7 CFU/mL for *Vibrio parahaemolyticus*. Approximately 1800 g of oyster meat per treatment was weighed in sterile bags and then inoculated with both cultures to obtain 10^6 CFU of each vibrio species per g of meat. After inoculation the meat was left in the bag for 15 min at room temperature under a bacteriological hood. Inoculation effectiveness was verified by triplicate analysis of samples consisting of 12 oysters per sample.

3.2.3 Oyster meat freezing

Air blast freezing (AB) and cryogenic freezing (CF) with liquid nitrogen were used as freezing methods in this study. Before freezing, the oyster meat was placed on aluminum trays containing approximately 25 g each. Air blast freezing was carried out in an air blast freezer (Master-Bilt Products, New Albany, Mississippi) at -20ºC with an average air velocity of 4.9 ± 1.9 m/s. The air velocity was measured in 25 different points (Taylor 3132 Jewelled anemometer) inside the freezer and then averaged. The oyster meat was frozen until the temperature at the center of the oyster meat reached -20ºC. The temperature of the circulating air and of the center of samples was monitored and measured using U12 stainless temperature loggers (Homo®, Onset Computer Corporation, Bourne, MA).

To cryogenically freeze the oyster meat, a cabinet-type cryogenic freezer (AirLliquide, Houston, Texas) employing liquid nitrogen with an operation temperature of -123.3ºC was used. Freezing was carried out until the geometrical center reached -20ºC. The temperature was monitored at one second intervals and recorded using a temperature data logger and type K thermocouples (Comark®, Comark Limited, Stevenage, Herts, UK). Cryogenically and air blast frozen oyster meat samples were packed in polypropylene trays containing 6 aluminum pans per tray, film sealed and stored at -20°C.

3.2.4 Thermodynamic considerations -Energy removal rate and freezing rate

The freezing rate (ºC/min) of the oyster meat was estimated as [(Final freezing temperature, Tf (ºC) - Initial freezing temperature, T_o (ºC))/Total freezing time (min)]. The amount of heat that has to be removed from the oyster meat in a particular time to decrease its initial internal temperature (T_o) to a predetermined final temperature (T_f) is the energy removal rate (Q).

Energy removal rate was calculated using heat capacity relations from thermodynamic laws with the following assumptions: (1) water was the only substance that would freeze, (2) the measured internal temperature was equivalent to the overall temperature of the oyster meat, and that (3) water crystallization into ice takes place at the internal freezing temperature T_{if}. The rate of heat transfer from the surface of the product to the cooling medium was calculated using equation (1):

$$Q = \frac{\Delta h}{t} \qquad\qquad [1]$$

where Q = the rate of heat transfer expressed in kJ/s, t = time taken for freezing in s and Δh is the product heat load (kJ) calculated as $\Delta h = w[C_{pu}(T_o - T_f) + L + C_{pf}(T_{if} - T_f)]$. In this equation w corresponds to the weight of frozen oyster meat (kg), T_o is the initial temperature of

the oyster meat, T_{if} is the initial freezing point temperature, and T_f is the final freezing temperature. T_o, T_{if}, and T_f, in both freezing processes were determined from the freezing curves of oyster meat constructed from the temperature recordings.

Latent heat (L) was calculated as $L = x_i L'$, where L' is the latent heat of fusion of water (333.6 kJ/ kg) and x_i is the weight fraction of ice given by $x_{i=} (x_{wu} - Bx_S)\left(\frac{T_{if}-T_f}{T_o-T_f}\right)$, where x_{wu} is the weight fraction of water, x_s is the weight fraction of solute, B is bound water/kg solute, which is expressed as $B = b - 0.5\frac{M_w}{M_s}$, where M_w and M_s are the molecular weight of water and molecular weight of solutes and b is the constant ($b = 0.32$ was reported for fish by Schwartzberg (1976), Pham (1987), Murakami and others (1989), and de Reinick (1996). The molecular weight of solutes was calculated as $M_S = 18.02 \left(\frac{X_W(1-x_{wu})}{x_{wu}(1-X_w)}\right)$, and X_w was the mole fraction of water of the oyster meat. X_w was calculated as, $ln(X_w) = -18.02 \left(\frac{L'(T_o-T_{if})}{RT_o^2}\right)$ where R was the ideal gas constant = 8.314 J/ mol K and temperature was expressed in K.

The specific heat (kJ/kg K) of the fresh and frozen oyster meats were calculated using Siebel equations (Singh, 1993).

$$C_{pu} = 0.837 + 3.349 X_w \qquad\qquad [2]$$

$$C_{pf} = 0.832 + 1.256\ X_w \qquad\qquad [3]$$

where C_{pu} = specific heat of fresh oyster meat (kJ/kg K); C_{pf} = specific heat of frozen oyster meat (kJ/kg K); and X_w = is the moisture fraction of fresh oyster meat.

3.2.5 Microbiological analysis

Microbiology analysis was performed on the oyster meat samples right after freezing (0 time), and after 1, 2, 3, 7, 30, and 60 days of frozen storage. The samples were allowed to thaw for 12 h inside a refrigerator maintained at 4°C before analysis. Identification and quantification

of *Vibrio vulnificus* and *Vibrio parahaemolyticus* species was performed by direct plating procedure for the enumeration of total and pathogenic *Vibrio vulnificus and Vibrio parahaemolyticus* in oyster meats as described by Cook D. and others (2000).

Each 450-g oyster meat sample (two trays) was divided into three sub-samples and analyzed independently. Each sub-sample was weighed in a Whirl-pak® bag (Nasco, Salida, CA, U.S.A.) having 150 g of oyster meat (an average of 12 oysters). The oyster meat was macerated (Stomacher 400, Tekman Co., Cincinnati, Ohio) for one minute in 450 mL of phosphate-buffered saline solution (PBS) to yield an oyster homogenate (dilution 1:3). One hundred microliters of serial tenfold dilutions (10^{-1} to 10^{-4}) was then pipetted into Petri dishes containing 12-15 mL of *Vibrio vulnificus agar* (VVA) prepared according to online U.S. Food & Drug Administration Bacteriological Analytical Manual (BAM, 2001). The Petri dishes were incubated inverted at 35ºC for 16 h. To confirm identity as *V. vulnificus* or *V. parahaemolyticus* colonies were blotted directly onto filter paper (Whatman #541). Following growth, two copies of each Petri dish were made by blotting with filter paper, one copy for the identification and quantification of *V. vulnificus* and the other for *V. parahaemolyticus*. Colony blots were lysed and the deoxyribonucleic acid (DNA) of each colony fixed *in situ*, then hybridized with gene probes specific for the assayed species. Hybridization was performed using 5 pmol of alkaline phosphatase conjugated 5' amine-C6 (designated "X") DNA probes targeting either the *V. vulnificus* cytolysin gene (*vvh*; 5'-XGA GCT GCT ACG GCA GTT GGA ACC-3') or the *V. parahaemolyticus* thermolabile hemolysin gene (*tlh*; 5'-XAA AGC GGA TTA TGC AGA AGC ACT G-3') (Integrated DNA Technologies, Coralville, Iowa). After washing and color development, enumeration was performed by visual counting of positive colonies (purple color).

3.2.6 Inactivation kinetics

Isothermal analysis of survival data was used to calculate D (decimal reduction time), and k (inactivation rate) values. D and k were determined by the linear regression from the expression of Singh (Singh, 1993)

$$\ln\left(\frac{N_t}{N_o}\right) = -kt \qquad or \qquad \log_{10}\left(\frac{N_t}{N_o}\right) = -\frac{t}{D} \qquad [4]$$

where N_o was the initial number of microorganisms and N_t was the number of microorganisms at time t. The calculated D-values from isothermal datasets were used to predict survival of microorganisms and compare with experimental results by following equation (Eq. 5).

$$\log N_t = \log N_o - \frac{t}{D_T} \qquad [5]$$

3.2.7 Freezing loss and frozen storage loss determination

The freezing weight loss of the frozen oyster meat during cryogenic (CF) and air blast (AB) freezing was determined from the weights of the oyster meat before and after freezing. After freezing, the samples were immediately weighed in a digital laboratory scale (Ohaus, model H-3830, NJ, USA.). The freezing weight loss was expressed as percentage as shown in the following formula (Eq.6):

$$\% Freezing\ loss = \frac{(weight\ of\ fresh\ oyster\ meat - weight\ of\ frozen\ meat)}{weight\ of\ fresh\ oyster\ meat} \times 100 \qquad [6]$$

Frozen storage weight loss was determined from the weights of the oyster meat after freezing and at the end of 1, 15, 30, 90, and 180 days of frozen storage. The samples were removed from storage and immediately weighed. The frozen storage weight loss was expressed as percentage as shown in the formula (Eq.7):

$$\%\ Frozen\ storage\ loss = \frac{(frozen\ weight - weight\ \text{after freezing})}{weight\ after\ freezing} \ x\ 100 \qquad [7]$$

3.2.8　Determination of microstructure of frozen oyster meat

Oyster meat samples were examined through light microscopy (LM) and scanning electron microscopy (SEM) to observe the effect of cryogenic and air blast freezing on the oyster meat, both on the surface as well as internally. These samples were compared to samples of meat from fresh oysters (control). Immediately after freezing, samples of oyster meat were collected from each freezing method and allowed to thaw for 12 h inside a refrigerator maintained at 4ºC.

Samples for LM were embedded in Tissue-Tek optimal cutting temperature compound (Sakura Finetek Inc.), and snap-frozen in liquid nitrogen. Sectioning was performed on a microtome-cryostat (Leica CM 1850 Frigocut). Samples were cut transverselly to the adductor muscle fibers to observe the spaces left by ice crystals in the tissue. After cryosectioning, cross-sections (16-µm thick) were observed under an optical microscope (Leica DMRXA, Leica Microsystems Inc., USA) using differential interference contrast (DIC).

The images were captured by a digital camera (SensiCam QE, Cooke Corporation, MI,USA) and treated using image analysis software (Slidebook 5, Intelligent Imaging Innovations, Inc., Denver, CO, USA) to standardize pixilation while calibration was performed using a micro rule. Cross-section area was used to compare and evaluate the effect of the freezing technique on the tissue and ice crystal formation. The cross-sectioned area refers to the surface area of the cross-section of an object (ice crystal or muscle fiber). For the purpose of this study, the surface of more than 50 objects of ice crystals voids and 50 objects of muscle fibers were evaluated by image processing and analysis software (ImageJ 1.44p, National Institutes of Health, USA).

For the SEM analysis, whole oyster meats were cut transversely in 2.5% glutaraldehyde in 0.1M cacodylate buffered fixative, pH 7.2, and then held in same fixative at room temperature for 2 h. All specimens were first washed with 0.1M cacodylate buffer 3X for 20 min each, and then washed with deionized water for 5 min twice. The samples were then dehydrated with a serial concentration of 50–100% ethanol, for 20 min each. Materials were critical point dried with liquid CO_2 in a Denton CPD, mounted on aluminum SEM stubs, coated with gold:palladium 60:40 in an EMS550X sputter coater, and imaged with JSM-6610 high vacuum mode SEM (JEOL Ltd., Tokyo, Japan).

3.2.9   Quality characterization of oyster meat

3.2.9.1 Proximate analysis of oyster meat

Fresh shucked oyster meat was analyzed for moisture, protein, lipid, and ash. The oyster meat was analyzed in triplicate for moisture and ash contents using the AOAC standard methods 930.15 and 942.05, respectively (AOAC International, 2005). Three batches of oyster meat obtained from 25 oysters were collected and homogenized separately in a Waring® commercial laboratory blender for 60 s at high speed. To calculate moisture content, approximately 5 g samples were dried at 105°C for 24 h in a draft oven. The weight loss was used to calculate the moisture content of the sample. A 3 g sample size was incinerated at 550°C for 24 h and the ash content was expressed as a percentage.

The lipid content was determined in triplicate using dichloromethyl ether with an FAS-9001 fat extractor analyzer (CEM Corporation, NC, USA). To measure protein, the nitrogen content was first determined in triplicate by the Dumas combustion method using a Leco TruSpec® Nitrogen Analyzer (LECO Corporation, St. Joseph, MI). The protein content was then calculated as percent nitrogen times 6.25.

3.2.9.2 Color of oyster meat

Evaluation of color was performed on the ventral body (belly section) of individual oyster meat (3 measurements per oyster meat, 3 oyster meat per sample) using a Hunter LabScan Colorimeter (Labscan XE, Hunter Associates laboratory, Inc., Reston, Virginia, USA). Standard background and calibration tiles were used: white tile (CIE illuminant D65 10 ° observer, 2002) and black tile (no company specification). An optical aperture of 1.7 cm was used. $L*$, $a*$, and $b*$ values were recorded (with $L*$ representing the lightness on a scale of 0 (dark) to 100 (white), $+ a*$ for redness, $- a*$ for greenness, $+ b*$ for yellowness and $- b*$ for blueness).

`Samples were analyzed after 1, 15, 30, 90, and 180 of frozen storage. Total color difference ($\Delta E*$) was also calculated to quantify the overall color difference of the different oyster meat samples compared to fresh oyster meat. Mean $L*$, $a*$, and $b*$ values were used to determine the $\Delta E*$ between CF, BF and fresh oyster meat, using equation (8)

$$\Delta E* = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}$$ [8]

3.2.9.3 Characterization of frozen oyster meat for moisture and lipid oxidation

Oyster meat samples were analyzed for moisture and lipid oxidation before freezing and after 1, 15, 30, 90, and 180 days of frozen storage. Oyster meat (600 g) of from each treatment was allowed to thaw for 24 h in a refrigerator maintained at 4°C and then homogenized for 60 s at high speed in a Waring® commercial laboratory blender. The homogenized samples were used for the analysis of moisture and thiobarbituric acid reactive substances (TBARS). Triplicate samples were analyzed for moisture content by drying in a draft oven at 105°C for 24 h (AOAC International, 2005).

TBARS analysis was assayed following the method of Lemon (Lemon, 1975) and expressing the results in mg of malondialdehyde (MDA) per kg of sample. All chemicals were

purchased from Sigma Chemical Co, St. Louis, MO, USA. Reagents were prepared following the protocol and the analysis was performed using 30 g of blended oyster meat. Extraction solution (60 mL) was mixed with blended oyster meat for 30 s in a Waring® commercial laboratory blender at low speed. The resulting homogenate was filtered through a Whatman No.1 filter paper. The clear filtrate was then mixed with the TBA solution, placed in boiling water for 60 min, cooled and the absorbance was measured at 530 nm using a U-3000 spectrophotometer (Hitachi, Tokyo, Japan).

3.2.10 Statistical Analysis

Means values from six measurements and/ or triplicate analysis were reported. Statistical analysis was done using the SAS (Statistical Analysis System) software (version 9.2) (SAS Institute Inc., Cary, NC, and U.S.A). Data was analyzed by Analysis of variance (ANOVA) following Tukey's studentized range test ($p < 0.05$).

3.3 Results and discussion

3.3.1 Energy Removal Rate, Freezing Rate and Freezing Time

Collected temperature data and related thermodynamic calculations that describe the freezing processes are contained in Table 3.1. Oyster meat cryogenically frozen (CF) with liquid nitrogen from 4ºC to -20.2ºC required 1.67 min. On the other hand, it required 27.9 min to freeze oyster meat in an air blast (AB) freezer operated with an average air velocity of 4.9 ± 1.9 m/s at similar initial and final temperatures. The calculated heat transfer rate for 1 kg of oyster meat in air blast and cryogenic freezing were 1.25 ± 0.20 and 23.73 ± 0.79 J/s, respectively.

The type of freezer clearly influenced the freezing time, freezing rate, and consequently the energy removal rate. The overall rate of freezing for any object is a function of the driving force and resistances involved in the process. CF oyster meat was frozen almost seventeen times

faster than AB. The reason is attributed to the enormous temperature driving force available in cryogenic freezing (Goswami, 2010). The freezing driving force was the difference in temperature between the cooling medium (operating temperature in the cabinet of -123ºC) and the oyster meat (4ºC). In contrast, the blast freezer employed air cooled to -20ºC which provided a lower temperature driving force and thus the freezing time was much longer. The freezing rate of AB oyster meat was 0.57 ºC/min and 9.58 ºC/min for CF oyster meat. The two freezing rates for air blast and cryogenic freezing were therefore in the ratio of 16.8:1. Freezing relates to the product quality since it considers the time for ice formation (Goswami, 2010).

Table 3.1 Thermodynamic related data and calculations for freezing oyster meat

	Air blast freezing (AB)	Cryogenic freezing (CF)
Initial temperature, T_o (ºC)	4.04 ± 0.5	4.00 ± 0.40
Initial freezing temperature, T_{if} (ºC)	$- 0.56 \pm 0.7$	$- 1.3 \pm 0.3$
Final freezing temperature, T_f (ºC)	$- 20.1 \pm 0.2$	$- 20.2 \pm 0.3$
Specific heat capacity of unfrozen oyster meat, C_{pu} (kJ/kg K)	4.18 ± 0.02	4.13 ± 0.10
Specific heat capacity of frozen oyster meat, C_{pf} (kJ/kg K)	2.03 ± 0.05	2.01 ± 0.02
kg bound water per kg solute, B	0.28 ± 0.002	0.28 ± 0.002
Weight fraction of ice, x_i	0.70 ± 0.006	0.67 ± 0.006
Latent heat, L (kJ/kg)	233.51 ± 2.12	224.93 ± 2.05
Product heat load, Δh (kJ)	174.321 ± 2.95	165.05 ± 1.95
Freezing time, (min)	27.9 ± 0.86	1.67 ± 0.52
Freezing rate, (ºC/min)	0.57 ± 0.08	9.58 ± 0.41
Heat removal rate, Q (J/s)	1.25 ± 0.20	23.73 ± 0.79

High rates of freezing generate a larger number of small ice crystals, while slow freezing gives a smaller number of large ice crystals. Slow freezing gives more time for the water

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molecules to move to the growing nuclei, resulting in large-size crystals (Damodaran and others, 2008). Large ice crystals pierce the cell membrane causing damage to the cells. This damage is greater at low freezing rates (Alizadeh and others, 2007).
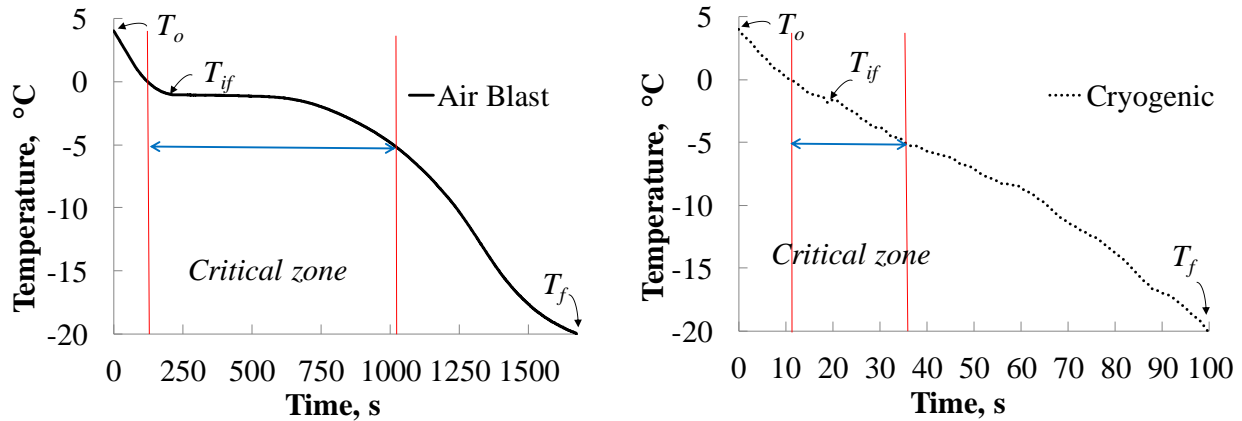
Figure 3.1: Freezing curves (left) air blast freezing and (right) cryogenic freezing

As can be seen in Figure 3.1, air blast freezing kept the oyster meat in the critical zone (0 to -5ºC) for a longer period of time when compared to cryogenic freezing (888 s and 24 s respectively). In addition to the formation of large ice crystals, an increase in protein denaturation and breakdown of lipids have been reported to occur in prolonged critical regions due to the concentration of solutes and enzymatic activity (Kolbe and others, 2007). In contrast, the very short critical zone observed in cryogenic freezing could lead to an increased rate of crystal formation dominating over the rate of crystal growth, and thus formation of small crystals was expected. In this regard, the size and the distribution of ice crystals during the cryogenic freezing of oyster meat may have had a less effect on cellular damage and product quality than in air blast freezing.

A high quality frozen product could be obtained by a better understanding of the freezing process. Freezing is an unit operation in which heat is extracted from a product, lowering product's temperature and converting most of the product's free moisture to ice. This needs to

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occur sufficiently fast so that the product will experience a minimum degradation of quality. Having specific and detailed information on the product's behavior during freezing, desired freezing time, freezing rate, and energy removal rate could help in the designing or selection of the right equipment to optimize production schedule and costs.

3.3.2 Survival of *Vibrio vulnificus* and *Vibrio parahaemolyticus* during freezing and frozen storage

The log reduction of viable cells from *Vibrio vulnificus* immediately after AB and CF freezing of oyster meat was approximately 0.95 and 1.39 respectively (day 0). On the other hand, a log reduction of 0.73 and 0.70 was observed for *Vibrio parahaemolyticus*, respectively, in AB and CF freezing processes (Table 3.2).

Population of *V. vulnificus* had a greater log reduction in CF oyster meat samples than in AB freezing. On the other hand, the freezing process (CF and AB) did not have an effect in the reduction of of *V. parahaemolitycus*. During frozen storage, inactivation of *V. vulnificus* occurred after 30 days regardless of the freezing process; however, survivors of *V. parahaemolyticus* were still detected at this time only in AB frozen samples. No detection of survivors was possible after 60 days of frozen storage at -20ºC in both AB and CF frozen samples.

These results suggest that exposing both *Vibrio spp* to low temperatures during freezing affected the survival of viable cells during frozen storage. The higher log reduction of *V. vulnificus* after cryogenic freezing suggests that this process caused more damage to the cells than air blast freezing. The low temperatures probably injured cells that were not able to survive and form colonies. The survival of *V. parahaemolyticus* after 30 days of frozen storage observed only in AB frozen samples suggests that CF affected this species as well; although no significant

difference in log reduction was observed immediately after freezing when comparing freezing processes.

A possible explanation for the response and inactivation of *V. vulnificus* and *V. parahaemolyticus* by different freezing processes as well as in frozen storage might be due to the combination of cold shock and the formation of intracellular ice crystals. As described by Bryan and others (1999), *V. vulnificus* lacks adaptive cold shock proteins which mediate the tolerance to cold temperatures and thus making it more sensitive to low temperatures. Critical cellular physiological processes and encoding of genes necessary for the survival of the cell cannot take place, and thus, it can be suggested that, during cryogenic freezing, the exposure to lower temperatures in shorter periods of time affects *V. vulnificus* more drastically than in air blast freezing.

Table 3.2 Effect of freezing and frozen storage at -20ºC in the logarithmic reduction of *Vibrio vulnificus* and *Vibrio parahaemolyticus* ($\log_{10}$ CFU/g) in oyster meat

Time, days	*Vibrio vulnificus*[x]			*Vibrio parahaemolyticus*[x]	
	Air blast	Cryogenic		Air blast	Cryogenic
0	0.95 ± 0.20^{Be}	1.39 ± 0.06^{Ae}		0.73 ± 0.17^{Af}	0.70 ± 0.32^{Ae}
1	1.24 ± 0.10^{Ade}	1.50 ± 0.03^{Ade}		1.16 ± 0.10^{Bfe}	1.86 ± 0.22^{Ad}
2	1.55 ± 0.18^{Ad}	1.90 ± 0.10^{Ad}		1.69 ± 0.20^{Be}	2.51 ± 0.14^{Acd}
3	2.17 ± 0.32^{Bc}	2.91 ± 0.21^{Ac}		2.38 ± 0.22^{Ad}	3.00 ± 0.10^{Ac}
7	3.76 ± 0.09^{Bb}	4.29 ± 0.05^{Ab}		4.03 ± 0.31^{Ac}	4.31 ± 0.26^{Ab}
30	Not detected*[Aa]	Not detected*[Aa]		4.74 ± 0.22^{Bb}	Not detected*[Aa]
60	Not detected*[Aa]	Not detected*[Aa]		Not detected*[Aa]	Not detected*[Aa]

Time = 0 refers to the time immediately after freezing, when samples reached -20ºC (effect of freezing)
[x]Initial inoculation level of 6.0 $\log_{10}$CFU/g for each vibrio species
[abcde]Means with different exponents in each column indicates significant difference
[AB]Means with different exponents in each row indicate significant difference
* Non-detectable levels ($\geq$ 6 log reduction)

V. parahaemolyticus has been reported to have a greater tolerance to low temperatures than *V. vulnificus* due to rapid and drastic morphological changes during cold stress (Chen and others, 2009). The authors observed that most of the rod-shaped cells of *V. parahaemolyticus* shrunk and became coccoid when stored at 4ºC. The reduction in size was believed to be a means of minimizing the requirements for cell maintenance, and protects non-spore-forming bacteria against environmental stresses. This could explain *V. parahaemolyticus'* 30-day survival during frozen storage only after air blast freezing. Freezing time during air blast freezing (almost 17 times slower than cryogenic freezing) coupled with the lower freezing rate may have allowed a higher number of cells to adapt and survive for a longer period of time. The shorter freezing time and higher freezing rate in cryogenic freezing might have not given this advantage which can be corroborated by the absence of survivors in samples frozen by this process after 30 days of frozen storage.

Although cold-shock adaptive responses play an important role in the inactivation of both *Vibrio spp.*, formation of intracellular ice during frozen storage could have had a greater effect in the reduction of bacterial counts. The size of the crystals is a function of initial crystal size, storage temperature, and storage time. At higher frozen storage temperature, such as in this study, ice crystal growth is faster (Seminario and others, 2011); and, ultimately, intracellular ice crystal growth and the damage to the cell might be the causes of inactivation of the *Vibrio spp.* in oyster meat during frozen storage, which is supported by studies authored by Shen and others (2009). According to Shen and others, the reduction of *V. parahaemolyticus* in shucked oysters is more effective at higher frozen storage temperatures. The results suggest that reduction to non-detectable levels was obtained in samples stored at -18ºC after 75 days, while at -30ºC of frozen storage, the population decreased from 5.46 log MPN/gram to 0.38 log MPN/gram in the

same period of time. For this study, the reductions of *Vibrio spp.* in oyster meat after freezing and during frozen storage was attributed to larger intracellular ice crystal growth formed in the cells at the frozen storage temperature of -20°C than at a lower temperature (-30°C), which caused greater cell damage by ice crystals at a higher than at a lower temperature.

3.3.3   Inactivation kinetics

First order isothermal analysis of survival data during storage at -20°C for each triplicate treatment yielded *D* and *k* values as shown in Table 3.3. The results show that there is no significant difference in *D* values for *V. vulnificus* for samples frozen by the different freezing processes. In contrast, *D* values calculated for *V. parahaemolyticus* were significantly different.

Table 3.3 First order isothermal analysis of inactivation of *Vibrio vulnificus* and *Vibrio parahaemolyticus* ($\log_{10}$ CFU/g) in oyster meat

	Vibrio vulnificus		*Vibrio parahaemolyticus*	
	Air blast	Cryogenic	Air blast	Cryogenic
D-value (days)	2.43 ± 0.16^A	2.27 ± 0.01^A	8.54 ± 0.18^A	2.11 ± 0.09^B
k (days^{-1})	0.95 ± 0.06^A	1.02 ± 0.01^A	0.27 ± 0.01^A	1.09 ± 0.05^B
R^2	0.988 ± 0.01	0.983 ± 0.01	0.832 ± 0.01	0.951 ± 0.04

[AB]Means with different exponents in each row indicate significant difference

Although *V. vulnificus* showed a significant difference in log reduction immediately after freezing between CF and AB freezing, the calculated *D* values suggest that the inactivation of *V. vulnificus* during frozen storage was not affected by the freezing process for this species and an average of 2.35 days were needed to produce a 1-log reduction of this species when stored at -20°C.

The significant difference found between the *D* values for *Vibrio parahaemolyticus* might suggest that the freezing process had an effect on survival of colonies during frozen storage

which corroborates that air blast freezing could provide opportunity for cold-shock adaptation to *V. parahaemolyticus* cells. Although the inactivation of *V. vulnificus* at cold temperatures has been reviewed by several authors (Andrews and others, 2000a; Bryan and others, 1999; Quevedo and others, 2005; Rodrick, 2009), there are very few studies quantifying the effect of freezing and frozen storage on the survival of *V. vulnificus*. Inactivation of *V. vulnificus* in phosphate-buffered saline solutions at low temperatures by Seminario and others (2011) shows that *D* values for *V. vulnificus* become smaller at higher frozen storage temperatures. At -10ºC, the *D* value was calculated as 2.22 days, while for -35 and -80ºC the corresponding *D* values were 27.52 and 549 days respectively. The larger *D* values at lower frozen temperatures was attributed to the reduced intracellular ice crystal growth which caused less cellular damage, and thus higher probability of survival. Published *D* values for *V. parahaemolyticus* during frozen storage were not found to compare with the results obtained from this study. However, the effectiveness of frozen and chilled storage on the survival of *V. parahaemolyticus* has been addressed (Chen and others, 2009; Muntadagarriga and others, 1995; Shen and others, 2009) and discussed previously in this document.

While the first order equation did a fairly good job of describing inactivation of *Vibrio vulnificus* during storage at -20°C regardless of the freezing process, as shown in Figure 3.2, it became evident that inactivation of *Vibrio parahaemolyticus* during storage did not follow first order inactivation kinetics after air blast freezing. The fitting of the curve for the inactivation of *V. parahaemolyticus* in samples frozen in the air blast freezer had the lowest correlation value, and probably an alternative model could be used to fit the experimental data.
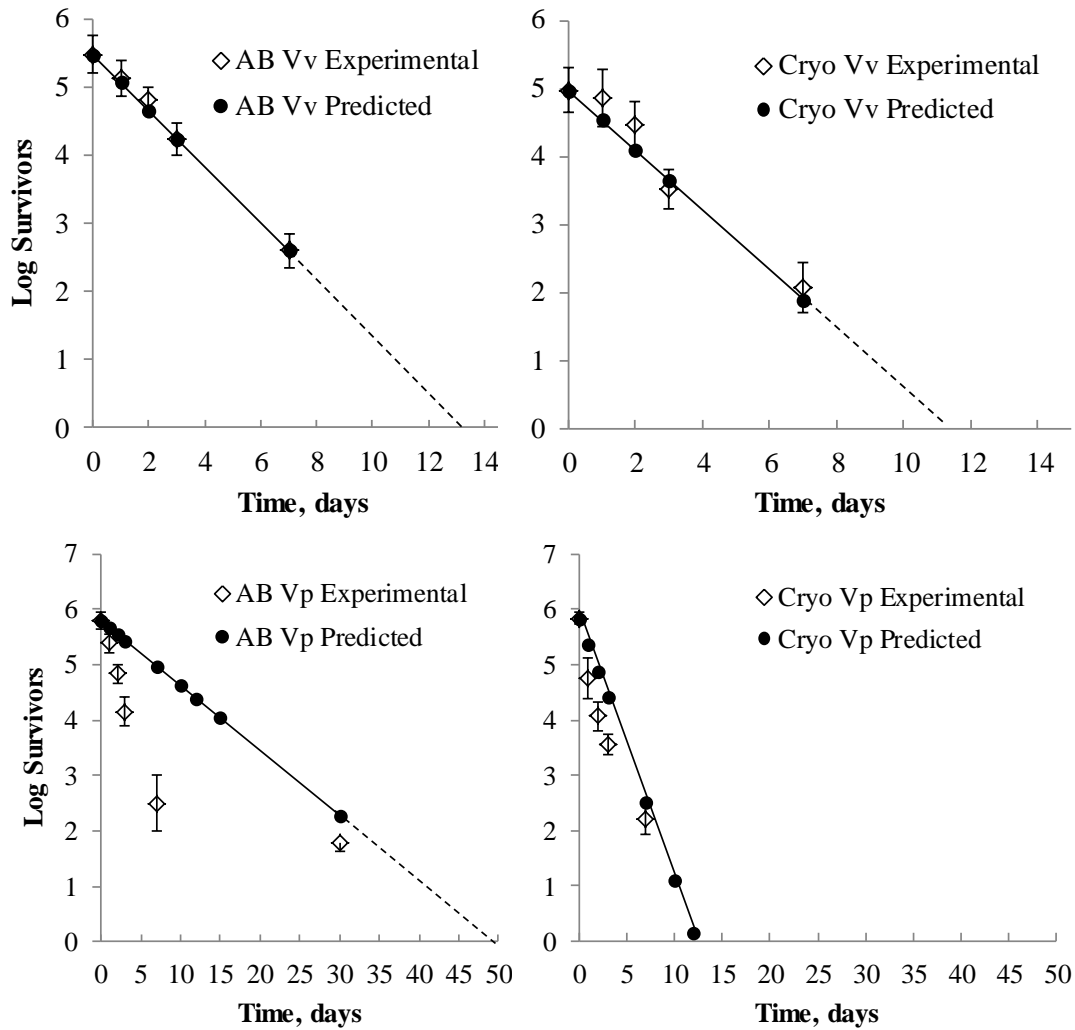
Figure 3.2: First order isothermal inactivation during frozen storage at -20°C of *V.vulnificus (Vv)* (top) and *V. parahaemolyticus(Vp)* (bottom) after cryogenic (Cryo) and air blast (AB) freezing

A prediction of the time needed to obtain a reduction from 6.0 log CFU/g to non-detectable levels of the *Vibrio spp*. in oyster meat during frozen storage can be calculated from the plots of the first order equation (Fig 3.2). According to the plots, a complete inactivation of *V. vulnificus* after air blast and cryogenic freezing could be expected to happen after 13 and 11 days respectively. On the other hand, after cryogenic freezing, *V. parahaemolyticus* could be inactivated after 13 days of frozen storage at -20°C, and between 50 and 60 days if frozen in an air blast freezer.

3.3.4 Freezing and frozen storage losses

The weight loss due to the freezing of oyster meat for AB and CF was $1.77 \pm 1.50\%$ and 0.76 ± 0.28 % respectively, calculated using the formula described in Eq. 6. Air blast frozen oyster meat samples lost 2.3 times more than those frozen cryogenically. During frozen storage, weight loss increased in both CF and AB frozen samples (Table 3.4). AB oyster meat samples showed a gradual increase in weight loss during the first 30 days of frozen storage, however, significant weight losses were observed after 90 days. In contrast, the weight loss from CF oyster meat samples was stable after 30 days of frozen storage, and significant weight losses were observed only after 90 days of frozen storage. After 180 days of frozen storage at -20ºC, AB frozen samples had the most weight loss (8.17%), which was 2.5 times higher than samples frozen with liquid nitrogen (3.17%).

Table 3.4 Weight loss of frozen oyster meat during frozen storage

Time, days	Frozen storage loss %	
	Air blast	Cryogenic
1	1.9 ± 0.2^{Ac}	0.33 ± 0.12^{Bc}
15	2.1 ± 0.3^{Ac}	0.51 ± 0.2^{Bbc}
30	3.1 ± 0.2^{Abc}	0.86 ± 0.6^{Bbc}
90	5.4 ± 3.1^{Aab}	2.3 ± 0.9^{Ba}
180	8.2 ± 0.7^{Aa}	3.2 ± 2.1^{Ba}

[abc]Means with different exponents in each column indicates significant difference
[AB]Means with different exponents in each row indicate significant difference

Weight losses during freezing and frozen storage of unprotected food products are due to surface ice sublimation (Campanone and others, 2006). During freezing, food surface dehydration occurs due to the difference in water vapor pressure. The temperature at the food's surface is higher than the environment temperature (freezer temperature) and thus the surface water vapor pressure is also higher. When a freezing process takes longer time to reduce this

difference in water vapor pressure, an extended dehydration occurs damaging the food surface, which corroborates the higher weight loss in oyster meat samples frozen in the air blast freezer.

On the other hand, weight loss during frozen storage is attributed to the temperature fluctuation as a result of the thermal cycling of refrigeration units. These temperature fluctuations affect food surface temperature. The changes in food surface temperature affect ice sublimation; therefore, affecting the cumulative weight loss of the product. Furthermore, the size and distribution of ice crystals can retard or accelerate the sublimation process (Searles and others, 2001). The freezing process dictates ice crystal morphology and size distribution. Small numbers of large ice crystals develop during slow freezing processes. In contrast, large numbers of small ice crystals occur during fast freezing (cryogenic freezing). Large ice crystals are unstable; they melt and recrystallize more easily resulting in moisture migration to the surface followed by ice formation. Temperature fluctuation causes the sublimation of the surface ice, making the moisture migration almost a continuous process. This effect could explain the larger weight loss observed in oyster meat samples frozen by air blast freezing during frozen storage.

Weight loss is a very important quality parameter in frozen food products that could be used to estimate economic losses of the product during frozen storage. Commercially, weight loss during frozen storage is more important than the one caused during freezing process (Campanone and others, 2005).

3.3.5 Observation of microstructure by scanning electron microscopy (SEM) and light microscopy (LM)

Microstructures of oyster meat after freezing using air blast and cryogenic freezing in comparison with fresh oyster meat (control) are shown in Figure 3.3. The smooth surface of fresh oyster meat observed on the SEM images was lost after freezing. In AB frozen samples,

the integrity of the surface was damaged and large deep cracks can be observed. The surface on

CF frozen oyster meat was less damaged after freezing.

SEM (surface)	Light microscopy (cross section)
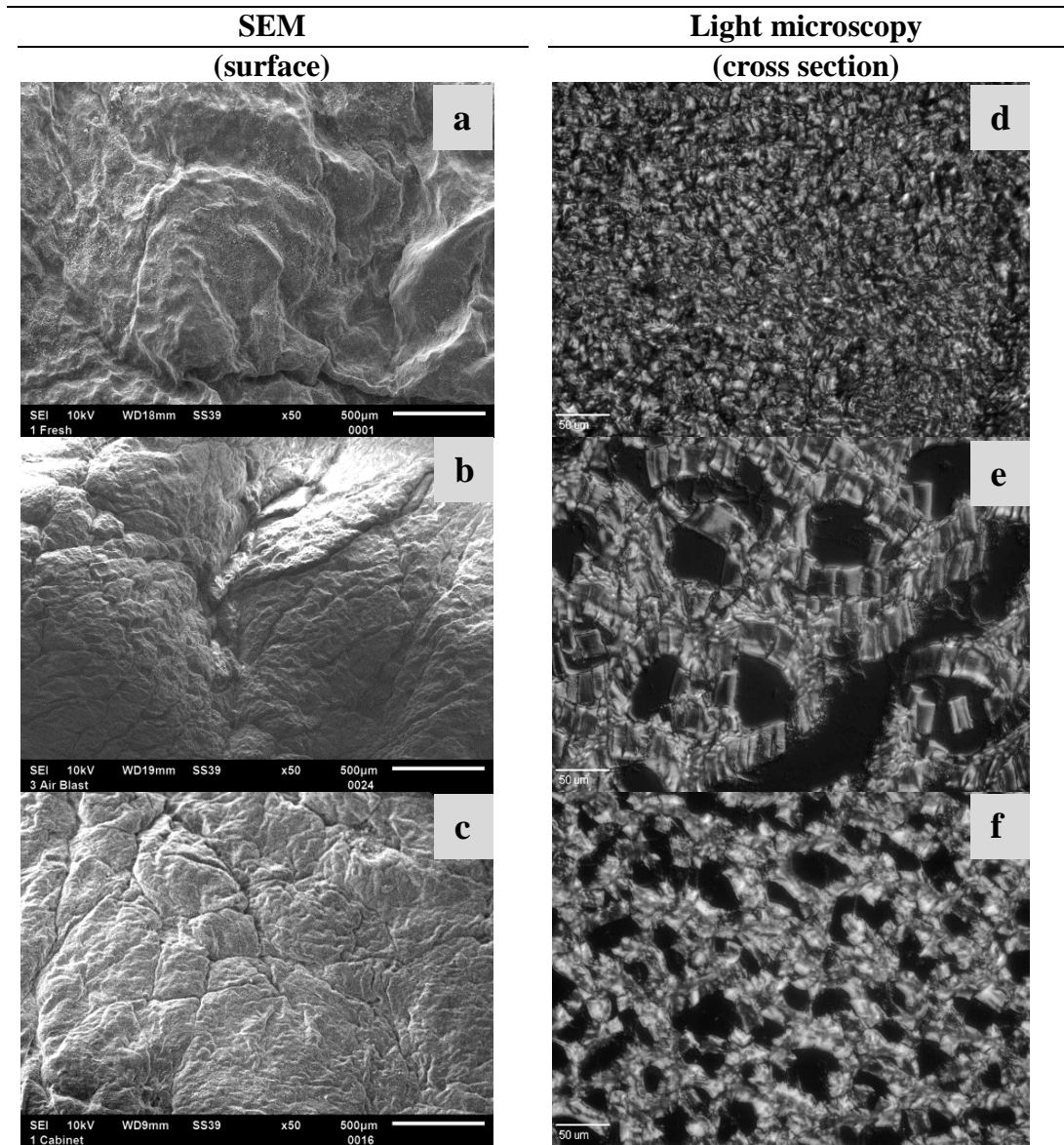
Figure 3.3: Scanning electron images of the surface (left) and light microscopy micrographs of the cross-section (right) of oyster meat after freezing. (a) SEM of control, (b) SEM of AB, (c) SEM of CF, (d) LM of control, (e) LM of AB, (f) LM of CF

According to Figure 3.3, the size of the cracks was smaller, and they appeared to be superficial without causing extensive disruption of muscle tissue compared to the air blast frozen samples. The change in appearance of the surface after freezing is attributed to sublimation of

ice, which leaves the product dehydrated, porous and spongy (Goswami, 2010).Sublimation of ice can affect the entire surface and penetrate deeply into the product, especially in slow freezing processes. Food products with high water content are more affected by this phenomenon reducing water holding capacity during thawing. In this regard, the rate of freezing has an important role in controlling the quality of frozen food products.The muscle fibers of the unfrozen fresh oyster meat demonstrated relatively uniform, compact, and regular shapes in the cross-section (Figure 3.3). The cross-section area of muscle fibers was $106 \pm 35 \ \mu m^2$, which was smaller than values reported for other seafood muscle fibers (Alizadeh and others, 2007). After freezing, the compact muscle fiber network was deformed by the formation of ice crystals. Observing the muscle fibers in oyster meat after air blast freezing, the majority of the area was occupied with the cross-section of ice crystals larger than the muscle fibers (Table 3.5). Slow freezing processes usually damage the texture of foods due to the large and extracellular ice crystals formed. In addition, considerable cellular shrinkage occurs in slow freezing processes due to osmotic behavior resulting in dehydration (Ken and others, 2004). This means that the muscle tissue was seriously deformed.

The light microscopy images from cryogenic freezing show evenly sized distribution between muscle fibers and ice crystal cross-sections. More numerous and smaller ice crystals were formed. Although there is a significant difference in the muscle fiber cross-section area between fresh and cryogenically frozen samples, it is clear that the muscle fibers' integrity was better preserved when compared to air blast frozen samples (Table 3.5).Rapid freezing, in comparison to conventional freezing processes, on the microstructure of muscle tissues in salmon (Alizadeh and others, 2007; Kaale and others, 2013), shrimp (Sriket and others, 2007), and pork (Martino and others, 1998), has demonstrated that the size and distribution of ice

crystals have a direct impact on the quality of the final product. Freezing processes with lower freezing rates, less than 1.7°C/min, produced larger as well as irregular ice crystals, resulting in the deformation of muscle tissue. Comparatively, the higher freezing rates (>8.33°C/min), significantly improved the microstructure of ice crystals (size, formation and location) which improved texture, lowering drip loss, and increasing sensory scores.

Table 3.5: Muscle fibers and ice-crystal equivalent cross-section area (μm^2) in oyster meat frozen by air blast and cryogenic freezing

	Fresh	Air blast freezing	Cryogenic freezing
Muscle fibers, μm^2	106 ± 35^C	708 ± 254^{Ab}	505 ± 167^{Ba}
Ice crystals, μm^2		4042 ± 2064^{Aa}	941 ± 522^{Ba}

[abc]Means with different exponents in each column indicates significant difference
[AB]Means with different exponents in each row indicate significant difference

3.3.6 Effect of freezing on the quality of oyster meat

3.3.6.1 Proximate analysis

The moisture and protein content of fresh oyster meat were found to be 88.32 ± 1.9 % and 8.16 ± 0.52 %, respectively. Fat content was found to be 1.54 ± 0.18 and the content of ash was 0.73 ± 0.10. The proximate composition of oyster meat is summarized in Table 3.6. Season variability has an effect on the composition of oysters due to metabolic activities resulting from complex interactions among food availability, environmental conditions, growth and reproductive cycles (Lira and others, 2013). Moisture values have been reported to be 75 -77% in oysters harvested during warmer months, with protein contents of 10.5-11.5%, and $2.6 - 2.8$ % in ash (Cruz-Romero and others, 2008; Linehan and others, 1999). On the other hand, Anthony and others (1983) and Lira and others (2013) reported higher values for moisture (82.8%), protein (13%), fat (2.5%), and ash (1.5%) in winter oysters. The moisture content measured in this study is higher compared to the cited articles; however, oyster meat utilized in

this study, shucked from oysters harvested between December and January, was larger and possibly retained more water.

Table 3.6: Proximate composition of oyster meat

	Composition (wet basis)
Moisture (%)	88.32 ± 1.9
Protein (%)	8.16 ± 0.52
Fat (%)	1.54 ± 0.18
Ash (%)	0.73 ± 0.10

3.3.6.2 Analysis of oyster meat color (*L*, a*, b*, ΔE**)

The color of oyster meat varies according to species, season, diet, and environmental conditions. Biological processes that take place during summer months (spawning) cause oysters to have a dark color, deviating from their normal creamy appearance. In frozen storage, color changes in seafood products can be attributed to degradation of pigments, enzymatic browning, and lipid oxidation (Songsaeng and others, 2010). In this study, the instrumental color analysis of fresh oyster meat presented the following values: L^* (lightness) of 54.72 ± 2.18, a^* (redness) of 1.02 ± 0.49 and b^* (yellowness) of 10.82 ± 1.24. Considerable color variation did not take place during frozen storage as observed in Table 3.7. Regardless of the freezing process, all color indicators presented only slight fluctuations.

Denaturation of myofibrillar and sarcoplasmic proteins have been reported to cause color changes in oyster meat during high-pressure (HP) processes by the increase of L^* values (Cruz-Romero and others, 2008). Several other studies on HP-treatment of oysters validate the increase in whiteness of oyster tissue with increasing pressure (Hsu and others, 2010; Murchie and others, 2005); however, subsequent evaluation of the color variation during frozen storage has not been performed. Color parameter variations during refrigerated storage of oysters in studies

performed by Fratini and others (2013) to test the effects of lagoon-farmed oysters with a finishing period in the sea were attributed to acclimation-shock, with no relation to the storage temperatures.

Table 3.7: Color (L^*, a^* and b^* values) of frozen oyster meat during frozen storage

Time, Days		L^*	a^*	b^*	ΔE^*
1	AB	51.16 ± 4.72^A	1.36 ± 0.98^A	10.79 ± 1.58^A	13.67 ± 4.32^A
	CF	52.09 ± 5.56^A	1.37 ± 0.63^A	11.61 ± 1.70^A	10.54 ± 5.34^A
15	AB	51.30 ± 5.02^A	2.41 ± 0.10^A	12.49 ± 0.11^A	13.57 ± 6.35^A
	CF	49.44 ± 3.18^A	1.05 ± 0.78^A	10.58 ± 0.81^A	15.35 ± 5.29^A
30	AB	53.34 ± 1.25^A	1.58 ± 0.73^A	13.41 ± 2.29^A	10.82 ± 3.62^A
	CF	53.99 ± 1.33^A	1.45 ± 0.71^A	12.03 ± 1.93^A	10.39 ± 2.96^A
90	AB	53.20 ± 4.43^A	1.77 ± 0.35^A	11.57 ± 0.72^A	10.60 ± 2.46^A
	CF	53.34 ± 1.25^A	1.58 ± 0.73^A	13.41 ± 2.29^A	10.82 ± 3.62^A
180	AB	48.60 ± 5.72^A	0.87 ± 0.45^A	10.45 ± 0.87^B	15.16 ± 7.61^A
	CF	51.04 ± 1.68^A	1.66 ± 0.62^A	15.02 ± 2.84^A	13.48 ± 4.29^A

[AB]Means with different exponents in each column indicate significant difference

When assessing the quality of frozen oysters by visual evaluation, no significant changes in the appearance of frozen oyster meat were reported by Songsaeng and others (2010) when comparing freezing processes. In this study, oyster meat was frozen by contact-plate (CPF) and individual quick freezing (IQF), followed by frozen storage at -20ºC for 12 months. However, IQF samples reported an increase in yellowness at a faster rate than that of CPF. This discoloration was attributed to lipid oxidation, but the direct assessment was not performed. According to Songsaeng and others (2010), formation of pigments was the result of ionic condensation of primary amino groups of protein with conjugated unsaturated aldehydes or similar reactive lipid oxidation products that are produced by the cleavage of unsaturated hydroperoxides.

The general color stability observed during frozen storage (Table 3.7) could probably be explained by the protection offered by the packaging. The characteristics of the package utilized in this study contributed to maintain quality properties by minimizing the negative effects of temperature fluctuation and air circulation during frozen storage. The oxygen (O_2) and carbon dioxide (CO_2) permeability of the trays (3,500 and 10,000 $cm^3/m^2atm/24h$ respectively), categorize the trays as a high barrier material. During frozen storage, in the case of unpackaged foods, these undergo mass transfer with the environment. Therefore, apart from water freezing, surface ice sublimes, altering the sensory characteristics of the products. This leads mainly to quality decay and to general appearance spoilage, for instance, changes in color, taste, and texture (Bøknæs and others, 2000; Campanone and others, 2006).

3.3.6.3 Moisture content of oyster meat during frozen storage

The moisture content of oyster meat frozen by air blast and cryogenic freezing processes did not changed significantly during the first 30 days of frozen storage. In AB frozen samples, a significant decrease in moisture was observed at day 90 which was expected since formation of ice was observed in the packaging. Ice began to accumulate in the inner side of the film at day 60 and continued to occur until the end of the study. In contrast, samples of oyster meat frozen cryogenically did not yield ice formation in the packaging. It can be assumed that the decrease of moisture content in air blast frozen samples probably was caused by the evaporation of water. The moisture content of the oyster meat during frozen storage is reported in Table 3.8. Evaporation of water in products stored at low temperatures is affected by several factors. Some of these factors are intrinsic to the food (surface exposure, insulation barriers such as fat layers, area to volume ratio, etc.), while others to the packaging and environmental conditions (Campanone and others, 2005). However, being that the freezing process was the only variant

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between sets of samples, it is probable that it had an influence in the moisture loss, related to the development and nature of ice crystals evidenced by the microscopic analysis of oyster meat tissues. The temperature fluctuation during the cycles of the refrigeration unit may have caused more sublimation resulting in loss of moisture from samples frozen by air blast.

Table 3.8: Moisture content (wet basis) of oyster meat during frozen storage

	1	15	30	90	180
AB	87.25±0.37[Aa]	87.16±1.22[Aa]	86.76±0.24[Aa]	81.57±1.99[Bb]	79.21±1.35[Bb]
CF	88.28±2.66[Aa]	87.91±2.28[Aa]	87.83±2.56[Aa]	88.01±1.96[Aa]	86.05±0.77[Aa]

[AB]Means with different exponents in each row indicate significant difference
[ab]Means with different exponents in each column indicates significant difference

Retention of moisture is important in the commercialization of oysters. Water loss affects the appearance and hence consumer acceptance, especially in oysters on the half shell where palatability is enhanced by the juiciness of the meat. In addition, excessive water loss causes concentration of solutes which may accelerate lipid oxidation and protein denaturation. Glazing oysters after freezing is a common practice in industry to protect the meat from dehydration during frozen storage.

3.3.6.4 Lipid oxidation of oyster meat during frozen storage

TBARS values increased during frozen storage in both air blast and cryogenic frozen oyster meat samples. The initial average value of TBARS in fresh oysters was 1.15 ± 0.70 mg MDA/kg which increased to 2.64 ± 0.27 and 2.35 ± 0.15 after one day of frozen storage in air blast and cryogenic frozen samples respectively. TBARS values increased more rapidly in AB frozen samples than in CF (Table 3.9) during frozen storage. Lipid oxidation (TBARS values) increased from 2.64 to 10.96 for AB samples, while in CF samples lipid oxidation was reported to increase from 2.35 to 5.15 at the end of storage. After 180 days of frozen storage, AB samples

had almost twice the amount of malonaldehyde (MDA) than CF samples. Significant differences in TBARS values between samples frozen by AB and CF were initially observed after 30 days of frozen storage. The difference in TBARS values between AB and CF samples were more obvious after 90 days of frozen storage. This time frame coincides with observations of increased moisture loss in AB samples which could explain the faster development of lipid oxidation. The higher moisture loss than in CF samples could produce an increased concentration of solutes (minerals, metals, etc.) that led to the acceleration of autooxidation of lipids. During lipid oxidation, unstable hydroperoxides form and decompose into shorter chain hydrocarbons and these final products are detected by the TBA test as TBARS.

Table 3.9: Lipid oxidation expressed as TBARS (mg MDA/kg) of oyster meat frozen by air blast and cryogenic freezing during frozen storage

	1	15	30	90	180
Air blast	2.64 ± 0.27^{Da}	3.29 ± 0.38^{Da}	5.93 ± 0.31^{Ca}	7.96 ± 0.92^{Ba}	10.96 ± 1.05^{Aa}
Cryogenic	2.35 ± 0.15^{Ba}	2.53 ± 0.47^{Ba}	3.19 ± 0.38^{Bb}	4.70 ± 0.64^{Ab}	5.15 ± 0.81^{Ab}

[AB]Means with different exponents in each row indicate significant difference
[ab]Means with different exponents in each column indicates significant difference

A uniformed criterion related to acceptable TBARS values in seafood, and especially in oysters, before objectionable odor/taste can be detected, as expression of lipid oxidation, has not been clearly established. Connell (1990) states that clean and pleasant flavors in fish are better perceived when MDA values do not exceed 1-2 mg/Kg. However, values of 8 mg MDA/Kg are acceptable for Schormüller (1968) and (Koral and others, 2010) in smoked salmon, and between 3-4 mg MDA /Kg is regarded as good quality limits for Yanar (2007) in catfish. TBARS values as high as 12 mg/Kg have been reported by Cruz-Romero and others (2008) in oyster meat after 20 days of storage in ice (2º C) with no reference to perception of unpleasant appearance, flavors or aromas.

The results in Table 3.9 clearly show that CF frozen oyster meat had lower TBARS values than AB samples which indicates that the freezing process had an influence in the inhibition of lipid oxidation. Hypothetically, CF frozen oyster meat probably would have a higher score in flavor evaluation studies from samples after 180 days of frozen storage; however, before 90 days, it is very probable that no differences in flavor could have been perceived. This hypothesis was not verified by sensory evaluations.

3.4   Conclusions

The reduction of *V. vulnificus and V. parahaemolyticus* to nondetectable levels after air blast and cryogenic freezing followed by frozen storage at -20ºC was possible. Cryogenic freezing was more efficient in reducing *V. parahaemolyticus* based on calculated *D* values. The shorter freezing time and lower temperatures in CF did not allow *V. parahaemolyticus'* colonies to adapt and survive as it was observed in AB samples. The freezing process had an effect on the inactivation of *V. vulnificus sp*. A greater log reduction in *V. vulnificus* was obtained in CF oyster meat immediately after freezing than in AB. This study demonstrates, once again, the advantage of cryogenic freezing in comparison to air blast freezing which is important in products with high moisture content. Microscopy observation of oyster meat samples revealed that the higher freezing rate in cryogenic freezing produced a large amount of smaller ice crystals homogenously distributed throughout the sample. In addition, less surface and tissue damage than in air blast frozen samples were observed. The lower freezing rate in air blast freezing produced larger ice crystals resulting in serious deformation of muscle tissue. The quality of oyster meat was affected by the damage produced by the formation of the ice crystals. This was reflected in larger weight and moisture losses during frozen storage in air blast frozen samples. In addition, the extent of lipid oxidation in oyster meat was also affected by the freezing

processes. TBARS values increased more rapidly in air blast than in cryogenic frozen samples. Color in all samples was not significantly affected.

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CHAPTER 4   STUDY OF THE EFFECTS OF MODIFIED ATMOSPHERE ON VOLATILE COMPOUNDS, AMINO ACIDS, AND QUALITY OF OYSTER MEAT (*CRASSOSTREA VIRGINICA*) PACKED IN MICROWAVABLE TRAYS DURING FROZEN STORAGE

4.1   Introduction

For generations, the oyster industry has been one of the economic engines in the coastal communities of the Gulf of Mexico. The National Marine Fisheries Service reported that 19.7 million pounds of oyster meat, valued at $62.3 million were harvested from the Gulf Coast region in 2010 (NOAA, 2011). There will always be a high consumer demand for oysters that are safe but still retain their original flavor, nutrient content, texture, and appearance. In addition, these oysters are expected to be additive-free as well as presenting a long shelf life. Modified atmosphere packaging (MAP), along with refrigeration, have become increasingly popular preservation techniques, which have brought major changes in storage, distribution and marketing of raw and processed products to meet consumer demands.

In recent years, customers' interest in fresh, mildly preserved, and conveniently packed seafood products has increased. As a result of this demand, application of MAP in seafood has been customized to account for the origin of the raw material, temperature and type of cold storage, gas mixtures, and packaging materials (Gunsen and others, 2010). Most recent reviews on the shelf life extension of fish and fishery products by modified atmosphere packaging (Conte and others, 2013; Del Nobile and others, 2012; Masniyom, 2011; Sivertsvik and others, 2002) compile in great detail the effects of MAP on the microbial reduction, retention of fresh quality characteristics, effect of the synergistic effect with storage temperature, and safety issues. However, the effect of MAP in seafood during frozen storage for extended periods has not been sufficiently addressed.

The few available studies of MAP and frozen storage have been developed to explore consumer demands for conveniently packaged seafood products (Bak and others, 1999; Bøknæs and others, 2000; Bono and others, 2012). The use of nitrogen has produced better color stability, lipid oxidation and texture in boiled shrimp stored at -17ºC for 12 months (Bak and others, 1999). Longer shelf life for chilled retail display was achieved in whiting, mackerel and salmon packed in $30\%N_2$:$40\%CO_2$:$30\%O_2$ and $60\%N_2$:$40\%CO_2$ respectively, when samples were stored at -30ºC for 3 days compared to MAP samples (same gas compositions) with no frozen storage. Sensory tests indicated that MAP samples with frozen storage were still acceptable after 7 days, which was 2 days longer than in samples with no frozen storage.

Combining MAP and frozen storage could probably maintain the overall quality of oyster meat more than frozen storage alone. The inclusion of a modified atmosphere should be beneficial during frozen storage to products undergoing microwave cooking. This combination could improve the retention of flavors, texture and appearance. The present study was mainly initiated to evaluate the effects of MAP in the quality retention of oyster meat in a ready-to-cook form, frozen cryogenically with liquid nitrogen and stored for six months at -20ºC. The effect of MAP on the shelf life was assessed primarily by comparing the volatile flavor compounds and key quality tests.

4.2   Materials and methods

4.2.1   Oyster meat handling, packaging and storage conditions

Fresh oysters were obtained from a local seafood company from Baton Rouge, Louisiana. The oysters were shucked and the meats were cryogenically frozen until reaching an internal temperature of -20°C using liquid nitrogen in a cryogenic chamber (Ultra-freeze, Air Liquide Industrial U.S. LP). After freezing, oyster meat samples were packaged in microwavable trays. The capacity of the trays was 1,392 mL having the dimensions of 195 X 140 X 51 mm with a

thickness of 0.5 mm. The oxygen (O_2) and carbon dioxide (CO_2) permeability of the trays were 3,500 and 10,000 $cm^3/m^2atm/24h$, respectively. Trays were sealed with a flexible packaging film with O_2 and CO_2 permeability of 140 and 350 $cm^3/m^2atm/24h$ respectively. Each tray containing approximately 300 g was packed in a modified atmosphere. Three sets of samples were prepared, (1) 100% CO_2 (MAP-CO_2), (2) 100% N_2 (MAP-N_2), and (3) 100% air (AIR) as control. The ratio between the volume of the gas and weight of oyster meat (G/P ratio) was 5:1 (v/w)(1,392 mL/300g). Food grade CO_2, N_2, and air gases were used to pack the product in a Multivac T-200 tray sealer (Multivac Inc, Kansas, MO). All samples were stored at -20 ± 1°C in a freezer for 6 months and three trays from each treatment were removed periodically at 1, 15, 30, 90, and 180 days for evaluation. Analyses were performed at these times, unless otherwise stated.

4.2.2 Headspace Gas Analysis

Headspace gas composition was measured at each sampling time using an oxygen/carbon dioxide analyzer (Quantek Instruments, model 902D, Grafton, MA). The gas analyzer was programmed to extract three mL of gas from the package's headspace for analysis.

4.2.3 Physical and chemical tests

Oysters were thawed for 24 h at 4°C and analyzed for color, which was measured on the ventral body of individual oysters, (L^*, a^*, b^*, ΔE^*), moisture content (%) (AOAC International, 2005), and TBARS (mg malonaldehide/kg sample)(Lemon, 1975).

4.2.4 pH determination

pH was measured using a pH meter model SB70P (VWR, Radnor, PA, USA) after homogenizing 5 g of sample in 50 mL deionized water for 10 s in a Waring® commercial laboratory blender. Three measurements were carried out on each sample, and the results were expressed as the average of them.

4.2.5 Drip loss

Drip loss of oyster meat was estimated according to the method of Liu and others (2013). After thawing, the oyster meat was removed from the trays and the liquid was collected and weighed. Drip loss was expressed as g of collected liquid / 100 g of weight of oyster meat before freezing (raw oyster meat).

4.2.6 Texture analysis

Texture was measured using a texture analyzer (Instron model 5544, Norwood, MA) equipped with a 10-blade Kramer shear attachment. The analysis was performed using a 2 kN load cell in compression mode at 2 mm/s. Thawed oyster meat was used for the texture analysis by filling the shear cell up to two thirds of its capacity and positioned opposite to the alignment of the blades. Data were collected and analyzed using the texture analyzer software (Merlin v. 5.31). The maximum force needed to cut trough was recorded as compressive strength (MPa).

4.2.7 Amino acid analysis

Amino acid profiles were determined by the AAA Service Laboratory Inc., Boring, OR. A freeze-dried sample was hydrolyzed with 6N HCl and 2% phenol at 110ºC for 22 h. Amino acids were quantified using a Beckman 6300 analyzer with post-column ninhydrin derivatization. Only the most common 16 amino acids were analyzed in this study and reported as milligrams of amino acid/gram of protein (mg aa/g protein). This analysis was performed at 30, 90, and 180 days of frozen storage.

4.2.8 Volatile profile

Volatiles developed by each treatment were obtained by headspace solid-phase micro extraction (HSSPME) and separated in a gas chromatograph (GC) (CP 3800 Varian), equipped with a flame ionization detector, on a capillary column coated with SPB-5 stationary phase (60m x 0.25 mm, 0.25 mm film thickness) (Supelco, Inc). The extraction was performed following the

protocol by Zhang. and others (2010) with some modifications. Oyster meat (50 g) was homogenized using a Waring® commercial laboratory blender with 50 mL of 0.04 g/mL sodium chloride solution. The sample was transferred to a 125-mL double neck flask and 1-mL of methyl isobutyl ketone (2 ppm) was added as internal standard followed by HSSPME exposure for 30 min at 25ºC to extract the volatiles. During time of exposure, the sample was gently agitated by flushing nitrogen into the flask through the lateral neck. Volatiles were collected using a 75 µm (partially crosslinked phase) carboxen/polydimethylsiloxane fiber (Sigma-Aldrich, needle size 23 ga, for use with manual holder). Finally, oyster volatiles were thermally desorbed by inserting the fiber into the GC injector set at 250°C for 30 s in splitless mode using helium as carrier gas at a column linear flow of 1.5 mL/min. The initial temperature of the GC oven was held at 40ºC for 5 min. Then, the temperature was increased by 8ºC/min to a final temperature of 200ºC and held for 1 min. The mass spectrometer (MS) (Saturn 2000 MS, Varian) detector was operated at an ionization voltage of 70 eV and ion source temperature of 200ºC. The volatile compounds were identified by comparison of the mass spectra and retention time of their standards and National Institute of Standards and Technology data base library. This analysis was performed in fresh oyster meat and after 180 days of frozen storage.

4.2.9 Statistical analysis

The collected data were analyzed using SAS version 9.2 (SAS, Version 92, SAS Institute Inc., Cary, NC., USA). One-way analysis of variance (ANOVA) was used to detect statistical differences ($P \leq 0.05$) Means values from six measurements and/ or triplicate analysis were reported. Statistical following Tukey's studentized range test ($p < 0.05$).

4.3   Results and discussion

4.3.1   Headspace composition

MAP-N_2 and control (AIR) packages maintained their shape during frozen storage. In MAP-CO_2, the trays started to lose shape (imploding) at day 60 and all MAP-CO_2 packages were completely collapsed after 150 days. Changes in the headspace composition in MAP-CO_2 packages were noticeable after 30 days of frozen storage (Table 4.1). In addition, an increase in oxygen composition was observed which continued rising until the end of the storage period for both MAP-N_2 and MAP-CO_2. The trays used in this study consist of high barrier structures to oxygen and carbon dioxide, however, the oxygen transfer rate (OTR) of the film is much lower, and thus diffusion of oxygen into the package through the film from surrounding air may have caused the increasing in oxygen composition.

Table 4.1: Headspace gas composition during frozen storage

		Days of Storage at -20°C				
		0	15	30	90	180
	Assay					
AIR	%O_2	19.9 ± 0.2^A	19.2 ± 0.2^B	19.8 ± 0.2^A	18.9 ± 0.2^B	15.3 ± 0.2^C
	%CO_2	1.2 ± 0.1^B	1.2 ± 0.1^B	1.2 ± 0.1^B	1.5 ± 0.2^A	0.6 ± 0.1^C
	%N_2	78.9 ± 0.2^B	79.6 ± 0.2^B	79 ± 0.5^B	79.6 ± 0.3^B	84.1 ± 0.5^A
MAP-CO_2	%O_2	0.3 ± 0.1^D	0.3 ± 0.1^D	0.7 ± 0.1^C	1.2 ± 0.1^B	2.7 ± 0.2^A
	%CO_2	99.1 ± 0.8^A	99.1 ± 0.8^A	99.3 ± 0.5^A	99.4 ± 0.5^A	95.5 ± 0.6^B
	%N_2	0.6 ± 0.1^B	0.6 ± 0.1^B	0 ± 0.0^C	0 ± 0.0^C	1.8 ± 0.4^A
MAP-N_2	%O_2	0.3 ± 0.1^B	0.3 ± 0.1^B	0.5 ± 0.2^B	0.7 ± 0.2^B	1.7 ± 0.2^A
	%CO_2	0.3 ± 0.2^B	0.3 ± 0.1^B	0.2 ± 0.2^B	0.6 ± 0.2^A	1.1 ± 0.3^A
	%N_2	99.4 ± 0.2^A	99.4 ± 0.2^A	99.3 ± 0.3^A	98.7 ± 0.2^A	97.2 ± 0.3^B

[ABCD]Mean values in the same row with different exponents indicate significant difference
AIR = control (compressed air), MAP-CO2 = 100% carbon dioxide, MAP-N2 = 100% nitrogen

Changes in headspace oxygen composition were also observed in AIR and MAP-N_2 but these were reported at the 180-day evaluation, which means that the permeation of oxygen into

the packages occurred in the last 90 days of frozen storage. As in MPA-CO_2, the increase in oxygen concentration could have occurred by diffusion of oxygen through the film in MAP-N_2 samples.

MAP-N_2 reported an increase in the concentration of carbon dioxide; however, possible sources for this increase are uncertain. Diffusion form surrounding air is not likely to have occurred due to the very low concentration of CO_2 in air (0.03% v/v) (Leakey, 2009) and is a current topic of investigation.

In nonrespiring foods, variation in headspace composition during chilled storage, especially in carbon dioxide, is attributed to microbial activity (growth or death) and absorption into the tissue. Yesudhason and others (2010) and Dufresne and others (2000) observed an increase in CO_2 concentration due to an increase of aerobic mesophilic bacteria in air-packed seer fish stored between 0 and 2°C. In contrast, the gradual decrease in CO_2 during frozen storage in MAP samples with high CO_2 composition was attributed to absorption of CO_2 into the tissue (Arvanitoyannis and others, 2012b; Sivertsvik and others, 2004). This phenomenon is commonly reported in MAP studies utilizing concentrations of CO_2 higher than 60% (Arvanitoyannis and others, 2012a; Sivertsvik and others, 2002; Yesudhason and others, 2010). In the current study, the collapsing of MAP-CO_2 samples was expected; however, determination of the time needed to happen was uncertain due to the decrease in solubility of CO_2 at low temperatures.

4.3.2 Color (L^*, a^*, b^*, ΔE^*) of oyster meat

MAP had no significant effect on L^*, a^*, b^* values during frozen storage with the exception of day 1 as shown in Table 4.2. At day 1, the L^* values for MAP-N_2 and MAP-CO_2 were significantly different which could be attributed to surface dehydration caused by the

freezing process. Although instrumental color analysis did not show any differences in color between treatments during frozen storage, a visual difference in lightness in MAP-CO_2 samples was observed when compared to AIR and MAP-N_2. The whiteness in MAP-CO_2 analyzed after 90 and 180 days of frozen storage had a visual contrast to samples from other treatments. The number of analyzed samples was increased in the attempt to verify the difference, however, the results from L^* (lightness) values showed no significant difference between treatments. In this study, the instrumental color analysis of fresh oyster meat presented the following values: L^* (lightness) of 54.72 ± 2.18, a^* (redness) of 1.02 ± 0.49 and b^* (yellowness) of 10.82 ± 1.24.

Color stability during frozen storage in MAP seafood has also been reported by Bak and others (1999). Boiled shrimp packed in nitrogen atmosphere and stored at -17ºC for 12 months showed constant concentrations of astaxantin, which gives the distinct red color in shrimp; however, the reduction of astaxantin occurred in packages containing oxygen (control) causing a change in color. It was suggested that the non-significant variations in color and appearance of shrimp was due to the exclusion of oxygen in the package which did not trigger the chemical reactions for the degradation of astaxanthin and fatty acid oxidation. Color changes during frozen storage occur often by the development of yellow pigment formation as result of the reaction between protein and oxidized lipids (Masniyom, 2011). The color changes of cuttlefish were accompanied with the development of rancid odors during frozen storage (Thanonkaew and others, 2006). Thiansilakul and others (2010) reported that the off-color development in seabass (*Lates calcarifer*) and red tilapia (*Oreochromis mossambicus* × O. *niloticus*) were correlated with lipid oxidation during 15 days of iced storage in samples with 60% CO_2 composition .

The results from instrumental color measurements suggest that oyster meat performed well during frozen storage regardless of the gas composition. The non-significant differences in

color could probably provide information about lipid oxidation in oyster meat. In this regard, the extent of oxidation probably was not high enough to produce color changes as suggested in previous studies in seafood during frozen storage. In addition, the properties of the packaging, could have contributed to maintain color stability by minimizing the negative effects of temperature fluctuation and air circulation during frozen storage decreasing surface dehydration (Bøknæs and others, 2000; Campanone and others, 2006).

Table 4.2: Color evaluation of oyster meat during frozen storage

	Treatments	Days of storage				
		1	15	30	90	180
$L*$	AIR	52.75 ± 3.42^{ABa}	50.99 ± 5.39^{Aa}	49.70 ± 2.58^{Aa}	49.26 ± 1.11^{Aa}	49.22 ± 2.07^{Aa}
	MAP N$_2$	48.03 ± 1.00^{Ba}	51.66 ± 2.77^{Aa}	46.05 ± 6.99^{Aa}	50.69 ± 9.19^{Aa}	52.69 ± 5.20^{Aa}
	MAP CO$_2$	58.22 ± 4.94^{Aa}	53.81 ± 7.27^{Aa}	49.17 ± 1.50^{Aa}	46.55 ± 1.03^{Aa}	54.28 ± 4.65^{Aa}
$a*$	AIR	2.20 ± 0.96^{Aa}	1.71 ± 0.57^{Aa}	1.91 ± 0.82^{Aa}	1.63 ± 1.20^{Aa}	1.58 ± 0.33^{Aa}
	MAP N$_2$	1.33 ± 0.32^{Aa}	2.07 ± 1.10^{Aa}	1.25 ± 0.65^{Aa}	0.89 ± 0.40^{Aa}	0.72 ± 1.47^{Aa}
	MAP CO$_2$	0.77 ± 0.96^{Aa}	2.43 ± 0.38^{Aa}	2.70 ± 1.06^{Aa}	2.17 ± 2.02^{Aa}	0.79 ± 0.64^{Aa}
$b*$	AIR	12.55 ± 1.66^{Aa}	11.30 ± 1.91^{Aa}	13.77 ± 2.10^{Aa}	11.25 ± 2.40^{Aa}	12.23 ± 1.87^{Aa}
	MAP N$_2$	9.92 ± 0.65^{Aa}	10.37 ± 0.88^{Aa}	9.46 ± 2.86^{Aa}	10.93 ± 2.19^{Aa}	10.52 ± 4.01^{Aa}
	MAP CO$_2$	10.65 ± 1.75^{Aa}	12.08 ± 2.55^{Aa}	11.34 ± 1.64^{Aa}	11.24 ± 1.57^{Aa}	14.74 ± 1.72^{Aa}
$\Delta E*$	AIR	12.33 ± 4.02^{ABa}	13.79 ± 6.62^{Aa}	15.49 ± 4.38^{Aa}	14.23 ± 2.01^{Aa}	14.23 ± 3.31^{Aa}
	MAP N$_2$	16.76 ± 1.93^{Aa}	13.17 ± 4.92^{Aa}	18.28 ± 7.54^{Aa}	12.99 ± 8.82^{Aa}	11.49 ± 5.76^{Aa}
	MAP CO$_2$	4.84 ± 4.51^{Bb}	11.14 ± 4.96^{Aab}	15.15 ± 0.45^{Aa}	16.89 ± 2.51^{Aa}	10.35 ± 3.17^{Aab}

[AB]Means with different exponents in each column indicate significant difference
[ab]Means with different exponents in each row indicates significant difference

4.3.3 Moisture content of oyster meat

The moisture content of oyster meat showed no significant differences in AIR, MAP-CO_2, and MAP-N_2 during frozen storage. In addition, a decrease in moisture content in all treatments was not observed when compared to the initial moisture content (Figure 4.1). MAP-CO_2 and AIR samples displayed erratic behavior in moisture content which was not observed in MAP-N_2. The initial average moisture content in oyster meat was $88.32 \pm 1.90\%$ decreasing to

87.45 ± 1.24%, 88.19 ±1.56%, and 88.59 ±1.76% in AIR, MAP-N_2 and MAP-CO_2, respectively after 30 days of frozen storage. At the end of the storage period, the moisture contents of the treatments were 87.53 ± 1.19%, 87.19 ±1.41%, and 89.25 ±1.05% in AIR, MAP-N_2 and MAP-CO_2, respectively.
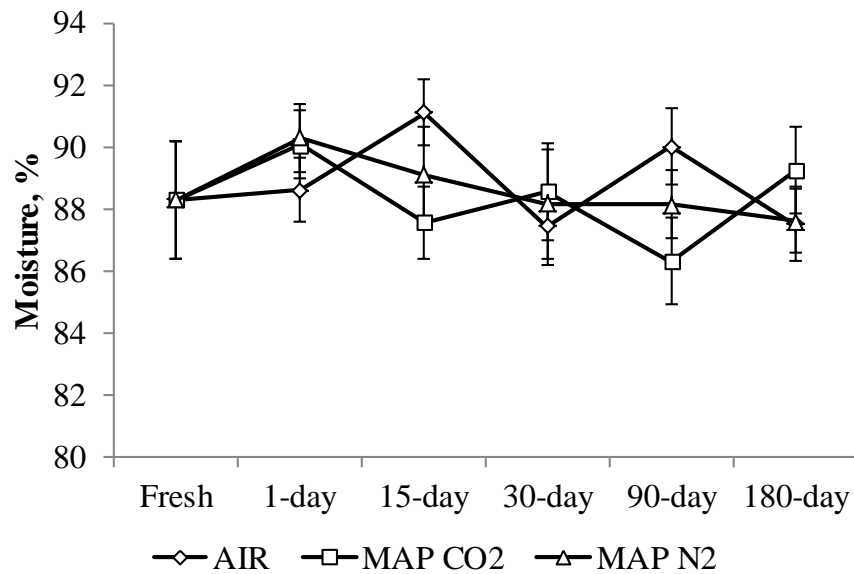
Figure 4.1: Moisture content (w/w%) of oyster meat in AIR, MAP-CO_2, and MAP-N_2 during frozen storage at -20ºC

The erratic variation in moisture content, especially in AIR was attributed to the formation of frost inside the packaging. The development of frost was inconsistent. The frost was observed to appear and disappear throughout frozen storage in most of the AIR trays but not in MAP-CO_2 or MAP-N_2. The increase in moisture content at those periods of time could possibly be caused by the melting of the frost followed by re-absorption of water by the tissue, which affected the outcome of the results. Temperature fluctuation is a common problem during frozen storage, as it produces excessive frost in air-packed products due to ice sublimation, reducing the moisture content and giving an overall appearance of poor quality (Bak and others, 1999).

4.3.4 pH of oyster meat

Significant change in pH was observed in MAP-CO_2 during storage (Figure 4.2). The initial pH values of oyster meat were close to neutrality (pH = 6.82 ± 0.02). The measured pH values in MAP-CO_2 slowly decreased to 6.42 ± 0.07 after 30 days. At the end of the storage period (180 days), pH values for MAP-CO_2 were reported as 6.12 ± 0.06. On the other hand, there was no significant difference in pH values between AIR and MAP-N_2, however, a decrease was observed over time reaching values of 6.67 ± 0.05 and 6.57 ± 0.06 respectively at the end of storage.
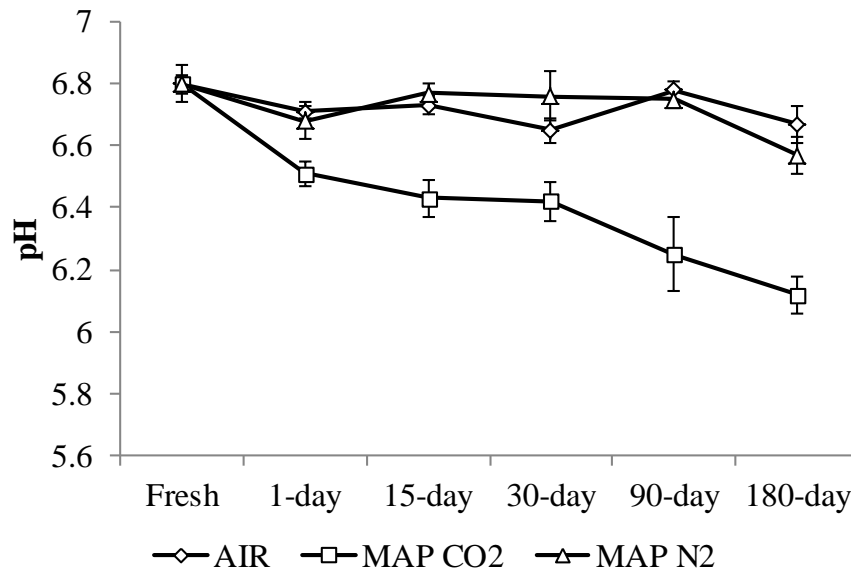
Figure 4.2: Evolution of pH in oyster meat in AIR, MAP-CO_2, and MAP-N_2 during frozen storage at -20ºC

Provincial, Gil, Guillén, and others (2010) reported a decrease in pH values in MAP samples of sea bass stored at 4ºC with high contents of CO_2 in the package. The drop in pH was attributed to the absorption of CO_2 on the fillet surface and a subsequence ionization of the carbonic acid. Similar results were previously reported by Tiffney and others (1982) where a decrease in pH values, as low as 6.09 in fish in 100% CO_2, was attributed to the absorption of

CO_2 into the fish's tissue verified by the collapsing of the packaging and decrease in headspace concentration. In contrast, a rise of pH values has also been reported to occur in MAP seafood during storage (Pastoriza and others, 1996). Alkaline compounds like ammonium, trimethylamine and other biogenic amines are produced and accumulate as a consequence of bacterial growth causing muscle damage (Ruiz-Capillas and others, 2005).

The initial collapsing of MAP-CO_2 packages observed at day 60 of frozen storage was possibly an indication of absorption of CO_2 into oyster meat and, coincidentally, the pH values were reported lower according to Figure 4.2. The measurement of pH is an important quality indicator. A sharp and quick decrease in tissue pH could suggest reduction of the CO_2 proportion in the gas mixture, however, an increase in pH could be attributed to deterioration by spoilage bacteria.

4.3.5 Drip loss of oyster meat

Drip loss refers to the most loosely bound water in muscle, and it is mainly associated with the structure of muscle and muscle cells, denaturation and degradation of proteins, and the rigor state of muscle (Huff-Lonergan and others, 2005). According to the results, MAP did not have an effect on drip loss. There were no significant differences between AIR, MAP-CO_2, and MAP-N_2 (Table 4.3). During storage, the drip loss continued to increase gradually to the point that significant differences were observed between the first and last day of frozen storage. In several MAP studies in seafood stored between 0 and 2ºC, it has been found by various authors that, the higher the levels of CO_2, the higher the drip loss (Masniyom, 2011; Pastoriza and others, 1996). This may be due to a greater loss of the water holding capacity of muscle protein at lower pH values. Dalgaard and others (1993) observed an increase in drip loss during modified atmosphere (MA) storage of cod fillets. Pastoriza and others (1998) observed

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increased exudation in MA-packaged fish after 7 days of storage. Fey and others (1982) found increased drip losses for red hake, chinook salmon and to a lesser extent, sockeye salmon stored in 60% CO_2:21% O_2:19% N_2 compared to air-packaged products.

Table 4.3: Drip loss (g drip liquid/100g sample) of oyster meat during frozen storage

Treatments	Days of storage at -20°C				
	1	15	30	90	180
Air	0.33±0.15[Ab]	0.43±0.12[Aab]	0.51±0.12[Aa]	0.67±0.10[Aa]	0.76±0.12[Aa]
MAP N_2	0.38±0.14[Ab]	0.43±0.04[Aab]	0.39±0.14[ABab]	0.66±0.10[Aa]	0.70±0.15[Aa]
MAP CO_2	0.38±0.12[Ab]	0.47±0.05[Aab]	0.55±0.08[Aab]	0.63±0.16[Aa]	0.77±0.12[Aa]

[AB]Means with different exponents in column row indicate significant difference
[ab]Means with different exponents in each row indicates significant difference

All the above mentioned MAP studies were performed in seafood products stored at temperatures above freezing where the effect of the gases could produce more observable and measurable changes. Information on the effect of MAP on drip loss in seafood during frozen storage could not be found to compare to the results obtained from this study. This could be explained by the fact that drip loss during frozen storage of muscle tissues is studied in relation to the effects of freezing processes and temperature fluctuation during frozen storage. Under this premise, the extent of liquid loss from any frozen food after thawing has been attributed to the rupture of cell structure and tissue by ice crystal growth during freezing (Goswami, 2010; Songsaeng and others, 2010). Furthermore, storage at frozen temperature may lower the water holding capacity of tissue. In addition, drip loss has been reported to increase as storage time increases caused by damage to muscle fibers by ice crystals resulting in leakage of various organelles during thawing (Benjakul and others, 2003). An accumulative drip loss below 2%

(w/) during frozen storage is regarded as very satisfactory (Kolbe and others, 2007) which means that all treatments performed well during frozen storage.

4.3.6 Lipid oxidation of oyster meat

AIR samples resulted in pronounced lipid oxidation during the last 90 days of frozen storage as compared to packaging in modified air. This can be seen from Fig. 4.3, where the TBARS in oyster meat packed in AIR were significantly higher than TBARS in MAP-CO_2 and MAP-N_2. For samples packed in modified air, there was no significant change in TBARS observed after 15 days of frozen storage.
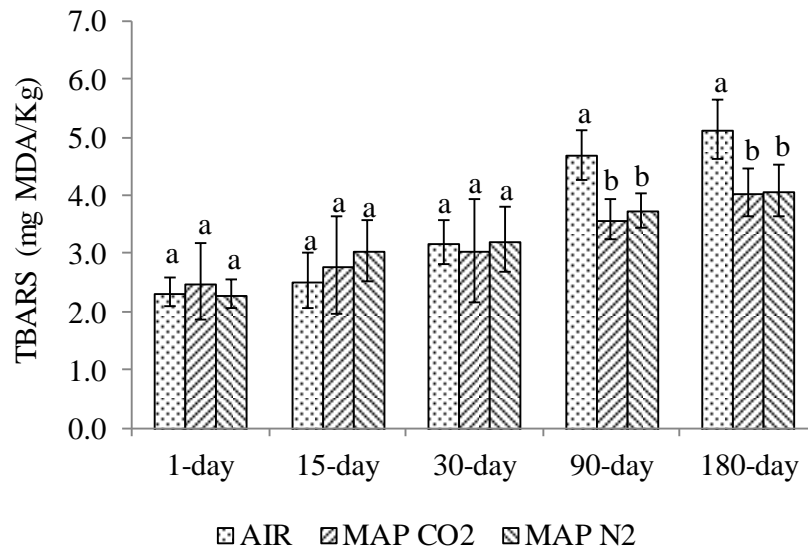
Figure 4.3: Lipid oxidation measured by determination of TBARS (mg malonaldehyde/kg) of oyster meat packed in AIR, MAP-CO_2, and MAP-N_2 during frozen storage at -20° C

The lower levels of TBARS recorded in the MAP-CO_2 and MAP-N_2 after 90 days of frozen storage were probably due to the exclusion of oxygen. The higher level of O_2 in AIR probably caused an increase of oxidative processes. This observation is also in agreement with the results reported in the case of shrimp stored at -15°C using low oxygen concentrations (Bono and others, 2012).

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Lipid oxidation is one of the most relevant degradation processes involved in seafood deterioration which is caused by endogenous activities and microbial enzymes. Fatty acids, particularly polyunsaturated fatty acids, are affected by oxidation, producing off-odors and off-flavors unpleasant to the consumer (Fernandez and others, 1997). TBARS values in seafood before objectionable odor/taste can be detected, as expression of lipid oxidation seems to vary according to seafood species. Consumption limits for TBARS have been reported to be 7-8 mg MDA/kg and a TBARS value less than 3 mg MDA/kg indicates a perfect quality material (Schormüller, 1968). Connell (1990) states that clean and pleasant flavors in fish are better perceived when malonaldehyde (MDA) values do not exceed 1-2 mg/kg. No referral to perception of unpleasant appearance, flavors or aromas was reported by Cruz-Romero and others (2008) in oyster meat after 20 days of storage in ice (2ºC) with TBARS values as high as 12 mg/kg

4.3.7 Texture of oyster meat

Compressive strength (CS) was tested in oyster meat as an index of tenderness/firmness. The CS value for fresh oyster meat was measured as 18.1 ± 1.5 Mpa. The results showed that AIR samples had lower CS values as compared to MAP-CO_2 and MAP-N_2 only during the first 30 days of frozen storage (Table 4.4). After this period of time, similar CS values for AIR and MAP-N_2 were reported, showing no significant differences. There was an interaction between MAP-CO_2 and storage time for oyster meat after 30 days. The CS of oyster meat increased with increasing storage time much higher than in AIR and MAP-N_2. Both AIR and MAP-N_2 samples after 180 days of frozen storage had lower CS than MAP-CO_2, but not as low as fresh oyster meat.

Texture is a valued indicator of freshness for oysters. In this study, the increase in CS observed in oyster meat in 100% CO_2 could possibly be related to temperature fluctuation during frozen storage and absorption of CO_2 into the tissue.

Table 4.4: Compressive strength (Mpa) as measurement of texture in AIR, MAP-CO_2, and MAP-N_2 oyster meat during frozen storage

Treatments	Days of Storage at -20° C				
	1	15	30	90	180
AIR	19 ± 1.7^{Ab}	19 ± 1.6^{Bb}	22 ± 2.1^{Bb}	27 ± 1.8^{Ba}	29 ± 3.7^{Ba}
MAP-CO_2	19 ± 1.2^{Ac}	24 ± 1.8^{Ab}	26 ± 1.2^{Ab}	32 ± 2.6^{Aa}	36 ± 3.8^{Aa}
MAP-N_2	18 ± 1.1^{Ab}	22 ± 1.5^{Aab}	24 ± 1.8^{Aab}	27 ± 2.3^{Ba}	29 ± 2.9^{Ba}

[AB]Means with different exponents in each column indicate significant difference
[abc]Means with different exponents in each row indicates significant difference

The toughening of seafood products during freezing and cold storage is well documented (Campanone and others, 2001; Sørensen, 2006). Changes such as protein denaturation, cross linking, loss in water holding capacity, and solubility are reported to affect the texture in fish during frozen storage. Salmon from frozen storage was firmer, less juicy and more fibrous (Refsgaard and others, 1998). Temperature fluctuation during frozen storage lead to moisture loss in the form of increased drip (Gormley and others, 2002) in a variety of frozen products which increased toughness.

MAP treatments with concentrations of CO_2 of 100% in raw whiting, mackerel and salmon (Fagan and others, 2004) had higher shear values than treatments with a mixture of 60% N_2:40% CO_2 which corroborates the results from Randell and others (1995) on the effect of gas/product ratio and CO_2 concentrations on the shelf life of MA fish. The toughening of samples was correlated to the decrease in pH by the absorption of CO_2 into tissues making it rubbery due to surface protein denaturation.

The decrease in pH observed in MAP-CO_2 samples might have produced a similar effect on the surface of oyster meat by the absorption of CO_2 which was reflected in the increased values of CS.

4.3.8 Amino acid profile of oyster meat

Essential amino acids were used for nutritional quality evaluation between MAP treatments during frozen storage. The amino acid compositions of oyster meat did not suffer significant changes in AIR, MAP-CO_2 and MAP-N_2 during frozen storage; however, lower contents of lysine (Lys) and arginine (Arg) were reported after 180 days in all treatments. Methionine (Met) content was also lower in AIR after 180 days (Table 4.5). The essential amino acids in oyster meat constituted approximately 31.4 – 32.6 per cent of total amino acids which is in agreement with results from Ridlington and others (1979), Ozden and others (2011), and the USDA (2013) in amino acid analysis of oysters. Glutamic acid (Glu) was found to be the most abundant amino acid in oyster. A 3-oz. serving of steamed oysters provides 1.55 g of aspartic acid (USDA, 2013). Glutamic acid is also known as glutamate and its primary role is that of a neurotransmitter that relays messages throughout your brain and influences memory. However, excessive amounts can result in diseases such as Amyotrophic lateral sclerosis (ALS), or Lou Gherig's disease (Aristoy and others, 2012). Aspartic acid (Asp) was the second most abundant amino acid in oyster meat. According to Topo and others (2009) consuming 3 grams of D-aspartic acid for 12 days significantly increased participants' testosterone levels, so this amino acid may be beneficial for strength training athletes or those deficient in testosterone. A 3-oz. serving of steamed oysters provides 1.55 g of aspartic acid (USDA, 2013).

Table 4.5: Amino acid profile (mg aa /g protein) of oyster meat during frozen storage

		30 days			90 days			180 days		
	Fresh	Air	MAP CO_2	MAP N_2	Air	MAP CO_2	MAP N_2	Air	MAP CO_2	MAP N_2
Asparagine	113.6^A	115.7^A	113.7^A	113.8^A	114.2^A	112.7^A	113.7^A	116.1^A	114.4^A	113.4^A
Threonine	48.5^A	48.2^A	48.2^A	49.1^A	48.2^A	48.8^A	47.4^A	48.5^A	48.1^A	47.9^A
Serine	46.9^A	46.8^A	46.1^A	46.7^A	46.8^A	46.3^A	46.7^A	46.6^A	46.8^A	47^A
Glutamic acid	164.6^A	164.8^A	166.2^A	164.7^{AB}	162.7^{AB}	160.2^{AB}	162.8^{AB}	163.4^A	159.7^{AB}	163^A
Proline	41.6^A	41.9^A	41.1^A	42.2^A	42.4^A	42.8^A	42.9^A	43.1^A	43.1^A	41.7^A
Glycine	45.7^{AB}	46.8^{AB}	46.8^{AB}	48.2^{AB}	50.9^{AB}	53.1^{AB}	50.8^{AB}	55.7^{AB}	57.6^A	54.4^{AB}
Alanine	57.9^B	57.2^B	55.4^B	57.1^B	60.4^B	58.1^B	58.2^B	68.2^A	64.5^{AB}	64.4^{AB}
Valine	50.6^A	51.1^A	50.5^A	50.4^A	50.5^A	51.1^A	50.3^A	49.1^A	48.4^A	48.4^A
Methionine	19.4^{AB}	19.1^{AB}	21.4^{AB}	22.4^{AB}	18.7^B	25.4^{AB}	24.9^{AB}	18^B	29.6^A	30.1^A
Isoleucine	47.8^A	46.8^A	46.2^A	47.1^A	46.3^A	46.5^A	45.7^{AB}	45.7^{AB}	45.1^{AB}	45.5^{AB}
Leucine	85.7^A	85.1^A	86.2^A	86.2^A	82.1^{AB}	84.5^{AB}	83.1^{AB}	80.8^B	80.3^B	81.1^B
Tyrosine	42.1^A	42.2^A	41.8^A	42.1^A	41.4^A	42.4^A	41.9^A	40.7^{AB}	40.9^{AB}	40.3^{AB}
Phenylalanine	41.7^A	41.6^A	42.1^A	42.1^A	40.8^A	41.2^A	41.3^A	40.5^{AB}	39.4^{AB}	38.6^{AB}
Histidine	20.5^B	22.8^B	23.4^{AB}	22.7^B	25.4^{AB}	26.2^A	26.7^A	26.5^A	27.2^A	27.2^A
Lysine	86.3^A	85.2^A	85.9^A	85.8^A	80.7^B	72.4^C	77.4^B	74.4^C	69.9^C	71.1^C
Arginine	87.1^A	84.7^{AB}	85.1^{AB}	79.4^C	88.5^A	88.4^A	86.2^A	83^C	85.6^{AB}	86.1^{AB}

^{AB}Mean values in the same row with different exponents indicate significant difference

Arginine (Arg), Lysine (Lys), and Leucine (Leu) were found in similar amounts constituting the next most abundant amino acids in oyster meat. Arginine aids in the production of nitric oxide, which helps blood vessels to dilate. This can make arginine helpful for conditions in which poor blood flow is responsible for adverse effects such as clogged arteries and vascular swelling (Sartoretto and others, 2013). Lysine aids in the absorption of calcium and can help make collagen, a structural component of skin and connective tissues. Diets low in protein could led to lysine deficiency, which can cause nausea, fatigue, slowed growth, reproductive disorders and anemia (Grygiel-Gorniak and others, 2012). Leucine has been reported to enhance endurance and promote increased strength and is marketed as a nutritional supplement for athletes due to its physiological effects (Crowe and others, 2006). The content of these amino acids in a 3-oz. serving of oysters is between 1.0 and 1.2 grams each (USDA, 2013).

4.3.9 Volatile profile components in AIR and MAP oysters

The analysis of aroma in oysters is a convenient means of checking their quality. The aromatic perception indicates to the consumer their state of freshness, necessary for the acceptability of the product. The identification of volatiles by the analytical determination of the individual compounds using gas chromatography coupled with mass spectrometry show an increase in acceptability to assess freshness in seafood (Fratini and others, 2012).

Analysis of volatile compounds by HSSPME showed slight variability between treatments. Table 4.6 lists the most potent volatile compounds found in fresh oyster meat and MAP samples after 180 days of frozen storage. The volatiles were grouped according to their most likely origin. A total of 22 volatile compounds were extracted in fresh oyster meat and AIR (180 days) which are in agreement with Zhang. and others (2010). In MAP-CO_2 and MAP-N_2, the extracted volatile compounds were 22 and 15 respectively. Higher number of volatile

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compounds were identified by Pennarun and others (2003), identifying 73 volatile compounds in raw oysters by vacuum hydrodistillation. A total of 52 were identified by Piveteau and others (2000) using a dynamic headspace volatile concentrator. The higher number of volatile components relied on the extraction and collection procedures which are time consuming and require expensive consumables. The analytical method developed by Zhang. and others (2010) which was followed in this study, provides quick, reliable, and sufficient information to characterize the fresh aroma characterization in oyster meat.

In the current study, among the identified volatiles shown in Table 4.6, the origin of 19 compounds was established. Most of them (10 volatile compounds) came from the degradation of fatty acids. Eight compounds came from the degradation of *n-3* fatty acids (Piveteau and others, 2000) and they were present in all treatments. Alcohols and ketones were in the highest proportions. The main ones were 1-penten-3-one, 1-penten-3-ol, and (*Z,Z*)-1,5-octadien-3-ol. This result is consistent with the large amount of *n*-3 poly unsaturated fatty acids (PUFAs) found in oysters along with the fact that *n*-3 PUFAs are very sensitive to oxidation. In oysters most of the volatiles arising from *n*-3 PUFAs are formed through enzymatic processes (Josephson, 1991). The 20:5 *n*-3 and 22:6 *n*-3, which are the main *n*-3 PUFAs in oysters, have been reported to be the most likely substrates for enzymatic oxidation producing mainly aldehyde and alcohol volatile compounds (Josephson and others, 1985).

Two compounds were identified which were the product of the oxidation of *n-6* PUFAs. Among them, pentanoic acid was found only in MAP-CO_2. The low proportion of these fatty acids in oysters accounts for the lower quantity of volatiles.

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Table 4.6: Volatile compounds (µg/100 g sample) identified in fresh oyster meat and MAP treatments

Compound	Origin	Fresh	After 180 days of frozen storage at -20º C		
			AIR	MAP-CO_2	MAP-N_2
PUFA oxidation					
1-penten-3-one	*n*-3	3.34[A]	3.16[A]	3.03[A]	3.11[A]
(*E,E,Z*)-1,3,5-octatriene	*n*-3	2.21[A]	2.16[A]	1.13[A]	2.32[A]
1-penten-3-ol	*n*-3	2.86[A]	2.44[A]	2.36[A]	2.52[A]
(*E,Z*)-2,6-nonadienal	*n*-3	1.13[B]	3.01[A]	2.02[A]	3.22[A]
(*Z*)-2-penten-1-ol	*n*-3	2.19[A]	2.54[A]	1.16[A]	2.16[A]
(*Z,Z*)-1,5-octadien-3-ol	*n*-3	3.40[A]	3.16[A]	2.18[A]	2.86[A]
3-methyl-2-butenal	*n*-3	1.16[A]	1.05[A]	1.22[A]	1.25[A]
14-octadecenal	*n*-3	1.21[A]	0.99[A]	1.12[A]	1.16[A]
pentanoic acid	*n*-6	0.0[B]	0.0[B]	1.55[A]	0.0[B]
1-octen-3-ol	*n*-6	1.63[A]	1.45[A]	1.88[A]	1.55[A]
fatty acid oxidation					
2,4-octadiene	Lipids	0.63[B]	2.16[A]	0.00[B]	1.85[B]
(*E,E,E*)-1,3,6-octatriene	Lipids	0.16[B]	3.18[A]	2.14[A]	1.02[A]
(*Z,Z,Z*)-1,3,5-octatriene	Lipids	1.1[B]	3.22[A]	2.66[A]	1.25[B]
3-octanone	Lipids	1.15[B]	4.22[A]	3.85[A]	0[B]
Amino acid degradation					
dimethyl disulfide	Amino acid	2.42[A]	0.45[B]	1.12[A]	1.86[A]
Carotenoid degradation					
toluene	carotenoids	1.15[A]	1.89[A]	1.25[A]	1.36[A]
6-methyl-5-hepten-2-one	carotenoids	0.54[A]	0.63[A]	0.88[A]	0.99[A]
Cedren – 13-ol, 8 –	carotenoids	3.58[A]	2.21[A]	2.85[A]	3.01[A]
Miscellaneous origin					
benzaldehyde	miscellaneous	2.54[A]	2.07[A]	2.33[A]	2.74[A]
3-pentanone	miscellaneou	0.52[A]	0.33[A]	0.32[A]	0.45[A]
p-xylene	polysaccharide	0.45[A]	0.33[A]	0.38[A]	0.38[A]

[AB]Mean values in the same row with different exponents indicate significant difference

Four aliphatic-hydrocarbon compounds arising from fatty acid (lipid) oxidation were identified but only 3 were present in MAP-CO_2. From these, (Z,Z,Z)-1,3,5-octatriene may have arisen from the dehydration from (Z,Z)-1,5-octadien-3-ol (Josephson, 1991).

Dimethyl sulfide was the only one volatile arising from amino acid degradation. Dimethyl sulfide is one of the most important aromas that characterize oysters, often described as the most odorant compound that relates to freshness (Nguyen and others, 2012)Dimethyl sulfide has been reported to derive from the oxidation of methanethiol which is a bacterial degradation product of methionine (Josephson and others, 1985; Zhang and others, 2010).

The identified volatiles coming from carotenoid oxidation were 3 compounds. The carotenoids may have originated from plant constituents in the diet of the oysters. Many seafood creatures have ripe seagrass seeds in their diet which contain these compounds, and hence they are found in the adult flesh (Piveteau and others, 2000).

Three volatile compounds having miscellaneous origins were identified. From these, benzaldehyde may have arisen from amino acid degradation (Piveteau and others, 2000). The synthesis of 3-pentanone could be possible in environments rich in lipids (Piveteau and others, 2000), and p-xylene may originate from degradation of polysaccharides (Akbar and others, 2012).

Trimethylamine (TMA) was not identified as a volatile compound in any of the treatments. TMA is a derivative of ammonia with a typical rotten fish odor at lower concentration and of an ammonia-like odor at higher concentrations (Yesudhason and others, 2010). Perception of TMA in seafood is often associated with decomposition and spoilage. With the appearance of TMA, a strong offensive and disgusting odor describes the deterioration

of seafood. In this study, such descriptors were not observed when opening the trays to prepare samples for the extraction of volatiles.

4.4   Conclusions

The present study demonstrated that oyster meat packed in modified atmosphere could have some advantages in maintaining its quality for longer periods of frozen storage at -20ºC. Nitrogen could give an overall better quality in relation to moisture content stability, lipid oxidation, package integrity, and the product's appearance than carbon dioxide and air.

Absorption of CO_2 in the oyster meat caused implosion of the package after 60 days of storage with a complete collapse after 150 days. In addition, the CO_2 absorption produced a decrease in pH which could negatively affect texture and appearance if storage was prolonged more than 180 days.

Although no significant differences were reported in most of the quality test performed between AIR and MAP-N_2 (color, pH, texture, amino acid and volatile profiles), the stability to temperature fluctuations during frozen storage could be the factor that favors MAP-N_2 over AIR for further development of microwavable oyster products.

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CHAPTER 5   INACTIVATION   OF   *VIBRIO   VULNIFICUS*   AND   *VIBRIO PARAHAEMOLYTICUS* IN CRYOGENIC FROZEN OYSTER MEAT USING STEAM VENTING TECHNOLOGY

5.1   Introduction

Vibrio vulnificus and *V. parahaemolyticus* are the pathogens most commonly associated with the consumption of raw oysters and have become the leading cause of many outbreaks (CDC, 2006, 2011, 2010a, 2010b, 2013). Several viable post-harvest techniques are available to reduce the bacterial load to innocuous levels. By nature, *Vibrio vulnificus* and *V. parahaemolyticus* are relatively susceptible to heat, and thus the utilization of microwave heating could be an alternative to conventional heating with the advantage of its rapid heating rate.

Microwave heating is usually not considered as a cooking method but a way to heat food products in a short period of time. The uneven heat distribution in microwave ovens is the main concern related to microbiological quality in food. Microbiological safety is often achieved by heating and thus if the food product is not adequately heated pathogenic microorganisms may survive. In addition to microbial safety concerns, microwave heating may induce texture damage due to uneven heating, poor yield due to moisture loss, and poor appearance (Mizrahi, 2012).

Self-venting technology has rapidly developed via several different approaches in steamed meals for microwave cooking. The packaging is designed so that steam builds during the cooking process. This technology utilizes the concept of pressurized steam cooking inside sealed plastic pouches or containers that have a self-venting release adaptation (Mast, 2000). Steam-venting technology for microwave cooking has been engineered to regulate cooking quality through controlled package expansion in conjunction with proprietary self-venting mechanisms (Fowle and others, 2005). During microwave cooking, food items absorb radio waves and are heated by dielectric heating. Rise in temperature, rapidly heats up the water

content of the food, producing steam. Positive pressure is created in the package by the production of steam and microwaving simultaneously resulting in reduced cooking time and evenly distributed heat (no hot spots). The package distends with the increase of the internal pressure and provides a visual indication that cooking is occurring while maintaining a temperature between 100ºC to about 105ºC (Mast, 2000). In addition, the production of steam and microwaving simultaneously results in reduced cooking time and evenly distributed heat which is an advantage in terms of food safety.

Microwave destruction of many microorganisms has also been reported, including: *Bacillus cereus, Campylobacter jejuni, Clostridium perfringens, E. coli, Enterococcus, Listeria monocytogenes, Staphylococcus aureus, Salmonella enteridis, Salmonella sofia, Proteus mirabilis* and *Pseudomonas aeruginosa, Aspergillus niger, Penicillium* and *Rhizopus nigricans* (Yaghmaee and others, 2005). Rodriguez-Marval and others (2009) evaluated the inactivation of *Listeria monocytogenes* in frankfurters to evaluate different power and time combinations of microwave oven heating to provide information to industry which would help to develop labeling instructions or guidelines for reheating. Similarly, research on the survival of *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7 on catfish fillets was performed by Sheen and others (2012) under different microwave power settings. However, applying microwave energy combined with steam venting technology to reduce or eliminate potential foodborne pathogens in oyster meat products has not been investigated.

Application of steam venting technology during microwave cooking of oyster meat could remediate the common food safety issues related to oysters, but also could remedy the negative effects of microwave heating, which usually leads to reduced palatability, moisture loss, and lower product yield. Therefore, the current research was focused on obtaining the necessary

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information for the inactivation of *Vibrio vulnificus* and *V. parahaemolyticus* in inoculated samples of oyster meat packed in microwavable trays sealed with a film having steam venting capabilities. The gathered information will be useful in the development of microwavable products having oyster meat as the main ingredient.

5.2   Materials and methods

5.2.1   Inoculation of oyster meat

Gulf oysters (*Crassostrea virginica*) were purchased from a seafood supplier in Houma, Louisiana and shucked to collect the meat. Three clinical strains of *Vibrio vulnificus* (ATCC® 27562, 7184, and 1007) and one strain of *Vibrio parahaemolyticus* (ATCC® 17802) obtained from the Food Safety/Food Microbiology laboratory at Louisiana State University Agricultural Center were used in this study. Separate cultures of *Vibrio vulnificus* and *Vibrio parahaemolyticus* were incubated on sodium chloride solutions of Bacto nutrient broth (Difco Laboratories, Detroit, MI) at 2% and 3% (wt. /wt.) respectively, at 37ºC for 12 h. Cells were allowed to reach stationary phase, as previously validated in Burnham and others (2009) until reaching 10^{6-7} CFU/mL in both *Vibrio sp.* Oyster meat was then collected and weighed (800 grams approximately) in sterile bags and then inoculated with both culture broths to obtain 10^{6} CFU of each vibrio species per gram of meat. The meat was left in the bag for 15 min at room temperature under a bacteriological hood. Inoculation effectiveness was verified by triplicate analysis of samples consisting of 12 oysters per sample.

5.2.2   Freezing of oyster meat

The oyster meat was cryogenically frozen until reaching an internal temperature of -20°C using liquid nitrogen in a cryogenic chamber (Ultra-freeze, Air Liquide Industrial U.S. LP). After freezing, oyster meat samples were immediately packaged in high barrier polypropylene (PP) microwavable trays in portions of 50 and 100 g, designated as 50F and 100F respectively.

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The trays were sealed with a flexible packaging film in a Multivac T-200 tray sealer (Multivac Inc, Kansas, MO) with nitrogen in the headspace. All samples were then stored in a household freezer (Frigidaire, Martienz, GA) for 12 h to simulate household conditions prior to analysis.

5.2.3 Temperature and pressure profiling during microwave cooking of oyster meat

Data collection of temperature and pressure during microwaving was performed in a Microwave Workstation (MW) (FISO Technologies Inc., Quebec, Canada) which included a 1100 watts microwave oven and 2450 MHz equipped with a turntable. Data were collected through the (MW) commander control. Four fiber optic temperature sensors (FOT-L-SD-C1, FISO Tech. Inc. Canada) and one fiber optic pressure sensor (FOP-C2-F2, FISO Tech. Inc. Canada) was used in the current study. Three of the temperature sensors were designated for measuring internal temperature in oyster meat and one for headspace temperature. For this purpose, frozen oyster meat was carefully perforated with a 2 mm stainless steel drill in the fattest portion before packing. For internal temperature measurement, the tip of the sensors (1, 2, and 3) were introduced in the center of the frozen oyster meat passing through the film and secured by special heat resistant septa to maintain the integrity of the. The oyster meat with the temperature sensors was randomly distributed inside the tray. For the headspace temperature and pressure measurements, the tip of the sensors (4 and 5, respectively) was carefully positioned in the center of the tray to avoid contact with the content in the package. The configuration of the temperature and pressure sensors in the package is showed in Figure 5.1. All tests were performed at 100% power with the turn table on. The measurement of the temperatures and pressure during microwaving were monitored and recorded until 90°C. This temperature was chosen as the maximum to avoid overcooking of the oyster meat.

The time and pressure at which the packaging was observed to vent were obtained from the data collected from the pressure sensor readings. The time of venting was determined by the quick pressure decrease from the pressure profile data during microwaving. In addition, the internal temperature of the oyster meat and package headspace temperature were measured.
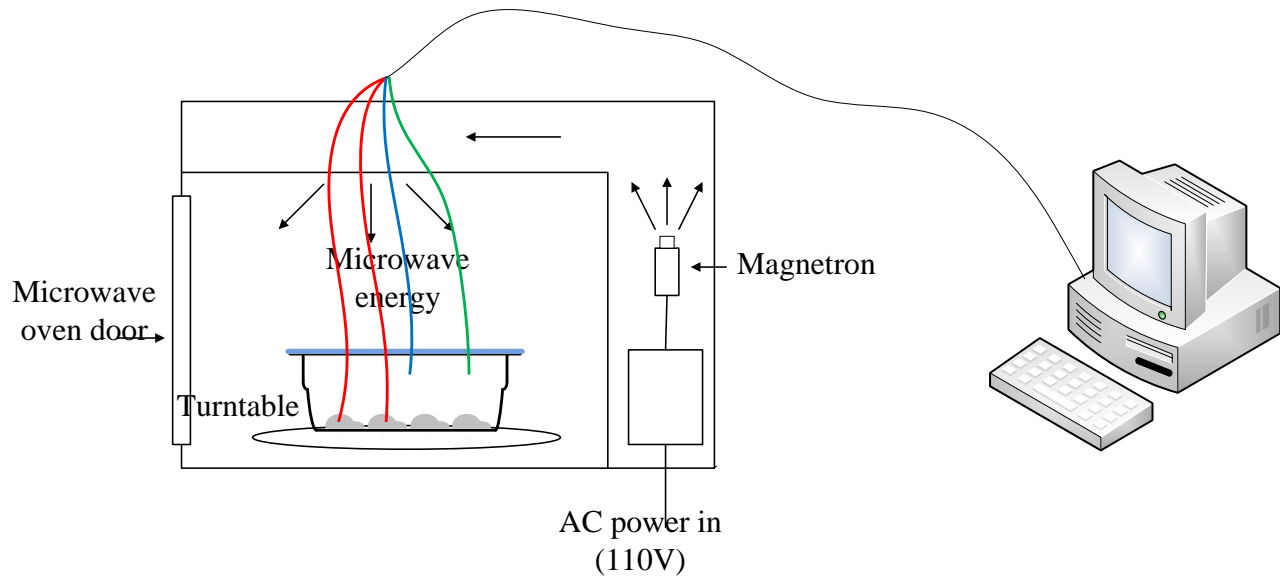
a)
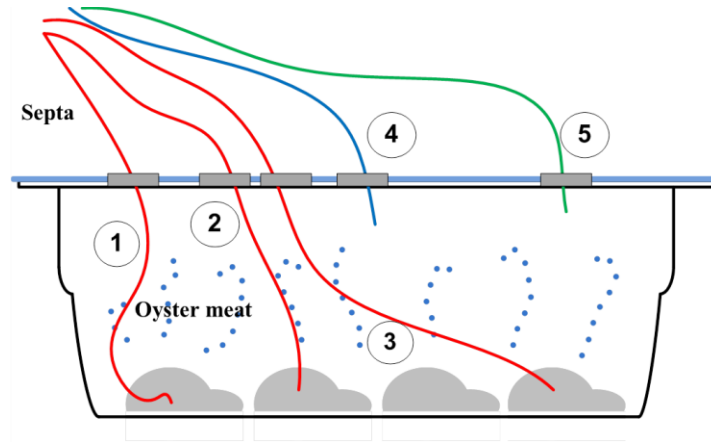
b)

Figure 5.1: a) Distribution of the internal temperature (1, 2, 3), pressure (4), and headspace temperature (5) sensors in the package, b) Schematic of the data collection using the Microwave Station

5.2.4 Reduction of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in oyster meat during microwave cooking

A household microwave oven equipped with a turntable, and with maximum power output of 1100W and 2450 MHz was used under a bacteriological hood. The trays containing 50g and 100g of oyster meat were microwaved for 0, 10, 20, 30, 40, 50, 60, 70, and 80s, at high power (100%). After microwaving, the trays were allowed to cool down for 2 min (standing time) for temperature equilibration. Following the standing time, the oyster meat was weighed in Whirl-pak® bag (Nasco, Salida, CA, U.S.A.) and phosphate-buffered saline solution (PBS) was added to yield an oyster homogenate of 1:3 (w/v). The mixture was homohenized (Stomacher 400, Tekman Co., Cincinnati, Ohio) for one minute and then analyzed for the identification and quantification of *Vibrio vulnificus* and *Vibrio parahaemolyticus* species by direct plating procedure for the enumeration of total and pathogenic *Vibrio vulnificus* and *Vibrio parahaemolyticus* in oyster meats (FDA/Gulf coast Seafood Laboratory Vp-ISSC-3) following Cook and others (2000). Trays containing 50 g and 100 g without film were used as controls to observe the effects of the film on the bacterial reduction. These samples were identified as 50NF and 100NF respectively.

5.2.5 Determination of moisture in oyster meat during microwave cooking

The moisture content of the oyster meat was measured during microwave heating at 10, 20, 30, 40, 50, 60, 70, and 80s. The trays were let stand for 1 min after each time segment before analysis. The oyster meat was extracted from the trays and homogenized for 30 s at medium speed in a Waring® commercial laboratory blender. The moisture content of the oyster meat was measured by drying 3 g of the homogenate in a draft oven at 105°C for 24 h (%) (AOAC International, 2005) in triplicate samples.

5.2.5   Statistical analysis

Means values from six measurements and/ or triplicate analysis were reported. Statistical analysis was done using the SAS (Statistical Analysis System) software (version 9.2) (SAS Institute Inc., Cary, NC, and U.S.A). Data was analyzed by Analysis of variance (ANOVA) following Tukey's studentized range test ($p < 0.05$).

5.3   Results and discussion

5.3.1   Temperature and pressure profiling during microwave cooking of oyster meat.

Heat treatments are important methods to provide safe foods. Conventional heat treatments involve the application of steam and recently microwave treatments have been studied and applied as they are considered to be fast, clean and efficient. Optical fiber sensing is an excellent tool to measure the temperature during microwave treatments. Optical fiber temperature and pressure sensing during the microwave cooking of 50 g of oyster meat is shown in Figure 5.2.

This figure depicts the evolution of the internal temperature of 50NF and 50F samples of oyster meat and the headspace temperature and pressure of the container in 50F during microwaving. During microwave cooking at 100% power, 50F's internal temperature profile showed a steep increase between -1℃ (20s) and 95º C (55s) and then it fluctuated between 95 and 97℃ until microwaving cooking was stopped. The heating caused thawing of the oyster meat to occur in a few seconds and then the rapid increase in internal temperature took place which is attributed to the absorption of microwaves and their interaction with the high water content of the oyster meat (Galema, 1997).
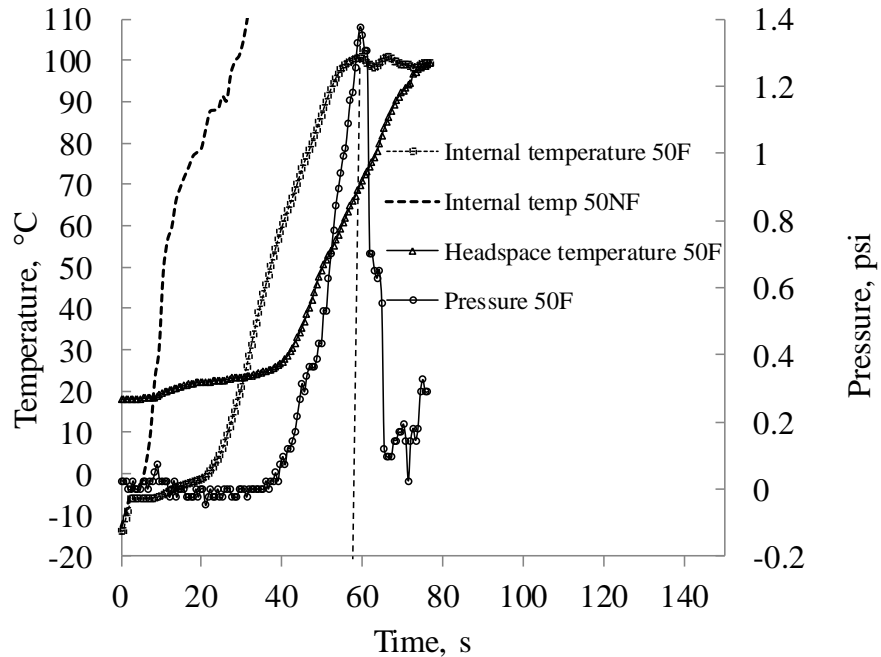
Figure 5.2: Temperature and pressure profiles of 50 g oyster meat during microwave cooking

Regarding the headspace temperature, it starts to increase when steam has been produced by the heating effect of the microwaves on the oyster meat, and it continues to rise and equalizes to that of the internal temperature. Headspace and internal temperature got very close when the container was saturated with steam during the last 10 seconds of microwaving (Fig 5.2). At this point the temperature of the oyster meat is the same both internally and upon the surface. When comparing to the internal temperature profile of 50NF (control) the effect of the film on the gentle development of internal temperature is evident. 50NF showed very drastic heating profiles in internal temperature reaching 90°C in 24 ± 3 s. The quick rise in internal temperature in 50NF was probably attributed to rapid absorption of microwaves causing intense dehydration and further over heating of the solids. It can be suggested that the film served as a protective barrier towards rapid moisture loss by decreasing the amount of microwaves absorbed in the oyster meat and thus ensuring a smoother increase of temperature.

The pressure profile during microwave cooking of 50F can also be observed in figure 5.2. The internal pressure in the container started to increase as steam was produced by the dielectric heating effect of the microwaves on the oyster meat. It can be observed that there is a clear correlation between the headspace temperature and internal temperature after 40 s of microwaving. The headspace pressure started to increase when enough steam was produced, which was observed by the shift in slope in the headspace temperature profile. The head space pressure continued to rise until reaching a breaking point at 1.4 psi after 61 ± 3 s. At this point, the package's film vented due to the weakening of the internal layer of the film. The internal temperature of 50F was 99 ± 3ºC when venting occurred. During these trials, the steam venting occurred at the corners of the trays. Knoerzer and others (2009), Zhang and others (2001), and Risman and others (1987) reported that high intensity electromagnetic fields occur in the corner and edges of containers which increase the temperatures in those areas. It can be suggested that higher temperatures developed in the corners, weakening the film and producing rupture of the film (venting). After venting occurred, the release of steam did not affect the headspace temperature, which continued to increase according to Figure 5.2. This could mean that steam continued to be produced and released in a control manner until the microwaving process was stopped.

After venting, the headspace pressure decreases to values close to the zero-reference pressure (atmospheric pressure). The sporadic rising of headspace pressure after venting was provided by the special properties of the film (Polymers, 2010). The internal layer of the film used in this study aligned with the border of the tray when the amount of steam released decreased due to cycling of the microwave oven. This allowed the film to make contact with the

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tray sealing it again. However, venting occured at a lower pressure due to disruption of the original seal (Polymers, 2010).
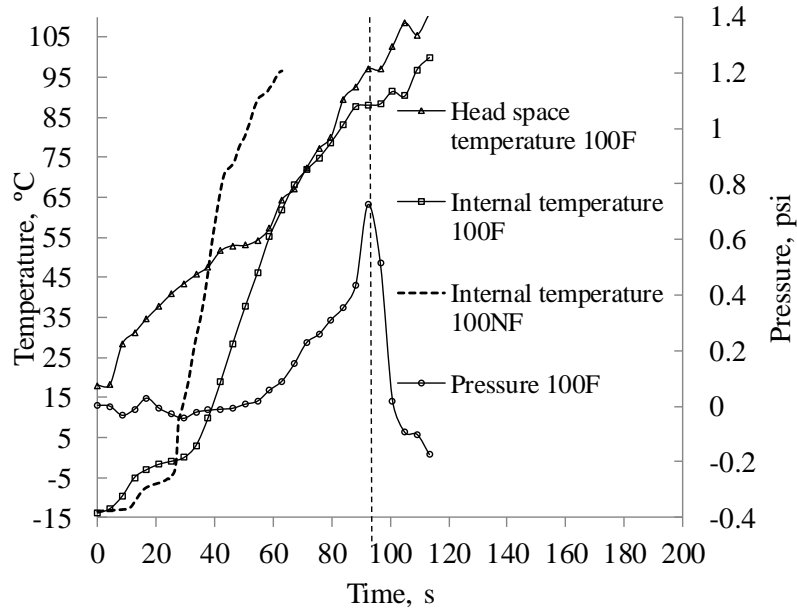
Figure 5.3: Temperature and pressure profiles of 100 g oyster meat during microwave cooking

Internal and headspace temperatures as well as pressure profiles for 100F and 100 NF showed the same trend as 50F and 50NF with some variations (Fig 5.3). 100NF showed similar rapid increase in temperature as in 50NF, however; the time to reach 90º C in 100F was 56 ± 2 s which is approximately 2.5 times longer than 50 NF.

In addition, the venting time and pressure were observed to occur at 94 ± 3 s and 0.73 ± 0.3 psi respectively. The venting time in 100F took 1.53 times longer than in 50F while venting pressure was 52% lower than in 50F. Comparisons of treatments' important microwave cooking events related to their temperature and pressure profiles are summarized in Table 5.1. The prolonged time required in 100F to reach similar temperature values as that of 50F could be explained by the double amount of oyster meat in the tray. Microwave heating times are directly related to the amount of product being heated (Geedipalli and others, 2007). Vadivambal and

others (2010) reported that doubling the quantity of a product causes an increase in heating time of at least 50% to obtain similar internal temperature values. This is related to the amount of energy absorbed in the product. In a microwave oven most of the microwave power is directly absorbed by the food being heated (Vadivambal and others, 2010). The rate of heating (heating power) is, therefore, fixed by the power of the oven. The rate of temperature increase in the food is therefore inversely proportional to the mass of food multiplied by the specific heat capacity of the food. This suggests that a double mass of food halves the rate of temperature rise, and the food would take as twice as long to reach the same temperature (Vadivambal and others, 2010).

Table 5.1 Results of microwave cooking of oyster meat in steam venting packages

Microwave cooking	50 g	100 g
Venting, s	61 ± 3^B	94 ± 3^A
Internal temperature at venting, ºC	99 ± 3^A	88 ± 4^B
Head space temperature at venting, ºC	68.1 ± 8.4^B	97 ± 3^A
Venting pressure , psi	1.4 ± 0.3^A	0.73 ± 0.3^B
Time to reach 90ºC (Internal temp),s	59 ± 4^B	98 ± 4^A

[AB]Means with different exponents in each row indicate significant difference

5.3.2 Inactivation of *Vibrio vulnificus Vibrio parahaemolyticus* in oyster meat during microwave cooking in steam venting packaging

The steam venting packaging had an effect on the time needed to inactivate both *Vibrio spp*. The log reduction of *V. vulnificus* for 50NF and 50F during microwave cooking is shown in Table 5.2. The reduction of *V. vulnificus* to nondetectable levels occurred after 20 s of microwave cooking of 50NF. In contrast, it took 50 s to produce the same effect in 50F samples (Table 5.2). However, in both treatments, the inactivation was believed to be caused by temperature. The internal temperatures of the oyster meat as well as the temperature in the headspace for 50F are also included in this table. It can be observed that the internal temperature

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in 50NF was 48 ± 3ºC at 10 s and reached 80 ± 3ºC by 20 s of microwave cooking. *V. vulnificus*

is a temperature sensitive bacterium with an optimum growing temperature of approximately

37ºC in appropriate levels of pH (6.5-7.0) and salinity (2%) (Ama and others, 1994; Andrews

and others, 2000).

Table 5.2 Effect of microwave cooking on the logarithmic reduction of *Vibrio vulnificus*[x] (log_{10} CFU/g) in 50 g of oyster meat

Time, s	Log reduction (50NF)	Internal temperature, ºC	Log reduction (50F)	Internal temperature, ºC	Headspace temperature, ºC
0	1.72 ± 0.16[Ac]	-14 ± 2	0.57 ± 0.21[Ae]	-14 ± 2	17 ± 3
10	3.86 ± 0.28[Ab]	48 ± 3	0.61 ± 0.20[Ae]	-5 ± 2	19 ± 3
20	Not detected*[Aa]	80 ± 3	1.66 ± 0.23[Bd]	-1 ± 2	22 ± 4
30	Not detected*[Aa]		2.44 ± 0.32[Bc]	21 ± 2	23 ± 4
40	Not detected*[Aa]		3.62 ± 0.19[Bb]	60 ± 2	27 ± 4
50	Not detected*[Aa]		Not detected*[Aa]	87 ± 2	49 ± 6
60	Not detected*[Aa]		Not detected*[Aa]	100 ± 2	71 ± 3
70	Not detected*[Aa]		Not detected*[Aa]	99 ± 2	93 ± 3

  Time = 0 refers to the time before microwaving, after 12 h in household freezer
  50NF and 50F denote 50 g of oyster meat with no film and with film, respectively
  [x]Initial inoculation level of 6.0 log_{10}CFU/g
 [abcde]Means with different exponents in each column indicates significant difference
 [AB]Means with different exponents in each row indicate significant difference
* ≥ 6 log reduction denotes nondetectable levels

   Kaspar and others (1993) reported a reduction of 90% in *V. vulnificus* numbers when

samples were held at 45ºC for six days, which indicated that the survival of *V. vulnificus* could

be reduced at temperatures above 37ºC. Heating oysters for 10 min in water at 50 C was

adequate to reduce *V.vulnificus* to a nondetectable level (Cook and others, 1992). This indicated

that the temperatures reached during microwaving cooking were enough to inactivate *V.

vulnificus* in 50NF. In 50F the internal temperature at 40 s reached 60 ± 2ºC which may caused

inactivation. The significant log reduction observed between 20 and 30 s in 50F was possibly

the result of other factors other than those related to thermal effects, which will be addressed

later in this document.

Inactivation of *V.vulnificus* in 100NF and 100F showed a similar pattern as in 50F and 50NF. *V.vulnificus* was inactivated after 50 s of microwave cooking in 100NF, and after 60 s in 100F (Table 5.3). The log reduction of *V. vulnificus* in 100F presented an interesting case. It was observed that significant reduction occurred after 20 s, although the internal temperature was low. The inactivation of *V. vulnificus* in 100F could be explained using the headspace temperature profile. The exposure to higher temperatures developed in the headspace at 20, 30, 40, and 50 s (38 ± 3, 44 ± 3, 50 ± 3, and 53 ± 2ºC, respectively) possibly caused the inactivation; especially in this study, where the inoculation of the *Vibrio spp.* was performed at the surface of the oyster meat.

Table 5.3 Effect of microwave cooking on the logarithmic reduction of *Vibrio vulnificus*[x] ($\log_{10}$ CFU/g) in 100 g of oyster meat

Time, s	Log reduction (100NF)	Internal temperature (100NF), ºC	Log reduction (100F)	Internal temperature (100F), ºC	Head space temperature (100F), ºC
0	0.33 ± 0.17[Ae]	-14 ± 1	0.58 ± 0.21[Ae]	-14 ± 2	18 ± 2
10	0.61 ± 0.10[Ae]	-13 ± 3	0.58 ± 0.22[Ae]	-8 ± 2	30 ± 2
20	1.69 ± 0.20[Ad]	-7 ± 3	0.62 ± 0.22[Be]	-1 ± 3	38 ± 3
30	2.38 ± 0.22[Ac]	15 ± 4	2.35 ± 0.17[Bc]	0 ± 2	44 ± 3
40	4.03 ± 0.31[Ab]	57 ± 4	3.71 ± 0.19[Bc]	15 ± 3	50 ± 3
50	Not detected*[Aa]	80 ± 3	4.88 ± 0.18[Bb]	37 ± 3	53 ± 2
60	Not detected*[Aa]	93 ± 3	Not detected*[Aa]	57 ± 4	59 ± 2
70	Not detected*[Aa]		Not detected*[Aa]	70 ± 3	70 ± 3

Time = 0 refers to the time before microwaving, after 12 h in household freezer
100NF and 100F denote 100 g of oyster meat with no film and with film, respectively
[x]Initial inoculation level of 6.0 $\log_{10}$CFU/g
[abcde]Means with different exponents in each column indicates significant difference
[AB]Means with different exponents in each row indicate significant difference
* ≥ 6 log reduction denotes non-detectable levels (< 30 MPN/gram)

As well as *V. vulnificus*, *Vibrio parahaemolyticus* also was temperature sensitive, however it showed more heat resistance according to the results from microwaving cooking (Table 5.4). Inactivation of *V. parahaemolyticus* in 50NF occurred at 30 s (102 ± 5ºC) which was 10 s more than *V. vulnificus*. Similarly, nondetectable levels were reported at 50 s (87 ±

2ºC) in 50F, again, 10 s more than in *V. Vulnificus*. The inactivation of *V. parahaemolyticus* in

100 g of oyster meat showed similar patterns as shown in Table 5.5. In 100 NF, the inactivation

of *V. parahaemolyticus* occurred after 50 s of microwave cooking which correlated to an internal

temperature of 80ºC, while reduction to nondetectable levels was reported after 60 s in 100F

(57ºC).

Table 5.4 Effect of microwave cooking on the logarithmic reduction of *Vibrio parahaemolyticus*[x] (log$_{10}$ CFU/g) in 50NF and 50F

Time, s	Log reduction (50NF)	Internal temperature, º C	Log reduction (50F)	Internal temperature, º C	Head space temperature, º C
0	0.43 ± 0.18^{Ac}	-14 ± 2	0.43 ± 0.09^{Ad}	-14 ± 2	17 ± 3
10	0.63 ± 0.28^{Ac}	48 ± 3	0.45 ± 0.10^{Ad}	- 5 ± 2	19 ± 3
20	4.51 ± 0.17^{Ab}	80 ± 3	1.48 ± 0.11^{Bc}	-1 ± 2	22 ± 4
30	Not detected*^{Aa}	102 ± 5	1.61 ± 0.07^{Bc}	21 ± 2	23 ± 4
40	Not detected*^{Aa}		3.76 ± 0.16^{Bb}	60 ± 2	27 ± 4
50	Not detected*^{Aa}		Not detected*^{Aa}	87 ± 2	49 ± 6
60	Not detected*^{Aa}		Not detected*^{Aa}	100 ± 2	71 ± 3
70	Not detected*^{Aa}		Not detected*^{Aa}	99 ± 2	93 ± 3

Time = 0 refers to the time before microwaving, after 12 h in household freezer
50NF and 50F denote 50 g of oyster meat with no film and with film, respectively
[x]Initial inoculation level of 6.0 log$_{10}$CFU/g
[abcd]Means with different exponents in each column indicates significant difference
[AB]Means with different exponents in each row indicate significant difference
* ≥ 6 log reduction denotes non-detectable levels

Johnston and others (2002) report that *V. parahaemolyticus* had higher heat resistance

properties than *V. vulnificus* after cold shock treatments   The authors observed that *V.*

parahaemolyticus underwent morphology changes that might have contributed to its longer

survival when exposed to higher temperatures, as high as 70ºC. The authors observed that most

of the rod-shaped cells of *V. parahaemolyticus* shrunk and became coccoid when stored at 4ºC.

The reduction in size was believed to be a means of minimizing the requirements for cell

maintenance, and protects non-spore-forming bacteria against environmental stresses. It is

possible that the exposure to low temperatures during freezing and short cold storage before

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microwaving could have provided similar characteristics to *V. parahaemolyticus* in this study, and thus it could explain the resistance to inactivation during microwave cooking compared to *V. vulnificus*.

Table 5.5 Effect of microwave cooking on the logarithmic reduction of *Vibrio parahaemolyticus*[x] ($\log_{10}$ CFU/g) in 100 g of oyster meat

Time, s	Log reduction (100NF)	Internal temperature (100NF), ° C	Log reduction (100F)	Internal temperature (100F), ° C	Head space temperature (100F), ° C
0	0.33 ± 0.18^{Ae}	-14 ± 1	0.58 ± 0.17^{Af}	-14 ± 2	18 ± 2
10	0.61 ± 0.10^{Ae}	-13 ± 3	0.72 ± 0.11^{Af}	-8 ± 2	30 ± 2
20	1.69 ± 0.20^{Ad}	-7 ± 3	1.66 ± 0.23^{Ae}	-1 ± 3	38 ± 3
30	2.38 ± 0.22^{Ac}	15 ± 4	2.44 ± 0.32^{Ad}	0 ± 2	44 ± 3
40	4.03 ± 0.31^{Ab}	57 ± 4	3.62 ± 0.19^{Ac}	15 ± 3	50 ± 3
50	Not detected*^{Aa}	80 ± 3	4.32 ± 0.20^{Bb}	37 ± 3	53 ± 2
60	Not detected*^{Aa}	93 ± 3	Not detected*^{Aa}	57 ± 4	59 ± 2
70	Not detected*^{Aa}		Not detected*^{Aa}	70 ± 3	70 ± 3

Time = 0 refers to the time before microwaving, after 12 h in household freezer
100NF and 100F denote 100 g of oyster meat with no film and with film, respectively
[x]Initial inoculation level of 6.0 $\log_{10}$CFU/g
[abcdef]Means with different exponents in each column indicates significant difference
[AB]Means with different exponents in each row indicate significant difference
* $\geq$ 6 log reduction denotes nondetectable levels

The mechanisms of destruction of microorganisms through the action of microwaves are controversial. It has been stated that inactivation of microorganisms by microwave is entirely by heat, through the same mechanisms as other biophysical processes induced by heat, such as denaturation of proteins, nucleic acids or other vital components, as well as disruption of membranes (Datta and others, 2000). However, the current study produced bacterial reduction at sub lethal temperatures during microwave cooking contradicting the former statement. The destruction of microorganisms during microwave heating has been linked to non-thermal effects, as a lower temperature is shown to cause killing of microorganisms (Dumuta-Codre and others, 2010).

Four predominant theories have been used to explain non-thermal inactivation by microwaves or "cold pasteurization": selective heating, electroporation, cell membrane rupture, and magnetic field coupling. The selective heating theory states that solid microorganisms are heated more effectively by microwaves than the surrounding medium and are thus killed more readily. Electroporation is caused when pores form in the membrane of the microorganisms due to electrical potential across the membrane, resulting in leakage. Cell membrane rupture is related in that the voltage drop across the membrane causes it to rupture. In the fourth theory, cell lyses occurs due to coupling of electromagnetic energy with critical molecules within the cells, disrupting internal components of the cell (Kozempel and others, 1998). Although there is a controversy about the mechanisms of microwave-induced death of microorganisms, there is no doubt about the destructive effect of microwaves.

5.3.3 Moisture of oyster meat during microwave cooking in steam venting packages

The utilization of steam venting packaging had an effect on the moisture behavior of oyster meat during microwave cooking. The moisture in 50NF samples was observed to decline very rapidly from the initial moisture value of 89.46 ± 0.67 % (Figure 5.4). After 25 s of microwave cooking 50NF samples were reported to have 39.2 ± 2.6% of moisture, at which time the internal temperature reached 90ºC. There was also a decrease in moisture content in 50F. As microwave cooking progressed, moisture content in 50F showed a steady decrease; however, the values of moisture content did not reached the values reported in 50NF. The moisture content of 50F was 75.67 ± 4.22 % when the internal temperature of the oyster meat reached 90ºC. The internal temperature of 90º C was reached after 60 s.

The moisture content in 100NF and 100F followed similar trend as in 50NF and 50F during microwave cooking. Moisture content in 100NF at an internal temperature of 90ºC was 40.6 ±

2.3%. This value is close to the one obtained in 50NF, with the difference that 100NF moisture content was reached after 60 s of microwave cooking instead of 25 s in 50NF.
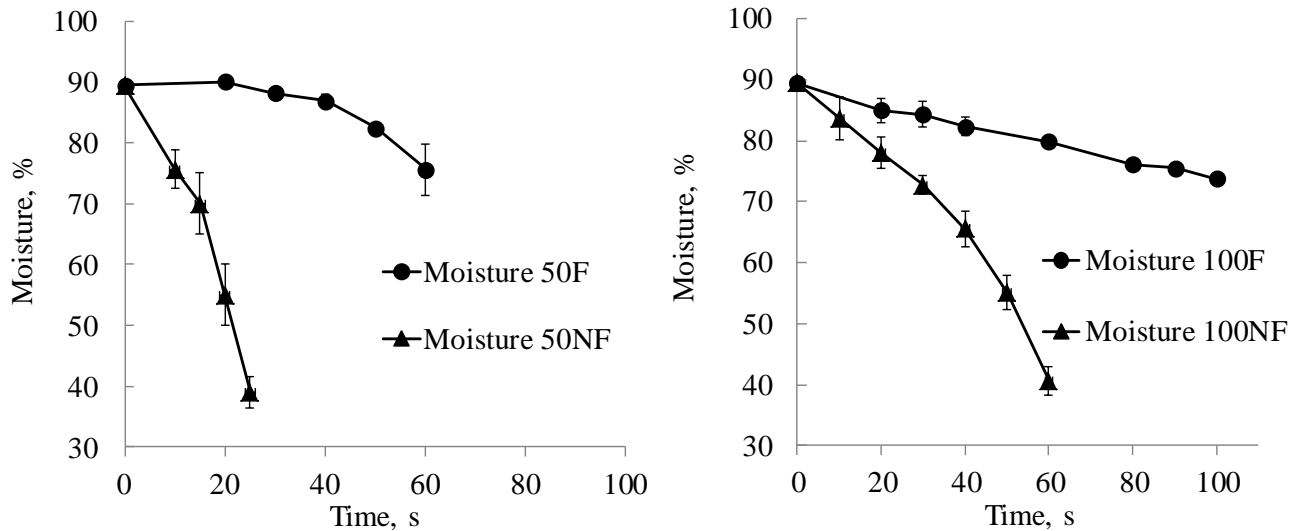

Figure 5.4: Moisture content (wet basis) 50F and 50NF during microwave cooking

The faster decrease in moisture in 50NF and 100NF could be explained by the absorption of microwave energy in the meat. This effect can be verified by the sharp increase in internal temperature shown in Figures 5.1 and 5.2 for the treatments without film (50NF and 100NF). This absorption occurred so rapidly that the meat was severely damaged. The abrupt increase in temperature caused disruption of tissue, and in some cases, the damage was so extensive that some pieces burst. The higher standard deviation in moisture content observed in 50NF and 100NF was caused by the effects mentioned above. In contrast, samples with film (50F and 100F) did not suffer damage.

It is probable that the steam produced during microwave cooking and trapped in the package by the film, could dissipate the direct absorption of microwaves by the meat. Microwaves excite water molecules by the effect of dielectric heating (Meier and others, 1998), and steam , which is water vapor, could absorb some of the microwave energy. As a consequence, steam could be heated causing an increase in the headspace temperature. The

increase in headspace temperature would not depend completely on the generation of steam by the heating of the product. As a result, a more gradual and uniform heating effect would occur, this could explain the higher moisture retention in 50F and 100F without damaging the meat.

5.4   Conclusions

Inactivation of *Vibrio vulnificus* and *Vibrio parahamolyticus* in cryogenically frozen oyster meat was successfully accomplished using steam venting packaging while maintaining satisfactory moisture content levels. Total inactivation of both species was achieved after 50 s and 60 s of microwave cooking at 100% power in 50 g and 100 g of oyster meat, respectively. The temperature range necessary to reduce the pathogens to non-detectable levels were obtained by correlating the data collected by the Microwave Station and the results from microbiological test. During microwave cooking, it was established that the inactivation of both pathogens in oyster meat can be achieved at internal temperatures between 70ºC and 80ºC.

Optical fiber sensing was an excellent tool to measure the temperature during microwave treatments. Precise measurement of temperatures for the inactivation of the targeted bacteria was possible. Thus, the use of optical fiber sensors resulted to be useful to develop protocols for product development.

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CHAPTER 6   DEVELOPMENT OF A MICROWAVABLE PRODUCT HAVING OYSTER MEAT AS THE MAIN INGREDIENT USING STEAM VENTING TECHNOLOGY

6.1   Introduction

Microwaveable foods are a group of ready-made, prepackaged, frozen, or prechilled products that can be consumed with minimal preparation. Conventional heat treatments involve the application of steam and recently microwave treatments have been studied and applied as they are considered to be fast, clean and efficient.

Self-venting technology has rapidly developed via several different approaches to be applied in steamed meals for microwave cooking. Steam-venting technology for microwave cooking has been engineered to regulate cooking quality through controlled package expansion in conjunction with proprietary self-venting mechanisms (Fowle and others, 2005). In addition, the production of steam and microwaving simultaneously results in reduced cooking time and evenly distributed heat. From the food safety point of view, microwave cooking using steam venting technology could assure the inactivation of harmful organisms. In general, each thermal process must be considered in its own right and it is important to choose the organism most likely to carry the greatest risk. However, control and uniformity of temperature is crucial to achieve lethality. At a temperature of 70ºC for two minutes the outcome is roughly the same as 43 min at 60ºC and less than 10 seconds at 80ºC based on calculations for *Clostridium botulinum*, related to *F, Fo*, and *z*-values (Fowle and others, 2005).

In seafood products, steaming is a process that usually provides desirable characteristics. Steaming is a gentle, fat-free cooking method that retains the natural moisture of foods. This feature makes it an excellent choice for preparing delicate meals, especially those having oysters as main ingredient. It is believed that with the emerging category of value added meal solutions the development of oyster-based products will create new opportunities for oyster processors to

expand their market. Therefore, the current research provides a protocol for the development of a microwavable product formulation that can retain high moisture, improve product yield, and maintain textural qualities in oyster meat as main ingredient.

6.2   Materials and methods

6.2.1   Experimental design and sample preparation

The guidelines to a microwavable product having oyster meat as the main ingredient were based on the results obtained from studies in chapter V in the inactivation of *Vibrio vulnificus* and *Vibrio parahaemolyticus* packed in microwavable trays with steam venting capability.

Steam venting packages containing frozen oyster meat and frozen vegetables were developed based on the following criteria:

1.   The internal temperature of the oyster meat should reach 90ºC to assure food safety within 300 s of microwave cooking

2.   The oyster products should maintain most of the fresh oyster's meat quality characteristics related to the maximum retention of moisture in the meat, good appearance, and texture.

The experimental process design is depicted in the Figure 6.1. The process of elimination/optimization of the microwavable product was performed by the evaluation of three parameters: a) overall product weight loss during microwaving, b) weight loss of oyster meat, and c) maximum retention of moisture in the meat.

6.2.2   Freezing of oyster meat

Fresh oyster meat was obtained from a local seafood store in Baton Rouge, LA., and transported on ice to the Food Processing Pilot Plant, Louisiana State University Agricultural Center. The oyster meat was drained and immediately arranged on aluminum trays which were

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previously covered in aluminum foil and a freezer paper was used to cover the oyster meat. A cabinet type cryogenic freezer with liquid nitrogen (Air liquid, Houston, Texas) was used for cryogenic freezing. Three thermocouples (Comark®, Comark Limited, Stevenage, Herts, UK) connected to a data logger were used to monitor the temperature during freezing. The thermocouples were inserted at the geometrical center of the oyster meat. The oyster meat was frozen until the temperature at the center of the oyster meat reached -20ºC. After freezing, the meat was collected in Ziploc bags and stored in a freezer at -20ºC.
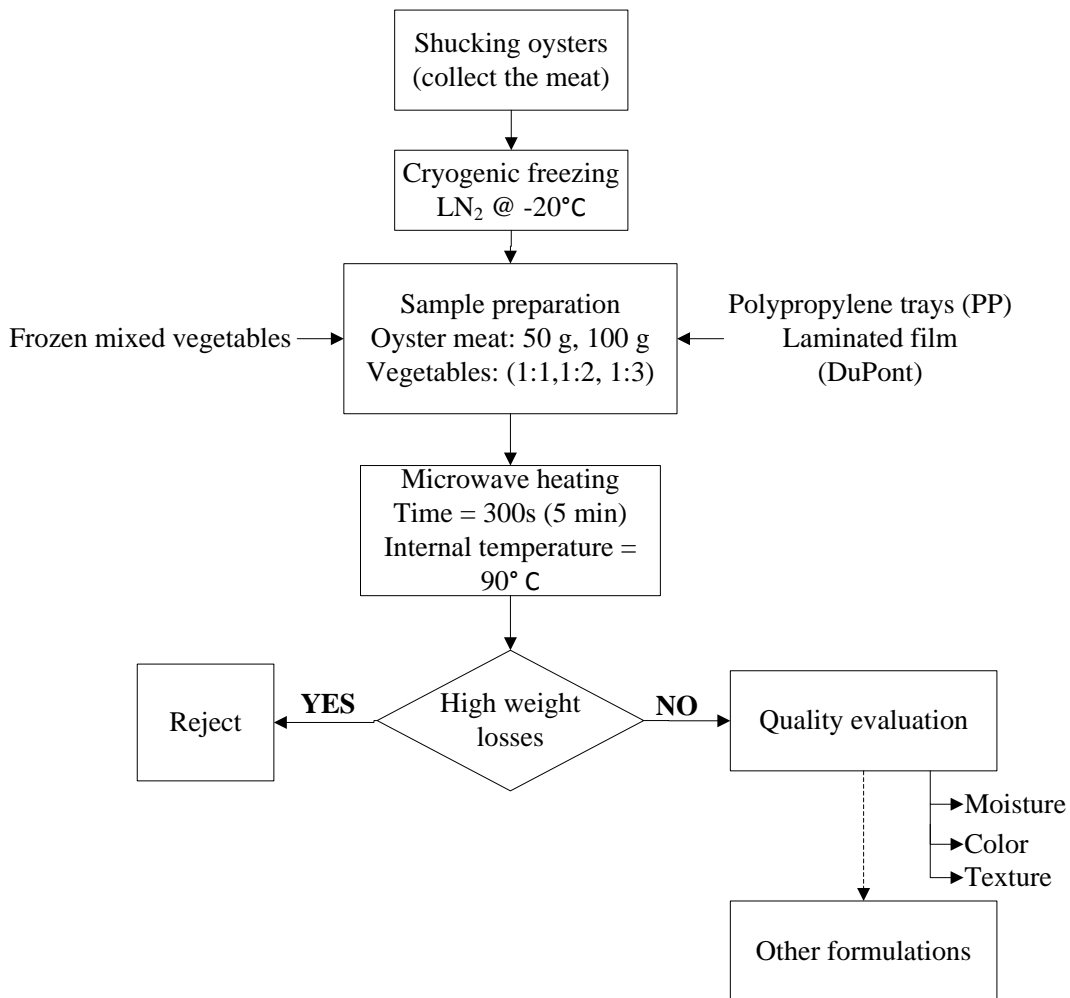

Figure 6.1: Flow diagram for the development of microwavable products containing oyster meat and frozen vegetables

6.2.3 Development of steam venting packages containing oyster meat and frozen vegetables

To retain the maximum amount of moisture in the oyster meat, preliminary experiments demonstrated that the inclusion of at least one more ingredient was necessary. This ingredient would serve as main water source for microwave cooking. Frozen mixed vegetables (MV) were utilized for this purpose and were purchased in a local store in Baton Rouge, Louisiana, and transported on ice to the Food Processing Pilot Plant at Louisiana State University Agricultural Center and stored at -20º C. The mixture of vegetables contained carrots, broccoli, green beans, and corn. Six combinations of oyster meat (OM) and MV were evaluated. Trays were prepared with proportions of OM to MV of 1:1, 1:2, and 1:3 based on 50 g and 100 g of OM.

6.2.4 Temperature and pressure profiling during microwave cooking of formulations

As a preliminary step, the internal temperature profile of OM was obtained for each of the six combinations. Data collection of temperature and pressure during microwaving was performed in a Microwave Workstation (MW) (FISO Technologies Inc., Quebec, Canada) which included a 1100 watts microwave oven and 2450 MHz equipped with a turntable. Data were collected through the (MW) commander control. Fiber optic temperature sensors (FOT-L-SD-C1, FISO Tech. Inc. Canada) and one fiber optic pressure sensor (FOP-C2-F2, FISO Tech. Inc. Canada) were used in the current study. In addition to the internal temperature of the oyster meat, microwave cooking events such as venting time, pressure, and headspace temperature were collected. For each combination, triplicate data were collected in three repetitions and averaged.

6.2.5 Preparation of samples

Frozen MV and cryogenically frozen OM were weighed in trays and sealed in a Multivac T-200 tray sealer (Multivac Inc, Kansas, MO) using nitrogen as headspace gas. The trays were stored at -20ºC. Before testing, the trays were allowed to equilibrate for 24 h in a household

freezer (Frigidaire, Martienz, GA) to simulate household conditions. All trials were performed in sets of five samples in three repetitions.

6.2.6 Product weight loss during microwave cooking

The product (oyster meat + vegetables + tray with film) was weighed before and after 50, 80, 110, 170, 200, 230, and 300 s of microwave cooking. Two min of standing time were allowed after each period of time before weighing. The weight loss was calculated following the formula (Eq.1):

$$\%Weight\ loss = \frac{(weight\ of\ product\ before\ microwaing - weight\ of\ product\ after\ microwaving\ )}{weight\ of\ product\ before\ microwaving} \ x\ 100 \qquad [1]$$

6.2.7 Oyster meat weight loss

After weighing of the product to measure weight losses, the oyster meat was extracted from the trays and weighed separately during the same period of time (50, 80, 110, 170, 200, 230, and 300 s of microwave cooking). The weight loss of the oyster meat was calculated by using the formula (Eq.2):

$$\%Weight\ loss = \frac{(weight\ of\ frozen\ meat - weight\ of\ meat\ after\ microwaving\ )}{weight\ of\ frozen\ meat} \ x\ 100 \qquad [2]$$

6.2.8 Moisture content of the oyster meat

After the weighing of the oyster meat extracted from the trays, the meat was homogenized for 30 s at medium speed in a Waring® laboratory blender and 5 g of the homogenate were used for the analysis of moisture in triplicate. The moisture content (%) of the oyster meat was measured by drying in a draft oven at 105°C for 24 h (AOAC International, 2005).

6.2.9 Effect of steam venting technology properties in the quality of oyster meat

Two sets of 5 trays each were prepared with the combination 100:200. Trays were identified as 100:200F (with film) and 100:200NF (no film). The trays were microwaved for 50, 80, 110, 170, 200, 230, and 300 s and let stand for 2 min after each period of time. After the standing time, the meat was extracted from the trays and analyzed for moisture, color, and texture.

6.2.10 Color of oyster meat

Color was measured during microwave cooking at 50, 80, 110, 170, 200, 230, and 300 s. The surface color of the oyster meat was measured with a Hunter LabScan Colorimeter (Labscan XE, Hunter Associates laboratory, Inc., Reston, Virginia, USA) on the ventral body of individual oysters. Color was recorded as *L*, a*, b**. Triplicate samples were analyzed.

Total color difference (*ΔE**) was also calculated to quantify the overall color difference of the microwaved heated oyster meat compared to fresh oyster meat using the formula (Eq.3):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

[3]

6.2.11 Texture analysis of the oyster meat

Texture of the oyster meat was measured at 50, 80, 110, 170, 200, 230, and 300 s of microwave cooking. A texture analyzer (Instron model 5544, Norwood, MA) equipped with a 10-blade Kramer shear attachment was used to measure texture. The analysis was performed using a 2 kN load cell in compression mode at 2 mm/s. After two min of standing time, the oyster meat was removed from the trays and used for the texture analysis by filling the shear cell up to two thirds of its capacity and positioned opposite to the alignment of the blades. Data were collected and analyzed using the texture analyzer software (Merlin v. 5.31). The maximum force needed to cut through was recorded as compressive strength (MPa).

6.2.12 Statistical analysis

The collected data were analyzed using SAS version 9.2 (SAS, Version 9.2, SAS Institute Inc., Cary, NC., USA). One-way analysis of variance (ANOVA) was used to detect statistical differences ($P \leq 0.05$) following Tukey's studentized range test ($p < 0.05$).

6.3   Results and discussion

The amount of product, venting time, and the time required to reach an internal temperature of 90ºC (T90) are correlated. As the quantity of product increases, venting time increases and T90 increases as well. Venting of the film in packages containing 50 g of oyster meat and frozen vegetables occurred at 82 ± 5, 102 ± 6, and 120 ± 9 s in 1:1, 1:2, and 1:3 MV proportions, respectively. Similarly, T90 in packages containing 50 g of oyster meat with different MV proportions increased with increasing amount of product. The internal temperature in these packages reached 90ºC after 90 ± 5 (1:1), 110 ± 9 (1:2), and 155 ± 8 (1:3). The same trend of increasing venting time and the time to reach an internal temperature of 90ºC was observed in packages containing 100 g of oyster meat. Venting of the film in packages containing 100 g of oyster meat and frozen vegetables occurred at 127 ± 3, 145 ± 5, and 179 ± 7 s in 1:1, 1:2, and 1:3 MV proportions, respectively. The internal temperature of these packages reached 90ºC after 190 ± 4 (1:1), 258 ± 3 (1:2), and 336 ± 10 (1:3), of microwave cooking.

According to Geedipalli and others (2007), the amount of product being heated in a microwave oven is directly related to microwave heating time. During microwave heating most of the microwave power is directly absorbed by the food being heated. The rate of heating (heating power) is, therefore, fixed by the power of the oven. The rate of temperature increase in the food is therefore inversely proportional to the mass of food multiplied by the specific heat capacity of the food. This suggests that a double mass of food halves the rate of temperature

rise, and the food would take as twice as long to reach the same temperature (Vadivambal and others, 2010).

The amount of product and the internal temperature at venting are inversely proportional (Table 6.1). The internal temperature at venting shows a decrease with increasing amount of product. This information is very important for the future development of heating instructions and warnings to the consumer. The venting of the package could be perceived as if the product is ready to eat.

Table 6.1 Microwave cooking events and conditions at venting time

Oyster meat (g)	Mixed vegetables (g)	Venting time (s)	Internal temperature (ºC)	Pressure (psi)	Headspace temperature (ºC)	Time to reach 90ºC (s)
50 g	50 (1:1)	82 ± 5^E	88 ± 4^A	1.5 ± 0.5^A	77 ± 8^A	90 ± 5^E
	100 (1:2)	102 ± 6^D	72 ± 6^B	1.2 ± 0.4^A	74 ± 3^A	110 ± 9^D
	150 (1:3)	120 ± 9^C	69 ± 6^B	1.2 ± 0.2^A	74 ± 3^A	155 ± 8^C
100 g	100 (1:1)	127 ± 3^C	51 ± 1^C	1.2 ± 0.6^A	73 ± 4^A	190 ± 4^F
	200 (1:2)	145 ± 5^B	40 ± 5^D	0.7 ± 0.1^A	74 ± 1^A	258 ± 3^B
	300 (1:3)	179 ± 7^A	8 ± 1^E	0.6 ± 0.2^A	47 ± 4^B	336 ± 10^A

[ABCDE]Means with different exponents in each column indicate significant difference

According to the results (Table 6.1), the headspace temperature at time of venting shows no significant differences between OM:MV combinations, with the exception of 100:300. Venting occurred when the headspace reached and average of 74ºC in all OM:MV combinations (100:300 not included). Similarly there was no significant difference observed between OM:MV and venting pressure. This means that, independently of the OM:MV combination, venting occurred when the headspace temperature reached 74ºC (average) at 1.1 psi (average) which suggested that venting time is dependent of the headspace and pressure conditions. The deviation found in the 100:300 combination could be attributed to the reduced headspace in the

package due to the higher amount of product in the tray which could have affected the film properties and thus the development of the venting conditions.

The relationship found between the venting time and the temperature in the headspace and pressure at which it happens was unknown; and it complements the technical information for the film used in this study.

6.3.1 Product weight loss during microwave cooking

Trays with 100 g of oyster meat reported lower weight loss than those having 50 g of oyster meat during the 300 s of microwave cooking (Figure 6.2). The highest weight loss occurred in trays with 50:50 OM:MV (47 ± 4.4%). Bubbling was observed during microwave cooking of 50:50, 50:100, and 100:100 samples. This effect was observed approximately after 160 s in 50:50, 220 s in 50:100, and after 200 s in 100:100. Bubbling was accompanied by the production of foam, probably produced by presence of soluble protein from the oyster meat. The production of foam was more abundant in 50:50 which overflowed the package and thus produced the highest weight loss. Bubbling and/or production of foam were not observed in the other combinations. Excluding the bubbling of liquid in 50:50, 50:100, and 100:100, the cause of weight loss in the packages in the other combinations could not be concluded by the observation of these graphs. However, from the customer point of view, high probability of rejection would occur for trays containing 50:50, 50:100, and 100:100. The excessive weight loss and poor performance would be very obvious.

6.3.2 Oyster meat weight loss during microwave cooking

The calculation of the weight loss of oyster meat provides more information about the overall weight loss observed in the product (OM + MV + tray/film). The oyster meat lost

between 70% and 75% of its weight in all packages containing 50 g of oyster meat as main ingredient (Figure 6.3).
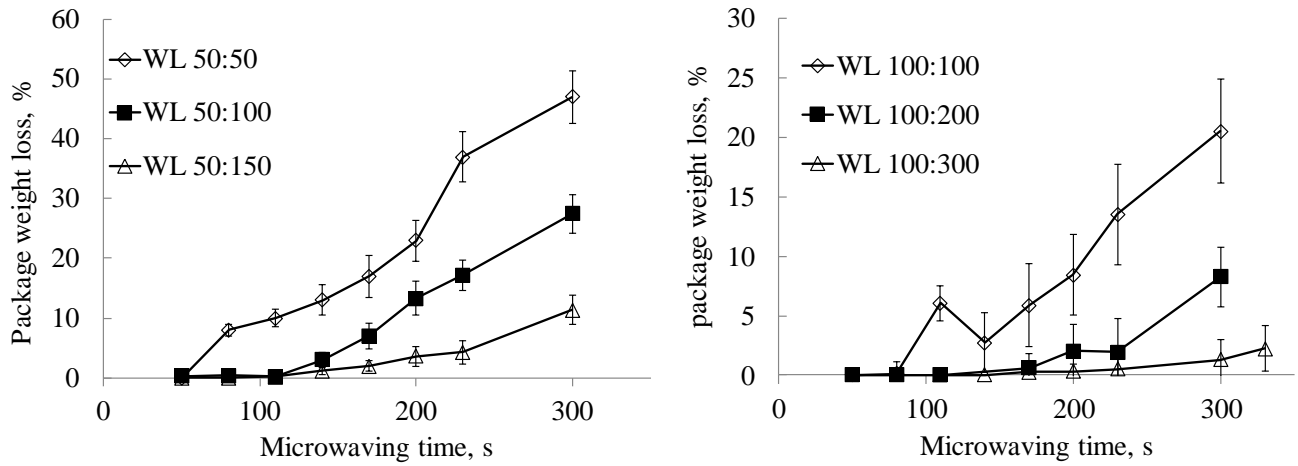

Figure 6.2: Weight loss (%) of the product during microwave cooking

The addition of vegetables did not contribute to retain moisture in the meat which was possibly lost during microwave cooking. The same situation could be observed for trays containing 100 g of meat and 100 g of vegetables. In contrast, trays containing 100 g of meat with 200 and 300 g of vegetables performed well during microwave cooking showing lower oyster meat weight loss. The meat weight loss in the combination of 100 g of meat with 200 g of vegetables was 33.89 ± 5.5 % after 300 s of microwave cooking. On the other hand, the oyster meat in the combination of 100 g of meat and 300 g of vegetables lost 25.71 ± 4.9% of its weight which was the lowest of all treatments.

The heating efficiency in a microwave oven is determined by the dielectric properties in addition to thermal properties of foods (Sipahioglu and others, 2003). Dielectric properties are the most important physical properties associated with microwave heating since the dielectric behavior of foods affects their heating characteristics. Specifically, the dielectric constant (ε_r),

describes the ability of a material to store energy when it is subjected to an electric field (Sosa-Morales and others, 2010).

Products with higher dielectric constant heat faster by the effect of microwave energy. The dielectric constant of vegetables and fruits decreases as temperature increases. Most of the water in vegetables and fruits exists as free water, and the dielectric constant of free water decreases with temperature (Mudgett, 1995). In addition, the higher the moisture content, the higher the dielectric constant.
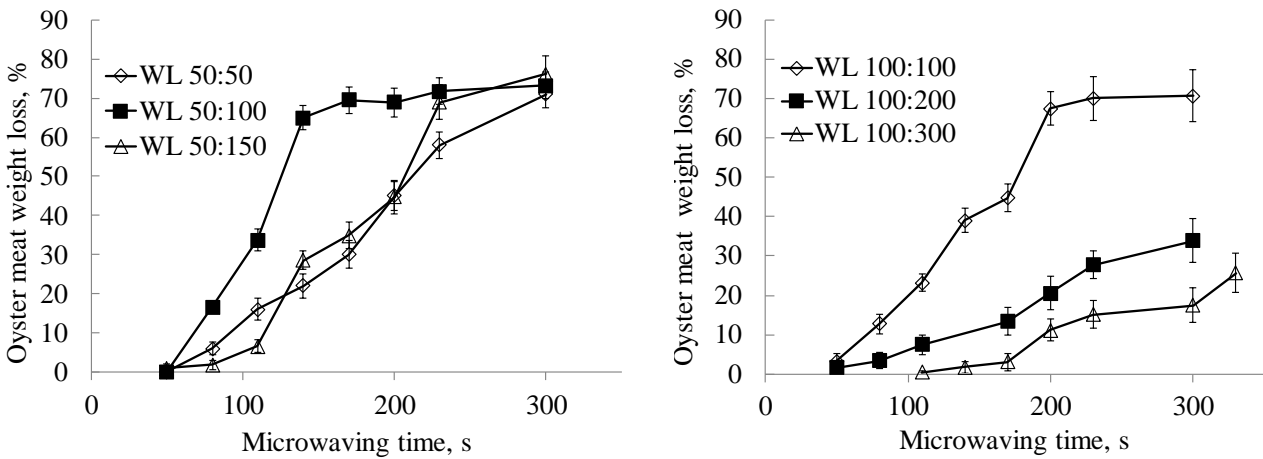

Figure 6.3: Oyster meat weight loss (%) in the package during microwave cooking

The vegetables in MV had close dielectric values, which range between 40 to 70 (Sipahioglu and others, 2003). At 10ºC, the corresponding ε_r is 70, while at 90ºC, the ε_r is 40. The decrease in the dielectric constant with increasing temperature agrees with Mudgett (1995). Oyster meat has been reported to have ε_r values of 65 (10ºC) and 50 (50ºC) (Hu and others, 2005).

According to the results, equal proportion of meat to vegetable had a disadvantage during microwave cooking. The difference in the dielectric constant at equal proportions of OM:MV could explain the elevated weight loss in 50:50, and 100:100. As microwave cooking

progressed, MV heated faster at the beginning due to its higher ε_r; this is advantageous for OM because MV produced the initial steam. However, with increasing temperature MV's ε_r decreases to a possible lower value than OM's ε_r which caused OM to heat faster and possibly reaching temperatures that could cause greater water loss.

In OM;MV proportions other than 50:50 and 100:100, the driving force for heating was mostly governed by the higher amount of vegetables. The higher the proportion, the more steam was produced by the vegetables which protected OM from greater moisture loss.

6.3.3 Moisture content in oyster meat during microwave cooking

The moisture in oyster meat was measured in both 100:200 and 100:300 combinations (Figure 6.4) which were the combinations that were not rejected due to high weight losses during microwave cooking. According to the results, the combination 100:300 showed steady moisture content during microwave cooking. The initial average moisture content of 89.46 ± 0.67% decreased to 87.54 ± 1.2%, while samples from the combination 100:200 had a moisture content of 84.1 ± 1.17%.

At this stage of the product development process, a decision was made between these last two alternatives. Although 100:300 obtained the best results in all tests performed, this combination required 336.3 ± 10 s to reach the safety temperature of 90°C which is out of the range of the established 300 s as a maximum time for microwave cooking. Consequently, 100 g of oyster meat and 200 g of vegetables was the optimum combination that produced acceptable results.
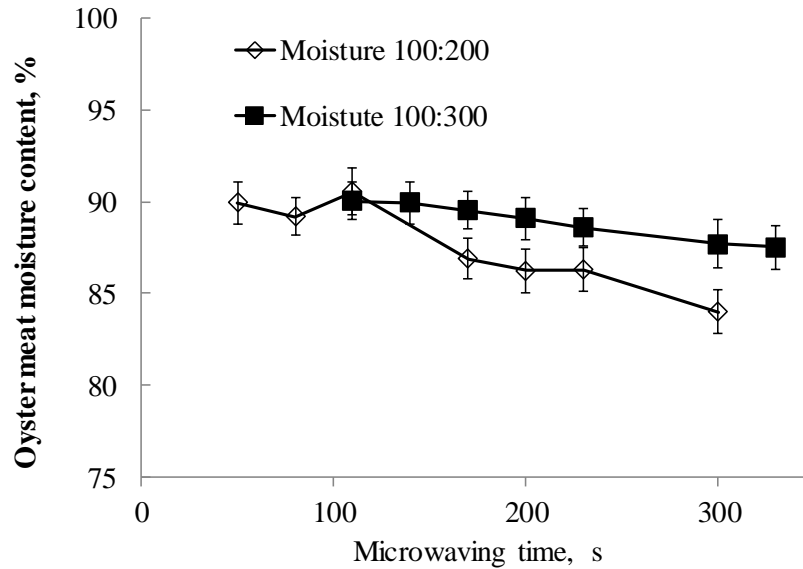
Figure 6.4: Oyster meat moisture (%) in the package during microwave cooking

6.3.4 Effect of steam venting technology properties in the quality of oyster meat

The moisture in 100:200NF had a noticeable decrease during microwave cooking. After 300 s, the moisture was $53.7 \pm 8.9\%$ (Figure 6.5). In addition, large variation in the results was observed between samples, reflected by the standard error bars. This variation could be the effect of uneven heating. Some meat pieces showed extreme dehydration, which was also observed in some vegetables located at the corners of the trays. The moisture in 100:200F had very slight variation. After 300 s of microwave cooking the measured moisture was $85.2 \pm 2.5\%$. The 100:200F had very good appearance. The vegetables looked as if they were fresh steamed and the oyster meat was plump and juicy.

In general, cooking of meat products by microwave heating increases cooking losses as compared with conventional cooking and results in a significant difference in the texture of the product (Gundavarapu and others, 1998). Product toughening during microwave cooking has been related to loss of moisture (Ohlsson and others, 1982). This suggest that 100:200F samples should be less tougher than 100:200NF due to lower moisture loss. However, according to Bih

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(2003), moisture escaping from product as a steam may be partially condensed on colder surfaces and thus may add sogginess.
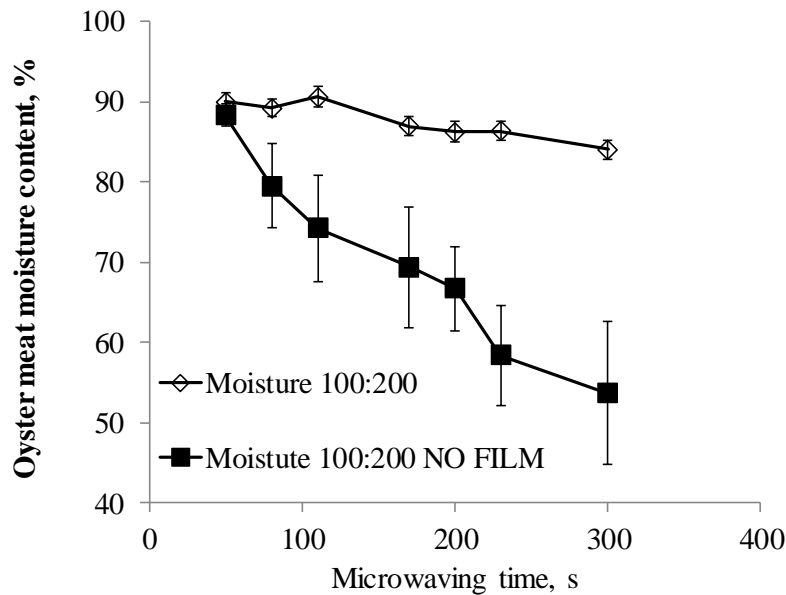

Figure 6.5: Comparison in oyster meat moisture content (%) in 100:200 trays with and without film

6.3.5 Color of the oyster meat during microwave cooking

During microwave cooking of meats, color changes are attributed to protein denaturation (cooked color). The proteins denature and recombine, or coagulate, and meat becomes opaque and whitish (Mizrahi, 2012). In this study, the instrumental color analysis of fresh oyster meat presented the following values: L^* (lightness) of 64.72 ± 2.18, a^* (redness) of 1.02 ± 0.49 and b^* (yellowness) of 10.82 ± 1.24. The L^* values in 100:200NF decreased during microwave cooking.

After 110 s of cooking, the cream color changed to yellow-brown which was verified by the in b^* values (Table 6.2). The color of the oyster meat in 100:200F had a whiter tone when compared to fresh oyster meat (visual). The L^* values for 100:200F showed an increase between 50 s and 200s, however; the values decreased at the end of the cooking period. The overall color

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change ΔE^*, which correlates to the color of fresh oyster meat was lower in 100:200F samples. This suggests that the color in 100:200F was closer to the color of fresh oyster meat.

Table 6.2 Color during microwave cooking

Time, S		L^*	a^*	b^*	ΔE^*
50	100:200 F	58.29±1.44[A]	3.24±1.62[A]	15.99±2.49[A]	6.98±4.32[A]
	100:200 NF	54.16±3.42[A]	3.93±1.82[A]	10.36±1.54[B]	8.66±4.43[A]
80	100:200 F	65.83±2.38[A]	3.53±0.69[A]	18.91±1.43[A]	9.67±6.35[B]
	100:200 NF	49.44±3.18[B]	3.01±1.01[A]	20.36±1.56[A]	19.06±6.18[A]
110	100:200 F	60.67±2.29[A]	2.44±0.0.44[A]	15.51±2.19[B]	7.09±2.62[B]
	100:200 NF	52.19±4.16[B]	2.56±1.23[A]	22.16±2.16[A]	17.71±3.58[A]
170	100:200 F	66.65±3.04[A]	2.87±0.53[A]	16.25±1.80[B]	7.19±2.46[B]
	100:200 NF	47.14±4.25[B]	1.85±1.56[A]	23.51±2.19[A]	19.98±5.62[A]
200	100:200 F	66.17±2.81[A]	2.85±0.28[A]	17.44±1.53[A]	8.11±7.61[B]
	100:200 NF	41.18±4.26[B]	0.73±1.42[A]	12.42±2.66[B]	24.04±6.18[A]
230	100:200 F	59.92±3.38[A]	2.89±1.07[A]	17.63±2.55[A]	8.95±7.61[B]
	100:200 NF	38.84±5.86[B]	0.23±1.02[A]	6.02±3.12[B]	28.53±6.16[A]
300	100:200 F	57.53±3.60[A]	3.08±0.92[A]	18.06±0.73[A]	16.81±7.61[A]
	100:200 NF	31.22±7.29[B]	-0.12±0.82[B]	7.44±3.01[B]	31.38±7.42[B]

[AB]Means with different exponents in each column indicate significant difference

6.3.6   Texture of oyster meat during microwave cooking

As mentioned above, textural changes during microwave cooking were directly related to moisture loss.   Compressive strength (CS) was tested in oyster meat as an index of tenderness/firmness. The CS value for fresh oyster meat was measured as 18.1 ± 1.5 Mpa. The results show that 100:200NF samples had higher CS values as compared to 100:200F after 80 s of microwave cooking. There was an interaction between CS and microwave cooking time. The CS of oyster meat increased with increasing cooking time much higher in 100:200NF than 100:200F which can be correlated to the drastic moisture loss observed in Figure 6.4. However,

organoleptic evaluation of 100:200NF oyster meat revealed that some oysters had a rubbery

consistency which suggests that the meat was over cooked. These oysters were usually found at

the edges and corners of the trays. The larger CS values in 100:200NF were mainly the result of

moisture loss.

Table 6.3 Texture of oyster meat (compressive strength, MPa) during microwave cooking

	Microwave cooking time, s						
	50	80	110	170	200	230	300
100:200 FILM	22 ± 2^{Acd}	24 ± 1^{Bcd}	23 ± 3^{Bc}	27 ± 2^{Bc}	29 ± 2^{Bc}	36 ± 3^{Ab}	53 ± 3^{Ba}
100:200 NO FILM	24 ± 2^{Af}	32 ± 4^{Ae}	37 ± 4^{Ae}	56 ± 7^{Ad}	88 ± 8^{Ac}	107 ± 24^{Ab}	168 ± 2^{Aa}

[AB]Means with different exponents in each column indicate significant difference
[abcdef]Means with different exponents in each row indicates significant difference

In 100:200F, protein denaturation during microwave cooking probably affected CS

values more than the effect of the moisture loss. During visual inspection of 100:200F, no over

cooked meat was found.

6.4   Conclusions

The development of steam venting microwavable products having frozen oyster meat as

the main ingredient was successfully achieved. From the different combinations of oyster meat

and mixed vegetables, product combination of 1:1 having either 50 g or 100 g of oyster meat

resulted in high product weight losses during microwave cooking due to the weight loss of the

oyster meat while reaching an internal temperature of 90°C. Although the combination of 100 g

of oyster meat and 300 g of mixed vegetables obtained the best results during the first stages of

the product development process, this combination required 336.3 ± 10 s to reach the safety

temperature of 90°C which is out of the range of the established 300 s as a maximum time for

microwave cooking. The optimum combination resulted from 100 g of oyster meat and 200 g of mixed vegetables which had low meat weight loss, good moisture retention, and textural properties closer to that of fresh oyster meat at the targeted internal temperature of 90ºC. The venting time for this combination occurred at 145 ± 5 s of microwave cooking and reached an internal temperature of 90ºC after 258 ± 3 s. More importantly, the internal temperature of 90ºC is considered high enough to inactivate pathogens naturally occurring in oysters such as *Vibrio vulnificus* and *Vibrio parahaemolyticus*.

This study also provided important information regarding the properties of the film that can be added to the technical specifications. The results showed that the film vented when the headspace temperature was 74ºC and the pressure reached 1.1 psi. In addition, it was also established that an important parameter in the formulation of microwavable products with oyster meat as the main ingredient, is the water balance from the meat related to other ingredients.

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CHAPTER 7   SUMMARY AND CONCLUSIONS

This study evaluated the feasibility of using steam venting technology in the development of a microwavable product having frozen oyster meat as the main ingredient. As first objective, cryogenic and air blast freezing techniques were evaluated in the reduction of specific pathogens that are of concern in the consumption of oyster meat (*Vibrio vulnificus* and *Vibrio parahaemolyticus*). From these techniques, cryogenic freezing was more effective in the reduction of oyster-related pathogenic bacteria to nondetectable levels. Moreover, the faster rate of freezing in cryogenic freezing resulted in considerable less muscle damage in the oyster meat improving moisture, and decreasing weight loss during frozen storage, as well as reducing lipid oxidation during frozen storage.

A quick frozen product may lose its advantages if not stored at proper conditions. In this regard, the evaluation of the effects of modified atmosphere (MA) during long periods of frozen storage in the quality of oyster meat was studied. Oyster meat packed in 100% nitrogen resulted with lower lipid oxidation than oyster meat packed in air after 180 days of frozen storage. In addition, the rich nitrogen environment provided protection against the effects of temperature fluctuation during frozen storage which was reflected by stability in moisture content in the oyster meat.

The study demonstrated that steam venting technology could be used to inactivate pathogenic bacteria in frozen oysters and oyster products cooked in the steam packages. The combined effect of microwaves and steam generated conditions under which lethality of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in oyster meat was assured, while maintaining important sensory properties in the meat.

VITA

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