Application of Alternative Technologies to Eliminate *Vibrios* spp. in Raw Oysters

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ABSTRACT

High pressure processing (HPP) and gamma irradiation were applied to inactivate *Vibrio vulnificus* (MO624) and *Vibrio parahaemolyticus* (O3:K6 TX2103) in pure culture and in inoculated live oysters. *Vibrio* pure culture and inoculated oysters were exposed to pressures of 207 MPa (30 kpsi) to 552 MPa (80 kpsi) for 0 min to maximum of 20 min. More than 5.4 log reductions of *V. vulnificus* occurred at 345 MPa for 0 min in oysters; 345 MPa for 2 min can achieve 4 log reductions on *V. parahaemolyticus*. Dosage of 1 kGy γ-irradiation was proved to be effective in producing *Vibrio* free oysters with comparable organoleptic quality to raw oysters.

Thermal conductivity of shucked oysters was measured to be 0.58 to 0.68 W/m°C, as temperature increased from 0 to 50 °C, using a line heat source probe. The specific heat was measured by differential scanning calorimeter methods. It increased from 3.80 to 4.05 kJ/kg °C, when temperature rose from 10 to 50 °C. The thermal diffusivity was calculated employing the data of thermal conductivity, specific heat and density of shucked oysters. The results showed that, under the tested temperature range, thermal properties did not change significantly with temperature. The dielectric constant and loss factor of oysters were determined by an open-ended coaxial line probe connected to a network analyzer at frequency of 30 MHz to 3000 MHz from 1 to 55 °C. The penetration depth of dielectric heating was calculated to be 1.1 cm with the dielectric constant of 55 and loss factor of 14.

A two-dimensional mathematical model was established to simulate the heat transfer of microwave heating using a fish gel. Finite difference method was utilized to solve partial

differential heat transfer equations. The model was able to predict the temperature distribution in heated fish gel with an accuracy of ± 8 °C. Applying the developed mathematical model, the lethality of *Vibrio* spp., artificially inoculated in live oysters, was estimated collectively by integrating the individual localized lethality of designated heating units. The predicted lethality was compared with microwave enumeration data on *Vibrios* in oysters. The observed maximum log reductions by microbial enumeration were 4.4 and 3.4 for *V. vulnificus* and *V. parahaemolyticus,* respectively. The lethality calculated by integrating temperature profiles was acceptable. The discrepancy between the estimated lethality and microbial test was attributed to the simplified model construction.

The quality of processed oysters, including color, aroma and texture properties, was evaluated instrumentally by a digital image system, an electronic nose and universal testing machine. The performance of two electronic nose systems on their abilities to detect oyster aroma and classify the aroma data into distinct groups was evaluated using a trained sensory panel and microbial tests. Cyranose 320 system has demonstrated potential as a quality assessment tool due to its sound correlation with microbial quality data and sensory evaluation scores. According to the quality measurement results, high pressure processing conditions were recommended to be at 345 MPa for less than 3 min and 379 MPa for less than 1.5 min. Deterioration of the quality was distinct for oyster meats exposed to 60 °C or above by thermal processing. The critical thermal processing condition was identified to be 55 °C for 2 min. With careful control, microwave processing could be considered as a candidate for seafood processing to reduce potential bacterial hazard but still retain the quality of the product.

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CHAPTER 1

Introduction

Oysters are perishable food products having a short shelflife even under refrigerated conditions. An oyster has unique flavor and texture which can be easily altered by traditional heating or freezing methods. However, consumer preferences for raw oysters require critical attention, because of spoilage bacteria and foodborne pathogens present in them. The presence of *Vibrio vulnificus and Vibrio parahaemolyticus* in raw oysters poses many health hazards to consumers, and recently there is an increasing concern with the safety of raw oyster consumption.

Oysters are filter feeders that can concentrate pathogens. Approximately 5,000 foodborne infections annually were caused by *Vibrios* other than *V. vulnificus* in the United States (Mead and others 1999), with *V. parahaemolyticus* being the leading cause of these infections. The spread of *V. parahaemolyticus* infection in California, Oregon, Washington, Connecticut, New Jersey, New York and Texas in 1997, 1998 and 1999 was associated with the consumption of raw oysters (CDC, 1997, 1998, 1999). Abdominal pain, vomiting and diarrhea are frequently observed symptoms; other typical symptoms of the illness include fever, chills, nausea and hypertension (Oliver 1989). Septicemia, gastroenteritis and wound infection were related to *V. vulnificus* infections. In 1998, due to *V. vulnificus* infection, 18 deaths out of 30 reported cases were reported in Los Angles, California (Tacket and others 1999).

Variety of methods, including depuration, relaying, Ameripure process, acid marinade and freezing have been developed to reduce the bacterial load. However, none of these methods can completely eliminate the bacteria due to their ability to remain

attached to oyster tissues (Jones and others 1991). The demand of consumers for safe and fresh oysters, which maintain their appearance, aroma, texture and nutritional qualities, is the driving force of searching novel processing technologies.

Microwave

Microwave has been widely used in reheating, tempering and cooking of food products. It is generally accepted that it inactivates microorganisms through thermal mechanisms, which totally depend on a time-temperature relationship. However, microwave heating occurs rapidly throughout the product rather than through conventional heat transfer from the product surface to interior (Mallikarjunan and others 1996). The entire process is fast compared to conventional heating with the same increase of temperature. Since *Vibrio* spp. are heat sensitive in nature, employing microwaves to replace the conventional heating could be an option. In fact, mild heat treatment (50 °C) was reported to be effective in control of *Vibrios* in oysters (Ama and others 1994).

Non-uniformity in microwave cooking has been well-documented and has become the major issue when applying microwave radiation in food processing. However, research has shown that by applying properly selected heating patterns, microwave heating can provide safe product with comparable sensory quality (Mallikarjunan and others 1996). Therefore, in order to be able to employ this technology, the mechanism of dielectric heating and critical processing condition needs to be identified.

High Pressure Processing

High pressure processing (HPP) has gained tremendous attention as a means of non-thermal processing of raw or fresh food products for effective bacterial reduction without causing significant changes in their organoleptic and nutritional qualities (Styles and others 1991, Berlin and others 1999). The process is, in fact, not 'heatless' because it will increase the temperature of the hydraulic medium (mostly water) approximately 3 °C per 100 MPa through adiabatic heating during the pressurizing time. But, the temperature of the product will return to its original state after depressurization. Therefore, ambient temperature processing becomes possible. It acts instantaneously and uniformly throughout a mass of food independent of size, shape and food composition.

Since the first attempt using high hydrostatic pressure to kill bacteria in 1899 by Hite, research on HPP to extend the shelf life of food has never stopped. Extensive work has been done after 1980. The targeted bacteria were *E. coli O157:H7* (Patterson and others 1995; Patterson and Kipatrick 1998); *Salmonella* spp. (Metrick and others 1989) and *L. monocytogens* (Styles and others 1991). *V. parahaemolyticus* was substantially more sensitive to HPP, being eliminated in clam juice using a pressure of 173 MPa for 10 min (Styles and others 1991). According to Berlin and others (1999), pathogenic *Vibrio* spp. was susceptible to HPP at pressure levels from 200 to 300 MPa. Extensive work using HPP to process oysters has been reported by Calik and others in 2001. They determined the optimum processing condition to be 345 MPa-0.5 min for *V. parahaemolyticus* in pure cultures and 345 MPa-1.5 min for *V. parahaemolyticus* inoculated in whole oysters, at which 8 log reductions can be achieved. A great benefit of

HPP on oysters was the opportunity to produce shucked oysters. This is because pressure can release the abduct mussels causing direct separation of oyster meat from shells.

Although increasing pressure level and time of exposure can significantly extend the shelflife, the potential for changes of the sensory quality of processed food become greater. This is especially true for raw, high protein food where pressure-induced protein denaturation will be visually evident. HPP can also cause structural changes in structurally fragile foods such as strawberries or lettuce. Cell deformation and cell membrane damage can occur to food exposed to high pressure resulting in a softer tissue.

Irradiation

Food irradiation is referred to "ionizing radiation" using a limited number of radiant energy such as cobalt-60 and high-energy electronic beam. The selection of radiant energy ensures high penetrating power without producing radioactivity in treated foods. Microorganisms are destroyed mainly due to molecular structure alteration caused by interaction with hydrogen peroxide, which acts as a strong oxidizing agent and biological poison. Since it does not generate significant heat in foods, food irradiation is also termed as "cold sterilization".

A low dose of gamma rays has proven effective in reducing spoilage and pathogenic microorganisms in a variety of seafood products. Novak and others (1966) found that oysters treated with 2 kGy gamma ray had a shelf life of 23 days under refrigerated conditions. Studies have shown that a dose level of 1 kGy or less was sufficient to reduce pathogenic bacteria like *E. Coli* and *Vibrio spp*. to undetectable levels depending upon specific seafood tested (Grodner and Land 1990; Grodner and Hinton

1985). Hinton (1983) also reported 1 kGy can significantly reduce *Vibrio cholera* in oyster homogenates by irradiation.

Although a low dose irradiation seems to be promising in controlling pathogenic and spoilage microorganisms in seafood products, the effect of irradiation on the sensory quality and consumer acceptance of such product was still a major concern for seafood industries (Shiu 2000). Irradiation induced color and aroma changes are evident when high doses are applied.

Combination treatment

As stated, if applying any technology singularly cannot achieve the required safety and maintain acceptable sensory qualities of oysters, using multiple hurdle technology concept may be considered. Combination of HPP and irradiation can produce the superior advantage of non-thermal processing; combination of high pressure processing with microwave treatment can produce the synergetic effect of HPP under elevated temperatures. Significant research has shown that microbial inactivation is not a simple additive reaction of applied treatment types (Crawfood and others 1996; Raso and others 1998). For example, Paul and others (1997) succeeded in eliminating *staphycoccci* (10^4 CFU/g) to undetectable levels in lamb meat by applying combined HPP and gamma irradiation, compared to only 1-log reduction found using both technologies singularly at the same treatment level.

Objectives

The hypothesis of this research is that the proposed high pressure, gamma irradiation and microwave heating technologies can effectively reduce *Vibrio* spp., providing a high quality oyster product that is close to its raw state. If not, a multiple hurdle technology approach will be employed. The primary objective of this study is to develop procedures for oyster products which will ensure the inactivation of *V. vulnificus* and *V. parahaemolyticus* and still maintain acceptable sensory qualities of raw oysters. The specific objectives are:

(1). Study the effect of high pressure and irradiation techniques on inactivation of *V. vulnificus* and *V. parahaemolyticus* in oysters.

(2). Study the effect of microwave pasteurization on inactivation of *V. vulnificus* and *V. parahaemolyticus* in oysters. Identify the heating patterns and critical processing conditions that will provide comparable quality product to raw oysters with reduced pathogen level.

(3). Study the microbial inactivation kinetics by microwave, high pressure and irradiation process

(4). Evaluate the quality of oyster products processed by microwaves, high hydrostatic pressure, and gamma irradiation using instrumentation. Validate the instrumental data using sensory evaluation.

CHAPTER 2

Literature Review

The presence of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in raw oysters has caused food poisoning outbreaks nationwide. The seafood industry is facing a recent petition by a consumer interest group to FDA to establish performance standards requiring zero tolerance of *V. vulnificus* in molluscan shellfish sold to consumers. Viable post-harvest technologies need to be developed to ensure product safety as well as to maintain the raw state of the product after processing. High pressure processing, irradiation processing and microwave processing are emerging new technologies that can be considered as an alternative to process oysters.

2.1 Oyster Biology

Oysters can be found in marine environments, where they form large beds in the tidal zone that extend up to 30 m towards warm waters. Along with mussels and scallops, oysters are among the invertebrate called pelecypods that are included in the phylum molluscan (clams, snails, squids and octopus). There are more than 100 species of oysters worldwide (Perkins 1995). Based on the breeding habit, internal and external structure, oysters are categorized into three genera, *Ostrea*, *Crassostrea* and *Pycnodonta* (Youge 1960). In the U.S., Eastern oysters, also named American oyster (*Crassotrea virginica*), Pacific oyster (*Crassostrea gigas*) and Olympia oyster (*Ostrea lurida*) are commercially available (Cook 1991). In 1997, the estimated U.S. commercial landings of oysters were about15 million pounds worth $39 million (NOAA 2003). In 2002, the total combined eastern oyster catch was 17 million pounds with a value of over $ 42 million (National Marine Fisheries Service 2004). The most recent survey showed that total consumer

expenditures for seafood products were estimated at $55.2 billion yearly, with approximately $121 million represented by commercially landed oysters each year (Weiher 2004).

Oysters are filter feeders. They eat by filtering food from surrounding waters through their gills. In ideal conditions, an oyster can pump 5 gallons of water within 1 h (Perkins 1995). Therefore, food particles, such as diatoms, algae and microorganisms, can be highly concentrated inside the oyster tissue, resulting in contaminated oysters if the water is not clean. The general anatomy of an oyster is demonstrated in Fig. 2.1.

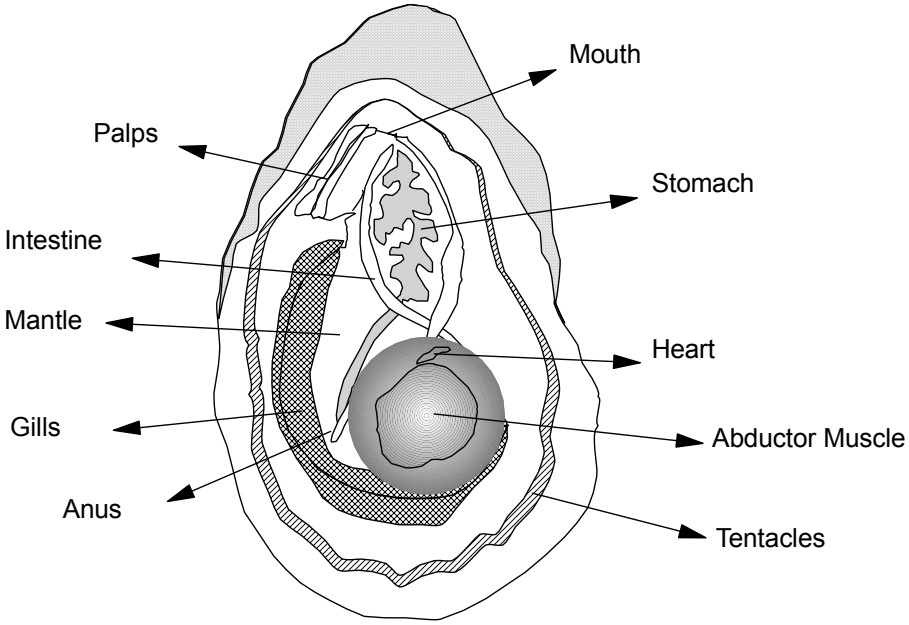

Fig. 2.1: Oyster anatomy

2.2 Chemical, Physical and Microbiological Properties of Oysters

2.2.1 Chemical properties

Like most seafood, oysters are highly nutritional. Depending on sex, maturity, water temperatures, food supply, stress and other environmental parameters, 100 g of raw oyster contains 77.4-90.2 g moisture, 5.6-10.0 g protein, 1.9-4.7 g carbohydrate, 0.7-2.4 g

total fat, 0.7-2.9 g ash, 37-58 mg cholesterol and 75 calories (Sidwell and others 1974). It provides 100% of VB_{12} and zinc and about 35% iron according to USRDA (United States Recommended Daily Allowance). Fresh oyster meat has a creamy color and clear liquid (Perkins 1995).

2.2.2 Thermal properties

Thermal properties of oysters, such as thermal conductivity, specific heat, thermal diffusivity, are important parameters due to their fundamental significance in calculating and modeling heat transfer during thermal processing of oysters, such as blanching, canning, drying, frying and freezing. Values of thermal properties vary with chemical composition, physical structure, state of the substance and temperature. For biological materials, factors affecting the thermal properties are cellular structure, density, moisture content and temperature of the sample materials (Fellows 2000).

2.2.2.1 Thermal conductivity

Thermal conductivity (k) is defined as the quantity of heat (q) which flows through two surfaces in unit time, across unit area (A), under unit temperature gradient, dt/dx. In mathematical terms, thermal conductivity is the proportional factor in the steady state of heat flow (Eq. 2.1) or Fourier's law for heat conduction (Johnson 1999).

$$q = kA\frac{dt}{dx} \qquad ..(2.1)$$

Most commonly used method to measure thermal conductivity is line heat source method, which applies transient state heat transfer. The basic idea is to apply a steady heat flux to the sample and measure the temperature rise at some point resulting from

applied heat flux. A simple temperature and time relationship including the target thermal conductivity can be established. The line heat source method was first suggested by Schleirmacher (1988) and later used by Van der Held and Van Drumen (1949). A modification of this technique was the development of the thermal conductivity probe. The theory of the line heat source is to assume a line heat source of constant strength in an infinite homogeneous body at uniform temperatures. Under these conditions, the temperature at any point of the body will be a function of several variables including time and thermal conductivity (Sweat and Haugh 1974). The solution of the heat transfer equation has been given by several authors (Hopper and Lepper 1950; Nix 1967; Chang and others 1980; Mohsenin 1980; Murakami and others 1996). For the change in temperature at the point close to the line heat source between time t_1 and t_2, induced by a heat input q per unit length of the heater, Eq. 2.2 was used to calculate the thermal conductivity.

$$T_1 - T_2 = \frac{q}{4\pi \times k \times \ln(t_1/t_2)} \quad .. (2.2)$$

where T_1 and T_2 are temperature corresponding to the time t_1 and t_2. Therefore, thermal conductivity can be obtained by the slope of the straight line of temperature versus time in a semi logarithmic plot. A schematic of thermal conductivity probe is presented in Fig. 2.2.

Numerous research has been conducted on measuring the thermal conductivity of food products using the line heat source method because it is rapid and easy to use. As for seafood products, in early 1974, Annama and Rao first measured thermal conductivity of prawn and sardine. Kumbhar and others (1981) examined various fish species using line heat source probe. Sanz and others (1987) reported their work of thermal conductivity

measurement on pike perch. Rahman and Potluri in 1991 tested the thermal conductivity of dry and fresh squid using this method. Recent work on other seafood products included: raw, blanched and cooked shrimp and scallops (Murakami1996); shrimp meat between -30 °C to 30 °C (Karunakar and others 1998); butterfly and popcorn shrimp (Ngadi and others 2000); catfish (Zheng and others 1998) and dried tuna (Rahman and others 2002).

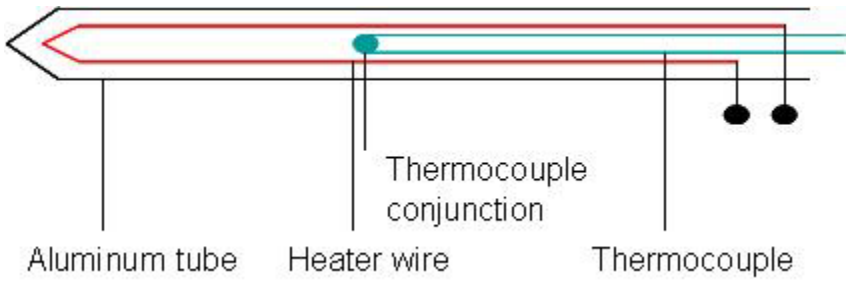

Fig. 2.2: The schematic of a line heat source thermal conductivity probe

Thermal conductivity of unfrozen foods or biological materials having high moisture content has a value close to water, which is 0.597 W/m°C at 20 °C. Experimental results showed that, in addition to moisture content and fat content, other compositions also had considerable effects on thermal conductivity (Chio and Okos 1986). For frozen food, thermal conductivity also depended on products' fiber orientation (Sweat 1975; Chio and Okos 1986). Also, thermal conductivity was found to be closely related to the product's bulk density, stated by Shrivastava and Datta (1999). They reported that thermal conductivity was reduced when the bulk density became less because the air is a poor heat conductor. In addition, the freezing rate, freezing and

thawing times and freezing conditions did have certain impacts on thermal conductivity of the frozen food products (Wang and Kolbe 1980).

2.2.2.2 Specific Heat

Specific heat (Cp), also called heat capacity, is defined as the ratio of the heat absorbed by a unit mass to the concomitant rise in temperature (Johnson 1999). It has a value ranging from 3.0 kJ/kg K to 4.0 kJ/kg K for aquatic materials (ASHRAE 1977). The most commonly used methods to measure specific heat include method of mixtures or the direct water immersion method and differential scanning calorimeter method.

The method of mixtures is conducted by placing a specimen of known mass and temperature into a calorimeter of known specific heat and water of known mass and temperature (Rahman 1993). The unknown specific heat can be calculated from the heat balance equation after an equilibrium condition has reached. Eq. 2.3 was generally used to calculate the average specific heat of the sample (Mohsenin 1980).

$$C_s = \frac{C_s W_w (T_e - T_w) - C_c W_c (T_i - T_e)}{W_s (T_i - T_e)} \qquad ..(2.3)$$

where C_s, C_w and C_c is specific heat of sample, water, and calorimeter, (kJ/kg K), respectively; W_w, W_c and W_w is weight of water, calorimeter, and sample, (kg), respectively; T_e is the equilibrium temperature of the mixture, (K); T_w is initial temperature of water, (K); T_i is initial temperature of sample and calorimeter, (K). In order to avoid heat loss, an adiabatic calorimeter was developed by maintaining the temperature of the calorimeter at the same temperature as the surroundings.

Applying mixtures method, specific heat and enthalpy were measured on ocean perch by Balaban and Pigott (1992) and on other 27 types of fish and processed meat by

Pham and others (1993). Several empirical equations were developed to estimate the specific heat of seafood products with respect to their moisture content and temperature. Rahman (1993) summarized his work on the specific heat measurement of fresh seafood, including calamari, cuttle, prawn, octopus and squid, and concluded that the range was between 3.29 kJ/(kg K) and 3.79 kJ/(kg K). The semi-empirical model was stated to be able to give a better prediction of the specific heat.

The principle of employing a differential scanning calorimeter (DSC) to measure specific heat was discussed by Mohsenin (1980). If a sample and a reference are heated at a known rate in a controlled environment, the increase in sample and reference temperature will be about the same (depending on specific heat differences), unless a heat-related change takes place in the sample, where sample either release or absorbs heat. In DSC, the temperature difference between sample and reference from such a heat change is directly related to the differential heat flow. A thermo-gram was generated recording the differential heat flow (mW) over a specific temperature level of interest. The specific heat can be calculated by

$$Cp = \left[\frac{60E}{Hr}\right]\frac{\Delta H}{m} \qquad ..(2.4)$$

where Cp = specific heat, (J/g °C)

E = cell calibration coefficient at the temperature of interest, dimensionless.

Hr = heating rate, (°C/min)

ΔH = difference in heat flow between sample and blank curves (ran without sample) at the temperature of interest, (mW)

m = sample mass, (mg)

The quantity 60E/Hr is a constant under a given set of experimental conditions. Its value can be determined by running a standard material of known specific heat under conditions identical to those of the unknown sample. Usually, a sapphire (Al_2O_3) is used for this purpose.

Since Wang and Kolbe (1991) first used this rapid method to obtain thermal properties such as initial freezing point, enthalpy and apparent specific heat, much research has been conducted to measure the specific heat of seafood. For example, Radhakrishnan (1977) measured the thermal properties of seafood from 5 to 35 °C. Ten types of seafood products were tested including bluefish, croaker, mackerel, salmon seabass, shrimp, spot tilapia trout, and tuna. She reported that the specific heat values ranged between 3.1 and 3.8 kJ/(kg K) and temperature did not have significant impact on the thermal properties. Karunakar and others (2002) also measured the specific heat of shrimp meat between -30 °C and 30 °C using DSC. Their data confirmed that the Schwanzberg model (Succar and Hayakawa 1984) provided comparatively better accuracy for predicting the apparent specific heat. Ngadi and others (2003) studied the effect of moisture content and fat content on enthalpy and specific heat change in butterfly shrimp at temperatures ranging from -40 °C to 30 °C using DSC. They reported that the specific heat increased to a peak value at a critical temperature close to -1 °C due to the phase change. Above this critical temperature, specific heat and enthalpy showed a linear relationship with respect to temperature.

Although the thermal properties of many agriculture products have been investigated, data on oysters were rare. It was reported that the specific heat above freezing and below freezing was 3.77 and 1.93 kJ/ (kg °C), respectively, for oyster meat

and 3.52 and 1.84 kJ/(kg °C) for oysters in shells. The latent heat of fusion for both oyster products was reported as 290 and 267 J/g, respectively (ASHRAE 1977). Reliable thermal property data is essential for process design and optimization in thermal processing of oysters.

2.2.3 Dielectric properties

The dielectric properties determine how materials interact with an electromagnetic field. They are of great interest in the use of microwave or radio frequency energy for heating and for sensing the moisture content and density of agricultural materials (Nelson 1999). The dielectric properties in most applications can be defined in terms of the relative complex permittivity (ε), $\varepsilon = \varepsilon' - j\varepsilon'' = |\varepsilon| e^{-j\delta}$, where the real part, ε', is called the dielectric constant; the imaginary part, ε", is called dielectric loss factor; and δ is the loss angle of the dielectric, where $\tan\delta = \varepsilon''/\varepsilon'$ is defined as the loss tangent or dissipation factor (Nelson 1999). The dielectric constant (ε') describes the ability that a material to store an electromagnetic energy while the dielectric loss factor associates with the ability a material to absorb the electromagnetic energy and convert that energy into heat. In general, the term "permittivity" represents the relative complex permittivity, that is , the permittivity relative to free space, or the absolute permittivity divided by the permittivity of free space (ε_o), where, $\varepsilon_o = 8.854 \times 10^{-12}$ F/m (Nelson 1999).

Microwave is an electromagnetic wave that may be reflected, transmitted, and absorbed by the food items subjected to it. The fraction of microwave power reflected by a food material back into surrounding medium can be estimated by

$$P_{ref} = \left(\frac{\sqrt{\varepsilon'} - 1}{\sqrt{\varepsilon'} + 1} \right)^2 \quad ..(2.5)$$

The microwave power that is not reflected is transmitted into the material. The transmitted energy can be calculated by Eq. 2.6. For most food materials, ε' ranges from 50 to 70, resulting in approximately 20% of the energy transmitted into food items (Engelder and Buffler 1991).

$$P_{Trans} = 1 - P_{ref} \quad ..(2.6)$$

Microwave power transmitted into the bulk of the material can be absorbed and converted to heat. The heating power for unit volume of the food item is associated with the dielectric loss factor, microwave frequency, and electric field intensity and can be calculated using Eq. 2.7. It can be seen that the food materials having a large dielectric loss factor can heat up rapidly; meanwhile, the dielectric constant has a significant contribution to the electric field intensity, therefore, affecting the heat absorption. However, the actual temperature increase caused by the microwave power also relies on thermal parameters, such as specific heat (Nelson 1999).

$$P_{abs} = 5.56 \times 10^{-4} \times f \times \varepsilon'' \times E \quad ..(2.7)$$

where P = absorbed microwave power (W/cm^3)

f = microwave frequency (GHz)

ε" = dielectric loss factor of food material

E = electric field intensity of given volume (volts/cm)

As microwave power is absorbed by the food material, the strength of the microwave beam diminishes gradually as it travels through the product. The distance over which the power decreases by 37% (1/e) is called the penetration depth (d_p)

(Engelder and Buffler 1991). If the heated product has a thickness that is bigger than $d_{p,}$ heating occurs only at the product surface leaving a cold core, whose temperature may increase primarily by slow heat conduction.

Studies have shown that dielectric constant and loss factor were closely related to microwave frequency, temperature, density, moisture content, salt content and state of the food items. There are several measuring techniques available to obtain ε values (Kent 1987). Of these, three methods are the most popular, including open-ended coaxial probe, transmission line, and resonant cavity (Engelder and Buffler 1991). Among them, open-ended coaxial probe was reported to be the most appropriate technique for food products. This method is not restricted by the shape and state of the materials; it can measure both the liquid and solid state products, and it is easy to clean and reuse. The principle of the open-ended coaxial probe method is the same for other measurement techniques. It involves (1) the generation of a microwave signal at frequencies of interest, (2) the direction of the signal at/through the testing materials, (3) the measurement of the changes in the signal caused by the material and (4) the computation of ε from those detected changes by computer program. The directing and detecting/measuring the signal changes are usually completed by microwave network analyzers (Engelder and Buffler 1991). The typical setup to measure ε using this method is demonstrated in Fig. 2.3. The probe is made of a coaxial line that ends abruptly at the tip, making direct contact with the material under test (Gabrel 1989; Stuchly and Stuchly 1980). Signal reflected from the interface is captured and utilized to calculate the dielectric properties.

Due to their fundamental significance in the heating achieved in a microwave field, dielectric properties have received great attention over the years. For food

materials, an important contribution was the publication of the food map, which depicts the effects of dielectric constant and dielectric loss factor on the penetration depth (Engelder and Buffler 1991). This map displays the inter-relationship of ε', ε" and dp at a microwave frequency of 2450 MHz for about 10 types of food products including meat, fish, fruit, vegetables, and soups. Meanwhile, a number of studies have been completed to measure the dielectric properties of food products. For instance, measurement of dielectric properties on whey protein gel, ground whole wheat flour, and apple juice by Nelson and Bartley (2001); corn, wheat, and rice by Nelson (1994); fresh fruits and vegetables by Nelson and others (1994); cereal grains and seed by Trabelsi and others (2001); beef and beef products by Tran and Stuchly (1987) and cheddar cheese by Green (1997).

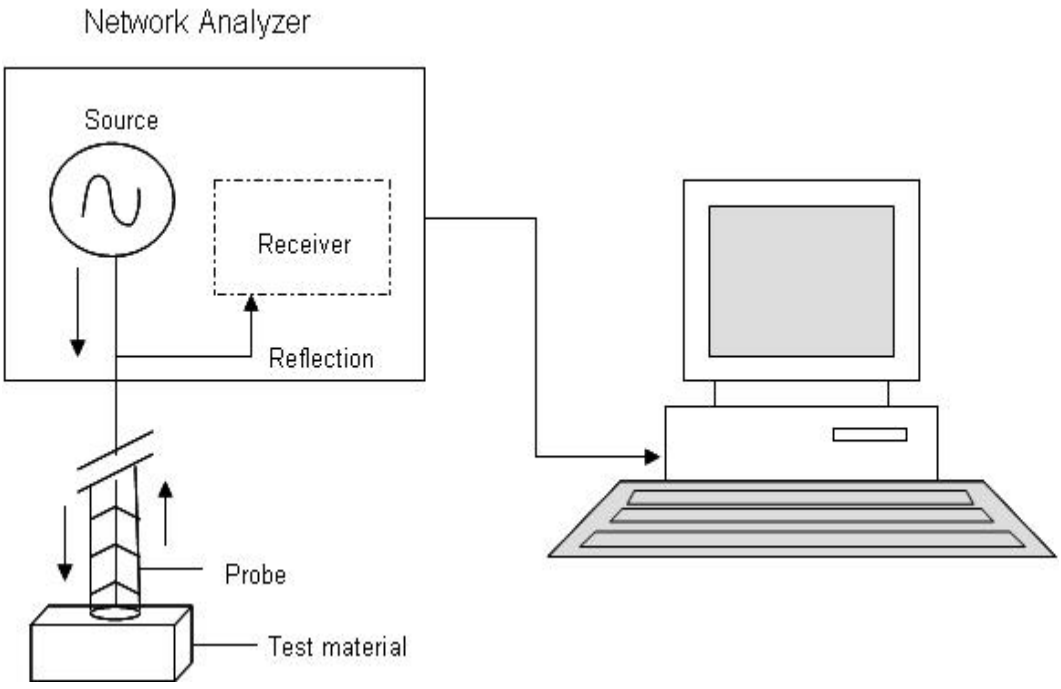

Fig. 2.3: Typical system to measure dielectric properties using open-ended coaxial probe

Prediction models have been developed to estimate the change of ε with temperature and food composition (Sun and others 1995; Yaghmaee and Durance 2002). However, the data on the dielectric properties of seafood products are limited. Available data were found for salmon, shrimp, and catfish (Tran and Stuchly 1987; Tanaka and others 1999; Zheng and others 1998).

2.2.4 Microbiological properties

2.2.4.1 Outbreaks

Oysters are filter feeders that contain a high level of microbial flora, not only resulting in eventual spoilage but also posing a threat to public health. Recently, the presence of *Vibrio vulnificus and Vibrio parahaemolyticus* bacteria in raw oysters has raised increased concerns on the safety of raw oyster consumption.

V. vulnificus and *V. parahaemolyticus* are two naturally occurring estuarine bacteria that can accumulate in oysters and cause illness in consumers. The outbreak of diseases like typhoid, dysentery, cholera, hepatitis and other forms of gastroenteritis were linked to the consumption of oysters (Brown and Dorn 1977; Earampamoorthy and Koft 1975; Wood 1976; Linton and others 2003). Fever, chills, nausea and hypotension are the most commonly seen symptoms and the incubation period before the onset of symptoms ranged from 7 hrs to several days. Bacteria target victims who have chronic disease, usually liver disease, and are immune compromised (Oliver 1989).

Multi-state outbreaks of viral gastroenteritis related to the consumption of oysters were reported in Louisiana, Maryland, Mississippi and North Carolina in 1993, involving 280 cases of illness (CDC 1993a). The spread of *V. parahaemolyticus* infection in the

Pacific Northwest in 1997 was associated with the consumption of raw oysters. A total of 209 cases were reported in California, Oregon, Washington in the United States and British Columbia in Canada due to the consumption of raw oyster (CDC 1997). Similar outbreaks were recorded in Connecticut, New Jersey, and New York (CDC 1999). Infection with *V. parahaemolyticus* generally induces gastroenteritis type of symptoms such as diarrhea, vomiting and chill, and in a majority of the cases, death would rarely occur. Business closures and loss of business due to this bacterium can be economically devastating to the oyster industry. In 1998, 368 people became ill in Texas due to *V. parahaemolyticus* infection from the consumption of raw shellfish. Galveson Bay was closed to shellfish harvesting on June 26, 1998. A majority of the victims were found in Texas, but health officials also confirmed victims in Florida, Oklahoma, Georgia, Tennessee, Colorado, and California (CDC 1998).

Infection of *V. vulnificus* can cause serious illnesses including septicemia, gastroenteritis, and wound infection. From 1981 to 1992, out of 125 persons infected with *V. vulnificus*, 44 (35%) was died in Florida (CDC 1993b). From 1992 to 1996, three deaths in Los Angeles, California were related to *V. vulnificus*. In 1998, due to *V. vulnificus* infection, 18 deaths out of 30 reported cases were recorded in Los Angeles, California (CDC 1998).

2.2.4.2 *Vibrio vulnificus* and *Vibrio parahaemolyticus*

The *Vibrio* family is characterized as gram-negative bacteria having a curved rod shape, usually 0.5-0.8 μm wide and 1.4-2.6 μm long. They are motile in an aqueous environment with a singular polar flagellum. They are facultative anaerobes capable of

respiratory and fermentative metabolism. They can ferment glucose without gas production. Most of them produce catalase and oxidase. Expect *V. cholerae* and *V. mimicus*, *Vibrio* species do not grow in media lacking 2-3% sodium chloride, therefore they are referred to as "halophilic" (Baumann and others 1986).

First isolated in 1953 in Japan, *V. parahaemolyticus* has been found in many species of fish, shellfish and crustaceans (Rodrick 1991). It grows readily at temperature from 22-42 °C but do not grow below 4 °C or above 45 °C. Optimum temperature is from 30 to 35 °C. The growth continues in acid conditions pH ranging from 5 to 11. It can tolerate up to 8% of sodium chloride concentrations (Baumann and others 1986). *V. parahaemolyticus* is temperature and pressure sensitive. It was reported to be more subjective to salt concentration after heating at 40 °C for 30 min, because of the lost selective permeability of the cell membrane (Emswiler and others 1976). Delmore and Crisley (1979) reported D values of 0.14 min at 85 °C, 0.66 min at 51 °C and 0.29 min at 55 °C in clam homogenate. The first research that studied the effect of pressure on the growth and viability of *V. parahaemolyticus* was carried out by Schwarz and Colwell in 1974. They found that 5 log reductions were achieved for holding seawater-yeast broth inoculums for three days at 101 MPa at 25 °C. However, 6 log reductions were observed under the pressure of 172 MPa for 10 and 30 min at 23 °C. This treatment successfully destroyed *V. parahaemolyticus* in clam juice and in buffer (Hoover and others 1989).

Isolates of *V. parahaemolyticus* can be differentiated from each other by serotyping; 13 O groups and 71 K types have been identified so far (Iguchi and others 1995). Serotype *V. parahaemolyticus* (O3:K6) was recently brought into attention due to its abrupt appearance and responses for many outbreaks worldwide (Wong and others

2000). It was reported to have significantly increased susceptibility to environmental stress such as low temperature survival, high resistance to mild acid and low salinity. The D value at 50 °C varied from 2.95 min to 4.26 min depending on the location of the isolation and time of the isolation (Wong and others 2000).

V. vulnificus is also a halophilic marine *Vibrio* having a similar phenotype to *V. parahaemolyticus*. It has been isolated from a wide range of environmental and food sources including water, sediment, plankton, oysters, clams, and crabs (Oliver and others 1983). It often grows in warm water with salinity between 0.7% and 1.5% (Kelly 1982). Temperatures lower than 8.5 °C or above 35 °C can significantly suppress the growth of *V. vulnificus* (Kaspar and Tamplin 1993). Compared to *V. parahaemolyticus*, *V. vulnificus* is more susceptible to heat, acid and pressure. The available data reporting the D values of *V. vulnificus* varied significantly. It was reported that the D value was 50 min at 45 °C and 10 sec at 51 °C (Anon 1996), without mentioning the strain name and the media, in which the decimal reduction time was measured. Cook and Ruple (1992) measured the D values of *V. vulnificus* to be 2.4 min at 47 °C and 1.15 min at 50 °C in buffered saline. *V. vulnificus* (ATCC 27562) was reported to have a D value of 3.7 min at 47 °C and 1.15 min at 50 °C (Drake and others 2003). D values of *V. vulnificus* (M0624) were measured to be 48.25 min at 45 °C, 0.55 min at 49 °C, and 0.19 min at 51 °C in BHI (Brain Heart Infusion) broth by Kim and others in 1997.

2.3 Traditional Processing Techniques

2.3.1 Depuration

The commercial methods currently used to control *Vibrios* in oysters include: depuration, relaying, Ameripure process, acidic marinade, and pasteurization. Depuration is a dynamic process whereby the oysters are allowed to purge themselves of contaminants in tanks of sterilized or pathogen free seawater for two to three days (Fleet 1978). Ultraviolet irradiation is generally used to sterilize seawater. The number of total plate count and pathogens in Sydney rock oysters were found to decrease after depuration (Son and Fleet 1980). Relaying involves transferring shellfish from contaminated water to clean water for self-cleaning (Groubert and Oliver 1994). It has been found to reduce *V. vulnificus* in oysters (Kelly and Dinuzzo 1985; Motes and DePaola 1996). However, it has also been reported that in controlled purification, neither total *Vibrios* nor pathogenic *Vibrios* were removed due to their ability to remain attached to oyster tissues (Jones and others 1991). Similar results were reported by Tamplin and Capers (1992). In fact, increased *V. vulnificus* counts in oysters were observed when applying ultra-violet (UV) light to seawater during depuration at temperature greater than 23 °C. They also found that if *Vibrio* spp. is only in the oyster's filtration system, the depuration process is effective, but if *Vibrios* are in the tissues of the oyster itself, depuration does not work effectively. Meanwhile, Eyles and Davey (1984) observed the natural-occurring *Vibrios* response to depuration varied significantly from lab-cultured *Vibrios*, which were more susceptible to depuration process.

2.3.2 Ameripure process

The Ameripure process (developed by Ameripure Oyster Company, LA) involves first immersing the oysters in a warm-water bath and then dropping them into an ice-water slurry. Chen and others (1996) found that the Ameripure process yielded oysters comparable in flavor, texture and smell to untreated oysters, but a slight lightening of color was observed. It was reported that 50 °C and 10 min heat treatment was sufficient to achieve 5 log reductions on *V. vulnificus* in oysters (Cook and Ruple 1992). The Ameripure process is often refereed as pasteurization, but it focuses only on *Vibrios*.

2.3.3 Acid marinade

Acidic marinade method involves soaking oysters in vinegar for several hours. The pH of vinegar is 2.0 and most food-borne bacteria would not be able to grow under such acidic conditions. However, there have been several outbreaks of gastroenteritis induced by consumption of vinegar picked raw oysters. Moreover, the marinade process, using 4% acetic acid at 37 ^{o}C for 3 hr, showed a strong influence on the formation of free polyunsaturated fatty acids (PUFA) and the toxicity in the treated oysters (Shiu 1999).

2.3.4 Pasteurization

Pasteurization can destroy pathogens or spoilage organisms present in foods. However, pasteurization of seafood products resulted in unfavorable changes in taste and texture (Learson and others 1969). Fresh oysters have a unique texture and flavor that can be easily altered with processing involving high heat. Studies on pasteurized oysters were conducted by Chai and others (1991). Oysters were packed in flexible pouches and

heated in 82 °C water bath till the oysters reached the desired temperature. Oysters were then transferred to a 72 °C water bath and held for 8 min before rapid cooling in ice water. They were stored in sealed pouches in refrigerated conditions. The authors claimed that this method resulted in a product with comparable sensory quality to raw oysters, yet the bacterial flora was reduced dramatically. An optimum heating process of 8 min at 75-76 °C was determined to pasteurize oysters packed in flexible pouches based on sensory attributes studies on texture, flavor, and appearance (Chai and others 1991). However, this method was time consuming and costly due to the packing process in the middle of the thermal process.

2.3.5 Other processing technologies

Other studies showed that mild heat treatment could be an effective way to eliminate *Vibrios*. Ama and others (1994) reported a distinct inactivation occurred for *V. vulnificus* in phosphate buffer, oyster homogenate or fish homogenate at 50 °C, though no significant inactivation achieved at 40 °C for 40 min. Johnston and Brown (2002) reported that 70 °C and 2 min was adequate to completely eliminate *V. parahaemolyticus*. Freezing can also reduce *Vibrios.* Vacuum packed shucked oysters after freezing at -20 °C contained significantly less *V. vulnificus* by day 7, however, the bacterium was still detected after day 70 (Parker and others 1994). The response of the *V. parahaemolyticus* to cold shock (15 °C for 2-4 h) was studied by Lin and others in 2004. They found that cold shocked organisms were more susceptible to heat, H_2O_2 and lactic and acetic acid, however, they were able to survive better under the stress of violet radiation. Vasudevan and others (2002) concluded that although chilling (4 °C) and freezing (-18 °C) can

reduce or suppress the growth of *V. parahaemolyticus*, they were not reliable methods for bacteria control. The results from Croci and others (2002) demonstrated that ozone was not effective in *Vibrios* reduction.

A recent petition by a consumer interest group to FDA demanded zero tolerance for *V. vulnificus* in molluscan shellfish. This petition was the driving force in developing reliable alternative technologies to process oysters with increased safety, yet without sacrificing the raw taste and texture. This challenge brings high pressure processing, irradiation, and microwave processing into consideration due to their effectiveness in eliminating bacteria as well as retaining the quality of the products.

2.4 Alternative Technologies

2.4.1 High hydrostatic pressure processing

2.4.1.1 High pressure system

High pressure processing (HPP), also called high hydrostatic processing, has gained tremendous attention as a means of non-thermal processing raw or fresh food products to achieve effective bacteria reduction without causing significant changes in their appearance, flavor, texture and nutritional qualities (Styles and others 1991; Berlin and others 1999). A typical HPP system includes (1) a pressure vessel of cylindrical design, (2) two end closures, (3) a means for restraining the end closures, (4) a low pressure pump, (5) an intensifier which uses liquid from the low pressure pump to generate high pressure process fluid for system compression, and (6) system controls and instrumentations. Food items, either in solid or liquid state, with or without package, are subjected to pressure of 100 to 800 MPa. Process temperature can be as low as below 0

°C or over 100 °C. A food-approved oil or water containing FDA- and USDA-approved lubricants, anti-corrosion agents and sometimes, antimicrobial compounds are used as pressurizing fluid (Farkas and Hoover 2000). A schematic of a typical HPP system is presented in Fig. 2.4.

2.4.1.2 Advantage of HPP

HPP was initially claimed as non-thermal processing or "heatless" processing. However, research showed that food temperature will be increased at a rate of approximately 3 °C/100 MPa due to adiabatic heating (Farkas and Hoover 2000). The temperature will return to its original state during the decompression, where expanding of compressed food against a constraining liquid media causes it to work and lower the temperature. The increase of the temperature is greatly affected by the composition of the food system and the isothermal conditions maintained during the pressure holding period. Food items containing a large amount of fat will have a significant temperature increase.

One of the key advantages of HPP is the possibility to perform processing at ambient or even lower temperatures (Knorr 1999). This will help to retain the fresh and wholesomeness of raw materials and will facilitate the subsequent storage, transportation, and distribution. Another advantage of HPP is that pressure is transmitted in a instantaneous and uniform matter regardless of product size and geometry, which used to be the limiting factor in thermal processing, resulting in enhanced process flexibility and product quality (Knorr 1999). Due to the fact that pressure appeared to have nearly no impact on the covalent bonds (Tauscher 1998, 1999), HPP can retain product color, taste, flavor and nutrient content, all of which requires intact chemical transformations (Hoover

and others 1989). HPP is also rated as environmentally friendly process with nearly no waste produced.

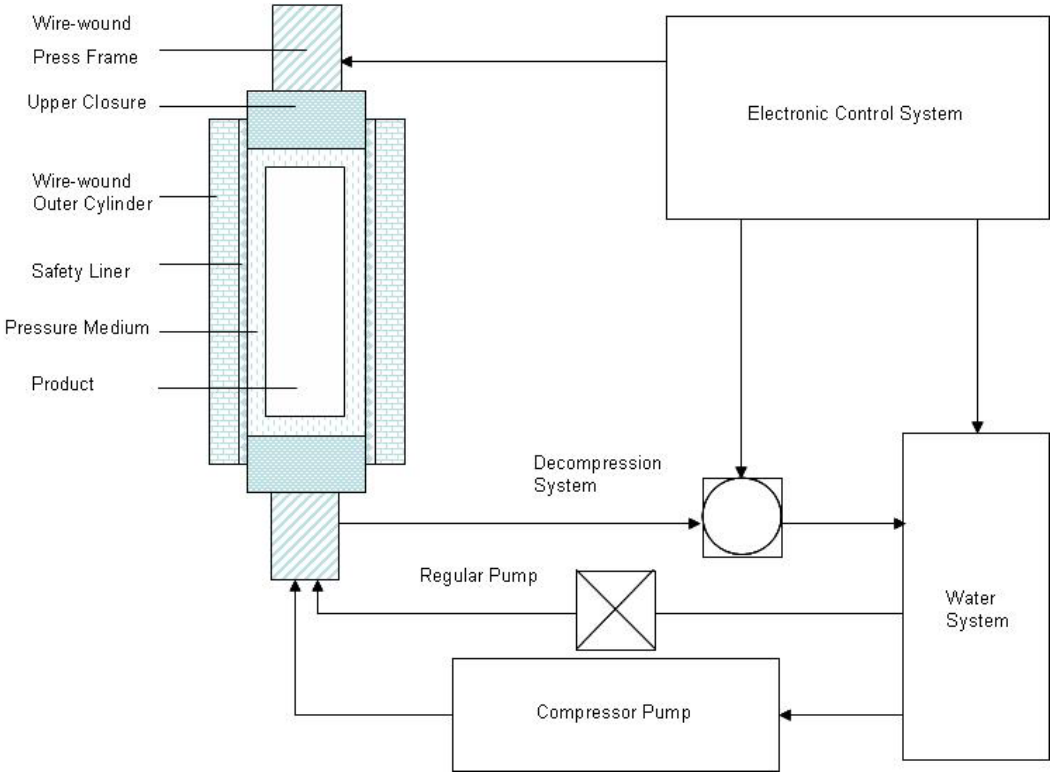

Fig. 2.4: Schematic of a typical HPP system

2.4.1.3 Application of HPP to inactivate bacteria in food system

Since the first attempt on application of HPP in foods in 1899 by Hite and his co-workers, subsequent research work was carried out to study the effect of HPP on the growth and survival of bacterial and spores by Larsen and others (1918) and Timson and Short (1965). Recent interest in HPP was stimulated by successful development of commercialized high pressure processed jam, jellies, and beverages by the Japanese (Hoover 1993). Numerous studies have been published on microbial and enzyme

inactivation, gel formation, protein denaturation, quality retaining and shelflife extension by HPP (Farkas and Hoover 2000; Knorr 1999; Tewari and Jayas 1999).

HPP can inactivate microorganisms by inducing changes to the cell morphology, cell membranes, biochemical reactions and genetic mechanisms (Hoover and others 1989; Smelt 1998). Pressure induced protein denaturation that affects the enzyme functionality in intercellular biochemical reactions and the altered cell permeability were the leading cause of cell death. Physical disruption on the surface of intact cells was observed at pressures greater than 500 MPa using a scanning electron microscopy. Zobell (1970) and Walsby (1973) observed slowed cell division and collapsed intercellular vacuoles at a pressure level of 0.6 MPa.

Styles and others (1991) investigated the resistance of *L. monocytogenes* to HPP. Nearly 6 log reductions occurred when bacteria in a buffer solution was exposed to pressure of 345 MPa for 20 min. Patterson and others (1995) published their results on the response of several vegetative types of food pathogens in buffer solutions to HPP. To obtain a 5 log reductions with a 15 min treatment, *Salmonella Typhimurium* required 350 MPa, *L. monocytogens* required 375 MPa, *Salmonella Enteritidis* 450 MPa, *E.coli O157:H7* and *S. aureus* needed 700 MPa. HPP of *L. monocytogenes* and *Salmonella Typhimurium* in fresh pork loin were studied by Ananth and others in 1998. D values at 414 MPa (25 °C) was determined to be 2.17 min for *L. monocytogenes* and 1.48 min for *Salmonella Typhimurium*. Carpi and others (1995) reported 3 min at 700 MPa extended the shelflife of smoked salmon cream from 60 days to 180 days with comparable sensory quality. In surimi, Miyao and others (1993) found that a pressure level of 300-400 MPa was sufficient to eliminate most fungi, gram-negative, and gram-positive bacteria.

However, applying 300-350 MPa pressure caused loosed or peeled-away skins for raw tomatoes, and a browning color for lettuce (Arroyo and others 1997). Similarly, Carlez and others (1994) stated that at pressure levels higher than 450 MPa, the color and texture change of freshly minced meat were evident even though this pressure level was not enough to completely suppress the growth of *Pseudomonads* and *Lactobaccilli*.

2.4.1.4 HPP inactivation of *Vibrios*

A number of studies have showed that, basically, *Vibrio* spp. is pressure sensitive (Styles and others 1991; Hoover 1993; Berlin and others 1999). A 10^6 CFU/ml population of *V. parahaemolyticus* in clam juice was inactivated by a 173 MPa pressure treatment for 10 min (Styles and others 1991). The results from Berlin and others (1999) indicated that pathogenic *Vibrio* spp. were susceptible to HPP at pressure levels from 200 to 300 MPa. Calik and others (2001) reported that HPP was an effective method in terms of reducing *Vibrio* spp. in whole oysters. In their study, environmental and clinical strains of *V. parahaemolyticus* in both pure cultures and in artificially inoculated live Pacific oysters (*Crassostrea gigas*) were exposed to HPP at 241 MPa to 345 MPa. The results indicated that 5 min was required to eliminate *V. parahaemolyticus* (O3:K6) at 310 MPa without sacrificing the sensory qualities. The optimum processing condition was determined to be at 345 MPa and 0.5 min for *V. parahaemolyticus* in pure cultures and 345 MPa-1.5 min for *V. parahaemolyticus* inoculated in whole oysters, at which 8 log reductions can be achieved. He and others (2001) also demonstrated the positive results of using HPP to extend the shelflife of shucked oysters. Another benefit of HPP on

oysters was producing clearly shucked oysters. This was because pressure can release the abduct mussels causing direct separation of oyster meat from the shells.

2.4.2 Irradiation

2.4.2.1 Gamma-irradiation and electronic beam irradiation

Food irradiation is also called “ionizing radiation” taking the form of γ-ray from isotopes or high-energy concentrated electron beam (Fellows 2000). The equipment of gamma-irradiation consists of two sealed stainless steel tubes (one inside the other - double encapsulated) called "source pencils". They are placed in a rack and the entire rack is immersed in a water chamber underground when not in use. During operation, a radioactive source is raised from the water pool. The food materials are processed by transporting them on conveyors through the radiation field in a circular path. Concrete walls and lead are used to enclose the processing area to avoid any leakage from radioactive sources. Stringent safety procedures are required. Cobalt-60 (^{60}Co) and caesium-137 (^{137}Cs) are most commercially used γ-radiation sources. They possess the largest penetration depth for all ionizations; however, the disadvantages of γ-radiation is its relatively un-evenly distributed energy and it can’t be switched off. Therefore, using ^{60}Co as a radioactive source requires an annual replacement of 12.3% for the γ-radiation to be maintained at consistent energy output (Wilkinson 1986).

Electronic beam (E-beam) irradiation is equipped with a machine source which can generate a high energy electron beam or X-ray (Kava 1996). A cathode is heated by a pulsed power network to provide electrons. Through an electron injector, electrons are directed into an evacuated tube accelerator to elevate their energy in a high voltage

electrostatic field. Electrons are concentrated and accelerated to 99% of the speed of light. Either the electrons are used directly on the food products or X-rays can be produced by bombarding a metal film. The electronic beam can be switched on and off. A conveyer or cart system moves the product to be irradiated under the electron beam at a predetermined speed to obtain the desired dosage. Products move in and out of the irradiation area continuously. E-beam has a limited penetration depth; therefore, product thickness has to be adjusted to a given level depending on density and electron energy. For example, e-beam energy can penetrate meat by a total of 9 cm with treatment on the top and bottom of a package. However, compared to γ-radiation, it has uniformly distributed energy and equipment handling is rather simple (Fellows 2000).

2.4.2.2 Determination of irradiation dose

Irradiation dose is measured by dosimeters, which have been made of various materials. The most commonly used are PVC (polyvinylchloride) dosimeters. These devices capture color change induced by release of hydrogen chloride due to irradiation (McLaughlin and others 1982). The unit, Grays (Gy), is generally used to describe how much irradiation has been received, where 1 Gy indicates the absorption of 1 J of energy per kilogram of food.

2.4.2.3 Application of irradiation in food

Irradiation has proved to be effective in inactivating microorganism and enzymes, killing insects and fungi, inhibiting sprout growing in root vegetables, delaying ripening of fruit and vegetables and improving the physical properties of foods (Kava 1996). The

different doses applied for food irradiation are listed in Table 2.1. Irradiation causes the structural change of cell membrane and malfunction of enzyme metabolic activity. More importantly, it affects DNA and RNA in cell nuclei, slowing or completely depressing the cell growth and replication (Fellows 2000). The D values (Table 2.2) of some food pathogens were reported by Olson (1998).

Table 2.1: Application of food irradiation (Source: Kava 1996)

Type of food	Irradiation dose (kGy)	Effect of treatment
Meat, poultry, fish, shellfish, baked products, some vegetables	20-70	Sterilization at room temperature.
Spices and seasonings	Up to 30	Insect control
Meat, poultry, fish	0.1-10	Delays spoilage, kill some foodborne pathogens and parasites
Strawberries and other fruits	1-5	Extend shelflife, mold control
Grains, fruits, vegetables	0.1-2	Inset control, partially replace fumigants
Bananas, avocados, mangoes, papayas, guavas	Up to 1.0	Delays ripening
Potatoes, onion, garlic ginger	0.05-0.15	Inhibits sprouting
Grains, dehydrated vegetables	Various doses	Desirable physical changes (for example, reduced rehydration time)

2.4.2.4. Application of gamma-irradiation in seafood

Low dose of gamma rays proved to be effective in reducing spoilage and pathogenic microorganisms in a variety of seafood products (Andrews and others 1998; Mallett and others 1991; Przybylski and others 1989). Venugopal and others (1987) reported that irradiation of refrigerated Indian mackerel with a dose of 1.5 kGy extended the shelflife up to 20 days. The results from Poole and others (1994) indicated that after examining nine species of fish products, 3 kGy seemed to be the threshold irradiation condition, above which adverse odor and flavor appeared. A dose level of 3 kGy proved to be sufficient in shelflife extension of Tilapia and Spanish mackerel with acceptable appearance, odor, texture and taste after 8 days. Elimination of *Salmonella* spp. was also observed at this dosage level (Abu-Tarboush, and others 1996). Meanwhile, D value of 0.11 kGy for *V. cholerae* in shrimps at 10 °C was reported by Hau and others (1992).

Table 2.2: D values of some food pathogens subjected to irradiation (Source: Kava 1996)

Pathogens	D values (kGy)	Irradiation temperature (°C)	Suspending medium
A. hydrophilia	0.14-0.19	2	Beef
C. jejuni	0.18	2-4	Beef
E. coli O157:H7	0.24	2-4	Beef
L. monocytogenes	0.45	2-4	Chicken
Salmonella spp.	0.38-0.77	2	Chicken
Staphlococcus aureas	0.36	0	Chicken
Yersinia enterocolitica	0.11	2.5	Beef
Clostridium botulinum (spores)	3.56	-30	Chicken

Irradiation of live oysters has been done for over 15 years (Andrews and others 2003). Novak and others (1966) found that oysters treated with 2 kGy had a shelflife of 23 days under refrigerated conditions. Studies have shown that a dose level of 1.0 kGy or lesser (Cobalt-60) will reduce environmental strains of *Vibrios* and *E. coli* to undetectable levels (Grodner and Hinton 1988; Grodner and Land 1990; Grodner and Watson 1989; Kilgen 1995). Results from Linton (1983) also demonstrated a rapid reduction of *V. cholera* in oyster homogenate with an irradiation dose of 1 kGy. Although a low dose of gamma-irradiation was found to be effective in reducing pathogenic and spoilage microorganisms in seafood products, the effect of irradiation on the sensory quality and consumer acceptance of such products was still a major concern for seafood industries (Shiu 2000). For example, a survey by Resurrection and others (1994) revealed that over 30% of consumers believed that irradiated food is radioactive, which is a concern to the food industry. The risk to workers and environmental issues are other concerns to be addressed.

2.4.2.5 Quality deterioration by irradiation

At a recommended dose level of less than 10 kGy, chemical changes are small when compared with other preservation technologies. However, both types of irradiation can break chemical bonds when absorbed, producing electrically charged particles, ions and radicals (Fellows 2000). In addition, reactive oxygen and its derivatives are produced in foods. Thiamin is lost due to its radiation sensitivity. Sensory properties may be altered in irradiated foods. Autoxidation of fats can occur and the result is rancid off-flavors (Kilcast 1996; Byrn and others 1999). Proteins with sulfur containing amino acid can

break down after the irradiation treatment and generate unpleasant off-flavors (Kilcast 1996; Ahn and others 2000). Dairy products, such as milk, can't be processed by irradiation because they produce off-flavors even at a very low irradiation dosage. Texture of food after irradiation may also change. The break down of carbohydrates like pectin will cause softening of fruit and vegetable tissue (Kader 1986). However, low dose γ-irradiation on seafood seemed to maintain acceptable organoleptic qualities. Chen and others (1996) published data on irradiated crab products (2 kGy or less) having similar odor and flavor comparable to control samples evaluated by sensory panels.

2.4.3 Microwave Processing

2.4.3.1 Mechanisms of dielectric heating

Microwave heating refers to the use of electromagnetic waves of certain frequencies to generate heat in a material (Metaxas 1996; Roussy and Pearce 1995). Typically, the application of microwave heating in food processing uses frequencies of 2450 MHz or 915 MHz as regulated by the Federal Communications Commissions (FCC). Microwave generates heat in two ways: dielectric and ionic (Datta and Davidson 2000). Dielectric heating by microwave involves dipole molecules, such as water, trying to align them in the electric field so that they oscillate at high frequencies to generate heat. Ionic heating is due to the oscillatory migration of ions in the food driven by high frequency alternate electric field. The heat generation can be quantified using Eq. 2.7. The importance of dielectric properties on microwave heating has been discussed in sec. 2.2.3.

Rapid heating that leads to less time to reach the desired temperature is the key advantage of microwave heating over conventional heating, resulting in less deterioration

of organoleptic qualities. It has also been reported, contradicting the general understating, that in certain situations, microwave can create more uniform heating than traditional heating (Datta and Hu 1992). The published data demonstrated that the accumulative lethality, F_o, had a more uniform profile volumetrically with regard to microwave heating compared to conventional heating, even though the temperature-time history looked nearly the same.

2.4.3.2 Factors affecting microwave heating

The factors influencing microwave heating included three aspects: food product itself, microwave equipment and operating conditions. Food shape, volume, mass, composition, thermal properties and dielectric properties are all critical parameters (Zhang and others 2001). Those factors determine not only the amount of energy generated, but also the spatial distribution of the absorbed energy, leading to corner and edge overheating, focusing and resonance (Datta and Davidson 2000). Microwave oven design, such as the size, the geometry of the cavity, the location of the magnetron, and the availability of devices that can improve the uniformity, can greatly affect the magnitude and spatial variation of the power absorption in the product (Zhang and others 2001). In addition, the operating conditions, for example, microwave input power and cycling pattern, operating frequency, relative location of the food in the heating cavity, equilibrium after microwave heating, can affect the heating rate and temperature distribution dramatically. The importance of equilibrium time on the bacterial inactivation has been emphasized by Fakhouri and Ramaswamy (1993). They reported a

significant amount of microbial inactivation occurred at equilibrium time, because equilibrium allowed a temperature re-distribution among hot and cold spots.

2.4.3.3 Application of microwave in food processing

Since the main function of a microwave is to generate heat, in theory, it can be applied in any food processing involving heat, such as drying, thawing, pasteurization and sterilization. The implementation of microwave in pasteurization or sterilization is a great challenge caused by non-uniform heating. Although commercially, there are only two industrial systems operating worldwide, the studies on microwave inactivation mechanisms have been carried on for nearly 60 years (Knutson and others 1988; Burfoot and others 1996; Kudra and others 1991; Cassanovas and others 1994; Vallamiel and others 1997; Zhang and others 2001). Numerous work has been published addressing the inactivation of food pathogens by microwaves including *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, pathogenic *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella* spp. in poultry, beef, fish, pork, milk and eggs (Rosenberg and Bogl 1987; Knutson and others 1987; Heddleson and others 1994; Tassinari and Dos 1997; Farber and others 1998; Yeo and others 1999). The amount of destruction of *Salmonella* spp. varied from 3.17 log CFU/ml in UHT milk to 0.44 log CFU/ml in beef broth. The greatest destruction of *L. monocytogenes* (2.39 CFU/ml) occurred in pudding, and least destruction incurred in cream sauce (1.63 log CFU/ml). There was no difference in the log reduction of *S. aureus* heated in five food products including UHT milk, beef broth, pudding, cream sauce, and liquid whole eggs (Heddleson and others 1996). However, Schiffmann (1992) pointed out the

microorganisms were more likely to survive in foods heated using microwaves than those by conventional methods.

2.4.3.4 Microwave inactivation kinetics

Regarding to the microbial inactivation kinetics, the controversy was focused on the existence of non-thermal effects of microwave heating aside from the lethal effects caused by heat (Datta and Davidson 2000). Basically, two mechanisms were proposed for inactivation of microorganisms. The first one states that death of microorganisms was purely due to heat induced protein denaturation, enzyme dysfunction, altered nucleic acids and disruption of membranes (Heddleson and Doores 1994). The second argues that, in addition to pure thermal effect, non-thermal effect also contributes inactivation of microorganisms. Theories developed to support the non-thermal effect statement include selective heating, electroporation, cell membrane rupture, and magnetic field coupling (Kozempel and others 1998). Selective heating hypothesizes that solid microorganisms are heated more effectively by microwaves than the surrounding medium and thus killed more readily. Electroparation is caused by pores formed in the membrane of the microorganisms due to electrical potential across the membrane, resulting in leakage. In a fourth theory, bacterial cell lysis occurs due to coupling of electromagnetic energy with critical molecules within the cells, disrupting internal components of the cells (Datta and Davidson 2000).

A series of studies supporting the non-thermal effects provided evidences on reduced D values in *B. stearothmophilus* spores, and the appeared lethal effects at sub-lethal temperatures on *S. aureasu*, by microwave heating compared to conventional

heating (Khalil and Villota 1988, 1989). Kozempel and others (2000), who employed a newly designed system that is capable of separating non-thermal effect from the thermal, found that microwave energy did not inactivate microorganisms in the absence of other stresses, for example, pH or heat, in fact, it simply magnified the thermal effects. In addition, Ramaswamy and others (2000) also agreed on the existence of a non-thermal effect, confirming that, compared to an equivalent heat treatment, microwave enhanced microbial inactivation by investigating the D values of *E. coli* K12 at 55, 60 and 65 °C. Aside from that, the majority of research has concluded that there was no non-thermal effect by microwave inactivation (Fujikawa and others 1992; Heddleson and Doores 1994). They further disputed that the reported non-thermal effect was due to the lack of accurate measurement of temperature-time history and its spatial variations (Datta and Davidson 2000). Currently, the general consensus is that microwave inactivation is essentially a thermal effect.

Therefore, the temperature profile at the coldest spot in the food system has become the key of the microbiological safety of the process. The accumulated lethality can be calculated by

$$F_0 = \int_0^t 10^{(T-250)/z}\, dt \qquad ..(2.8)$$

where T is the cold point temperature at any time t, z is z-value in °F. However, because the thermal and dielectric properties of food items are changing under microwave processing, the relative locations of cold and hot areas also change. In general, the hottest points migrate from the center to the surface of the product, further influencing the spatial heat distribution (Datta and Davison 2000). Thus, the coldest spot in microwave heating is very difficult to predict. Mathematical modeling can provide a solution to this problem.

2.4.3.5 Mathematical modeling of microwave heating

Mathematical modeling of microwave-cooked food products has been widely studied on simple geometry such as slabs (Datta 1991; Zhou and others 1992; Tong and Lund 1993; Housova and Hoke 2001), cylinders (Zhou and others 1992; Chen and others 1993; Mallikarjunan and others 1996; Cheng and others 1996) and spheres (Ohlsson and Risman 1978; Van-Remmen and others 1996) The finite difference (Gundavarapu and others 1995; Lee and others 2002) and finite element methods (Pandit and Prasad 2003; Tanaka and others 2001) are frequently used to solve fundamental differential equations. The coupling of electromagnetic and thermal effect of microwave processing has been investigated by Zhang and Datta (2000). Mathematical simulation can not only save tremendous time and money by reducing the experimental numbers, but also provide a scientific way of determine the temperature profile of any locations within the product scheme, including the coldest point. However, the limitation of traditional approach to calculate the lethality is: the lethality values generated from the time-temperature history of the coldest point is used and compared to the microbial enumeration data which have been obtained collectively. The end result under or over estimated the lethal effect. A more accurate approach is proposed by this author to calculate the lethality at each designated heating unit of the entire product scheme and get the lethality by the mass average of the localized lethality. Author assumes this way will get a more closed prediction of microbial inactivation by utilizing mathematical model approach.

2.5 Microbial Inactivation Kinetics Model

2.5.1 Bigelow model

The most general equation to describe microbial inactivation by heat, adopted from the first order reaction kinetics model, is:

$$-dN/dt = kN \qquad .. (2.9)$$

where N is the number of microorganism, t is time and k is reaction rate constant. In its integrated form, by assuming the rate constant would not change with respect to pressure or temperature, Eq. 2.9 becomes the traditional approach to describe the microbial survival as a function of time:

$$\ln(N/N_0) = -kt \qquad ..(2.10)$$

where N is microbial population at any time, t; N_0 is initial microbial population.

When D, a decimal reduction time, is defined as time required for 1-log cycle reduction in the microbial population at a specific temperature, the equation becomes:

$$\log(N/N_0) = -t/D \qquad ..(2.11)$$

Therefore, by comparing Eqs. 2.10 and 2.11, the relationship between decimal reduction time and the first-order reaction rate constant is:

$$k = 2.303/D \qquad ..(2.12)$$

2.5.2 Weibull Distribution

For a long time, first order kinetics dominated the microbial inactivation kinetics (Holdsworth 1997). When calculating the microbial inactivation parameters, the frequently observed non-linear survival curves presenting “shoulder” or “tailing” are neglected, and only the linear portion is considered. With the discoveries of many

emerging bacteria and processing technologies, it is the time to think of a question, "Do bacteria only die in a fixed mode"?

Peleg and Cole (1998) challenged the assumption of first order kinetics by questioning the in-sensitivity of linear regression data fitting and the temperature dependence of the generated models. They argued that the microbial survival curve actually reflects an accumulated form of resistance distribution of microbial population. Since the resistance of a microorganism can be influenced by many factors, its distribution form can have different forms, linear or non-linear with upward or downward concavity or sigmoid (Campanella and M. Peleg 2001). According to this concept, a Weibull distribution can be used to describe the survival curves and a linear semi logarithmic survival curve is just a special case of Weibull distribution with resistance shape factor equal to 1. The survival function can be defined as:

$$S(t) = \exp\left[-(\frac{t}{a})^{b}\right] \qquad ..(2.13)$$

where S(t) is the fraction of survival microorganisms. t is treatment time and a and b are the scale and shape factors. Mean t_c is defined as a measure of the microorganisms resistance and can be calculated by:

$$t_c = a\Gamma(1+b^{-1}) \qquad ..(2.14)$$

where Γ is the gamma function and t_c is mean t_c. Both a and t_c vary with processing conditions and can be considered as kinetic parameters (Fernández and others 1999).

2.5.3 Fermi's Model

For survival curves showing two regions, one with no or negligible effect of microbial death followed by rapid exponentially falling and with no effect of time

constrain, Fermi's equation, which was used to describe mechanical changes of bio-materials at and around their glass transition temperature, may be used to describe microbial death kinetics induced by processes such as irradiation (Peleg 1995).

$$S(V)=100/(1+\exp((V-Vc)/a)) \quad ..(2.15)$$

where S(V) is the percentage surviving of microorganisms, V is the process treatment, such as electric filed strength (kV/cm) in a pulse electric filed process, or dosage (kGy) in an irradiation process. Vc is the critical level of V where survival level is 50% and a is a parameter indicating the steepness of survival curve at Vc. Since V is generally much greater than Vc, Eq. 2.15 can be simplified to Eq. 2.16 with k (reaction rate) = 1/a:

$$S(V)=100/\exp(V/a) \quad ..(2.16).$$

2.5.4 Model for Reaction Rate Constant under High Pressure Processing

As mentioned before, non-linearity of survival curves generally occur under a process having a non-isothermal temperature profile. A differential equation whose coefficients are functions of the organism's survival pattern needs to be used instead of its integrated form. Since it is known that microbial death rate k follows a first order kinetics differential equation, Eq. 2.9 can be transformed to Eqs. 2.17 and 2.18:

$$\Delta N/\Delta t = -kN \quad ..(2.17)$$

$$k = -\frac{\Delta N}{\Delta t N} \quad ..(2.18)$$

where ΔN is the change of microbial survival number over the time, Δt. It is also known that the effect of pressure on the death rate constant k follows the Eyring's equation,

proposed by Johnson and Eyring (1974). This equation has a similar form of Arrhenius equation:

$$\ln k = \ln B - \frac{VaP}{R_g T} \qquad ..(2.19)$$

where k is the death rate constant, B is Arrhenius constant, Va is activation volume, R_g is gas constant, T is temperature of the pressure treatment and P is applied pressure. Substitute k from Eq. 2.18 into Eq. 2.19, a linear relationship between ln (k) and pressure (P) can be established. Employing linear regression, the unknown parameters Va and B in Eq. 2.19 can be found. Thus, death rate constant k and D values can be obtained at any pressure levels.

2.6 Quality Evaluation of Oysters

2.6.1 Microbial quality

Spoilage of shellfish involves a combination of complex, interrelated processes brought about by either biological reactions, mostly enzymatic and oxidative reactions, or by metabolic activities of microorganisms, resulting in off-flavors, texture deterioration and discoloration (Ashie and others 1996; Erikson 1997). Several methods can be used to determine the microbial quality of oysters. The National Shellfish Sanitation Program recommends that levels of both total bacteria and fecal coliforms be determined as a means of assessing the microbial quality of an oyster (Hood and others 1983). Additionally, bacteriological monitoring of live bivalve shellfish such as oysters can be based on the enumeration of *E. coli*, which is considered to be the best bacterial indicator of faecal contamination of water and foods (González and others 1999). An oyster is

classified as acceptable if there is less than 2.3 *E. coli* cells per g and if the total plate count is less than 10^5 cells per g (Huss and others 2000; Son and Fleet 1980).

2.6.2 Color Assessment

Visual effects are important in consumer acceptance towards a particular food item. Color can be defined as the energy distribution of the light reflected by or transmitted through a particular food product (Mallikarjunan and Hung 1997). In food chemistry, color is often measured using standard Commission International de l'Eclariage (CIE) *L*a*b** scales (Mallikarjunan and Hung 1997). This model of color measurement was designed to be device independent, creating consistent color regardless of the device used to create or output the image (Papadakis and others 2000). The parameters utilized in this model represent the luminance or lightness (L), the degree of redness or greenness ($\pm$ a), and the degree of yellowness or blueness ($\pm$ b) of the product measured (Mallikarjunan and Hung 1997). When the color measurement of a food product is made, a three numeric value data set is generated that uniquely describes the color of the sample. To give some physical sense to the *L*a*b** values, these three numbers represent a distinct point in three-dimensional space of the color solid that is seen in Fig. 2.5.

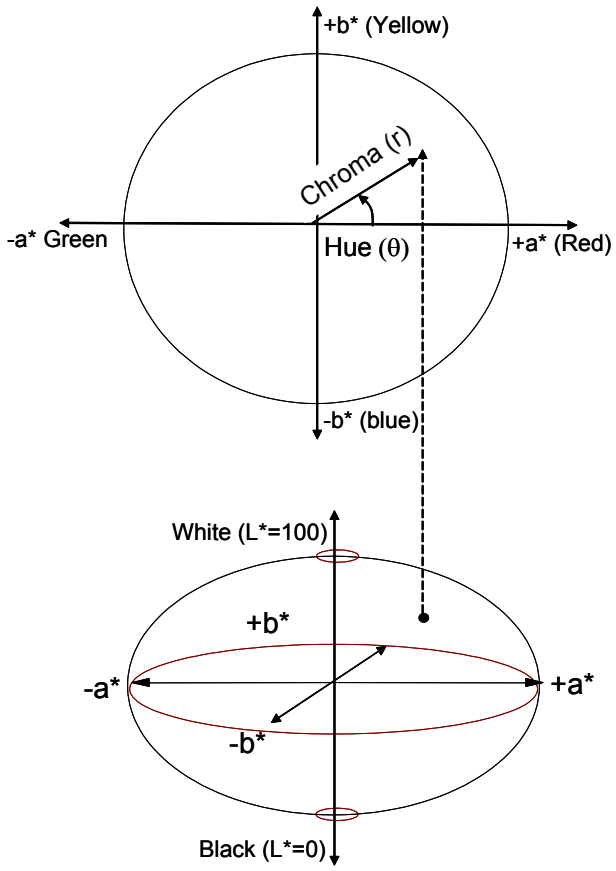

Fig. 2.5: Schematic representation of a color wheel and solid representing color-measurement system

2.6.3 Odor Analysis

2.6.3.1 Seafood aroma

Raw shellfish has little odor and has a flavor similar to that of fruit or seaweed. These odors and flavors are due to the action of unsaturated alcohols and aldehydes containing less than 10 carbon atoms. A major volatile alcohol, 1-octen-3-ol, plays a huge role in the mushroom-like odor of shellfish. 1-octen-3-ol, 1,5-octadien-3-ol, and 2,5-octadien-1-ol all contribute to the heavy, plant-like aroma found in oysters (Shahidi and Botta 1994). Much of the spoiled odor and taste of post-harvest seafood is due to bacterial enzymes or endogenous enzymes (Bremmer 2000). These biological reactions include the hydrolysis of urea to ammonia and carbon dioxide, as well as the hydrolytic

reaction of enzymatic and non-enzymatic oxidation (Bremmer 2000). Aldehydes are largely derived from auto-oxidation of lipids. The 4Z-heptenal compound is responsible for the burnt, stale or fishy aroma of oysters. (E,Z) 2,6-nonadienal, was found to be a volatile compound of fresh oysters as well. Both these compounds are responsible for the "shell-fishy" odor as a result of auto-oxidation (Shahidi and Botta 1994).

2.6.3.2 Instrumental Olfaction-Electronic nose

Electronic noses are composed of an array of chemical sensors, each tuned to a specific range of odorant molecules (Bartlett and others 1997). Different electronic nose technologies employ varying types of chemosensors. A specific chemosensor is a chemocapacitor (CAP). CAP functions by use of a conducting polymer electrode. Electric and physical properties of polymers vary when exposed to molecules of gaseous compounds. By taking measurements with and without the presence of gaseous molecules, the electrical and physical property changes are captured and interpreted to odor values (Pearce and others 2003).

Another relevant chemosensor technology is quartz-crystal microbalance (QCM). QCM is the simplest type of odor sensor using a piezoelectric device, such as quartz (Pearce and others 2003). The quartz crystal is covered with thin-filmed gold electrodes to create a functional sensor. By inducing an electric voltage on the single crystal quartz, an electric field is created that disfigures the material. If an AC signal is incident on the quartz crystal, it will oscillate at a resonant frequency (Pearce and others 2003). Changes in quartz crystal mass due to gas molecules create a frequency change and the degree of frequency change is proportional to the mass or abundance of specific gas molecules.

2.6.4 Texture assessment

The definition of texture has been modified over time, and the latest version was given by Meilgaard and others (1999). They define texture as the sensory manifestation of the structure or inner makeup of products in terms of their reaction to stress and tactile properties. The reaction to stress is measured as mechanical properties such as hardness/firmness, adhesiveness, cohesiveness, gumminess, springiness and viscosity by the kinesthetic sense in the muscles of the hand, fingers, tongue, jaw or lips. Texture of seafood, mainly fish, is commonly tested in the industry by the 'finger method". A finger is pressed on the skin, and depending on the dent or hole left on the fish muscle, the hardness of fish is evaluated. However, this method is subjective and requires highly experienced personnel to perform, therefore, it is not desirable (Sigurgisladottir and others 1997).

Objective measurement of the texture properties of seafood products has received considerable attention because the results contain more scientific values and can be duplicated (Segars and Johnson 1986). The primary techniques use puncture, compression, shear, cut, and tensile, described by Johnson and others (2001). Among them, the shearing force and compression methods are most recommended (Sigurgisladottir and others 1999). More often, instrumental measurements are a necessary complement to the sensory data, and it is suggested that non-destructive measurement are not good predictors of sensory texture, especially with products containing high protein (Hamann 1986). A variety of shearing and cutting devices are available, and the most commonly used are the Kramer shear compression cell and the

Warner-Bratzler device. One of the disadvantages of utilizing the Kramer cell is the requirement of large amount of samples (Sigurgisladottir and others 1999).

Many attempts have been made to correlate the texture properties with sensory evaluations. For example, Barroso and others (1998) suggested that Kramer cell compression was a more appropriate method for texture analysis of whole fish, and it correlated very well with sensory evaluations. According to Zapata and Price (1992), Instron compressing and the Kramer cell method demonstrated higher sensitivity in detecting the texture difference in Sucker fish than penetrometer. Chamberlain and others (1993) modified the Warner Bratzler device and found out a sound correlation between the texture properties and the fish species. Sigurgisladottir and others (1999) reported that the shear force method based on cutting with a knife edge blade was more sensitive than the puncture methods in differentiating texture properties such as hardness. Jonsson and others (2001) concluded that the Warner-Bratzler device was a more reliable method to measure the texture properties of raw salmon fillet compared to Kramer cell method. Unfortunately, not many studies have been carried out on texture property measurement of oysters.

Conclusions

High pressure, gamma-irradiation and microwave processing have demonstrated potential as appropriate technologies to enhance the safety of oysters. However, each mentioned technology has its limitations in terms of maintaining the product quality that is as close to their raw state as possible. The critical process conditions need to be identified and, if necessary, multiple hurdle technology needs to be employed to achieve the targeted bacteria reduction and quality retention.

References

Abu-Tarboush HM, Al-Kahtani HA, Atia M, Abou-Arab AA, Bajaber AS, El-Mojaddidi MA. 1996. Irradiation and post-irradiation storage at 2 ± 2 °C of Tilapia (*Tilapia nilotica ×T. aurea*) and Spanish mackerel (*Schomberomorus commerson*): sensory and microbial assessment. J Food Prot. 59(10):1041-1048.

Ahn DU, Jo C, Olson DG. 2000. Analysis of volatile components and the sensory characteristics of irradiated raw pork. Meat Sci. 54(3):209-215.

Ama AA, Hamdy M K, Toledo RT. 1994. Effects of heating, pH and thermo-radiation on inactivation of *Vibrio vulnificus*. Food Microbiol. 11:215-227.

Ananth V, Dickson JS, Olson DG, Murano EA. 1998. Shelflife extension, safety and quality of fresh pork loin treated wit high hydrostatic pressure. J Food Protect. 61(12):1649-1656.

Andrews LS, Ahmedna M, Grodner RM, Liuzzo JA, Murano PS, Murano EA, Rao RM, Shane S, Wilson PW. 1998. Food preservation using ionizing radiation. Inter. Rev. Environ. Contam. Toxicol. 154:1-53.

Andrews L, Jahncke M, Mallikarjunan K. 2003. Low dose gamma-irradiation to reduce pathogenic *Vibrios* in live oysters (*Crassostrea virginica*). J Aqua. Food Product Technol. 12(3):71-82.

Annama T, Rao CVN. 1974. Studies on thermal diffusivity and conductivity of fresh and dry fish. Fish Technol. 11(1):28

Anon. 1996. The International Commission on Microbiological Specifications for Foods. *Vibrio vulnificus* Microorganisms in Foods: 5 Microbiological Specifications of Food Pathogens. London: Blackie Academic and Professional. 436-439 p.

Arroyo G, Sanz PD, Prestamo G. 1997. Effect of high pressure on the reduction of microbial populations in vegetables. J Appl. Microbial. 82:735-742.

Ashie INA, Smith JP, Simpson BK. 1996. Spoilage and shelf-life extension of fresh fish and shellfish. Crit. Rev. Food Sci. & Nutr. 36(1&2):87-121.

ASHRAE. 1977. Handbook and Product Directory: Applications. American Society of Heating, New York: Refrigerating and Air-conditioning Engineers.

Balaban MO, Pigott GM. 1992. Thermal conductivity, heat capacity and moisture isotherm of ocean perch at different moisture levels and temperatures. J Aqua. Food Product Technol. 1(2):57-74.

Barroso M, Careche M, Borderias AJ. 1998. Quality control of frozen fish using rheological techniques. Trends Food Sci. Technol. 9:223-229.

Bartlett PN, Elliott JM, Gardner JW. 1997. Electronic noses and their application in the food industry. Food Technol. 51(12): 44-48.

Baumann P, Furniss AL, Lee JV. 1986. *Vibrios*. In: Sneath PA, editor. Bergey's Manual of Systemic Bacteriology. 9th ed. Maryland, Baltimore: Williams and Wilkins. Vol.2, 518-538 p.

Berlin DL, Herson DS, Hicks DT, Hoover DG. 1999. Response of pathogenic *Vibrio* species to high hydrostatic pressure. Appl. Environ. Microbiol. 65(6):2776-2780.

Bremmer H, Allan. 2000. Safety and quality issues in fish processing. Cambridge, UK: Woodhead Publishing Limited. 235p.

Brown LD, Dorn CR. (1977). Fish, shellfish and human health. J Food Protect. 40:712-717.

Burfoot D, Railton DJ, Roster AM, Reavell R. 1996. Modeling the pasteurization of prepared meal with microwaves at 896 MHz. J Food Eng. 31(1-2):117-133.

Byrn MW, Lee JW, Yook HS, Lee KH, Kim KP. 1999. The improvement of color and shelf life of ham by gamma-irradiation. J Food Protect. 62(10):1162-1166.

Calik H, Morrissey MT, Reno PW, An H. 2001. Effect of high pressure processing on *Vibrio parahaemolyticus* in pure couture and Pacific oysters. J Food Sci. 67(4):1506-1510.

Campanella OH, Peleg M. 2001. Theoretical comparison of a new and the traditional method to calculate *Clostridium botulinum* survival during thermal inactivation. J Sci. Food Agric. 81:1069-1076.

Carlez A, Rosec JP, Richard N, Cheftel JC. 1994. Bacterial growth during chilled storage of pressure-treated minced meat. Lebens Wiss Technol.,27:48-54.

Carpi G, Buzzoni M, Gola S, Maggi A, Rovere P. 1995. Microbial and chemical shelf-life of high-pressure treated salmon cream at refrigeration temperatures. Ind. Conserve. 70:386-397.

Cassonovas J, Anantheswaran RC, Shenk J, Puri VM. 1994. Thermal processing of food packaging waste using microwave heating. J Microwave Power EE. 29:171.

CDC 1993a. Multi-state outbreak of viral gastroenteritis related to consumption of oysters – Louisiana, Maryland, Mississippi, and North Carolina, 1993. MWR, 47(49):417-422.

CDC 1993b. *Vibrio vulnificus* infections associated with raw oyster consumption-Florida, 1981-1992. MMWR, 42(21):405-407.

CDC 1997. Viral gastroenteritis associated with eating oysters – Louisiana, December 1996 to January 1997. MMWR, 46(47):1109-1112.

CDC 1998. Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters – Pacific Northwest, 1997. MMWR, 43(22):457-462.

CDC 1999. Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters and clams harvested from Long Island Sound – Connecticut, New Jersey, and New York. 1998. MMWR, 48(03):48-51.

Chai T, Liang KT, Pace J, Schlimme DV. 1991. Effect of heat processing on quality of pasteurized oysters. J Food Sci. 49:331-333.

Chamberlain AI, Kow F, Balasubramaniam E. 1993. Instrumental methods for measuring texture of fish. Food Australia. 45:439-443.

Chang ES, Lai FS, Miller BS. 1980. Thermal conductivity and specific heat of grain dust. Trans. ASAE. 23:1303-1306, 1312.

Chen DD, Singh RK, Haghichi K, Nelson PE. 1993. Finite element analysis of temperature distribution in microwaved cylindrical potato tissue. J Food Eng. 18:351-369.

Chen YP, Andrews LS, Grodner RM. 1996. Sensory and microbial quality of irradiated crab meat products. J Food Sci. 61(6):1239-1242.

Chen YP, Park DL, Andrews LS, Grodner RM. 1996. Reduction of *Vibrio vulnificus* in Gulf Coast oysters by mild heat treatment [abstract]. In: IFT Annual Meeting Book of Abstracts; 1996 June 22-26; New Orleans, La. Chicago, III. : Institute of Food Technologist. p.134.

Cheng Y, Sakai N, Hanzawa T. 1996. Heat transfer analysis of flat cylindrical food model heated by microwave. J Jan. Soc. Food Sci. & Technol. 43(11):1183-1189.

Choi Y, Okos M. 1986. Effects of temperature and composition on the thermal properties of foods. In: Lemaguer M, Jelen P. Food Engineering and Process Applications. Vol.1. Elsevier Applied Science Publishers.

Cook DW, Ruple AD. 1992. Cold storage and mild heat treatment as processing aids to reduce the number of *Vibrio vulnificus* in raw oysters. J Food Protect. 55(12):985-989.

Cook DW. 1991. Microbiology of bivalves molluscan shellfish in Microbiology. In: Ward DR, Hackney CR, editors. Marine Food Products. New York: Van Nostrand Reinhold.

Crawford YJ, Murano EA, Olson DG, Shenoy L. 1996. Use of high hydrostatic pressure and irradiation to eliminate *Clostridium sporogenes* in chicken breast. J Food Protect. 59:711-715.

Croci L, Suffredini E, Cozzi L, Toti L. 2002. Effects of depuration of molluscan experimentally contaminated with *Escherichia coli*, *Vibrio cholerae* O1 and *Vibrio parahaemolyticus*. J Appl. Microbiol. 92(3):460-465.

Data AK. 1991. Mathematical modeling of microwave processing as a tool to study safety. ASAE International Winter Meeting; 1991 December 17-20; Chicago, IL. St. Joseph, MI: American Society of Agricultural Engineers. Paper No. 91-6614.

Datta AK, Davidson PM. 2000. Microwave and Radio Frequency Processing. In: Kinetics of Microbial Inactivation of Alternative Food Processing Technologies. IFT's response to task order #1, U.S. Food and Drug Administration: How to

quantify the destruction kinetics of alternative processing technologies. Chicago, IL: Institute of Food Technologist: J Food Sci. Suppl. 32-41 p.

Datta AK, Hu W. 1992. Quality optimization of dielectric heating processes. Food Technol. 46(12):53-56.

Delmore RP, Crisley D. 1979. Thermal resistance of *Vibrio parahaemolyticus* in clam homogenate. J Food Protect. 42:131-134.

Drake SL, Bang W, Jaykus LA, Drake MA, Green DP, Elhanafi E. 2003. Evaluation of growth and survival characteristics of *Vibrio vulnificus* expressing green florescent protein [abstract]. In: IFT Annual Meeting Book of Abstracts; 2003 July 13-16; Chicago, IL. Chicago, III.: Institute of Food Technologist.

Earampamoorthy S, Koft RS. (1975). Health hazards of bivalve mollusk ingestion. Ann. Intern. Med. 83:107-110.

Emswiler BS, Pierson MD, Shoemaker SP. 1976. Sub-lethal heat tress of *Vibrio parahaemolyticus*. Appl. Environ. Microbiol. 32:792-798.

Engelder DS, Buffler CR. 1991. Measuring dielectric properties of food products at microwave frequencies. Microwave World. 12(2):6-15.

Erickson MC. 1997. Lipid Oxidation: Flavor and nutritional quality deterioration in frozen foods. In: Erickson MC, Hung Y-C, editors. Quality in Frozen Food. New York, NY: Chapman and Hall. 141-173 p.

Eyles MJ, Davey GR. 1984. Microbiology of commercial depuration of the Sydney rock oyster, *Crossostrea commercialis*. J Food Protect. 47(9):703-706.

Fakhouri MO, Ramaswamy HS. 1993. Temperature uniformity of microwave heated foods as influenced by product type and composition. Food Res. Int. 26:89-95.

Farber JM, Daoust JY, Diotte M, Sewell A, Daley E. 1998. Survival of *Listeria* spp. on raw whole chickens coked in microwave ovens. J Food Protect. 61(11):1465-1469.

Farkas DF, Hoover DG. 2000. High pressure processing. In: Kinetics of Microbial Inactivation of Alternative Food Processing Technologies. IFT's response to task order #1, U.S. Food and Drug Administration: How to quantify the destruction kinetics of alternative processing technologies. Chicago, IL: Institute of Food Technologist: J Food Sci. Suppl. 47-64 p.

Fellows P. 2000. Irradiation. In: Food Processing Technology: Principles and Practice. 2nd ed. Boca Raton, Florida: CRC Press LLC. p 196-209.

Fernández A, Salmerón C, Fernández PS, Martínez A. 1999. Application of a frequency distribution model to describe the thermal inactivation of two strains of *Bacillus cereus*. Trends Food Sci. Technol. 10:158-162.

Fleet GH. 1978. Oyster depurations – a review. Food Technol. 30:444-454.

Fujikawa H, Ushioda H, Dudo Y. 1992. Kinetics of *Escherichia coli* destruction by microwave irradiation. Appl. Environ. Microbiol. 58(3):920-924.

Gabriel C, Grant EH. 1989. Dielectric sensors for industrial microwave measurement and control. Proceedings of High-frequency Microwave Conference; Amhem, KEMA, Netherlands.

González I, García T, Fernádex A, Sanz B, Hernández PE, MartíR N. 1999. Rapid enumeration of *Escherichia coli* in oysters by quantitative PCR-ELISA. J Appl. Microbiol. 86(2): 231-236.

Gordner RM, Watson MA. 1989. Low dose gamma irradiation of *Vibrio vulnificus* in blue crab (*Callineces sapidus*). Proceedings of the Fourteenth Annual Conference of the Tropical and Subtropical Fisheries Technological Society of the Americas. 128-133 p.

Green AD. 1997. Measurements of the dielectric properties of cheddar cheese. J Microwave Power EE, 32(1):16-26.

Grodner RM, Hinton A. 1988. Gamma irradiation of *Vibrio cholerae* in crayfish (*Procambarus clakii*). Proceedings of the Twelfth Annual Conference of the Tropical and Subtropical Fisheries Technological Society of the Americas. p 429-441.

Grodner RM, Land MA. 1990. Gamma irradiation of *Vibrio parahaemolyticus* in the blue crab (*Callanectes sapidus*). Conference Proceedings of Fifteenth Annual conference, Florida Sea Grant Program. Gainseville, FL.

Groubert TN, Oliver JD. 1994. Interactions of *Vibrio vulnificus* and the eastern oyster, *Crassostrea virginica*. J Food Protect. 57:224-228.

Gundavarpu S, Hung YC, Brackett RE, Mallikarjunan P. 1995. Evaluation of microbiological safety of shrimps cooked in the microwave oven. J Food Protect. 58(7):742-747.

Hamann DD. 1986. Rheological changes during thermally induced gelation of proteins: methods [Abstrct]. In: IFt Annual Meeting Book Abstract; 1986 June 4-10; Dallas, TX. Chicago III.: Institute of Food Technologist.

Hau LB, Liew MH, Yeh LT. 1992. Preservation of grass prawns by ionizing radiation. J Food Protect. 55:198-202.

He H, Adams RM, Farkas DF, Morrissey MT. 2002. Use of high-pressure processing for oysters shucking and shelflife extension. J Food Sci. 67(2):640-645.

Heddleson RA, Doores S. 1994. Factors affecting microwave heating of foods and microwave induced destruction of foodborne pathogens: a review. J Food Protect. 57(11):1025-1037.

Heddleson RA, Doores S, Anantheswaran RC, Kuhn GD. 1996. Viability loss of *Salmonella* species, *Staphylococcus aureus*, and *Listeria monocytogenes* in complex foods heated by microwave energy. J Food Protect. 59:813-818.

Heddleson RA, Doores S, Anantheswaran RC. 1994. Parameters affecting destruction of *Salmonella* spp. by microwave heating. J Food Sci. 59(2):447-451.

Hinton 1983. Survival of V. cholera in Seafood [dissertation]. Baton, Rouge, LA.: Louisiana State University. Available from: University Microfilms.

Hite BH. 1899. The effect of pressure in the preservation of milk. Bull. WV Univ. Agric. Exp. Sta., Morgantown. 58:15-35.

Holdsworth SD. 1997. Kinetics of thermal processing. In: Thermal Processing of Packaged Foods. UK: Chapman & Hall, London.

Hood MA, Ness GE, Rodrick GE, Blake NJ. 1983. Effects of storage on microbial loads of two commercially important shellfish species, *Crassostrea virginica* and *Mercenaria campechiensis*. Appl. Environ. Microbiol. 45(4): 1221-1228.

Hooper FS, Lepper AR. 1950. Limitation of thermal conductivity. Trans. ASAE, 56:309-324.

Hoover DG. 1993. Pressure effects on biological systems. Food Technol. 47(6):150-155.

Hoover DG. Metrick C, Papineau AM, Farkas DF, Knorr D. 1989. Biological effects of high hydrostatic pressure on food microorganisms. Food Technol. 43(3):99-107.

Housova J, Hoke K. 2001. Temperature profiles in microwave heated solid foods of slab geometry: influence of process parameters. Czech J Food Sci. 19(3):111-120.

Huss HH, Reilly A, Embarek PKB. 2000. Prevention and control of hazards in seafood. Food Control II. 11(2): 149-156.

Iguchi T, Kondo S, Hisatsune K. 1995. *Vibrio parahaemolyticus* O serotypes from O1 to O13 all product R-type lipo-polysaccharides: SDS-PAGE ad compositional sugar analysis. FEMS microbial. Lett. 130:287-292.

Jobin R, Jobin A. 2004. The Chincoteague National History Association. Available at http://www.assateague.com/nt-bival.html. Accessed Nov 14.

Johnson A. 1999. Heat transfer systems in Biological Process Engineering. In: An Analogical Approach to Fluid Flow, Heat Transfer, and Mass Transfer Applied to Biological Systems. New York: John Wiley & Sons, Inc.

Johnson FH, Eyring H, Jones-Stover B. 1974. The theory of rate processes in biology and medicine. New York: Wiley. p 82.

Johnston MD. Brown MH. 2002. An investigation into the changed physiological state of *Vibrio* bacteria as a survival mechanism in response to cold temperatures and studies on their sensitivity to heating and freezing. J Appl. Microbiol. 92(6):1067-1077.

Jones SH, Howel TL, O'Neill KR. 1991. Differential elimination of indicator bacteria and pathogenic *Vibrio* from eastern oysters in commercial controlled purification facility in Maine. J. Shellfish Res. 10:105-112.

Jonsson A, Sigurgisladottir S, Hafsteinsson H, Kristbergsson K. 2001. Textural properties of raw Atlantic salmon (*Slmo salar*) fillets measured by different methods in comparison to expressible moisture. Aquacult. Nutr. 7(2):81-89.

Kader AA. 1986. Potential applications of ionizing radiation in post-harvest handling of fresh fruits and vegetables. Food Technol. 40(6):117-121.

Karunakar B, Mishra SK, Bandyopadhyay S. 1998. Specific heat and thermal conductivity of shrimp meat. J Food Eng. 37(3):345-351.

Kaspar CW, Tamplin ML. 1993. Effects of temperature and salinity on the survival of *Vibrios vulnificus* in seawater and shellfish. Appl. Environ. Microbiol. 59:2425-2429.

Kava R. 1996. Irradiated Foods. 4th Edition. New York, NY: The American Council on Science and Health Publication.

Kelly MT. 1982. Effect of temperature and salinity on *Vibrio vulnificus* occurrence in a Gulf Coast environment. Appl. Environ. Microbiol. 44:820-824.

Kelly MT, Dinuzzo A. 1985. Uptake and clearance of *Vibrio vulnificus* from Gulf coast oysters (*Crasssostrea virginica*). Appl. Environ. Microbiol. 50:1548-1549.

Kent M. 1987. Electrical and dielectric properties of food materials. UK: Cost 90bis Production, Science & Technology Publishers.

Khalil H, Villota R. 1988. A comparative study of the thermal inactivation of *B. stearothermophilus* spores in microwave and conventional heating. In: Food Engineering and Process Application. New York, NY: Elsevier Applied Science Publishers. p 583-594.

Khalil H, Villota R. 1989. The effect of microwave sublethal heating on the ribonucleic acids of *Staphylococcus aureus*. J Food Protect. 52(8):544-548.

Kilcast D. 1995. Food irradiation: current problems and future potential. Int. Biodeter. Biodegr. 36(3,4):279-296.

Kilgen MB, Hemard MT, Duet D, Rabalais S. 1995. Collaborative evaluation of commercial irradiation for *Vibrio vulnificus* control in Louisiana oysters. Report to Louisiana State University Sea Grant Agency. p 1-15.

Kim CM, Jeong KC, Rhee JH, Choi SH. 1997. Thermal death times of opaque and translucent morph-types of *Vibrio vulnificus*. Appl. Environ. Microbiol. 63(8):3308-3310.

Knorr D. 1999. Process assessment of high-pressure processing of foods: an overview. In: Oliveira FAR, Oliveira JC, Hendrickx ME, Knorr D, Gorris LGM. Processing foods: quality optimization and process assessment. Florida: CRC Press LLC. p 249-267.

Knutson KM, Marth EH, Wagner MK. 1987. Microwave heating of food. Lebensm Wiss Technol. 20:101-110.

Knutson KM, Marth EH, Wagner MK. 1988. Use of microwave ovens to pasteurize mile. J Food Protect. 51(9):715-719.

Kozempel MF, Annous BA, Cook RD, Scullen OJ, Whiting RC. 1998. Inactivation of microorganisms with microwaves at reduced temperatures. J Food Protect. 61(5):582-585.

Kozempel M, Cook RD, Scullen OJ, Annous BA. 2000. Development of a process for detecting nonthermal effects of a microwave energy on microorganisms at low temperature. J Food Process Preserv. 24(4):287-301.

Kudra T, Van De Voort FR, Raghavan GSV, Ramaswamy HS. 1991. Heating characteristics of milk constituents in a microwave pasteurization system. J Food Sci. 56(4):931-934.

Kumbhar BK, Agarwal RS, Das K. 1981. thermal properties of fresh and frozen fish. Int. J Refrig. 4(3):143-146.

Larsen WP, Hartzell TB, Diehl HS. 1918. The effects of high pressure on bacteria. J Inf. Dis. 22:271-279.

Learson RJ, Ronsivalli LJ, Spracklin BW. 1969. Process criteria for producing radiation sterilization fish products. Food Technol. 23:85-91.

Lee DS, Shin DH, Yam KL. 2002 Improvement of temperature uniformity in microwave reheated rice by optimizing heat/hold cycle. Food Serv. Technol. 2(2):87-93.

Lin D, Yu RC, Chou CC. 2004. Susceptibility of *Vibrio parahaemolyticus* to various environmental stresses after cold shock treatment. Int. J Food Microbiol. 92(2):207-215.

Linton M, McClements MJ, Patterson MF. 2003. Changes in the microbiological quality of shellfish brought about by treatment with high hydrostatic pressure. Int. J Food Sci. Tech. 38:713-727.

Mallett JC, Beghian LE, Metcalf TG, Kaylor JD. 1991. Potential of irradiation technology for improved shellfish sanitation. J Food Safety. 11:231-242.

Mallikarjunan P, Hung Y-C. 1997. Physical and ultrastructural measurements. In: Erickson MC, Hung Y-C, editors. Quality in Frozen Food. New York, NY: Chapman and Hall. p 313-339.

Mallikarjunan P, Hung YC, Gundavarapu S. 1996. Modeling microwave cooking of cocktail shrimp. J Food Process Eng. 19:97-111.

McLaughlin WL, Jarret RD, Olejnik TA. 1982. Dosimetry. In: Josephson ES, Peterson MS, editors. Preservation of Food by Ionizing Radiation. Vol. I. Boca Raton, Florida: CRC Press LLC. p 189-245.

Mead PS, Slutsker L, Dietz V, McGaig LE, Bresee JS, Sapiro C, Griffin PM, Tauxe, RV. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607-625.

Meilgaard M, Civille GV, Carr BT. 1991. Factors influencing sensory verdicts. In: Sensory Evaluation Techniques. 2nd Ed. Boca Raton, Florida: CRC Press, Inc.

Metaxas R. 1996. Foundations of electro-heat: a unified approach. Chichester UK: John Wiley & Sons.

Metrick C, Hoover DG, Farkas DF. 1989. Effects of high hydrostatic pressure on heat-resistant and heat-sensitive strains of *Salmonella*. J Food Sci. 54:1547-1564.

Miyao S, Shindoh T, Miyamori K, Arita T. 1993. Effects of high pressurization on the growth of bacteria derived from surimi (fish paste). J Jpn. Soc. Food Sci. Tech. 40(7):478-484.

Mohsenin NN. 1980. Thermal properties of foods and agricultural materials. New York: Gordon and Breach Science Publishers.

Motes ML, DePaola A. 1996. Offshore suspension in relaying to reduce levels of *Vibrio vulnificus* in oysters. Appl. Environ. Microbiol. 62:3875-3877.

Murakami EG, Sweat VE, Sastry SK, Kolbe E, Hyskswa K, Datta A. 1996. Recommended design parameters for thermal conductivity probes for non-frozen food materials. J Food Eng. 27:109-123.

National Marine Fisheries Service. 2004. Fisheries Statistics and Economics Division. U.S Aquaculture Production: Oysters. Available from http://www.msstate.edu/dept/crec/aquaoysters.html. Accessed July 4.

Nelson S. 1994. Measurement of microwave dielectric properties of particulate materials. J Food Eng. 21(3):365-384.

Nelson S, Bartley PG. 2001. Frequency and temperature dependence of the dielectric properties of some food materials. In: 2001 ASAE Annual International Meeting; 2001 July 30-August 1, Sacramento, California; Paper number 01-6144.

Nelson SO. 1999. Dielectric properties measurement techniques and applications. Trans. ASAE. 42(2):523-529.

Nelson S, Forbus W, Lawrence K. 1994. Permittivity of fresh fruits and vegetables at 0.2 to 20 GHz. J Microwave Power EE. 29(2):81-93.

Ngadi MO, Chinnan MS, Mallikarjunan P. 2003. Enthalpy and heat capacity of fried shrimp at freezing and refrigeration temperatures. Lebensm.Wiss.Technol, 36(1):75-81.

Ngadi MO, Mallikarjunan P, Chinnan MS, Radhakrishnan S, Hung YC. 2000. Thermal properties of shrimps, French toasts and breading. J Food Process Eng. 23:73-87.

Nix GH, Lowery GW, Vachon RI, Tanger GE. 1967. Direct determination of thermal diffusivity and conductivity with a refined line-source technique. In: Process in Aeronautics and Astronautics: Thermo-physics of Spacecraft and Planetary Bodies. 20:865-878. New York: Academic Press.

NOAA. 2003. Personal communication from the National Marine Fisheries Service, Fisheries Statistics and Economics Division, Silver Spring, MD. Available from http://www.st.nmfs.gov/st1/fus/fus98/commercial/ld-aquc.pdf. Accessed Nov. 14.

Novak AF, Liuzzo JA, Grodner RM, Lovell RT. 1966. Radiation pasteurization of Gulf coast oysters. Food Technol. 20:201-202.

Ohlsson T, Risman PO. 1978. Temperature distribution of microwave heating- spheres and cylinders. J Microwave Power. 13(4):303-310.

Oliver JD. 1989. *Vibrio vulnificus*. In: Doyle MP, editor. Food borne bacterial pathogens. New York, NY: Marcel Dekker, Inc. p. 569-600.

Oliver JD, Warner RA, Cleland DR. 1983. Distribution of *Vibrio vulnificus* and other lactose-fermenting *Vibrios* in the marine environment. Appl. Environ. Microbiol. 45:985-988.

Olson DG. 1998. Irradiation of food. Food Technol. 52(1):56-62.

Pandit RB, Prasad S. 2003. Finite element of microwave heating of potato: Transient temperature profiles. J Food Eng. 60(2):193-202.

Papadakis SE, Abdul-Malek S, Kamdem RE, Yam KL. 2000. A versatile and inexpensive technique for measuring color of foods. Food Technol. 54(12):48-51.

Parker RW, Maurer EM, Chilers AB, Lewis DH. 1994. Effect of frozen storage and vacuum-packaging on survival of *Vibrio vulnificus* in Gulf Coast oysters (*Crassostrea virginica*). J Food Protect. 57:604-606.

Patterson MF, Kilpatrick DJ. 1998. The combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in milk and poultry. J Food Protect. 61(4):432-436.

Patterson MF, Quinn M, Simpson R, Gilmour, A. 1995. Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate buffered saline and foods. J Food Protect. 58(5):524-529.

Paul P, Chawala SP, Thomas P, Kesavan PC. 1997. Effect of high hydrostatic pressure, gamma-irradiation and combined treatment on the microbiological quality of lamb meat during chilled storage. J Food Safety. 16(4):263-271

Pearce TC, Schiffman SS, Nagle HT, Cardner JW. 2003. Handbook of Machine Olfaction. In: Electronic Nose Technology. KGaA Weinheim: WILEY-VCH Verlag GmbH & Co.

Peleg M, Cole MB. 1998. Reinterpretation of microbial survival curves. Crit. Rev. Food Sci. Nutr. 38:353-380.

Peleg M.1995. A model of microbial survival after exposure to pulsed electric fields. J Sci. Food Agric. 67:93-99.

Perkins BE. 1995. Aquacultured oyster products: inspection, quality, handling, storage and safety. Southern Regional Aquaculture Center (SRAC) publication. No. 434.

Pham QT, Wee HK, Kemp RM, Lindsay DT. 1993. Determination of the enthalpy of foods by an adiabatic calorimeter. J Food Eng. 21(2):137-156.

Poole SE, Mitchell GE, Mayze JL. 1994. Low dose irradiation affects microbiological and sensory quality of sub-tropical seafood. J Food Sci. 59:85-87.

Przybylski LA, Finerty MW, Gordner RM, Gerdes DL. 1989. Extension of shelf-life of iced fresh channel catfish fillets using modified atmospheric packaging and low dose irradiation. J Food Sci. 54:269-273.

Radhakrishnan S. 1997. Measurement of thermal properties of seafood [MSc thesis]. Blacksburg, Va.: Virginia Polytechnic Institute & State University. Available from: University Labaray.

Rahman MS, Potluri RL. 1991. Thermal conductivity of fresh and dried squid meat by line source thermal conductivity probe. J Food Sci. 56(2):582-583.

Rahman MS. 1993. Specific heat of selected fresh seafood. J Food Sci. 58(3):522-524.

Rahman MS, Saud AO, Mohmed AL. 2002. Pores and physico-chemical characteristics of dried tuna produced by different methods of drying. J Food Eng. 53(4):301-313.

Ramaswamy H, Koutchma T, Tajchakavit S. 2000. Enhanced thermal effects under microwave heating conditions. International Conference of Engineering and Food (ICEF-8). Puebla, MX.

Raso J, Palop A, Pagan R, Condon S. 1998. Inactivation of *Bacillus subtilis* spores by combining ultrasonic waves under pressure and mild heat treatment. J Appl. Microbiol. 85:849-854

Resurreccion, AVA, Galvez FCF, Fletcher SM, Misra SK. 1994. Consumer attitudes toward irradiated food: results of a new study. J Food Protect. 58(2):193-196.

Rodrick GE. 1991. Indigenous pathogenic Vibrionaceae. In: Wardand DR, Hackney C, editors. Microbiology of Marine Food Products. New York: Van Nostrand Reinhold. p 285-300.

Rosenberg U, Bogl W. 1987. Microwave pasteurization, sterilization, blanching and pest control in the food industry. Food Technol. 41(6):92-99.

Roussy G, Pearce J. 1995. Foundations and industrial applications of microwaves and radio frequency fields. New York: Wiley.

Sanz PD, Alonso MD, Mascheroni RH. 1987. Thermo-physical properties of meat products: General Bibliography and experimental values. Trans. ASAE. 30(1):283-289.

Schwarz JR, Cowell RR. 1974. Effect of hydrostatic pressure on growth and viability of Vibrio parahaemolyticus. Appl. Environ. Microbiol., 28:977-981.

Segars RA, Johnson EA. 1986. Instrumental measurement of the textural quality of fish flesh: effect of pH and cooling temperature. In: Kramer DE, Liston J, editors. Seafood Quality Determination, Proceedings of an International symposium; Nov.10-14; Anchorage, Alaska, USA. University of Alaska Sea Grant College Program.

Shadhindi F, Botta JR. 1994. Seafoods: Chemistry, Processing Technology and Quality. UK: Chapman and Hall.

Shiffmann RF. 1992. Major problems in heating foods in microwave ovens. Microwave world. 13(1):21-23,25.

Shiu S. 1999. Effect of high hydrostatic pressure (HHP) on the bacterial count and quality of shucked oysters [MSc thesis]. Corvallis, OR: Oregon State University. Available from: University Library.

Shrivastava M, Datta AL. 1999. Determination of specific heat and thermal conductivity of mushrooms (*Pleruotus floida*). J Food Eng. 39:255-250.

Sidwell V, Foncannon PR, Moore NS, Bonnet JC. 1974. Composition of the edible portion of raw (fresh or frozen) crustaceans, finfish and mollusks. Part I. Protein, fat, moisture, ash, carbohydrate, energy value and cholesterol. Mar. Fish. Rev. 36(3):21-35.

Sigurgisladottir S, Hafsteinsson H, Jonsson A, Lie ∅, Nortvedt R, Thomassen M, Torrissen O. 1999. Textural properties of raw salmon fillets as related to sampling method. J Food Sci. 64:99-104.

Sigurgisladottir S, Torrissen O, Lie ∅, Thomassen M, Hafsteinsson H. 1997. Salmon quality: Methods to determine the quality parameters. Rev. Fish. Sci. 5:1-30.

Smelt JPPM. 1998. Recent advances in the microbiology of high pressure processing. Trends Food Sci. Technol. 9:152-158.

Son NT, Fleet GH. 1980. Behavior of pathogenic bacteria in the oyster (*Crassostrea commercialis*), commercials during depuration, relaying and storage. Appl. Environ. Microbiol. 40(6):994-1002.

Stuchly MA, Stuchly SS. 1980. Coaxial line refection methods for measuring dielectric properties of biological substances at radio and microwave frequencies. IEEE, IM, 29(3):

Styles MF, Hoover DG, Farkas DF. 1991. Response of *Listeria monocytogenes* and *Vibrio parahaemolyticus* to high hydrostatic pressure. J Food Sci. 56(5):1404-1407.

Succar J, Hayakawa K. 1984. Parametric analysis for predicting freezing time of infinitely slab shaped food. J Food Sci. 49:468-477.

Sun E, Datta A, Lobo S. 1995. Composition-based prediction of dielectric properties of foods. J Microwave Power EE. 30(4):205-212.

Sweat VE. 1975. Modeling the thermal conductivity of meats. Trans. ASAE, 18(3):564-568.

Sweat VE, Haugh CG. 1974. A thermal conductivity probe for small food samples. Trans. ASAE. 17(1):56-58.

Takcet CO, Brenner F, Blake PA. 1999. Clinical features and an epidemiological study of V*ibrio vulnificus* infections. J Inf. Dis. 149:558-561

Tamplin ML, Capers GM. 1992. Persistence of *Vibrio vulnificus* in tissues of Gulf coast oysters exposed to seawater disinfected with UV light. Appl. Environ. Microbiol. 58(5):1506-1510.

Tanaka F, Mallikarjunan P, Yung YC. 1999. Dielectric properties of shrimp related to microwave frequencies: from frozen to cooked stages. J Food Process Eng. 22(6):455-468.

Tanaka F, Morita K, Maeda Y. 2001. Development of evaluation software of microbiological safety of food during microwave heating. J Jpn. Soc. Food Sci. & Technol. 48(7):514-519.

Tassinari R, Dos A. 1997. Effect of microwave heating on survival of *Salmonella typhimurium* in artificially contaminated ready-to-eat foods. J Food Safety. 17:239-248.

Tauscher BK. 1998. Effect of high pressure treatment to nutritive substances and natural pigments. In: Fresh Novel Foods by High Pressure VTT Symposium 186. Helsinki, Finland: Technical research Centre of Finland

Tauscher BK. 1999. High pressure and chemical reactions: effects on nutrients and pigments. Emerging Food Science and Technology. 1999 Nov 22-24: Tempere, Finland. Paper no. 58.

Tewari G, Jayas DS. 1999. High pressure processing of foods: an overview. ASAE Annual International Meeting. 1999 July 18-21; Toronto, Ontario, CA. Paper no. 99-6033.

Timson WJ, Short AJ. 1965. Resistance of microorganisms to hydrostatic pressure. Biotechnol and Bioeng.7:139-159

Tong CH, Lund DB. 1993. Microwave heating of baked dough products with simultaneous heat and moisture transfer. J Food Eng. 19:319-339.

Trabelsi S, Kraszewski A, Nelson S. 2001. On-line microwave dielectric characterization of cereal grains and seed. ASAE Annual International Meeting. 2001 July 30-August 1; Sacramento, California, USA. Paper number 01-6142.

Tran VN, Stuchly SS. 1987. Dielectric properties of beef, beef liver, chicken and salmon at frequencies from 100 to 2500 MHz. J Microwave Power EE. 22(1):29-33.

Van der Held EFM, Van Durmen, FG. 1949. A method of measuring the thermal conductivity of liquids. Phys. 15:865-881.

Van-Remmen HHJ, Ponne CT, Nijhuis HH, Bartels PV, Kerkhof PJAM. 1996. Microwave heating distributions in slabs, spheres and cylinders with relation to food processing. J Food Sci. 61(6):1105-1113.

Vasudevan P, Marek P, Daigle S, Hoagland T, Venkitanarayanan DS. 2002. Effect of chilling and freezing on survival of *Vibrio parahaemolyticus* on fish fillets. J Food Safety. 22(4):209-217.

Venugopal V, Alur MD, Nerkar DP. 1987. Storage stability of non-packaged irradiated Indian mackerel (*Rastrelliger kanagurta*) in ice. J Food Sci. 49:507-508.

Villamiel M, LopezFandino R, Olano A. 1997. Microwave pasteurization of milk in a continuous flow unit: Effects on the cheese-making properties of goat's milk. Milchwissenschaft. 52(1):29-32.

Walsby AE. 1973. Gas-filled structures providing buoyancy in photosynthetic and organism. In: Slengh MA, McDonald AG, editors. The effects of pressure on living organisms. New York: Academic Press Inc. p 233-287.

Wang, D. Q. and E. Kolbe 1990. Thermal conductivity of surimi measurement and modeling. J Food Sc. 55(5):1217-1221.

Weiher R. 2004. Economic Statistics for NOAA. 3rd ed. U.S. Department of Commerce, National Oceanic and Atmospheric Administration.

Wilkinson V. 1986. Food irradiation in practice. Proc. Inst. Food Sci. Technol. 19(4):169-170.

Wong HC, Liu SH, Wang TK, Lee CL, Chiou CS, Liu DP, Nishibuchi M, Lee BK. 2000. Characteristics of *Vibrio parahaemolyticus* O3:K6 from Asia. Appl. Environ. Microbiol. 66(9):3981-3986.

Wood PC. 1976. Guide to shellfish hygiene. WHO Offset Publication no. 31, WHO, Geneva.

Yaghmaee P, Durance TD. 2002. Predictive equations for dielectric properties of NaCl, D-sorbitol and sucrose solutions and surimi at 2450 MHz. J Food Sci. 67(6):2207-2211.

Yeo CBA, Watson IA, Stewart TDES, Koh VHH. 1999. Heat transfer analysis of *Staphylococcus aureus* on stainless steel with microwave radiation. J Appl. Microbiol. 87(3):396-401.

Youge CM. 1960. Oysters. London: Collins St. James's Palace.

Zapata JF, Price JF. 1982. Evaluation of texture and water holding capacity in cooked minced fish. J Food Proc. Pres. 6:189-195.

Zhang H, Datta AK. 2000. Electromagnetics of Microwave Heating: Magnitude and Uniformity of Energy Absorption in an Oven. In: Datta AK, Anantheswaran RC, editors. Handbook of Microwave Technology for Food Applications. New York: Marcel Dekker, Inc.

Zhang H, Datta AK, Taub IA, Doona C. 2001. Electromagnetics, heat transfer, and thermokinetics in microwave sterilization. J AIChE. 47(9):1957-1968.

Zheng M, Huang YM, Nelson SO, Bartley PG, Gates KW. 1998. Dielectric properties and thermal conductivity of marinated shrimp and channel catfish. J Food Sci. 63(4):668-672.

Zhou L, Puri VM, Anantheswaran RC. 1992. Evaluation of food quality and safety during microwave heating using finite element model. ASAE International Winter

Meeting. 1992 Dec. 15-17; Nashville, TN; St. Joseph, MI: American Society of Agricultural Engineers. Paper No. 92-6577.

ZoBell CE. 1970. Pressure effects on morphology and life process of bacteria. In: Zimmermann AM, editor. High pressure effects on cellular processes. New York: Academic Press Inc. p 85-130.

CHAPTER 3

Running head: Kinetic models to describe inactivation of *Vibrio* spp.

Comparison of Kinetic Models to Describe High Pressure and Gamma-Irradiation to Inactivate *Vibrio vulnificus* and *Vibrio parahaemolyticus* Prepared in Buffer Solution and in Whole Oysters

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Key words: Vibrio spp., oysters, inactivation kinetic models, high pressure processing, gamma-irradiation

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ABSTRACT

Comparisons of different models in inactivation kinetics were conducted on data obtained from high pressure and gamma-irradiation processing. *Vibrio vulnificus* (MO-624) and *Vibrio parahaemolyticus* (O3:K6 TX-2103) suspended in Phosphate-Buffered Saline (pH 7.4, 10^7 CFU/ml) were exposed to pressures from 207 to 379 MPa for 1 to 20 min. Inoculated whole oysters (10^6 CFU/g) were exposed to pressure from 276 to 379 MPa for 1 to 15 min. Pure cultures and inoculated oysters (10^6 CFU/g) were also irradiated (gamma-irradiation) at doses of less than 3 kGy. Four mathematical models including the Bigelow model, Arrhenius equation, Fermi equation and Weibull frequency distributions were applied to microbial survival data and performances of the different kinetic models were compared. Weibull frequency distributions can predict the high-pressure inactivation of *Vibrio* spp. with more accuracy in both pure cultures and inoculated oyster samples. The Fermi model provided a better description of gamma-irradiation inactivation kinetics compared with the traditional Bigelow model.

Keywords: *Vibrios* spp., oysters, inactivation kinetics models, high-pressure processing, gamma-irradiation

Non-thermal processing of foods using high hydrostatic pressure (HHP) or irradiation, helps retain nutritional and sensory properties, destroys pathogenic microorganisms and extends product shelf-life. The mechanism underlying the process and kinetic parameters associated with inactivation of bacteria, spores and enzymes have been described by Tewari and Jayas (*15*), Farkas and Hoover (*7*) and Sendra et al. *(14)*. Currently, in most non-thermal inactivation studies, D values and z values are still routinely used based on the assumptions that microbial destruction is exponential and that the logarithmic relationship between microbial inactivation and processing time is linear**;** however, recent reviews of non-linearity of semi-logarithmic survivor curves, indicates that first order kinetics, which dominate current microbial kinetics modeling, may not be appropriate for many non-thermal processes.

Peleg and Cole (*12*) challenged the assumption of first order kinetics by questioning the in-sensitivity of linear regression data fitting and the temperature dependence of the generated models (*6*). They proposed that the survival curve is a cumulative form of the resistance distribution of the exposed population. Thus, the semi-logarithmic survival curve can be of any shape, and the linear curve is a special case of the Weibull distribution of resistances with a shape factor of one (*11*). For a microbial resistance curve having a sigmoid shape, with respect to the forcing agent, a Fermi's equation, which is used to describe mechanical changes of bio-materials at and around their glass transition temperature, may be a better model to quantify the relationship (*10*). This model has been successfully applied to microorganisms exposed to pulsed electric fields. Several other important implications of this approach have been demonstrated

(*10*); however, the application of this method has not been applied to other non-thermal processing techniques.

The main objective of this study was to compare different inactivation kinetic models applied to microbial survivor curves for high pressure and gamma-irradiation treatments to eliminate *Vibrio vulnificus* (*V.v.*) and *V. parahaemolyticus* (*V.p.*) in pure cultures and in inoculated oysters.

MATERIALS AND METHODS

Clinical strains of *V. parahaemolyticus* (O3:K6 TX2103) and *V. vulnificus* (MO624) were used in this study. Pure cultures of *V.p. and V.v.* were incubated overnight in tryptic soy agar (TSA) with final concentration of 1% NaCl, and suspended in Phosphate-Buffered Saline (PBS, pH 7.4, *4*) to obtain initial concentrations of approximately 10^8 CFU/ml. Approximately, 2 ml of the pure cultures were placed in a heat-sealed 5.1 x 10.2 cm Kapak pouch (Kapak Corporation, Minneapolis, MN, USA) in duplicate. These pouches were then placed inside a second 16.5 x 20.3 cm Kapak pouch for pressure treatment. The pouch is made of laminated films of polyethylene terephthalate (PET) and linear low density polyethylene (LLDPE).

Whole eastern oysters (*Crassostrea virginica)* were also inoculated with *V.p.* (O3:K6 TX-2103) to initial concentrations of 10^6 CFU/g. Fifteen to twenty live oysters were cultured in 30.3 L of seawaters in a aquarium tank. In order to get high concentration of bacteria in each animal, 100 ml freshly cultured *V.p.* (TSA broth with 1% final NaCl concentration) were poured into the tank and mixed properly. An initial microbial concentration of 10^6 CFU/g in the oyster tissues was obtained after overnight exposure. The oysters were refrigerated and afterwards 3-4 whole oysters were put into

heavy duty (4.5 mm in thickness) 20.3 x 30.5 cm Kapak pouches for treatment. Duplicate pouches were placed and heat-sealed in another pouch with 10 ml of disinfectant. The pouches were not vacuum-packed, and there were small air bubbles present inside the pouches. High pressure treatments were conducted in duplicate. The samples were treated in a HHP vessel (EPSInc, Engineered Pressure Systems, National Forge, Co). This equipment has a chamber size of 22 L. The come-up time for the HHP unit varies according to the applied pressure. It was recorded that the come-up time was 5.7 to 8 min as pressure increased from 275 to 550 MPa. A 50:50 mixture of water and Houghto-safe 620 (water-glycol) was used as the pressurizing medium. The pure cultures of *V.p.* and *V.v.* and inoculated shelled oysters were treated with high pressure of 207, 241, 276, 310, 345 and 379 MPa (30-55 kpsi) for 0 to 20 min.

In the irradiation study, oysters were treated using a research size irradiator (Shepherd Model 484) with a radioactive source of Cobalt-60 at a dose rate of 900 rads per min. A dosimetry study was performed to determine the best location in the irradiation chamber for oysters in 4 L container to achieve the most consistent dose rate. Both pure culture and oysters received a dose level from 0-3 kGy. Irradiation processing was performed in quadruplicate for broth cultures and sextuplet for oyster samples.

Pure culture of *V.p.* and *V.v.* and oyster homogenate were serially diluted in PBS. Approximately 5 ml of pure *Vibrio* culture was transferred into a sample cup and plated onto TSA (with 1% NaCl final concentration) using spiral plater (Microbiology International, Frederick, MD). Plates were incubated at 35ºC for 18-20 h. Viable plate counts of *V.p.* and *V.v.* in oyster homogenate were determined by Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) (*4*) and Modified Cellobiose-Polymyxin B-Colistin (mCPC) (*4*)

plates using the hydrophobic membrane filtration method (*5*). In the irradiation study, for *V.p.* and *V.v.* determinations, instead of the hydrophobic membrane filtration method, a 3-tube MPN method was used (*4*). Additional identification of *V.v.* and *V.p.* were conducted using appropriate biochemical tests, (i.e., trypticase soy agar with 8% NaCl and API-test Strips 20E).

The death rate constant (k) and decimal reduction time (D) were calculated using differential first order kinetics equation and the microbial survival curve including the cumulative pressure come-up time. Four models including Bigelow, Arrhenius, Fermi and Weibull distribution were applied to microbial enumeration data and the performances of different kinetics model were compared. The accuracy factor (A_f), a measure of the precision of a developed model, was used to evaluate the different predictive models, as proposed by Ross *(13)* and expressed as:

$$A_f = 10^{\frac{\sum \log(predicted / observed)}{n}}$$

Where A_f is the accuracy factor; n is the number of observations used in the calculation.

RESULTS AND DISCUSSION

The microbial inactivation data of *Vibrio* spp. in pure culture and in oysters by HPP can be found in paper by Koo, et al. (*9*) (Appendix A.1-A.3). The D values of HHP treatment of *V.v.* and *V.p*. are presented in Tables 3.1, 3.2 and 3.3. For *V.p.* pure culture in PBS buffer, as the pressure increased from 207 to 310 MPa, D values decreased from 3.28 min to 1.04 min. Comparatively, D values calculated using the differential form of the first order kinetics equation and Arrhenius equation were greater at low pressure levels, while the discrepancy was reduced as the pressure increased. At the pressure level

of 310 MPa, the D value obtained by the Arrhenius equation was smaller compared with the one obtained from a traditional linear regression method. For *V.v.* in pure culture, D values decreased from 1.58 to 1.04 min as pressure increased from 207 to 276 MPa. Compared with D values obtained by the Arrhenius equation, similar phenomena were observed for the *V.v.* strain in pure cultures; however, D values calculated using a traditional linear regression model for *V.p.* concentrations in raw oysters were less compared with values from the Arrhenius equation at all measured pressure levels. Theoretically, the differential form of the first-order kinetics using the Arrhenius equation should provide a more accurate description of bacterial inactivation kinetics, because it considers the effect of the pressure come-up as a gradual effect instead of an instant jump-up process. Therefore, D values calculated by including the entire pressure come-up time into the calculation should be greater; however, due to the lack of data on the pressure come-up profile, especially at higher pressure levels, which have a longer pressure build-up time, the expected results were not demonstrated

V. v. (MO-624) was more sensitive to pressure treatments compared with *V.p.* (TX-2103) as shown by the smaller D values at the same pressure levels. This agrees the results from Berlin et al. (*3*). Our data suggest that oyster tissues may provide protection to *V.p.* as shown by the increased D values for *V.p.* found in inoculated oysters.

The Weibull frequency distribution provided the best fit in all instances, although parameters "a" and mean "t_c" (data not shown) did not demonstrate any trends to allow comparisons among various treatments. The Bigelow model provided the next best fit. There was disagreement with the conclusions made by Fernández et al. (*8*), who stated that parameters "a" and mean "t_c" can be considered as the characteristic time required

for inactivation, and can be used to compare the degree of resistance correlated to different microorganisms. The application of Weibull distribution function in high-pressure inactivation requires additional examination for the physical meaning underlying these parameters.

The deviation of the predicted values from experimental data using the Arrhenius approach was probably due to the lack of additional information on the pressure buildup profile in the beginning of the experimental time period.

The accuracy factor was a good index of indicating the precision of the predictive models. Although the correlation coefficient (R^2) was generally used to compare the performance of model fitting, it cannot be considered as an appropriate index in this study because the number of data points collected at various pressure levels was not the same. Processing times were reduced at higher pressure levels resulting in fewer data points, and therefore relatively higher R^2 values.

The microbial inactivation data of *Vibrio* spp. in pure culture and in oysters by gamma irradiation can be found in paper by Andrews, et al. (*2*) (Appendix A.4). The kinetics parameters for the irradiation treatment and the performance evaluation of various models are presented in Table. 3.4. *V. p.* (TX2103) was more resistant to irradiation compared with *V. v. (MO624),* which was consistent with the results on heat resistance, pressure resistance studies for *V.p.* and *V.v. (1, 3)*. However, the D values of *V.p.* in irradiated oyster tissues were less compared to *V.p.* in pure cultures. The reason is not evident and additional investigations are needed.

Fermi's equation had a slightly better prediction of the experimental data as indicated by the A_f values being closer to 1. Correspondingly, the regression parameter of

R^2 demonstrated the same trend. D_c, which is defined as the critical value of irradiation dosage when the survival level is 50%, was 0.60 kGy for *V. p.*, 0.50 kGy for *V. v.* and 0.57 kGy for *V.p.* in oysters. These values were found to have a sound linear correlation with D values obtained from the Bigelow model. Therefore, it maybe considered as a characteristic constant to compare resistance of microorganisms. The kinetics parameter "a" describes the steepness of the survival curve around the critical dosage. Implication of Fermi's equation to microbial inactivation kinetics by gamma irradiation was inspired by the research work of Peleg (*10*), who applied this approach to microbial survival in pulsed electric fields. The assumption of this analogy was that nearly all microorganisms are not affected by gamma irradiation until a certain dosage is achieved, is similar to the fact, that the responses of microorganisms to electric field was little affected by electric intensity of less than 4-8 kV/cm.

Weibull frequency distributions can predict the high-pressure inactivation of *Vibrio* spp. with more accuracy in both pure cultures and inoculated oyster samples. The Fermi model provided a better description of irradiation inactivation kinetics compared with the traditional Bigelow model. The obtained critical dosages showed good correlations with microbial resistance. However, the characteristic parameter from Weibull distribution did not allow for resistance comparison among HPP treatments.

Thus, the non-linear relationship of microbial survivor curves should be critically evaluated in non-thermal processing studies. Verification of characteristic parameters requires additional application of the developed models to determine the inactivation kinetics on different microorganisms, so that a database can be established to allow for parameter comparisons.

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REFERENCES

1. Ama, A.A., M.K. Hamdy, and R.T. Toledo. 1994. Effects of heating, pH and thermo-radiation on inactivation of *Vibrio vulnificus*. *Food Microbiol.* 11:215-227.

2. Andrews, L., M. Jahncke, and K. Mallikarjunan. 2003. Low dose gamma irradiation to reduce pathogenic *Vibrios* in live oysters (*Crassostrea virginica*). *J. Aquat. Food Prod. Technol.* 12:71-82.

3. Berlin, D.L., D. Herson, D.T. Hicks, and D.G. Hoover. 1999. Response of pathogenic *Vibrio* species to high hydrostatic pressure. *Appl. & Environ. Microbiol.* 65:2776-2780.

4. Elliot, E.L., C.A. Kaysner, L. Jackson, and M.L. Tamplin. 1998. *Vibrio cholerae, V. parahaemolyticus, V. vulnificus*, and Other *Vibrio* spp., *Bacteriological Analytical Manual*, 8th Edition, Revision A, 1998. Chapter 9.

5. Calik, H., M.T. Morrissey, P.W. Reno and H. An. 2002. Effect of high pressure processing on *Vibrio parahaemolyticus* strains in pure culture and pacific oysters. *J. Food Sci.* 67:1506-1510.

6. Campanella, O.H. and M. Peleg. 2001. Theoretical comparison of a new and the traditional method to calculate *Clostridium botulinum* survival during thermal inactivation. *J. Sci. Food Agric*. 81:1069-1076.

7. Farkas, D.F. and D.G. Hoover. 2000. High pressure processing. *J. Food Sci. Supplement*: *Kinetics of Microbial Inactivation of Alterative Food Processing Technologies.* 47-64.

8. Fernández, A., C. Salmerón, P.S. Fernández, and A. Martínez. 1999. Application of a frequency distribution model to describe the thermal inactivation of two strains of *Bacillus cereus*. *Trends Food Sci. Technol*. 10:158-162.

9. Koo J., X. Hu, M. L. Jahncke and K. Mallikarjunan 2002. Effect of high hydrostatic pressure on *Vibrio vulnificus* and *Vibrio parahaemolyticus* in pure cultures and whole eastern oysters. Presented in the Annual Meeting of Institute of Food Technologists, June 15-19, Anaheim, CA.

10. Peleg, M.1995. A model of microbial survival after exposure to pulsed electric fields. *J. Sci. Food Agric.* 67:93-99.

11. Peleg, M. 2001. On calculating microbial survival during thermal preservation processes. 21st Annual Conference on the Responsibilities of Thermal Processing Specialists. Nov. 13, 2001, Kissimmee, FL.

12. Peleg, M. and M.B. Cole. 1998. Reinterpretation of microbial survival curves. *Critical Rev. Food Sci. Nutri.* 38:353-380.

13. Ross, T. 1996. Indices for performance evaluation of predictive model in food microbiology. *J. Appl. Bacteriol.* 81:501-508.

14. Sendra, E., M. Capellas, B. Guamis, X. Felipe, M. MorMur, and R. Pla. 1996. Review: Food irradiation. General Aspects. *Food Sci. Technol. Intl.* 2:1-11.

15. Tewari, G. and D.S. Jayas. 1999. High pressure processing of foods: An overview. *Sciences-des-Aliments.* 19:619-661.

TABLE 3.1: Comparison of kinetics parameters for high-pressure treatment of *Vibrio parahaemolyticus* (O3:K6 TX2103) in PBS (pH 7.4)

Pressure	Bigelow			Arrhenius			Weibull			
(MPa)	D	R^2	A_f	D	R^2	A_f	a	b	R^2	A_f
207	3.28	0.94	1.11	4.03	0.93	1.31	0.95	0.87	0.91	1.05
241	1.82	0.89	1.92	2.43	0.93	2.13	0.36	0.77	0.88	1.04
276	1.26	0.95	1.12	1.47	0.93	1.23	0.58	0.98	0.72	1.09
310	1.04	0.88	1.27	0.89	0.93	1.28	1.96	2.49	0.90	1.06

Footnotes: D is the decimal reduction time (min); R^2 is the correlation coefficient; A_f is the accuracy factor; a and b are scale and shape factor of Weibull distribution.

TABLE 3.2: Comparison of kinetics parameters for high-pressure treatment of *Vibrio vulnificus* (MO624) in PBS (pH 7.4)

Pressure	Bigelow			Arrhenius			Weibull			
(MPa)	D	R^2	A_f	D	R^2	A_f	a	b	R^2	A_f
207	1.58	0.93	1.28	1.93	0.91	1.36	2.07	1.92	0.95	1.11
241	1.13	0.85	1.40	1.23	0.91	1.47	1.94	2.56	0.93	1.11
276	1.04	0.79	1.45	0.87	0.91	1.45	2.73	4.35	0.99	1.00

Footnotes: D is the decimal reduction time (min); R^2 is the correlation coefficient; A_f is the accuracy factor; a and b are scale and shape factor of Weibull distribution.

TABLE 3.3: Comparison of kinetics parameters for high-pressure treatment of *Vibrio parahaemolyticus* (O3:K6 TX2103) in raw oysters

Pressure	Bigelow			Arrhenius			Weibull			
(MPa)	D	R^2	A_f	D	R^2	A_f	a	b	R^2	A_f
276	3.27	0.94	1.09	4.19	0.78	1.23	1.36	0.98	0.87	1.05
310	2.68	0.86	1.11	2.96	0.78	1.12	0.95	0.91	0.83	1.05
345	2.02	0.94	1.13	2.09	0.78	1.11	1.11	1.11	0.78	1.05

Footnotes: D is the decimal reduction time (min); R^2 is the correlation coefficient; A_f is the accuracy factor; a and b are scale and shape factor of Weibull distribution.

TABLE 3.4: Comparison of kinetics parameters for irradiation of *Vibrio parahaemolyticus* (O3:K6 TX2103) and *Vibrio vulnificus* (MO624) *in* PBS (pH 7.4) and in raw oysters

	Bigelow			Fermi			
	D	R^2	A_f	a	D_c	R^2	A_f
V.p. in buffer	0.24	0.91	1.18	0.11	0.60	0.93	1.13
V.v. in buffer	0.19	0.84	1.26	0.09	0.50	0.88	1.24
V.p. in oysters	0.22	0.81	1.23	0.10	0.57	0.87	1.26

Footnotes: D is the decimal reduction time (min); R^2 is the correlation coefficient; A_f is the accuracy factor; a is the kinetics parameter of Fermi equation, describes the steepness of the survival curve around the critical dosage; D_c is the critical value of the gamma irradiation dosage where the survival level is 50%.

CHAPTER 4

Running head: Physical Properties of oysters

Thermal and Dielectric Properties of Shucked Oysters

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ABSTRACT

Thermal conductivity, thermal diffusivity and specific heat were measured for shucked oysters. Thermal conductivity increased from 0.577 W/m °C to 0.677 W/m °C as temperature increased from 0 to 50°C, measured by a line heat source thermal conductivity probe. Specific heat was measured using a differential scanning calorimeter. It increased from 3.795 kJ/kg°C to 4.047 kJ/kg°C when temperature was raised from 10 to 50 °C. Thermal diffusivity of oysters was calculated from thermal conductivity, specific heat and density values. Dielectric properties of oysters were determined by an open-ended coaxial cable connected to a network analyzer between 300 MHz to 3 GHz. At interested microwave frequencies of 915 MHz and 2450 MHz, it was observed that dielectric constant decreased (64.02 to 50.89 at 915 MHz and 59.10 to 47.67 at 2450 MHz), while the loss factor increased (13.84 to 20.14) at 915 MHz as temperature increased from 1°C to 55°C. Models were developed to describe the temperature effects on thermal and dielectric properties of shucked oysters.

1. INTRODUCTION

Oysters are delicacies named for its raw taste. In the U.S., the total combined eastern oyster catch in 2000 were 17 million ponds with a value of over $ 42 million (National Marine Fisheries Service, 2004). However, without proper processing, this perishable product can cause illnesses including fever, chills, nausea, hypertension and gastroenteritis related diseases by concentrated foodborne pathogens (Oliver, 1989; Tacket, Brenner & Blake, 1984; CDC, 1998).

In order to reduce the microbial load as well as to keep the raw texture and taste of oysters, many researchers worked on non-thermal or low temperature treatment of oysters.

Among them, microwave processing is considered to be a promising technology. However, its application is restricted by the rare information on the thermal or dielectric properties of oysters. Comparatively, many studies were carried out on the thermal and dielectric properties of various meat and seafood products (Rahman & Potluri, 1991; Murakami, Sweat, Sastry, Solbe, Hyskswa & Datta, 1996; Karunakar, Mishra & Bandyopadhyay, 1998; Ngari, Mallikarjunan, Chinnan, Radhakrishman & Hung, 2000; Tran and Stuchly, 1987; Zheng, Huang, Helson, Bartley & Gates, 1998 and Tanaka, Marllikarjunan & Hung, 1999). There was no data available on thermal conductivity and dielectric properties of oysters related to the temperature factors. Thus the objective of this study was to determine thermal properties, including thermal conductivity, thermal diffusivity and specific heat and dielectric properties of shucked oysters at the temperature range of 0°C to 55^{o}C. The selection of this temperature range is to ensure that the protein will not denaturate induced by over exposure of oyster meat to elevated temperatures (Scopes, 1994).

2. MATERIALS AND METHODS

2.1 Sample preparation

Shucked oysters (*Crassostrea virginica*) were obtained from the super market processed and packed by Sparrer Inc., Virginia. The average size of the oyster was 6-7 cm in length, 3-4 cm in width and 1-1.5 cm in thickness. Samples were stored in the refrigerator at 4°C before use.

2.2 Apparatus

The thermal conductivity was measured by an E-type line heat source thermal probe. The inner construction and configuration are similar to the one described by Sweat

(1975). The diameter of the probe was 0.8 mm and length was 100 mm. The heater wire has a resistance of 24.4 Ω.

Temperature of the oyster samples was controlled by soaking the samples into a constant temperature circulator (Haake A82, Model 000-7069, Germany) with a temperature stability of ±0.5°C. The heater circuit consists of DC power supply (BK Precision, model 1630, Taiwan) and digital multi-meter (Keithley, model 2000, USA). Temperature-time was continuously recorded by a data logger (21X Micro logger, Campbell Scientific Inc., USA) directly interfaced with a personal computer. Water containing 0.5 g/100g agar (Sigma, USA) and glycerol (Sigma, USA) were used as standard materials for probe calibration at 30°C and 25°C, respectively. Power supply was properly selected at 150 mA to ensure the minimum time and current range for sufficient temperature increase without affecting the value of the measured thermal conductivity significantly. Oyster samples were kept inside the temperature controller to measure the thermal conductivity at 0, 10, 20, 30, 40 and 50°C. The probe was immersed inside the oyster at perpendicular or parallel directions to oyster tissues, which gave the maximum length and width. The measurement was conducted in triplicate.

The permittivity of the shucked oysters was measured by an open-ended, 3.6 mm diameter, semi-rigid coaxial line probe with copper conductors, connected to a Network Analyzer (Model 85107A, Hewlett-Packard, Santa Clara, CA). The coaxial probe was fixed in a place by a stand arrangement. A sample holder (55 mm in diameter and 35 mm in depth) was placed on a vertical movable table. The temperature of the sample holder was controlled by a surrounding jacket, within which ethylene glycol was circulated.

The oyster sample was firstly placed inside a plastic bag and immersed in a constant temperature circulator until the center of the oyster reached the desired temperature. It was then confined in the stainless steel sample holder. The table was moved up slowly and the sample was brought in contact with the probe tip. Care was taken to avoid any air gap between sample and the probe tip without applying much pressure on the sample. Measurement was triggered and the network analyzer recorded the reflection coefficient at the probe-sample interface. Dielectric probe kit software (Model 85070A, Hewlett-Packard, Santa Clara, CA) was used to calculate the dielectric properties of the shucked oysters. Measurement was conducted in triplicate.

During the dielectric property measurement, a handheld digital thermometer (Mode HH23, Omega Engineering, Inc., Stamford, CT) was used to monitor the temperature of the oyster meat at the heating temperature range of 1°C to 50°C. Calibration of the system was done using air, metallic short-circuit and de-ionized distilled water at 25ºC. The frequency range was fixed 0.3 GHz to 3 GHz and the instrument captured data for every 5 MHz in that range.

The specific heat of the oyster was measured by a differential scanning calorimeter (TA Instruments DSC 2920). Pure water was selected as the standard reference material with a known specific heat value. The scanning rate was set at 3°C/min for running the baseline, water and oyster sample. The heat flow (mW) of samples was directly recorded by the computer over the temperature range of 10°C to 50°C.

The thermal diffusivity of oyster was directly calculated using equation

$$\alpha = K/Cp \bullet \rho \qquad ..(4.1)$$

where α = Thermal diffusivity of oysters (m^2/s)

K = Thermal conductivity of oyster (W/m °C)

Cp = Specific heat of oysters (J/kg °C)

ρ = Density of oysters (kg/m^3)

The density, thermal conductivity, specific heat and thermal diffusivity of each principle component with respect to the changes of temperature can be estimated using Choi and Okos's equation (1986). The density of the oysters was estimated by knowing ρ_t, the density of the ith component and mi, the mass fraction of the ith component using the formula:

$$\rho = \frac{1}{\sum_{i=1}^{n} \frac{m_i}{\rho_i}} \qquad ..(4.2)$$

where n = number of different components.

The thermal conductivity for a food material with n components can also be calculated from the weighted average of the thermal conductivity of the ith component K_i:

$$K = \sum_{i=1}^{n} V_i K_i \qquad ..(4.3)$$

where V_i = the volume fraction of the ith component, which can be calculated by

$$V_i = \frac{\frac{m_i}{\rho_i}}{\sum_{i=1}^{n} \frac{m_i}{\rho_i}} \qquad ..(4.4)$$

The specific heat of a food material with n components can be estimated by the following equation:

$$Cp = \sum_{i=1}^{n} m_i Cp_i \qquad ..(4.5)$$

where Cp_i = the specific heat of the ith component in food matrix.

Similarly, the thermal diffusivity of a food material with n components can be estimated by:

$$\alpha = \sum_{i=1}^{n} m_i \alpha_i \qquad ..(4.6)$$

where α_i = the specific heat of the ith component in food matrix.

3. RESULTS AND DISCUSSION

3.1 Thermal properties of oysters

The thermal conductivity data of oysters are presented in Fig. 4.1. As temperature increased from 0^oC to 50°C, thermal conductivity increased from 0.578 W/m°C to 0.677 W/m°C. The relationship between thermal conductivity and temperature can be described by the following regression model, with R square of 0.93.

$$K = 0.00002T^2 + 0.0013T + 0.5769. \qquad ..(4.7)$$

where K = Thermal conductivity (W/m°C)

T = Temperature (°C)

The moisture content of oysters was very high (86 g/100g). Compared with the thermal conductivity behavior of pure water at the same temperature range, it can be seen that as temperature increased, the thermal conductivity of water also increased from 0.575 W/m°C to 0.615 W/m°C. The increasing rate from this study was much higher than expected. Comparing with published data for other seafood products, it was found that the thermal conductivity slowly increased at the temperature range of 0 to 40°C although the moisture of those seafood products were 10 g/100g less than oysters used in this study

(Kumbhar, Agarwal & Das, 1981). However, some of other researchers (Sudhaharini, 1997 and Sweat, 1975) concluded that temperature has basically no influences on thermal conductivity values of meat products within the temperature range of 0 to 50°C.

Choi and Okos's (1986) mathematical models associating food properties with food components provided another way to obtain the thermal conductivity value. Except water (86 g/100g), the main components of oyster included protein (7.8 g/100g), fat (1.4 g/100g), carbohydrate (3 g/100g) and ash (1.8 g/100g). The calculated thermal conductivity values (using Choi and Okos's equations) increased from 0.532 W/m °C at 0°C to 0.598 W/m °C at 50°C.

The higher thermal conductivity value obtained from this study maybe attributed to: (1) The calibrated constant used to calculate the thermal conductivity was assumed to be the same under different temperatures. (2) During the time to reach the desired temperature, moisture was migrated from the oyster surface to the air so that the salt concentration was increased resulting in much higher thermal conductivity values. This hypothesis was made based on the shrinkage observed on the oyster surface. In order to test this hypothesis, the moisture content of oysters with and without thermal treatment was measured. The thermal treatment was conducted exactly as what have been done during the thermal conductivity measurement. It was found out that, statistically, there was no significant difference on the moisture content when oysters were kept in various temperatures for 30 min ($P<0.05$). Therefore, the proposed second reason to explain the measured high thermal conductivity was not valid.

The thermal conductivity of oysters at the direction of maximum width is also shown in Fig. 4.1. Statistically, there was no significant difference between the value

measured along the maximum width and maximum length direction ($P<0.05$). This results illustrated heat transfer was not influenced by the direction of oyster tissue fiber, which agreed with other studies (Hill, Leitman & Sunderland, 1967; Lentz, 1961). Those authors emphasized that the fiber orientation normally affected the thermal conductivity of frozen meat.

The specific heat of oyster meat increased from 3.795 kJ/kg°C to 4.047 kJ/kg°C when the temperature increased from 10°C to 50°C, which was higher than published data (Fredrick and Thomas, 1985). Compared with the specific heat values calculated by Choi and Okos (1986) (an increase from 3.844 kJ/kg°C to 3.859 kJ/kg°C), the rate of increase observed in this study was higher too. The thermal diffusivity of oyster meat increased from 1.460×10^{-7} m^2/s to 1.515×10^{-7} m^2/s as temperature increased from 10°C to 50°C, which was calculated using equation (1). The density was obtained using the methods described in Materials and Methods section. The data on specific heat and thermal diffusivity of oyster meat are presented in Table 4.1. The data which were calculated using estimating equations by Choi and Okos (1986) are presented in Table 4.2.

3.2 Dielectric properties of oysters

The dielectric properties of oysters are presented in Figs. 4.2 and 4.3. It was observed that as temperature increased from 1^oC to 55^oC, the dielectric constant decreased from 64.02 to 50.89 at 915 MHz and from 59.10 to 47.67 at 2450 MHz, respectively. This indicated that the sensitivity of dielectric constant to the temperature change was nearly the same at both interested microwave frequencies in present study. The decease of dielectric constant was prominent when temperature increased above 45^oC. Higher frequency resulted in the lower dielectric constant value. Two regression models can be used to

define the changes in the dielectric constant with temperature (Equation 4.7 and 4.8) and the R square values were 0.933 and 0.925, respectively.

$$e'_{(915MHz)} = -0.0039T^2 - 0.0341T + 65.377 \quad ..(4.8)$$

$$e'_{(2450MHz)} = -0.002T^2 - 0.0926T + 59.547 \quad ..(4.9)$$

where $e'_{(915MHz)}$ = Dielectric constant at 915 MHz

$e'_{(2450MHz)}$ = Dielectric constant at 2450 MHz

T = Temperature (^oC)

Although this decreasing trend agreed well with other published data on seafood and meat products (Kent, 1987; Tanaka Marllikarjunan & Yung, 1999), which represents the general behavior of high moisture content food materials, the decreasing rate of dielectric constant in this study was much higher than other published data within the same temperature range. The reason was not clear. It has been well documented that salt concentration was an influencing factor on the dielectric properties of foods (Kent, 1987; Tanaka Marllikarjunan & Yung, 1999). Since the salt concentration of the published dielectric constant data was not available, comparisons between the salt concentration of oysters used in this study and the publish data was unable to proceed.

The loss factor of oysters increased from 13.84 to 20.14 at 915 MHz as temperature increased from 1 to 55^{o}C. However, similar trend was not found at 2450 MHz. The loss factor decreased from 16.08 to 12.94 at about 40^{o}C and increased to 15.61 at 55^{o}C. Compared to dielectric constant, the loss factor demonstrated less temperature dependence at higher frequency of 2450 MHz. These results were also found for many other food products (Tanaka, Brenner & Blake, 1999 and Tong, Lentz & Rossen, 1994). However, a continuous increase of dielectric loss factor at both microwave frequencies was reported

for shrimps by Zheng, Huang, Helson, Bartley & Gates (1998) and for macaroni and cheese product by Nelson & Bartley (2000). This strongly indicated that dielectric properties not only depend on the temperature and frequency but also on the composition and physical and chemical state of the material (Nelson & Bartley, 2000). The rapid increase of dielectric loss factor at 915 MHz was probably related to the ionic reduction as temperature increased at frequencies lower than 1 GHz (Tanaka, Brenner & Blake, 1999).

Penetration depth was defined as the depth in the product at which the microwave power is decreased to 1/e (e = natural logarithm) of its value at the surface of the product. Mathematically, equation (4.10) can be used to quantify the penetration depth, an important indicator of microwave energy absorption.

$$D=\{\lambda_0/2\pi(2e')^{0.5}\}[\{1+(e''/e')^2\}^{0.5}-1]^{-0.5} \quad ..(4.10)$$

where D is penetration depth and λ_0 is the free space microwave wavelength, which has the value of 32.76 cm and 12.24 cm for 915 and 2450 MHz, respectively.

The calculated penetration depth values are presented in Fig. 4.4. As can be seen from the graph, the penetration depth reduced distinctively from 3.03 cm to 1.88 cm at 915 MHz, where the maximum reduction occurred at temperature of 40^{o}C. However, the penetration depth increased from 0.94 cm at 1^{o}C to the maximum of 1.1 cm at 40^{o}C and reduced to 0.87 cm at 55^{o}C under the frequency of 2450 MHz. These demonstrated that penetration depth was much more affected by temperature at low frequencies and low frequencies of microwave power generate much more penetration depth. Comparing the results of this study with the food map (penetration depth for a given dielectric constant and loss factor) at 25^{o}C (Engelder and Buffler, 1991), the penetration depth of raw oyster

meat of 1.1 cm was very close to the raw meat and shrimp group in the map with a dielectric constant of 55 and a loss factor of 14.

4. CONCLUSIONS

The thermal properties of oysters were not significantly affected by temperature changes within the range of 1 to 50°C. However, the dielectric properties were much more influenced by temperature. Frequency also played an importance role in the dielectric properties of oysters. Higher frequency resulted in a lower dielectric constant. The loss factor was more sensitive to frequency change than the variation in temperature. The critical temperature seems to be at 40°C for a dramatic change in dielectric properties indicated by a significant decrease of the penetration depth at 915 MHz.

Generally, measuring physical properties of food was an uneasy task. It was limited by the variations in measurement conditions and techniques and variations in food material itself. Therefore, data obtained from different researchers are difficult to compare, which makes the validation process rather difficult. However, the data of the thermal and dielectric properties of shucked oysters were in the same order of magnitude in comparison to the data in the literature and the results from this study will help understanding the heat transfer mechanism of oysters in an electromagnetic field.

REFERENCES

CDC (Centers for Disease Control and Prevention) (1998). *Vibrio vulnificus* infections associated with eating raw oysters. New York, 1998. Morb. Mortal. Wkly. Rep 48(3), 48-51.

Choi, Y. and Okos, M. (1986). Effects of temperature and composition on the thermal properties of foods. Food Engineering and Process Applications. Volume 1. Ed by Lemaguer, M and P. Jelen. Elsevier Applied Science Publishers.

Engelder, D. S. and Buffler, C. R. (1991). Measuring dielectric properties of food products at microwave frequencies. *Microwave World*, 12(2), 2-11.

Fredrick, W. W & Thomas, B. L. (1985). Processing Aquatic Food Products. A Wiley-Interscience Publication. John Wiley & Sons Inc., New York, NY.

Hill, J. E. Jr., Leitman, J. D. and Sunderland, J. E. (1967). Thermal conductivity of various meat. *Food Technology*, 21(8), 1143-1148.

Karunakar, B. Mishra, S. K. and Bandyopadhyay, S. (1998). Specific heat and thermal conductivity of shrimp meat. *Journal of Food Engineering*, 37, 345-351.

Kent, M. (1987). Electrical and dielectric properties of food materials. A COST 90bis Production. Science and Technology Publishers, London.

Kumbhar, B. K., Agarwal, R. S. and Das, K. (1981). Thermal properties of fresh and frozen fish. *International Journal of Refrigeration*, 4(3), 43-146.

Lentz, C. P. (1961). Thermal conductivity of meats, fats, gelatin gels and rice. *Food Technology*, 15, 243-247.

Murakami, E. G., Sweat, V. E., Sastry, S. K., Kolbe, E., Hyskswa, K., and Datta, A. (1996). Recommended design parameters for thermal conductivity probes for non-frozen food materials. *Journal of Food Engineering*, 27, 109-123.

National Marine Fisheries Service, Fisheries Statistics and Economics Division. (2004). U.S Aquaculture Production: Oysters. Retrieved July, 4, 2004 from http://www.msstate.edu/dept/crec/aquaoysters.html

Nelson, S. O. & P. G. Bartley, Jr. (2000). Measuring frequency- and temperature-dependent dielectric properties of food materials. Presented at 2000 ASAE Annual International Meeting, July 9-12. Paper No. 006096. ASAE, 2950 Niles Road, St. Joseph, MI 49085-9659 USA.

Ngari, M. O., Mallikarjunan, P., Chinnan, M. S., Radhakrishnan, S. and Hung, Y. C. (2000). Thermal properties of shrimps, French toasts and breading. *Journal of Food Process Engineering*, 23, 73-87.

Oliver, J. D. (1989). *Vibrio vulnificus*. In Foodborne Bacterial Pathogens. Ch:14, pp. 569-600. M. P. Doyle(Ed.). Marcel Dekker, Inc., New York, NY.

Rahman, M. S. and Potluri, R. L. (1991). Thermal conductivity of fresh and dried squid meat by line source thermal conductivity probe. *Journal of Food Science*, 56(2), 582-583.

Scopes, R. K. (1994). Protein Purifications: Principles and Practices. 3rd ed. Ringer-Verlag, New York, Inc., USA.

Sudhaharini, R (1997). Measurement of thermal properties of seafood. Master Thesis. Virginia Polytechnic Institute and State University (unpublished).

Sweat, V. E. (1975). Modeling the thermal conductivity of meats. *Transactions of ASAE*, 18(3), 564-568.

Tacket, C. O., Brenner, F. and Blake, P. A. (1984). Clinical features and an epidemiological study of *Vibrio vulnificus* infections. *Journal of Infection Disease*, 149, 558-561.

Tanaka, F., Marllikarjunan, P. K. and Hung, Y. C. (1999). Dielectric properties of shrimp related to microwave frequencies: from frozen to cooked stages. *Journal of Food Process Engineering*, 22, 455-468.

Tong, C. H., Lentz, R. R. and Rossen, J. L. (1994). Dielectric properties of pea puree at 915 MHz and 2450 MHz as a function of temperature. *Journal of Food Science, 59(1)*, 121-122, 134.

Tran, V. N. and Stuchly, S. S. (1987). Dielectric properties of beef, beef liver, chicken and salmon at frequencies from 100-2500 MHz. *Journal of Microwave Power*, 2(1), 29-33.

Zheng, M., Huang, Y. W., Helson, S. O., Bartley, P. G. and Gates, K. W. (1998). Dielectric properties and thermal conductivity of marinated shrimp and channel catfish. *Journal of Food Science*, 63(4), 668-672.

Table 4.1: Specific heat and thermal diffusivity of shucked oysters

Temperature (°C)	Thermal conductivity (W/m °C)	Specific heat (kJ/kg °C)	Density (kg/m^3)	Thermal diffusivity $(m^2/s) \times 10^{-7}$
10	0.590	3.795	1038	1.497
20	0.604	3.740	1037	1.557
30	0.618	3.968	1035	1.504
40	0.632	4.073	1032	1.504
50	0.647	4.047	1029	1.554

Table 4.2: Thermal properties of shucked oysters calculated using estimation equations by Choi and Okos (1986)

Temperature (°C)	Thermal conductivity (W/m °C)	Specific heat (kJ/kg °C)	Density (kg/m^3)	Thermal diffusivity (m^2/s) × 10^{-7}
10	0.548	3.844	1038	1.304
20	0.562	3.846	1037	1.357
30	0.575	3.850	1035	1.405
40	0.578	3.854	1032	1.448
50	0.598	3.859	1029	1.487

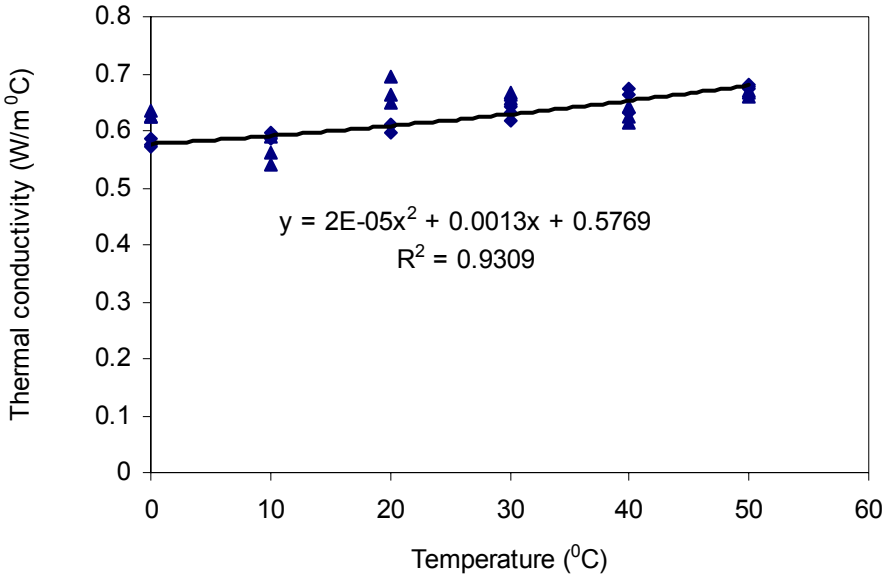

Fig. 4.1: Thermal conductivity of shucked oysters

Notes: ■ Indicates thermal conductivity measured along the oyster tissue with maximum length direction; ▲ Indicates thermal conductivity measured along the oyster tissue with maximum width direction.

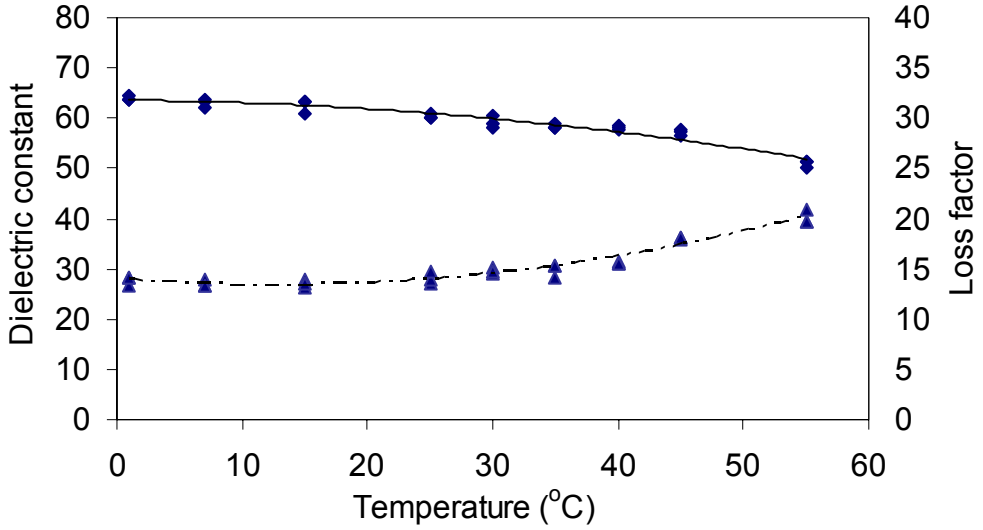

Fig. 4.2: Dielectric constant and loss factor of oyster meat at 915 MHz

Notes: ♦ Indicates dielectric constant; ▲ Indicates loss factor.

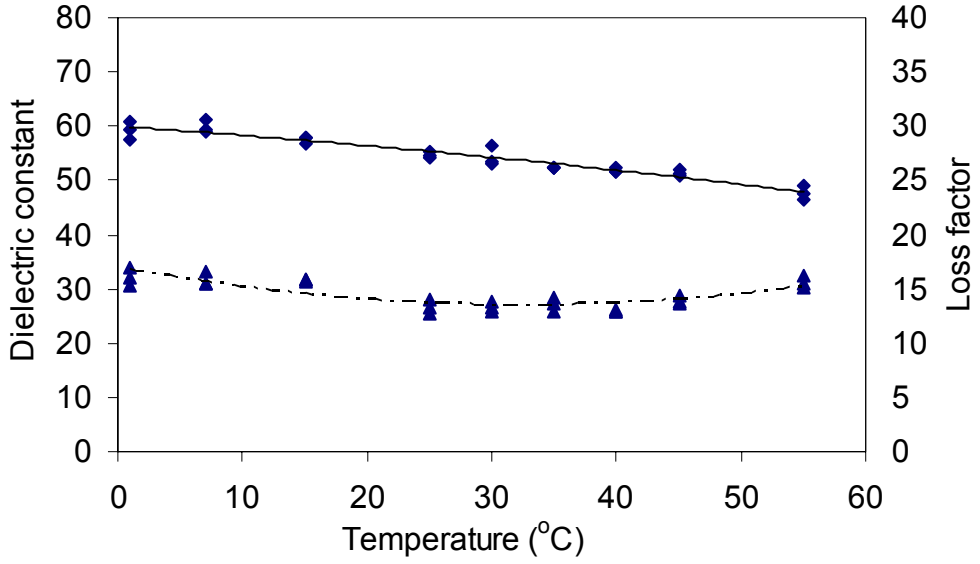

Fig. 4.3: Dielectric constant and loss factor of oyster meat at 2415 MHz

Notes: ♦ Indicates dielectric constant; ▲ Indicates loss factor.

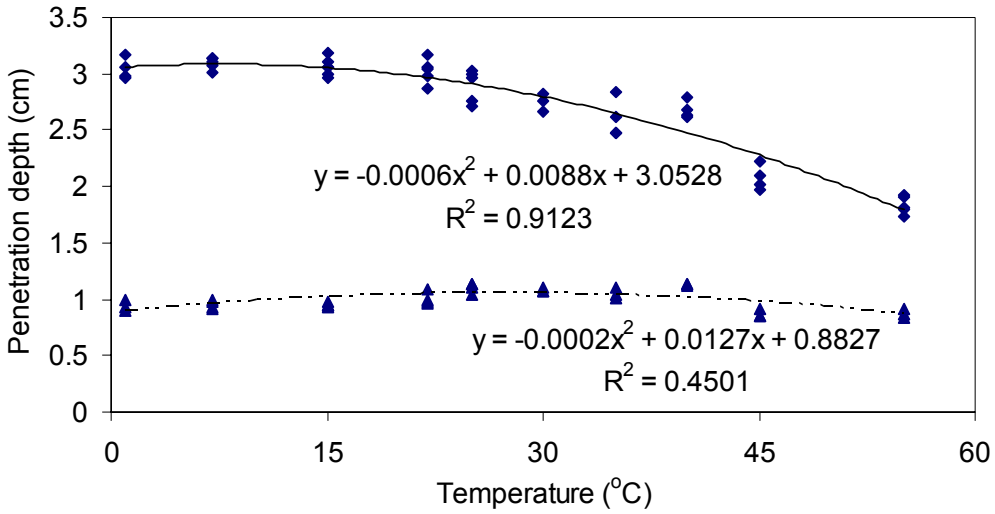

Fig. 4.4: Effect of temperature on penetration depth of microwave power on oyster meat

Notes: ♦ Indicates penetration depth at 915 MHz; ▲ Indicates penetration depth at 2415 MHz.

CHAPTER 5

Mathematical Modeling of Heat Transfer of Microwave Heated Fish Gel

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ABSTRACT

Mathematical modeling of fish gel as a replacement for seafood can be an alternative way to study the heating mechanisms of seafood products inside a microwave oven with many advantages such as timesaving and money saving. Fish gel was formed into a cylindrical shape in a beaker and heated using various combinations of temperature and microwave heating powers. Three fiber-optic probes were used to measure the temperature elevation at geometric center, surface, and bottom of the cylinder gel and headspace of the beaker. A two-dimensional cylindrical geometry model was established and a finite difference method was applied to solve the differential equations. The model can predict the heating profile within the temperature range of ±8 °C for different compositions of fish gel. Cold spots were observed at the bottom of the cylinder. There is a potential to use formulated fish gel to mimic seafood products for better understanding of microwave processing mechanisms.

Key Words: Heat transfer, Microwave, Modeling, Fish gel

1. INTRODUCTION

Non-uniformity in microwave cooking is the major concern when applying microwave in food processing and food preservation. Those generated cold spots provide a natural shelter for bacterial survival preventing the use of microwave in food industry especially for sterilization purpose. The cause of non-uniformity can be attributed to many factors including size, geometry, location, composition, electric and thermal properties of food materials as well as the microwave oven parameters, power supply and heating time etc. Among them, the heating pattern is the only factor that can be controlled so far to improve the heating uniformity of microwave cooked food products.

However, to study the combination effects of microwave power level, heating time and pattern is very time consuming and costly when dealing with expensive raw materials such as seafood products. Besides, the unique feature of microwave cooking requires very specified cooking procedures applied to each product. Therefore, mathematical modeling of microwave heating of fish gel instead of seafood product has the following advantages: The composition of the product can be easily simulated; The geometry of the product can be easily controlled compared to the irregular shape of seafood products and the thermal probe can be easily placed at desired locations.

Mathematical modeling of microwave-cooked food products has been widely studied as simple geometry such as slabs (Datta, 1991; Zhou et al., 1992 and Tong and Lund, 1993), cylinders (Zhou et al., 1992; Chen et al., 1993 and Mallikarjunan et al., 1996) and spheres (Ohlsson and Risman, 1978). These provided a sufficient theoretical background for this study.

The objectives of this study were to develop a mathematical model to describe the heating profile of fish gel, mapping the cold spots and hot spots within the product; to study the effects of shape and the composition of the fish gel on heating profiles and to validate the model by comparing the simulated results with the temperature profile of microwave heated raw oysters.

2. MATERIALS AND METHODS

2.1 Experimental setup

Fish gel was obtained from Protein Product Inc. Gel solution (100 ml) was formed into a cylindrical shape in a 200 ml glass beaker, with a diameter of 40 mm and a height of 50mm. Two types of gel were prepared, composed of protein (5 g and 10 g) and carbohydrate (1g and 2g). Sodium chloride (0.35 g) and potassium chloride (0.35 g) were added to strengthen two gel systems. The rest of the parts were filled with water. It was heated from a temperature of 7.5 °C at microwave power levels of 60, 70, and 80% for 36, 33 and 24 s continuously. The temperature was measured by 4 fiber-optic temperature probes (FISO Technologies, Inc., Quebec, Canada) at geometric center, surface, bottom of the gel cylinder and at the headspace of the beaker. The specific locations of the probes are shown in Fig. 5.1.

2.2 Microwave process

The mechanism of microwave heating mainly involved high electromagnetic frequency induced rapid rotation of polar molecules. Microwave is reflected in every direction inside the oven chamber and is absorbed by the product. Considering the heat

transfer process, there were basically heat generation by microwave and heat conduction since the gel was solidified at initial temperature of 7.5 °C. The amount of heat generated was directly related to the selected microwave oven power and the penetration depth, which was decided by the microwave frequency and the dielectric properties of the gel. During the heating process, there were several other phenomena occurring simultaneously:

a. Changed physical, thermal and dielectric properties as temperature increased.

b. Surface cooling occurred due to the low thermal conductivity of the surrounding air.

c. Surface evaporation, a mass transfer process, induced by increased temperature in a small closed headspace.

d. When temperature reaches beyond 37 °C, the gel starts to melt from a solid state to a liquid state, which complicates the heat transfer mechanics. This is because not only heat convection occurred at different locations but also the thermal and dielectric properties changed.

e. Changed volume of the cylindrical gel during the heating process.

2.3 Assumptions

To simplify the addressed issues, following assumptions were made:

a. The initial temperature of the gel was uniform.

b. There was no mass transfer during the heating process simultaneously.

c The changes of thermal, physical and dielectric properties with composition were considered by using Choi and Okos's equations (Choi and Okos, 1986) assuming a temperature at 20 °C.

d. The increased temperature inside the headspace was not considered.

e. There was no volume change of cylindrical gel.

f. Involved property changes due to the phase change were neglected.

g. Heat convection within the melted gel system was neglected due to its extreme high viscosity.

2.4 System parameters

The heat transfer parameters involved in microwave cooking included density (ρ), thermal conductivity (k), specific heat (Cp), thermal diffusivity (α), surface heat transfer coefficient (h) and dielectric properties (ε', ε") of the fish gel. Changes of density, thermal conductivity, and specific heat associated with temperature and product compositions were obtained based on the work of Hu and Mallikarjunan (2004) and Choi and Okos's mathematical models (Choi and Okos, 1986). Thermal diffusivity was calculated when density, thermal conductivity and specific heat at different temperatures were known. The surface heat transfer coefficient was assumed to be 25 for natural heat convection.

Dielectric properties at different temperatures were calculated by regression equation of dielectric properties of oysters, which were measured using an open ended coaxial-line probe, connected with a network analyzer (Hu and Mallikarjunan, 2001). Penetration depth of microwaves was obtained by the equation (Nelson et al., 1994):

$$\xi_p = \frac{\lambda_0}{2\pi(2\varepsilon')^{0.5}}\left\{\left[1+\left(\frac{\varepsilon''}{\varepsilon'}\right)^2\right]^{0.5}-1\right\}^{-0.5} \qquad ..(5.1)$$

2.5 Mathematical model

A two-dimensional cylinder geometry was used to develop the model. The finite difference equation was constructed in a R-Z plane. In R direction, it was evenly divided

into 5 segments with 4 mm of each, while in Z direction, there were 10 segments with 5 mm each. The whole cylindrical object was divided into 10 layers of each having 5 rings with different diameters (Fig. 5.2)

The governing equation for conductive heat transfer is:

$$\frac{\partial T}{\partial t} = \alpha\left(\frac{1}{r}\frac{\partial T}{\partial r} + \frac{\partial^2 T}{\partial r^2} + \frac{\partial^2 T}{\partial z^2}\right) + \frac{Q}{\rho Cp} \qquad ..(5.2)$$

The heat generation during microwave cooking is obtained by Lambert' law (Decareau, 1985) to describe microwave power absorption by food products:

$$Q = Q_0\left[\exp\left(\frac{-(R-r)}{\xi_p}\right) + \exp\left(\frac{-(Z-z)}{\xi_p}\right)\right] \qquad ..(5.3)$$

The boundary conditions for heat transfer in R direction and Z direction are:

$$-kA\frac{\partial T}{\partial r} = h_1 A(T_s - T_a) \qquad ..(5.4)$$

$$-kA\frac{\partial T}{\partial z} = h_2 A(T_s - T_a) \qquad ..(5.5)$$

The program was written using DESIRE software (Appendix B.1).

3. RESULTS AND DISCUSSION

3.1 Shape effect

The microwave heating profile of the cylinder gel is shown in Fig.5.3. After heating for 10 seconds, the temperature of the gel rapidly increased from 7.5 °C to 62 °C at the surface but it slowly increased to 37 °C at the bottom. As time elapsed, the temperature of each portion continuously increased. Generally, the center zone was believed to have a higher temperature than the outside layers. However, the results obtained from the experiment were opposite.

This heating profile was mainly caused by the heat generation term. In Z direction, as the locations move away from the bottom, there will be more microwave generated heat according to the Lambert's law resulting in higher temperatures. Similarly, in R direction, as the location deviate from the center axis, higher temperatures occurred. Comparatively, the conventional boundary conditions did not play major roles to influence the temperature at the boundary part of the cylinder gel.

When a dummy cylinder changed to a long and thin one, the heating profiles changed dramatically. These effects are illustrated in Figs.5.3 and 5.4. The temperature of the tall cylinder along the R direction varied slightly compared to the short cylinder. As heating proceeded, the non-uniformity in R direction was improved. However, the temperature variations along the Z direction became more obvious.

3.2 Model validation

The comparison of the simulation results with the experimental data are shown in Figs. 5.5 and 5.6. This particular gel contained CMC (1 g) and protein (10 g). It was heated under microwave power of 70% level for 30 s. Due to the unique heating mechanism of microwave oven, for the first 21 second, which is 70% of microwave duty cycle, the microwave power was generated with a full power; while for the rest of 9 s, the power stopped.

It can be observed that the predicted temperature at the geometric center of the cylinder gel had a similar increasing pattern though the maximum difference between the predicted and experimental data was about ±8 °C. The simulated temperatures at the surface linearly increased from 7.5 °C to 109 °C which can not predict the real increasing

trend on the gel surface (Fig. 5.6). Measured experiment data showed the temperature at gel surface grew rapidly from 14 °C to 87 °C within 10 s and being nearly constant at 93 °C for 10 s and dropped slowly to 77 °C.

The temperature discrepancy between the experimental data and simulation results on the surface can be partially attributed to the rapid temperature increase in the small headspace created by adding a plastic cover on the top of the beaker to hold the probes. It was caused by enhanced moisture migration from the surface of the gel to the headspace and the melting of the gel at the surface when temperature increased above 37 °C. However, the rapid increase of temperature at the bottom obtained in the simulation was not clear. Possible reasons are:

a. Unevenly distributed product temperatures. Typically, the geometric center had the lowest temperature, which was close to 7.5 °C while the surface had the highest (8 °C higher).

b. Changed physical, thermal and dielectric properties of fish gel after melting occurred.

c. Convective heat transfer occurred at different locations.

d. Mathematical equation used to calculate the heat generation by microwave, Lambert's Law, is not appropriate.

The temperature profile of the microwave-heated oysters was compared to the simulation results using fish gel, which had similar compositions as the oysters (Fig. 5.7). The model can describe the heating profile of the oysters well at the initial 20 s heating time, when the microwave power was on. When the microwave power was off, the simulated temperature immediately dropped; while the measured temperature seemed to have some hysteresis effect. This created the increased temperature difference between the

simulated temperatures and the real measurements. It also can be seen that the simulated results were slightly lower than the measured data. The reason could be partially attributed to iron concentration inside the oysters which was not included in the mathematical model.

3.3 Composition effect

Temperature response of the fish gel heated by microwave oven varied with protein, carbohydrate, and water contents. Table 5.1 shows the effect of composition of fish gel on the values of the heat transfer parameters. Assuming at a constant ambient temperature, the thermal conductivity of fish gel decreased from 0.56 $Wm^{-1}°C^{-1}$ to 0.39 $Wm^{-1}°C^{-1}$ as protein content increased from 10% to 50% while water content decreased from 88% to 48%. At the same time, specific heat of fish gel decreased from 4400 Jkg^{-1} $°C^{-1}$ to 3300 $Jkg^{-1}°C^{-1}$ and density increased from 1034 kgm^{-3} to 1166 kgm^{-3}. Although each heat transfer parameter changed drastically, the combined effect on thermal diffusivity did not change quite much. Besides, gel density and specific heat, which directly affected the microwave heat generation term, did cancel out the positive effect of thermal diffusivity value. This was true for the change of carbohydrate concentration affecting the temperature distribution of the fish gel.

4. CONCLUSIONS

The non-uniformly distributed temperature of fish gel after microwave cooking is greatly affected by the geometry of the product. Composition of the product had fewer influences on the heating profiles. Simplified model can predict the temperature profile of fish gel within the range of 8 °C. But it did not work efficiently at certain locations due to

the complexity of the process. However, there are potentials to use this model to simulate microwave processing of oysters. Further research work needs to be conducted on modifying the models to deal with the phase change occurred at different locations and to include iron concentration into the model to get more accurate simulation results.

NOTATION

α	Thermal diffusivity of fish gel, m^2s^{-1}
ε'	Dielectric constant of fish gel
ε''	Dielectric loss factor of fish gel
ξp	Penetration depth, m
λ_o	12.237 cm at 2450 MHz
ρ	Density of fish gel, kgm^{-3}
A	Cross section area, m^2
C_p	Specific heat of fish gel, $Jkg^{-1}\ ^oC^{-1}$
h	Surface heat transfer coefficient, $Wm^{-1}\ ^oC^{-1}$
h_1	Surface heat transfer coefficient at R direction, $Wm^{-2}\ ^oC^{-1}$
h_2	Surface heat transfer coefficient at Z direction, $Wm^{-2}\ ^oC^{-1}$
k	Thermal conductivity, $Wm^{-2}\ ^oC^{-1}$
Q	Heat generation, Wm^{-3}
Q_o	Unit heat generation, Wm^{-3}
t	Time, s
T	Temperature, ^oC
T_a	Ambient temperature, ^oC
T_s	Surface temperature, ^oC
r	Radial position, m
R	Radius of fish gel, m
z	Axial distance, m
Z	Thickness of fish gel, m

REFERENCES

Chen, D. D, Singh, R. K., Haghichi, K. and P. E. Nelson 1993. Finite element analysis of temperature distribution in microwaved cylindrical potato tissue. Journal of Food Engineering, 18: 351-369.

Choi, Y. and M. Okos 1986. Effects of temperature and composition on the thermal properties of foods. Food Engineering & Process Applications. Volume 1. Ed by Lemaguer, M. and Jelen, P. Elsevier Applied Science Publishers.

Datta, A. K. 1991. Mathematical modeling of microwave processing as a tool to study safety. Paper No. 91-6614. ASAE International Winter Meeting, American Society of Agricultural Engineers, St. Joseph, MI.

Decareau, R. V. 1985. Microwaves in the Food Processing Industry. Academic Press., Orlando, FL, USA.

Hu, X and P. Mallikarjunan 2004. Thermal and dielectric properties of shucked oysters. Upcoming in Journal of Food Science and Technology/LWT in Jan, 2005 issue.

Mallikarjunan, P., Hung, Y. C. and S. Gundavarapu 1996. Modeling microwave cooking of cocktail shrimp. Journal of Food Process Engineering, 19:97-111.

Nelson, S. O., Forbus, W. R., JR. and R. C. Lawrence 1994. Microwave permittivity of fresh fruits and vegetables from 0.2 to 20 GHz. Transaction of ASAE, 37(1):183-189.

Ohlsson, T. and P. O. Risman 1978. Temperature distribution of microwave heating-spheres and cylinders. Journal of Microwave Power, 13(4):303-310.

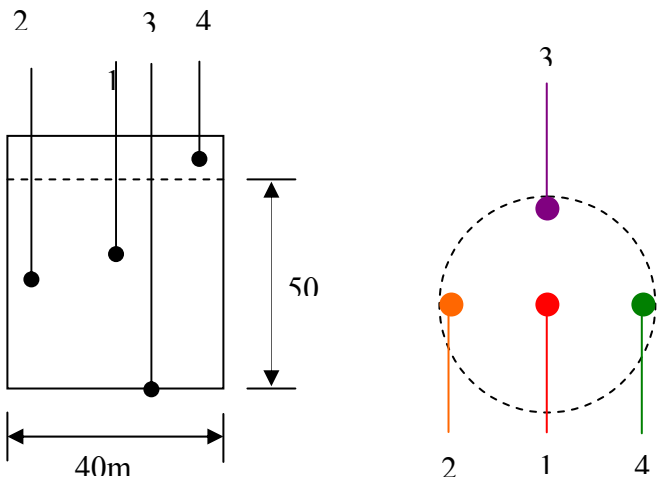
Tong, C. H. and D. B. Lund 1993. Microwave heating of baked dough products with simultaneous heat and moisture transfer. Journal of Food Engineering, 19:319-339.

Hu, X. and K. Mallikarjunan (2001). Thermal and electric properties of shucked oysters. Proceedings of the 7th Conference of Food Engineering (COFE), Nov. 5-9, Reno, NV, USA.

Zhou, L., Puri, V. M. and R. C. Anantheswaran 1992. Evaluation of food quality and safety during microwave heating using finite element model. Paper No. 92-6577. ASAE International Winter Meeting, American Society of Agricultural Engineers, St. Joseph, MI.

Table 5.1: Effect of composition on heat transfer parameters

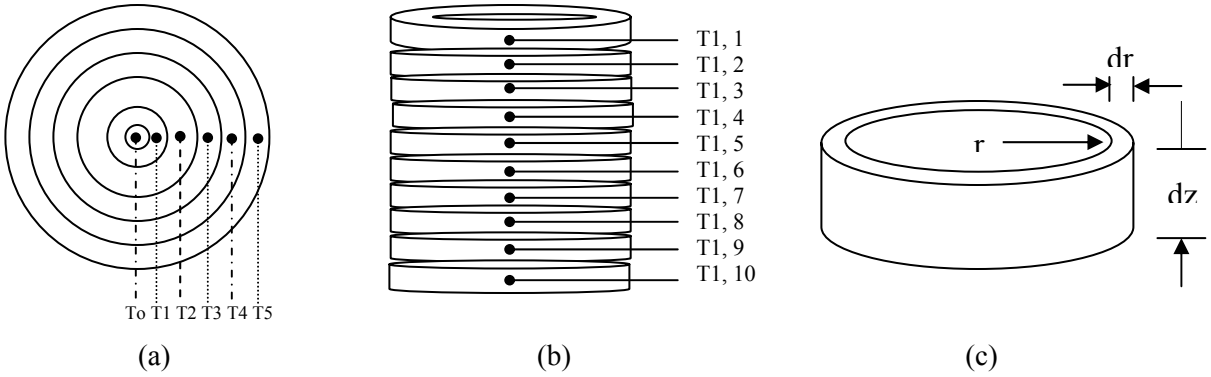
Gel composition (%)			Thermal parameters				
Protein	Carboh -ydrate	Water	K ($Wm^{-1}°C^{-1}$)	ρ (kgm^{-3})	Cp ($Jkg^{-1}°C^{-1}$)	$\alpha \times 10^{-6}$ (m^2s^{-1})	$\rho \times cp \times 10^{6}$ ($J\ m^{-3}°C^{-1}$)
10	2	88	0.555	1034	4392	0.122	4.54
30	2	68	0.475	1100	3854	0.112	4.24
50	2	48	0.395	1166	3316	0.102	3.87
10	2	88	0.555	1034	4392	0.122	4.54
10	20	70	0.487	1143	3828	0.111	4.38
10	40	50	0.412	1263	3200	0.110	4.04



(a) (b)

Fig. 5.1: The location of temperature probes

Note: (a): The side view of the cylindrical shaped fish gel. (b): The top view of the cylindrical shaped fish gel. Number 1 to 4 indicates the location of the 4 fiber optic probes.



(a) (b) (c)

Fig. 5.2: Finite difference element

(a): In R direction, the fish gel was segmented into 5 lays with different radius. T0 to T5 represents the temperature at the center of these layers. (b): In Z direction, the fish gel was segmented into 10 layers. This figure illustrates the first layer right next to the core of the cylindrical fish gel in R direction. T represents the temperature at the center of each layer. The second number followed the T indicates the number of the layer away from the top surface. (c): Enlarged finite difference element.

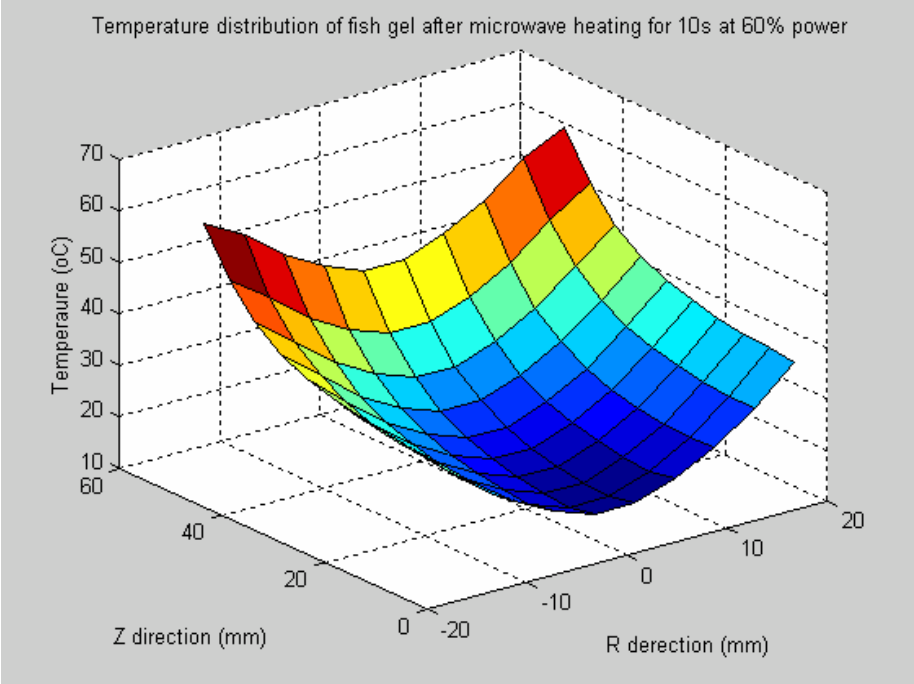


Fig. 5.3: Temperature distribution of fish gel after microwave treatment for 10 s at 60% power level

Note: Dimension of the fish gel: diameter = 40 mm, height = 50 mm.

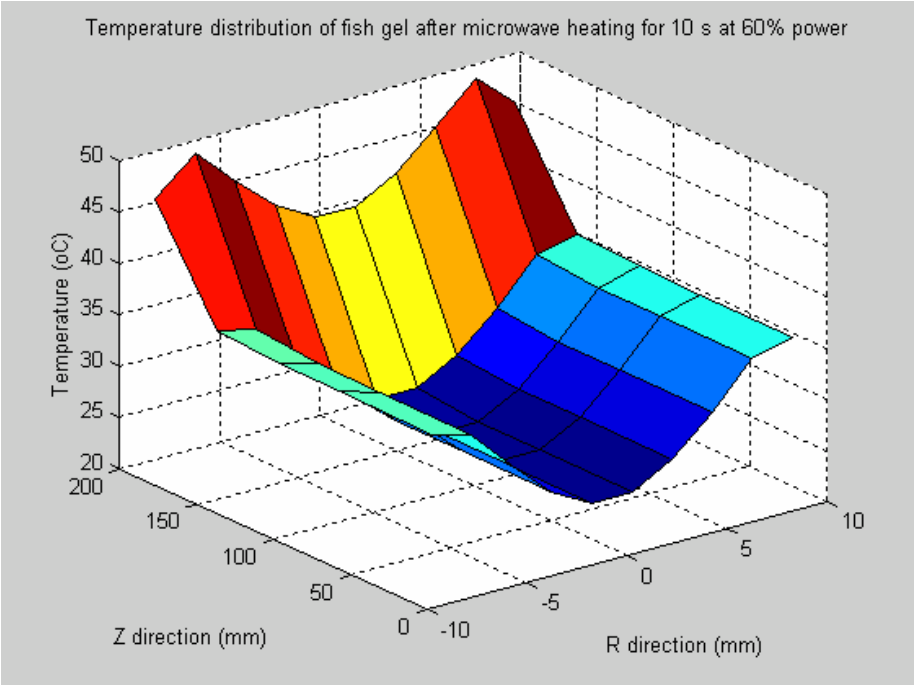


Fig.5.4: Temperature distribution of fish gel after microwave treatment for 10 s at 60% power level

Note: Dimension of the fish gel: diameter = 20 mm, height = 200 mm.

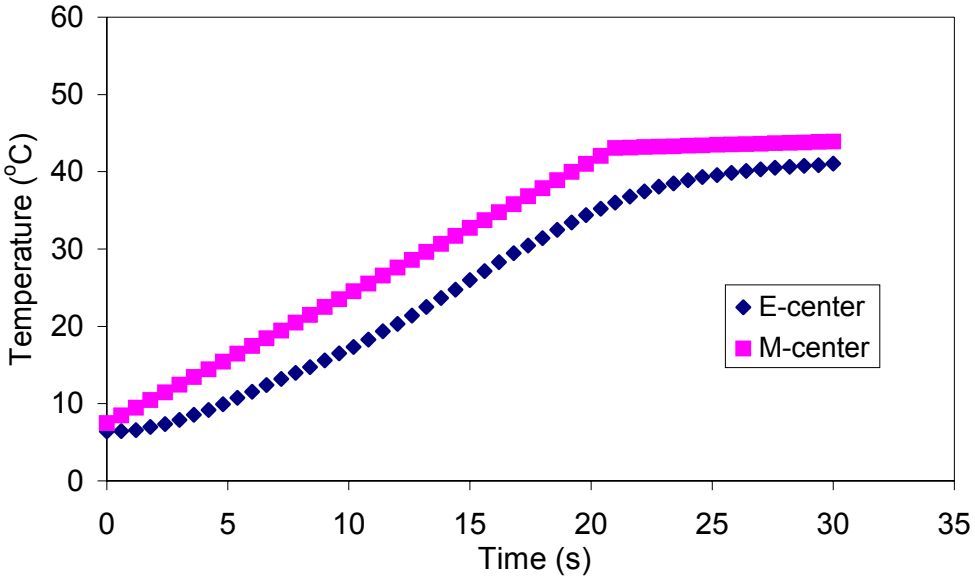


Fig. 5.5: Temperature profile of fish gel after microwave heating for 30 s at 70% power (Geometric center)

Note: E-center indicates the experimental data on temperature profile; M-center indicates the measured temperature profile.

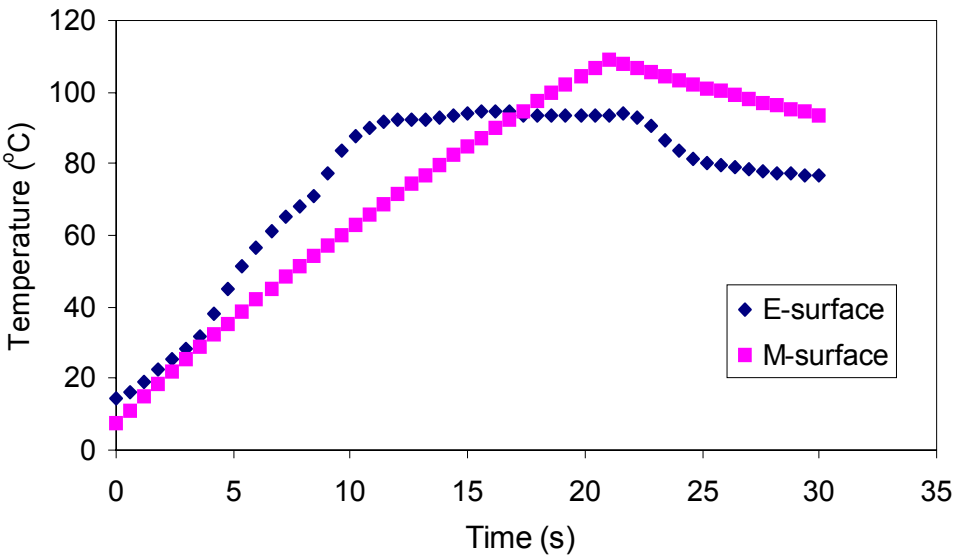


Fig. 5.6: Temperature profile of fish gel after microwave heating for 30 s at 70% power (Surface)

Note: E-surface indicates the experimental data on temperature profile; M-surface indicates the measured temperature profile.

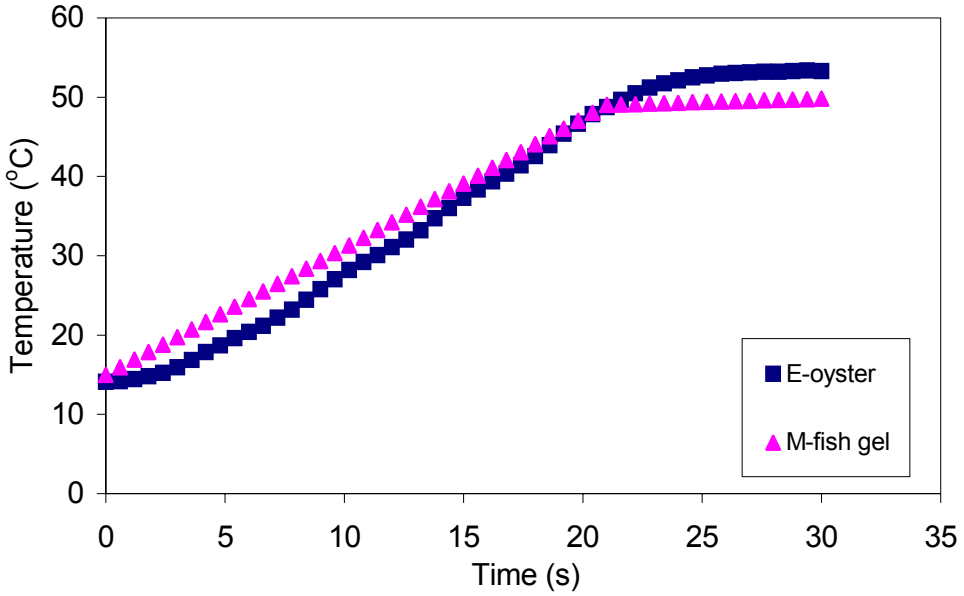


Fig. 5.7: Temperature profile of microwave heated oysters and simulation results from fish gel

Note: E-oyster indicates the experimental data on temperature profile; M-fish gel indicates the measured temperature profile.

CHAPTER 6

Inactivation of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in Whole Oysters by Microwave

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Running Head: Microwave Inactivation of *Vibrio* spp. in oysters

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ABSTRACT

Live oysters inoculated with *Vibrio vulnificus* (10^6 CFU/g) and *Vibrio parahaemolyticus* (O3:K6, 10^7 CFU/g) were heated in a microwave oven. Inoculated samples were treated with appropriate combinations of time and microwave power levels to have a final temperature between 55 and 60 °C, and maintain for 2 min to allow temperature to equilibrate within the product. D and z values for *V. vulnificus* and *V. parahaemolyticus* were determined using a water bath. A two-dimensional mathematical model was established to obtain the temperature profile. The lethality of microwave treatment was calculated by integrating the temperature profile at each designated heating element within the product and the first-order microbial inactivation kinetics were applied. The predicted results were compared with the lethality data from microbial tests and from the real-time temperature history measurement. The observed maximum log reduction by microbial enumeration was 4.4 and 3.4 for *V. vulnificus* and *V. parahaemolyticus*, respectively. The lethality calculated by integrating temperature profiles was acceptable. The application of mathematical model to predict the microwave inactivation collectively needs to be critically considered, due to the non-uniform heating mechanism of the microwave oven and limited real time measurement devices.

Keywords: Microwave, inactivation, *Vibrio* spp., oysters, lethality

INTRODUCTION

The presence of *Vibrio vulnificus* (*V.v.*) and *Vibrio parahaemolyticus* (*V.p.*) in shellfish, particularly in raw oysters, has become the leading cause of many outbreaks of gastroenteritis and wound disease, resulting in an increased concern of seafood safety from consumers (CDC, 1998; 1999). In order to enforce the safety, it is essential to employ viable post-harvest techniques to reduce the bacterial load to a safe-to-consume level. Since the *Vibrio* spp. are relatively susceptible to heat in nature, applying microwave radiation maybe an alternative compared to conventional heating, due to its rapid heating rate.

Numerous studies have addressed the effectiveness of bacterial inactivation by microwave radiation. The well-documented non-uniformity heating can be attributed to many factors including size, geometry, composition, electric and thermal properties of food materials as well as the microwave oven parameters, power supply, heating time, etc. (Berek and Wickersheim, 1988). In addition, continuous debate on possible non-thermal effects of microwave processing has been lasting for many years. Several studies suggested that there was a non-thermal effect for inactivation of microorganisms or enzymes when food was exposed in high frequency electromagnetic field, because the heat generated by microwave itself was inadequate to create the lethal effect (Juneja and Sofos, 2001; Mertens and Knorr, 1992; Pothakamury et al., 1993; Taichakavis and Ramaswamy, 1996). The theories underlining non-thermal effect inactivation were also developed and reviewed by Knorr, et al., 1994. However, many others argued that no additional lethality was found in destroying microorganisms using microwave radiation aside from the temperature-time relationship as in thermal processing (Mudgett, 1982, Decareau, 1985 and Welt, et al.,

1994). A third opinion was that, compared to conventional cooking, microorganisms were more likely to survive in foods cooked using microwaves (Schiffmann, 1992).

The disagreement on the non-thermal inactivation effect by microwave radiation was essentially a problem of how to accurately measure the thermal lethality by microwave heating. In general, several temperature probes were inserted in certain locations of the food system being heated. Due to the limited number of the temperature probes, the locations were usually determined according to heat transfer principles so that the hot spots and the cold spots were always been monitored. The calculation of the thermal inactivation was based on the inactivation on the cold spots to avoid overestimate of the lethality for the safety reason. Sometimes, a mixed final temperature was used. These estimations are objective and prone to error. Firstly, recent review by Datta and Davidson (2000) suggested that the cold and hot spots can shift during microwave processing due to the changing thermal and dielectric properties during microwave heating. Secondly, the difference of the localized lethality can vary dramatically. For example, Zhou and Puri (1992) reported a maximum of 8 log reduction difference in the bacterial destruction between the hot and cold spots in a simulated three dimensional cylindrical potato system using finite element model. Therefore, a more accurate approach might be to calculate the lethality of each segmented heating element individually by using the localized temperature profile, and then get the mass average of the lethality of the entire product. This can be easily accomplished by employing a mathematical model. Since the lethality from the microbial test was a collective measurement, which is an average lethality of the total processed mass, the measurement of microwave inactivation also needs to be a collective effect. By doing so, the comparison of both would be more logical.

Limited study has been carried out to address the issue mentioned above. The main objectives of this work were to study the mechanism of microwave inactivation of *V.v.* and *V.p.*, inoculated in oysters; to validate the collective approach of obtaining the lethal effect of *V.v.* and *V.p.* by microwave processing, using mathematical models.

MATERIALS AND METHODS

Measurement of Inactivation Kinetics Parameters

Fresh oysters used in this study were obtained from Bevans's Oyster Inc., Virginia. They were cultured in artificial seawater in three 10 gl aqua-tanks and fed with fresh algae before the experiment. The seawater was prepared by dissolving commercial sea-salt in water and allowing it to sit for three days before use. Air was supplied to each aqua-tank.

Clinical strains of *V. p.* (O3:K6 TX2103) and *V.v.* (MO624) were used in this study. They were inoculated into Tryptone Salt Broth (T_1N_1: Tryptone Soy Agar with 1% Salt) and incubated at 35 °C for 18-20 hr. One loop of *Vibrio* spp. cultures were transferred to freshly prepared T_1N_1 broth and continuous incubation at 35 °C for another 18-20 hr. The bacterial counts can achieve over 10^8 CFU/ml for both *V.v.* and *V.p.*.

In order to get D and z value of *V.v. and V.p.*, seven to eight oysters (roughly 125 g) were shucked into sterilized stomacher bags. Manual massages were applied to the bags until all oyster tissues were smashed completely. The maximum pH of the oyster homogenate was measured to be 6.25 and the minimum to be 6.02. The autoclaved oyster homogenate (9 ml) was dispensed into 25 ml test tubes. The test tubes were adjusted to the temperatures of interest by equilibrating them in a water bath for 30 min. Then two pre-warmed test tubes were inoculated with 1 ml *V.v. and V.p.* pure culture, respectively. They

were removed from the water bath at designated time intervals and cooled in ice-water. Tests were conducted at 45, 50, 55, and 60 °C with 4 replications. The controlled samples were prepared by removing the oyster homogenate tubes from the water-bath immediately after the inoculation. The thermal treatment conditions were summarized in Table 6.1.

Series dilutions were made immediately using PBS (Phosphate-buffered saline, pH=7.4) on control and thermal treated oyster homogenates. They were spread plated onto T_1N_1 agar. Plate counts were performed after the plates were incubated at 35 °C for 18-24 hr. Duplicate places were made on each *Vibrio* strain. D values were calculated from the linear regression line of the number of survival bacteria vs. time in a semi-logarithmic plot at each temperature. Z values were determined from the plot of Log D values against temperature.

Inoculation of Oysters

Three aqua-tanks were set under the bio-safety facility, each containing 8 L of sea water. Submersible heating element was utilized to maintain the temperature of the seawater at 25 °C for each tank. Thirty oysters, 10 each, were placed into one tank 4-5 hr before the inoculation. The purpose of doing this was to allow oysters to adapt to the warm environment. Dead oysters were discarded. Fresh algae were fed to encourage oysters to keep pumping water. Large amount of air was supplied using air pump. Freshly prepared pure *Vibrio* cultures (100 ml) were poured into each tank and slight stir was applied. After 20-24 hr exposure, the *Vibrio* counts can achieve 10^6 CFU/ml for *V.v.* and 10^7 CFU/ml for *V.p.* in oyster tissues. Sanitation procedures were strictly carried out on seawater and all contaminated equipment. Concentrated hyper-sodium chloride was poured into culture

tanks (5000 ppm) and allowed the tanks being soaked for 3-4 hr before the seawater was discarded into the sewage system. Alcohol (75%) was applied to further clean all contaminated equipment and water tanks that were not autoclavable.

Microwave Processing

Thirty oysters were divided into 5 groups. Depending on the size and weight of each oyster, 5-6 oysters were placed in one group to make a mass of approximately 1000 g. Oysters were kept in a microwavable glass container, which has a cylindrical shape. The diameter of the container is about 39 cm and the depth of the container is about 15 cm. In order to have more uniformed heating, 1000 g water (20°C) was added to the container. They were heated by a microwave oven (Sharp, 1000w/R-21HT, Japan). The power level and heating time were determined by a preliminary study, which can bring a final temperature of oysters between 55 and 60 °C. The detailed processing conditions are listed in Table 6.2. The temperature profile was recorded by employing three fiber optic probes (FISO Technologies, Inc., Quebec, Canada). After microwave treatment, oysters were immediately kept in ice to cool down to room temperature.

***Vibrio* spp. Enumerations**

Processed oysters were shucked into sterilized stomacher bags. Hand massage was applied to help smashing oysters. Dilution (1:10) was made by adding PBS of exactly same amount as oyster tissues into stomacher bags, followed by transferring 20 ml solution (1:1 dilution) into 80 ml PBS dilution bottles. The 3-tube MPN method was used to enrich *Vibrios* (Elliot et al., 1998). After 18-24 hr incubations at 35 °C, the turbid APW (Alkaline

Peptone Water) tubes were streaked to Modified Cellobiose-Polymxin B-Colistin Agar (mCPC) plates for identification of *V. v.* and to Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS) plates for identification of *V.p.*. Additional bio-chemical confirmation tests of *V. v* and *V. p* were performed using trypticase soy agar with 8% salt and API-test Strips 20E (Elliot et al., 1998).

Mathematical Modeling

A two dimensional cylindrical model was used to simulate the heat transfer process based on the work of Hu and Malllikarjunan (2004a) (Appendix B.2). The finite difference equations were constructed in a R-Z plane. The cylinder had a diameter of 39 cm and a height of 6.8 cm. In R direction, it was evenly divided into 5 segments; while in Z direction, there were 10 segments. The whole cylindrical object was divided into 10 layers of each having 5 rings with different diameters. Therefore, a total of 50 heating elements were established. The heat transfer was attributed to conduction within the oysters, convection between the product surface and surrounding air, and heat generation by microwaves. The boundary conditions were governed by heat convection. Lambert's law (Decareau, 1985) was used to estimate the heat generation by microwave.

The simplified model neglected the heat absorption by oyster shells and by surrounding air in the microwave oven. Multi-volume cavities between oysters was ignored therefore the oysters were considered a uniform and continuous distributed meat homogenate. The initial temperature of oysters and surrounding air were measured to be 20 °C. Since the final temperature of heated products was controlled to reach 55 to 60 °C only, the mass transfer, resulting from the evaporation on the surface of the product, was

also ignored. The employed heat transfer parameters, such as thermal conductivity, specific heat, density, were measured in the work of Hu and Mallikarjunan (2004b). The dielectric properties were estimated by the quadratic model developed by Hu and Mallikarjunan (2004b).

When temperature profile of each designated heating element was determined, the lethality (Li) was performed mathematically using eq. 6.1 (Holdsworth, 1997). Then the total lethality was calculated using the mass average of the obtained individual lethality. The specific eq. 6.2 was listed below.

$$L_i = \int 10^{(T-T_R)/Z} dt \tag{6.1}$$

where L i= lethality of individual element

T = localized temperature at time t

T_R = reference temperature

Z = thermal resistance constant

t = process time

$$L = \frac{\sum_{i=1}^{n} L_i \times M_i}{M} \tag{6.2}$$

where L=total lethality

Mi= mass of individual element

i = number of the element

M= total mass of the heating object

RESULTS AND DISCUSSION

Identify Thermal Inactivation Parameters

Decimal reduction time (D values) were calculated based on microbial enumeration data in a water bath using Bigelow model (Holdsworth, 1997), which considers the microbial inactivation as a first order kinetics reaction, resulting in a linear relationship between the logarithmic number of microbial survival and processing time. The data were presented in Fig. 6.1. The calculated D values at 45 °C, 50 °C, 55°C and 60 °C are presented in Table 6.3.

The available data in literature reporting the D values of *V. v.* varied significantly. D values were reported to be 50 min at 45 °C and 10 sec at 51 °C without mentioning the strain name and the media, in which the decimal reduction time was measured (Anon, 1996); 2.4 min at 47 °C and 1.15 min at 50 °C in buffered saline (Cook and Ruple, 1992); 2.2 min at 47 °C, 0.83 min at 50 °C and 0.21 min at 52 °C in oyster homogenate (Dombroski et al., 1999). *V.v.* (ATCC 27562) was reported to have D values of 3.7 min at 47 °C and 1.15 min at 50 °C (Drake et al., 2003). These values were all significantly less than the values determined from this study ($P<0.05$). Even with the same *Vibrio* strain, D values of *V.v* measured from this study were still higher compared to the data measured by Kim et al., in 1997, who determined that D values of *V. v.* (M0624) were 48.25 min at 45 °C, 0.55 min at 49 °C and 0.19 min at 51 °C, though BHI (Brain Heart Infusion) broth was used as the measurement medium.

Comparatively, the thermal resistance data on *V. p.* were less available. Delmore and Chrisley (1979) reported the D values of 0.66 min for *V.p.* at 51 °C and 0.29 min at 55 °C in clam homogenate. However, on the study of the thermal resistance of *V.p.* (O3:K6),

the D value at 50 °C varied from 2.95 min to 4.26 min depending on the location of the isolation and time of the isolation (Wong et al., 2000).

The variability of the data on the thermal resistance of *V.v.* and *V. p.* can be mainly attributed to the differences on the bacterial strain, the medium used to suspend the bacteria and the microbial enumeration method. It is a general believe that the bacteria will be more resistant to physical or chemical stress if they are grown in nutrient broth than in simple aqueous media. Therefore, the obtained D values on *V.v* and *V.p* were reasonable in this study. Compared to the data measured at similar conditions, D value of *V.v.* at 50 °C was 0.53 min less than reported from the Ministry of Health (Anon, 1996) when the pH of the oyster homogenate was 6.2. It can be seen that *V.p.* was significantly more resistant to thermal stress than *V.v.* at every target temperature.

The z values of *V.v.* and *V.p.* in oyster homogenate were identified to be 5.8 °C and 6.2 °C, respectively. This result demonstrated that, potentially, *V.v.* is more sensitive to the change of the temperature than *V.p.* during the thermal inactivation. Those values are relatively high compared to regular non-spore forming pathogenic bacteria such as *Escherichia coli O157:H7* and *Listeria monocytogenes* (Ahmed et al., 1995; Bremer and Osborne, 1995). It was reported that *V.v.* (M0624) had z value of 2.5 °C in BHI broth (Kim et al., 1997). The high value obtained in this study was probably due to the strong protection effect from oyster homogenate, which contains 6-10% protein and 0.7-2.4% fat.

Microwave Inactivation of *Vibrio* spp.

The microwave inactivation data are presented in Fig. 6.2. It can be seen that although the applied microwave energy was same with designated power level and time

combination, the end results from microbial inactivation varied. For *V.v.*, 100% power and 4.2 min radiation created 4.4 log reductions, while no significant difference was found between the log reductions occurred at 50% and 70% power level ($P<0.05$). Power level of 30% along with the longest processing time of 13.9 min resulted in 3.8 log reductions. However, for *V.p.*, as power level increased from 30% to 100%, the number of log reductions also increased from 2.5 to 3.4. Andrews et al. (2000) reported a 5 log reduction on both *V.v.* and *V.p.* in oysters when heated at 50 °C for 10 min. Hesselman et al. (1999) also conducted experiments on thermal inactivation of *V.v.*. They found out that 50 °C and 4 min can significantly reduce *V. v.* up to 4 log cycles in oysters. Therefore, the inactivation rate of *V.v.* and *V.p.* obtained from this study was reasonable. In addition, the results confirmed that *V.v.* was more susceptible to microwave treatment compared to *V.p.*, which was fairly consistent with the published data on the resistance of *V.p.* to thermal processing, high pressure and irradiation over *V.v.* (Kim et al., 1997; Wong et al., 2000, Hu et al., 2003).

The resistance of *V.v.* and *V.p.* to microwave treatment using real time-temperature measurement by thermal probes is presented in Table 6.4. For *V.v.*, basically, the number of log reductions matched with the microbial enumeration data. At three microwave power level, 30%, 70% and 100%, the microbial analysis showed slightly higher values than the predicted ones. However, interesting observation was that for *V.p.*, data collected from the microbial study were higher at each microwave processing condition, and statistically, the value in power level 50%, 70% and 100% was significant ($P<0.05$).

The discrepancy of the lethality between the values obtained from real temperature measurement and from the microbial enumeration was essentially a problem of how to

quantify the lethality induced by thermal effect. Since the temperature profiles were measured at limited locations, they can not fully represent the temperature elevation in the entire product, although two min equilibrium time was given. The risk factor is the non-uniform heating mechanism by dielectric hating. In addition, the discrepancy would be more distinct with bacteria having higher z values, which is the temperature required to achieve 90% inactivation, due to their increased sensitivity to thermal effect, if the calculation of the lethal effect was made on the temperature profile of the coldest spot. In fact, this has been confirmed from this study.

In order to perform a more logic comparison with microbial enumeration data, which have been measured collectively, the lethal effect on the bacteria by microwave treatment needs to be measured collectively too. Instead of using the real time- temperature history collected at limited locations, it was proposed to utilize the temperature profile at each location of the product to get localized lethality and integrate the localized lethality to obtain the entire lethal effect. The results are showed in Table 6.4.

The inactivation results from mathematical model showed, although the input microwave energy was, the lethality of *V.v.* and *V.p.* was highest at 30% power level for 13.9 min, followed by 100% power level for 4.2 min, resulting in nearly 3 log reductions for *V.v.* and 2 log reductions for *V.p.*. Microwave power of 50% for 8.3 min and 70% for 6 min generated 2.7 log reductions for *V.v.* and 1.7 log reductions for *V.p.*. Compared to the results from microbial enumeration, the mathematical model, which was constructed on the first order thermal inactivation kinetics, seemed to underestimate the lethality of *V.v.* and *V.p.* by microwave processing at each processing condition. However, the results observed from the mathematical model did reflect the same correlation relations between the

lethality and the microwave processing conditions as the ones revealed by the microbial test results except for at 100% power level. In microbial test, 100% power and 4.2 min created the highest lethality rate among all. However, it was not reflected by the results calculated using mathematical model.

The underestimation of the predicted lethality by mathematical model can be mainly attributed to the neglected heat absorption by oyster shells. In the established model, the mass and volume of the shells were replaced with homogenously distributed oyster meat. Due to the different thermal and dielectric properties, heat absorbed by oyster tissues was much more than that absorbed by shells. When having the same energy input, this would result in a reduced final temperature of the oysters of the counterparts in the original model. Ignoring heat absorbed by shells would also influence the temperature distribution in oysters by affecting the boundary conditions and heat conduction within oyster homogenate. In addition, the ignored multi void space among each oyster also created some errors in predicting the temperature profiles.

CONCLUSIONS

Microwave radiation can eliminate *Vibrio* spp. in raw oysters effectively. The maximum inactivation, 4.4 and 3.4 log reductions for *V.v.* and *V.p.*, respectively, was observed at 100% microwave power level for 4.2 min. The lethality calculated using the real time-temperature measurement was less than the results obtained from microbial test. The established mathematical model was able to predict the lethality of microwave processed food items within an acceptable level. However, the simplified model can not achieve an accurate estimation, because microwave radiation of food products with

irregular shape and unevenly distributed mass was a very complex process involving many varying parameters.

Although certain discrepancy occurred between the lethality values calculated utilizing the mathematical model and microwave enumeration, the concept of predicting the lethality of microwave processed product collectively needs to be critically considered. The accuracy of the prediction was entirely depended on how accurate the temperature distribution profile can be achieved. Until that stage, the debate, on whether pure thermal inactivation kinetics can fully explain for the achieved inactivation or whether there could be a non-thermal impact by microwave radiation, can be stated with more confidence.

REFERENCES

Ahmed, N. M., Conner, D. E. and Hulfman, D. L. 1995. Heat resistance of *Escherichia coli* O157:H7 in meat and poultry as affected product composition. J. Food Sci. 60:606-610.

Andrews, L. S., Park, D. L. and Chen, Y. P. 2000. Low temperature pasteurization to reduce the risk of *vibrio* infections from raw shell-stock oysters. Food Addit. Contam. 17(9):787-791

Anon. 1996. *Vibrio vulnificus.* In *Microorganisms in Foods: Microbiological Specifications of Food Pathogens*. The International commission on Microbiological Specifications for Foods. pp. 436-439. Blackie Academic and Professional, London.

Berek, H. E. and Wickersheim,, K. A. 1988. Measuring temperatures in microwavable packages. J. Packag. Technol. 2:164.

Bremer, P. J. and Osborne, C. M. 1995. Thermal death times of *Listeria monocytogenes* in green shell mussels (*Perna canaliculus*) prepared for hot smoking. J. Food Protect. 58:604-608.

CDC 1998. Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters – Pacific Northwest, 1977, MMWR. 43(22):457-462.

CDC 1999. Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters and clams harvested from Long Island Sound – Connecticut, NJ, 1998, MMWR. 48(3):48-51.

Cook , D. W. and Ruple, A. D. 1992. Cold storage and mild heat treatment as processing aids to reduce the number of *Vibrio vulnificus* in raw oysters. J. Food Protect. 55(12):985-989.

Datta, A. K. and Davidson, P. M. 2000. Microwave and Radio Frequency Processing. In *Kinetics of Microbial Inactivation of Alternative Food Processing Technologies: IFT's response to task order #1, U.S. Food and Drug Administration: How to quantify the destruction kinetics of alternative processing technologies*. J. Food Sci. Suppl., pp32-41. Institute of Food Technologies, Chicago, IL, USA.

Decareau, R. V. 1985. Microwave in the Food Processing Industry. Academic Press Inc. Orlando, FL.

Delmore, R. P., Jr. and Chrisley, F. D. 1979. Thermal resistance of *Vibrio parahaemolyticus* in clam homogenate. J. Food Protect. 42(2):131-134.

Dombroski, C. S., Jaykus, L. A., Green, D. P. and Farkas, B. E. 1999. Use of a mutant strain for evaluating processing strategies to inactivate *Vibrio vulnificus* in oysters. J. Food Protect. 62(6):592-600.

Drake, S. L., Bang, W., Jaykus, L. A., Drake, M. A., Green, D. P. and Elhanafi, E. 2003. Evaluation of growth and survival characteristics of *Vibrio vulnificus* expressing green florescent protein. 2003 IFT Annual Meeting, July 13-16, Chicago, IL.

Elliot, E.L., Kaysner, C.A., Jackson, L. and Tamplin, M.L. 1998. *Vibrio cholerae, V. parahaemolyticus, V. vulnificus*, and Other *Vibrio* spp. In *Bacteriological Analytical Manual*, *8th Edition.* Revision A, 1998.

Hesselman, D. M., Motes, M. L. and Lewis, J. P. 1999. Effects of a commercial heat-shock process on *Vibrio vulnificus* in the American oyster, *Crassostrea virginica*, harvested from the Gulf Coast. J. Food Protect. 62(11):1266-1269.

Holdsworth, S. D. 1997. Thermal Processing of Packaged Foods, pp. 80-85, Blackie Academic and Professional, Chapman & Hall, London, UK.

Hu, X. and Mallikarjunan, P. 2004a. Mathematical modeling of heat transfer in microwave heated fish gel. Int. Agric. Eng. J. 13(3):93-100.

Hu, X and Mallikarjunan, P. 2004b. Thermal and dielectric properties of shucked oysters. Upcoming in J. Food Sci. Tech./LWT in Jan, 2005 issue.

Hu X., Mallikarjunan, P., Koo, J., Andrews, L. S. and Jahncke, M. L. 2003. Comparison of kinetics on high pressure and irradiation inactivation of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in buffer solution and whole oysters. 2003 IFT Annual Meeting: Book of Abstracts. July 13-16, Chicago, IL. Institute of Food Technologist, Chicago, IL.

Juneja, V. K. and Sofos, J. N. 2001. Control of foodborne microorganisms. In: *Magnetic fields as a potential non-thermal technology for the inactivation of microorganisms,* pp. 399-418, Marcel Dekker Inc., New York, USA.

Kim, C. M., Jeong, K. C., Rhee, J. H. and Choi, S. H. 1997. Thermal death times of opaque and translucent morph-types of *Vibrio vulnificus*. Appl. Environ. Microbiol. 63(8): 3308-3310.

Knorr, D., Geulen, M., Grahl, T., and Sitzmann, W. 1994. Food application of high electric field pulses. Trends food Sci. Tech. 5(3): 71-75.

Mertens, B. and Knorr, D. 1992. Developments of non-thermal processes for food preservation. Food Technol. 46(5):124-133.

Mudgett, R. E. 1982. Electrical properties of foods in microwave processing. Food Technol. 36, 109-115.

Pothakamury, U. R., Barbosa-Canovas, G. V. and Swanson, B. G. 1993. Magnetic-field inactivation of microorganisms and generation of biological changes. Food Technol. 47(12):85-93.

Schiffmann, R. F. 1992. Major problems in heating foods in microwave ovens. Microwave World, 13(1):21-23, 35.

Taichakavis, S. and Ramaswamy, H. S. 1996. Inactivation kinetics of pectin methylesterase: thermal vs microwave heating in batch mode - evidence of non-thermal effects. 1996. 1996 IFT Annual Meeting: Book of Abstracts, p. 49. June 22-26, 1996. New Orleans, LA. Institute of Food Technologist, Chicago, IL.

Welt, B. A., Tong, C. H., Rossen, J. L. and Lund, D. B. 1994. Effect of microwave radiation on inactivation of *Clostridium sporogenes* (PA 3679) spores. Appl. Environ. Microbiol, 60(2): 482-488.

Wong, H. C., Liu, S. H., Wang, T. K., Lee, C. L., Chiou, C. S., Liu, D. P., Nishibuchi, M. and Lee, B. K. 2000. Characteristics of *Vibrio parahaemolyticus* O3:K6 from Asia. Appl. Environ. Microbiol. 66(9):3981-3986.

Zhou, L. and Puri, V. M. 1992. Evaluation of food quality and safety during microwave heating using finite element model. ASAE International Winter Meeting, Dec. 15-18, 1992. Nashville, Tennessee. Paper number: 925677.

Table 6.1.

Thermal processing conditions for oyster homogenate to determine thermal inactivation parameters

Temperature (°C)	Time (min)					
45	0	20	40	60	80	100
50	0	2	4	6	8	10
55	0	0.5	1.0	1.5	2	2.5
60	0	0.2	0.4	0.6	0.8	1.0

Table 6.2.

Microwave processing conditions on inoculated live oysters

Heating pattern	Power setting (%)	Power level (W)	Heating time (min)	Power Input (kJ)
I	30	300	13.9	250
II	50	500	8.3	
III	70	700	6.0	
IV	100	1000	4.2	

Table 6.3.

D values of *V. vulnificus* (*V.v.*) and *V. parahaemolyticus* (*V.p.*) at temperature from 45 °C to 60 °C

Temp. (°C)	45	50	55	60
V.v.	59.9	3.23	0.641	0.142
V.p.	75.8	4.68	0.960	0.250

Table 6.4.

Lethality (log reductions) of *V. vulnificus* and *V. parahaemolyticus* in live oysters by microwave radiation obtained using microbial enumeration and mathematical model

	V. vulnificus			*V. parahaemolyticus*		
	Microbial test	Temperature Measurement	Mathematical Model	Microbial test	Temperature Measurement	Mathematical Model
30%-13.9min	3.83±0.10	3.66±0.35	3.01	2.51±0.06	2.39±0.19	2.10
50%-8.3 min	3.01±0.09	3.30±0.21	2.64	2.62±0.10	1.96±0.12	1.69
70%-6.0 min	2.98±0.08	2.69±0.16	2.65	2.79±0.14	1.68±0.10	1.80
100%-4.2 min	4.38±0.19	4.06±0.06	2.90	3.39±0.13	2.50±0.05	1.95

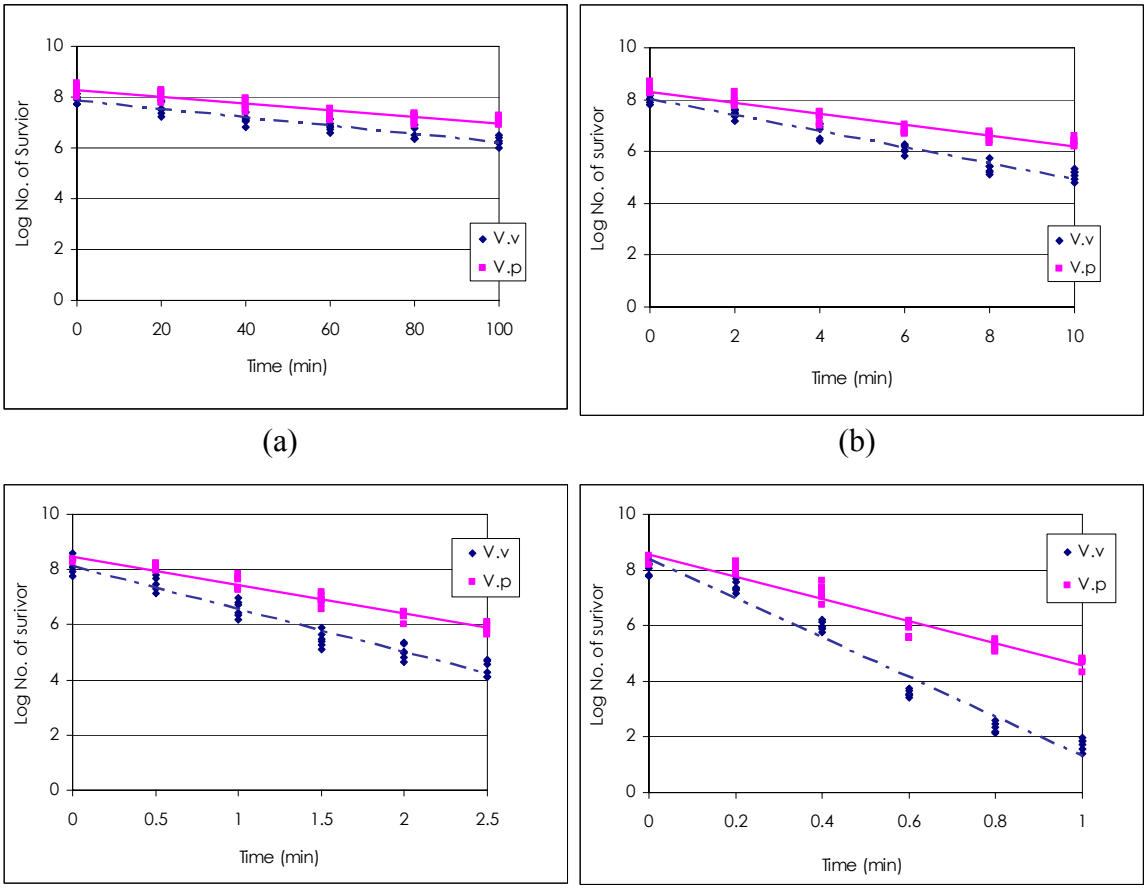

Fig. 6.1: Survival curves of *V. vulnificus* (*V.v.*) and *V. parahaemolyticus* (*V.p.*), where (a) 45 °C, (b) 50 °C, (c) 55 °C and (d) 60 °C

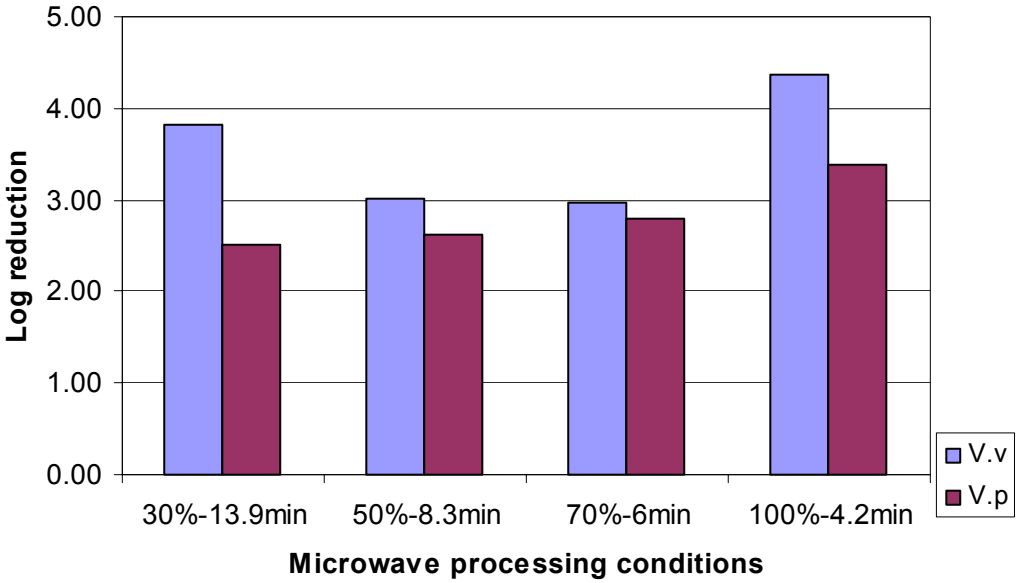

Fig. 6.2: Comparison of microwave inactivation of *V. vulnificus* (*V.v.*) and *V. parahaemolyticus* (*V.p.*) in raw oysters

CHAPTER 7

Development of Non-destructive Methods to Evaluate Oyster Quality by Electronic Nose Technology

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ABSTRACT

The effectiveness of two different electronic nose systems to assess the quality of oysters was studied on live oysters stored at 4 and 7° C for 14 days. Electronic nose data were correlated with a trained sensory panel evaluation by Quantitive Description Analysis (QDA) and with microbial enumeration. Oysters stored at both temperatures exhibited varying degrees of microbial spoilage, with bacterial load reaching 10^7 CFU/g at day 7. Cyranose 320 electronic nose system was capable of generating characterized smell print to differentiate oyster qualities of varying age (100% separation). The validation results showed that Cyranose 320 can identify the quality of oysters in terms of storage time with 93% accuracy. Comparatively, the correct classification rate for VOChek electronic nose was only 23%. Correlation of electronic nose data with microbial counts suggested Cyranose 320 was able to predict the microbial quality of oysters. Correlation of sensory panel scores with electronic nose data revealed that electronic nose has demonstrated potential as a quality assessment tool by mapping varying degrees of oyster quality.

Keywords: Electronic nose, oysters, quality, shelflife, sensory analysis, QDA

INTRODUCTION

Oysters are a valuable commodity in both the United States and the rest of the world. In 2001, 22,449 metric tons of oyster meats were commercially landed and produced by way of aquaculture in the United States. The value of these oysters was estimated to be $120.8 million (National Marine Fisheries Service 2004). Oysters have extremely short shelflife because they are filter feeders and contain a high level of

microbial flora, not only resulting in eventual spoilage, but also posing a threat to public health. The outbreak of diseases like typhoid, dysentery, cholera, hepatitis and other forms of gastroenteritis were linked to the consumption of oysters (Earampamoorthy and Koft 1975; Wood 1976; CDC 1998, 1999).

The spoilage of shellfish involves a combination of complex, interrelated processes brought about by either biological reactions, or by metabolic activities of microorganisms, resulting in off-flavors, rancidity, texture deterioration, discoloration, and other changed characteristics (Gorga and Ronsivalli 1982; Hebard and others 1982; Johnsen and Kelly 1990). The indication of spoilage of the oysters is generally first recognized by their off-flavors. Although instrumentation method can provide a detailed description of the source and concentration of the off-flavor, it needs a complicated extraction process which is tedious, time consuming and insensitive to low-level compounds less than 1 ppb (Johnsen and Lloyd 1992).

Current techniques to assess the quality of oysters heavily rely on sensory evaluation panels (Du and others 2002). Such a method of evaluation is subjective, imprecise, and results are scarcely repeatable. Even under conditions such as specified sensory methods, defined terminologies, controlled sample preparations and sensory environment, trained panelists still face the difficulties of evaluating off-flavors due to adaptation and fatigue. It is generally expensive, time consuming and lack of constancy in terms of training and maintaining the scientific accuracy of panelists. Unreliable sensory panels drive the seafood industry to augment sensory evaluation panels with an accurate instrument that can perform reproducible objective measurements quickly and reliably (Pearce and others 2003). The electronic nose is a new development in sensor technology

that can facilitate accurate assessment. In addition, handheld electronic noses are desirable because of ease of use, ruggedness, low power consumption, low cost, stability, and reliability (Pearce and others 2003).

Electronic noses have many applications in food industry (Tokuşoğlu and Balaban 2004). Seafood applications include evaluating raw and cooked catfish fillets and tilapia fillets (Korel and others 2001a, 2001b), trout and cod fillets (Pearce and others 2003), raw yellow-fin tuna (Du and others 2001a; Newman and others 1999), mahi-mahi fillets (Du and others 2001b), shrimp and fresh salmon fillets (Luzuriage and Balaban, 1999a, 1999b; Du and others 2002) and fresh prawns and cod roe (Holmgerg and others 1994). In these works, the ability to objectively measure the odor of the seafood stored at variable temperatures was tested and the results indicated that both the microbial counts and the sensory scores from trained panelists can be correlated with the electronic nose data. An additional application of the electronic nose system in conjunction with a machine vision system for quality assessment of oysters was reported by Tokuşoğlu and Balaban (2004). The system achieved 100% separation on electronic nose data by storage time and sensory evaluation results. A predictive model of odor changes with regard to varying storage time and temperature was developed.

Besides seafood products, e-nose was reported in assessing the quality of ground pork (Holmgerg and others 1994), “Boar taint” odor in pork fat (Annor-Frempong and others 1998) and ground beef (Turhan and others 1998). It has also demonstrated its ability with multiple applications in grading coffee, screening tomatoes, evaluating the adulteration of whisky and wine, classifying grains and aged Parmesan cheeses (Alpha 1995; Moul and others 1998; Aparico and others 2000; Josson and others 1997; Russell

1995; Rogers and others 1995). Other than food products, e-nose has been reported involved in food packaging and medical applications by Deventer and Mallikarjunan (2002).

Current research of electronic nose on various food or non-food applications indicated that e-nose could be a potential candidate for quality control. Although research on exploring the possibilities of using electronic nose to monitor the sensory quality of oysters has been started by Tokuşoğlu and Balaban in 2004, their chemo sensor (Model 4000, EEV) may not be the best for high moisture products like oysters. This is because the polar coating of these sensors is very sensitive to water molecule, resulting in sensor drift, less repeatability and reproducibility. The two e-noses systems used in this study belong to polymer composite sensor (Cyronose 320) and quartz crystal microbalance sensor (QMB4). They are both less sensitive to moisture and QMB is in fact the sensor system which is not affected by water or other polar compounds.

The objectives of this study were to develop a methodology to "sniff" the oysters using two different e-nose systems and to evaluate the potential of e-nose as an assessment tool for the quality control of oysters.

MATERIALS AND METHODS

Oyster Samples and Storage Conditions

Experiments were conducted with shell stock oysters obtained from Bevan's Oyster Company, Inc. and Bon Secour Fisheries, Inc., harvested in June, 2004, off the coast of Louisiana. Oysters were immediately stored in 4° C and 7 ° C refrigerators after receiving. They were sampled at 0, 3, 7, 10, and 14 day respectively. On the day of testing, twenty-

five oysters from each temperature were hand shucked carefully to preserve the body structure, specifically the abductor muscle.

Microbial Analysis

Five shucked oysters were transferred into sterilized stomacher bags including their body liquors. Series dilutions were made using Phosphate Buffered Saline (PBS, pH 7.4 Phosphate Buffered Saline, 10X Powder Concentrate, Fisher Scientific, Fairlawn, NJ, USA) solution. The bags were massaged by hands such that oysters are uniformly broken up and suspended into PBS solution. Two replications were conducted and for each sample replication, duplicate plate counts were performed.

In order to assess the total microbial enumeration, 100 µL from each appropriate dilution was plated onto Total Plate Count (TPC) agar (Fisher Scientific/Difco, Fairlawn, NJ, USA). To assess the total coliform and *E. Coli* enumeration, 1 mL from each appropriate dilution was plated onto 3M™ Petrifilm™ *E. Coli*/Coliform Count Plates (3M™, St. Paul, MN, USA). Duplicates from each dilution for each respective temperature category were completed as well. All plates and petrifilms were incubated at 35°C for a period of 24 hours (FDA, 2001). After incubation, the total numbers of colony forming units (CFU) were counted and recorded.

Electronic Nose Analysis

Electronic Nose analysis utilized individually sealed glass jars for headspace analysis (Fig. 7.1). A hole was punched into the lid top to allow an entry point for the insertion of the sniffing needle of the electronic nose. A small piece of sticky foam

(Darice, Inc., Strougsville, OH, USA) purchased from the local crafts aisle was used to cover and seal the hole. The oyster samples in their respective jars were placed in ambient temperature for 30 minutes. This incubation time allowed for the volatile compounds of the oysters to concentrate into the headspace of the jar.

Two electronic noses were employed for headspace analysis. The first was the Cyranose320, equipped with 32 polymer-conducting sensors, by Cyrano Sciences (Pasadena, CA, USA). A second nose, the AppliedSensor VOCcheck (Warren, NJ. USA), utilizes four quartz-crystal microbalance piezoelectric sensors. The operating conditions were pre-determined to have the maximum sensor response signal (Appendix A.5). For each measurement, 30 s and 16 s were needed to collect data from Cyranose 320 and VOCcheck system, respectively. Ten oyster samples, representing oysters of varying age at each storage condition, were exposed to both electronic nose systems.

Sensory Panel Analysis

Sensory characteristics of color, texture, and odor for each varying oyster samples were evaluated by a trained sensory panel consisting of 13 members recruited from the Biological Systems Engineering Department at Virginia Tech. Panelists were undergraduate, graduate students, faculties, and staffs ranging from 21 to 55 years of age. Panelists underwent three training sessions of 30 to 40 minutes each in approximately one month period of time.

Panelists were given standard food or non-food samples to identify the sensory characteristics of oysters respect to color, texture, and odor and classify them into varying degrees. Each of these sensory attributes was divided into four different categories ranging from a fresh oyster (minimum score) to an extremely spoiled oyster (maximum score).

The descriptors for each of these attributes over the range of freshness were developed based on the research work of He and others (2002) and summarized in Table 7.1. Panelists were also instructed how to sniff the oysters and how to use an unstructured line scale to provide sensory scores. The performance of each individual panelist was evaluated before participating in the real sensory evaluation.

Each Panelist evaluated fifteen oysters indicating various storage days of day 0, 3, 7, 10 and 14 with three replications. Samples were assigned arbitrary numbers and randomized. Results were marked on the evaluation scorecard and retraced with numerical numbers later on. Two evaluation sessions were conducted for oysters stored at 4 °C and 7 °C, respectively (Appendix C.1, C.2). Ground coffee powders were provided to refresh the sense of panelists. Diluted lemon juice and hand sanitizers were also provided to clean hands after sensory analysis.

Electronic Nose Verification

Verification of varying oyster quality, as determined by training the electronic nose, was executed for each e-nose system. Multiple oyster samples representing each storage time of specific temperature were exposed to e-nose. Performance was evaluated using statistics based upon the accuracy of correct oyster identification.

Data Analysis

Statistical analysis was performed by SAS (SAS Institute Inc.) using Principal Components Analysis (PCA) and Canonical Discriminant Analysis (CDA). CDA is a multivariate statistical technique which can be used to determine relationships between

qualitative variables with quantitative variables, for example, the objective sensory scores and electronic nose sensor response. It creates new variables by taking special linear combinations of the original variables to classify samples into appropriate populations. One advantage of this method is to allow a researcher to visualize the actual distances between the populations under investigation in a reduced dimensional space. Those functions are constructed by maximizing the F-ratio of the analysis of the variance (Johnson 1998). In general, the first two functions are sufficient to identify samples into corresponding categories. Storage time, microbial data and sensory panel scores were correlated to electronic nose sensor readings. Two predictive functions were obtained and they were employed to generate variables for creating plots to illustrate the ability of the electronic nose to separate the data in two dimensional space.

RESULTS AND DISCUSSION

Microbial Analysis

The logarithmic plot of the microbial load increase of oysters over the course of the two-week storage time was illustrated in Fig. 7.2. The displayed log number of bacterial was the average value of 4. For 4 °C, the total plate counts increased from initial 6.6×10^4 CFU/g to 5.5×10^6 CFU/g. The total coliform increased from 6.3×10^4 CFU/g to 2.7×10^6 CFU/g. In comparison, for oysters under 7 °C storage, the total plate counts increased from 6.6×10^4 CFU/g to 9.7×10^8 CFU/g over two weeks' time. The total coliform grew 1000 times reaching 10^7 CFU/g after 14 days storage. *E. Coli* was not detected for oyster samples under both storage temperatures. The microbial enumeration data also showed oysters stored at 7˚ C yield a higher overall bacterial load as compared to oysters stored at

4˚ C. Bacterial growth rate declined significantly at day ten for samples stored at both temperatures. Total coliforms stayed relatively constant after day seven at 4 ˚C. A continuous increase was noticeable for 7˚ C samples.

Total colony forming units (CFU/g) for both storage temperature oysters reached 10^7 by day seven. According to the International Commission on Microbiological Specifications on Foods (ICMSF 1986), fresh seafood should contain no more than 10^7 CFU/g microorganisms. Based upon this standard, oysters became spoiled at day seven under 7 ˚ C storage, while oysters stored under 4 ˚ C were still considered to be microbiologically safe after two week period .

Sensory Analysis

Results obtained from sensory panel testing revealed the human perception of oyster quality. An average score for each sensory characteristic was established for each oyster storage day. Average scores for oysters stored at 4° C and 7° C in relation to storage time are shown below in Table 7.2. Results showed that as storage days increased, the odor, appearance and texture of oysters degraded, indicated by increased sensory scores. Similar trend was observed for each sensory attribute and for oyster quality index at both storage temperatures. This further indicated that panelists were correctly trained to identify oysters of varying quality.

For oysters stored at 4 °C, although an increasing trend was observed for odor, appearance and texture scores as oysters aged, statistically, no significant difference (90% confidence level) was found for sensory scores of odor between storage days 1 and 3; or days 7 and 10. Same results were demonstrated for the appearance scores. The sensory score of texture demonstrated significant difference (90% confidence level) at day 10.

Similarly, for oysters stored at 7°C, sensory panelists were able to detect the differences in odor and appearance starting from day 7. The significant difference in texture was noticeable at day 10 only (90% confidence level).

Results also revealed that oyster appearance was the most offensive to sensory panelists. Panelists generally gave higher scores to appearance compared to odor and texture. Because odor, appearance, and texture were ranked together for each individual oyster, an offensive appearance could bias a panelist's perception of another sensory attribute. In contrast, sensory panelists were least sensitive to texture changes. When both storage conditions were compared, quality of the oysters stored at 7° C was generally ranked worse, which was demonstrated by higher sensory scores assigned to oysters stored at 7° C for the majority of attributes on each storage day.

Electronic Nose Analysis

Correlation of E-nose sensor readings to storage time

The ability of both Cyranose320 and VOCcheck electronic noses to differentiate between oysters of varying age was tested. Canonical Discriminant Analysis (CDA) analysis was preformed on the sensor readings to build a training set and classify observations into categories. Fig. 7.3 illustrates plots representing data obtained from the Cyranose320 measured for oysters at 4 °C and 7°C, respectively.

Excellent separation (100%) was observed between clusters for oysters stored at 4 °C using Cyranose 320. It provided a clear visual representation of a distinguishable e-nose response to varying oyster quality. The separation among clusters was not distinct for 7° C storage (90%). Zero and three day old oysters were moderately grouped into the same

cluster. This grouping is acceptable considering that the relative age difference between samples is small. However, similar smell print or recognition pattern was visualized for oysters stored at two temperature conditions, demonstrated by the relative location of each separated group appeared in those two plots.

Results from the VOCcheck for each storage temperature are shown in Fig. 7.4. Compared to Cyranose 320, separation by VOCcheck e-nose was less distinct. The data points were scattered for samples of the same age. The separation degrades at the higher storage temperature, indicated by more scattered data points and mixed cluster in CDA plots. The reason could be attributed to the sensitivity of different sensor building technology to unique oyster odor. Also, since there was a self- employed carbon filter in Cyranose 320 system, it may have a better control of the surrounding air. This is critical to the hand-hold electronic nose system with no standard exterior air supply served for a base-line purge.

Correlation of to E-nose Reading to Microbial Enumeration

Microbial data for storage temperatures of 4 and 7˚C were complied and subsequently correlated individually with Cyranose320 readings. To categorize the microbial data for the electronic nose correlation, total plate counts of equal or less than $10x10^5$ CFU/g were grouped as A, while total plate counts between $10x10^5$ to $10x10^6$ CFU/g were grouped as B and total plate counts greater than $10x10^7$ CFU/g were grouped as C. CDA plots of the correlation between microbial data and the electronic nose sensor readings are presented in Fig. 7.5.

As seen in the CDA plot of Fig. 7.5, there was a good correlation between the Cyranose320 sensor readings and the microbial load of oyster samples. This was demonstrated by distinct clustering of the microbial loads. Thus, Cyranose320 can be considered a relatively quick, noninvasive means of determining the microbial load of oyster samples. On the other hand, since VOcheck did not demonstrate a clear separation between oysters of varying age, no correlation analysis was conducted.

Correlation of E-nose Reading to Sensory Perception

Odor sensory scores for each temperature and corresponding storage day were grouped into three classes according to the standard used by the U.S. Food and Drug Administration (FDA) to evaluate the odor of fishery products (CFR 1997). However, the results of the sensory analysis showed that panelists intended to use the central portion of the line structured scale, resulting in clustered scores ranging from 5 to 10. This is generally indicated by the capriciousness against timidity error in sensory evaluations (Meilgaard and others 1991). In order to obtain useful information out of the sensory evaluation, scores were grouped into three classes according to FDA standards proportionally. Class I represents a sensory score ranging from 4 to 7, Class II from 7 to 9, and Class III from 9 to 12. All sensory scores for odor were correlated with corresponding electronic nose readings as determined by Cyranose320. Fig. 7.6 depicts a CDA plot of the correlation between the Cyranose320 readings and odor sensory scores at 7 °C. It showed good separation between classes Class I (98%), Class II (100%) and Class III (95%). This demonstrated that with proper training, electronic nose Cyranose 320 was capable of replacing human subjects to assess the sensory quality of oysters.

Due to the fact that the panelists can not distinguish the sensory attribute of the oysters between days 1 and 3, days 7 and 10, stored at 4 °C, only two classes of sensory score were generated. Therefore multiple statistical analysis was only performed on oysters stored at 7°C. Again, the correlation between the electronic nose readings from VOCcheck and sensory data was not conducted because previous results showed that it failed to categorize the oysters into correct groups of oysters in varying ages.

Electronic Nose Verification

Verification of each of the training sets was carried out using the Canonical Discriminant Analysis. The correct verification rate for each training set is listed in Table 7.3. It is evident that the correct verification rate was much greater for Cyranose320 as opposed to the VOCcheck. For the Cyranose320, the correct verification rate was 7% greater for a storage temperature of 4°C as compared to 7°C. Conversely, for the VOCcheck, the correct verification rate was 100% greater for a storage temperature of 7°C as compared to 4°C. The fact that the Cyranose320 was more capable than the VOCcheck in verifying oysters into a given training set substantiates the previous data collected. For each set of data, the Cyranose320 system has been superior in separating the data into different groups.

CONCLUSIONS

The methodology developed in this study to evaluate the odor of oysters using two different electronic nose systems was able to produce an accurate smell-print of oysters stored at two temperatures for various time periods. Recognition patterns were more distinguishable for the Cyranose320 system than for the VOCcheck system. The study

found that both storage temperature and time play important roles in the spoilage of oysters based on microbial enumeration. Sensory panel evaluation of odor, appearance, and texture proved very useful in creating and monitoring a comprehensive quality profile of oysters. Correlation of microbial enumeration with Cyranose 320 electronic nose data proved promising in identifying microbial activity based on odor. Good correlation of sensory panel scores with this e-nose system indicated that Cyranose 320 can be considered a qualified candidate for quality identification of oysters because it reflected the human sensory perception on oysters.

On the other hand, VOCcheck was unable to classify the quality of oysters of different age according to their odors. The efficacy of one type of sensor technology over the other was affected by many factors such as the consistency of the sensing signals, the sensitivity to specific sample order profiles and the operating conditions. Selection of the e-nose technology requires critical considerations based on specific applications.

REFERENCES

Alpha MOS.1995. The electronic nose: towards a global standard of fish freshness, odor and volatiles identifications and quantification by electronic noses. Belle mead, NJ: Alpha M. O. S. America, Inc. p.5.

Annor-Frempong IE, Nute GR, Wood JD, Whittington FW, West A. 1998. The measurement of the responses to different odor intensities of "Boar Taint" using a sensory panel and an electronic nose. Meat Sci. 50(2):139-151.

Aparico R, Rocha SM, Delgadilo J, Morales MT. 2000. Detection of rancid defect in virgin olive oil by the electronic nose. J Agric Food Chem. 48(3):853-860.

CDC. 1998. Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters – Pacific Northwest, 1997. MMWR. 43(22):457-462.

CDC. 1999. Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters and clams harvested from Long Island Sound – Connecticut, New Jersey, and New York, 1998. MMWR. 48(3):48-51.

CFR. 1997. Code of Federal Regulations. Title 50, Wildlife and Fisheries Part 265. Washington, DC: The Office of the Federal Register, National Archives and Records Administration, U.S. Government Printing Office.

Deventer DV, Malikarjunan P. 2002. Comparative performance analysis of three electronic nose systems using different sensor technologies in odor analysis of retained solvents on printed packaging. J Food Sci. 67(7):1:14.

Du WX, Kim J, Cornell JA, Huang TS, Marshall MR, Wei CI. 2001a. Microbiological, sensory and electronic nose evaluation of yellow fin tuna under various storage conditions. J Food Protect. 64(12):2027-2036.

Du WX, Kim J, Huang TS, Cornell JA, Marshall MR, Wei CI. 2001b. Chemical, microbiological and AronmaScan evaluation of mahi-mahi fillets tuna under various storage conditions. J Agri. Food Chem. 49(1):527-534.

Du WX, Lin CM, Huang TS, Kim J, Marshall MR, Wei CI. 2002. Potential application of the electronic nose for quality assessment of salmon fillets under various storage conditions. J Food Sci. 67(1):307-313.

Earampamoorthy S, Koft RS. 1975. Health hazards of bivalve mollusk ingestion. Annals Int. Med. 83:107-110.

FDA. 2001. Bacteriological Analytical Manual Online, Aerobic Plate Count. Available from http://vm.cfsan.fda.gov/~ebam/bam-3.html. Accessed on June 28 2004.

Gorga C, Ronsivalli LJ. 1982. International awareness for quality seafood: A survey. Mar. Fish. Rev. 44(2):11-16.

He H, Adams RM, Farkas DF, Morrissey MT. 2002. Use of high-pressure processing for oysters shucking and shelflife extension. J Food Sci. 67(2):640-645.

Hebard CE, Flick GJ, Martin RE. 1982. Occurrence and significance of trimethylamine oxide and its derivatives in fish and shellfish. In: Martin RE, Flick GJ, Hebard CE, Ward DR. Chemistry and Biochemistry of Marine Food Products. Westport, Connecticut: AVI Pub. Co. p 149-304.

Holmberg M, Winquist FSH, Lundtrom I. 1994. An electronic nose used for food control. Alpha M. O. S. 1st International Symposium on Olfaction and Electronic Noses, Toulouse, France.

ICMSF. 1986. Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Specific Applications 2nd ed. Toronto: University of Toronto Press.

Johnsen PB, Kelly CA. 1990. A technique for the quantitative sensory evaluation of farm-raised catfish. J Sens. Stud. 4:189-199.

Johnsen PB, Lloyd SW. 1992. Influence of fat content on uptake and depuration of the off-flavor 2-methylisoborneol by channel catfish. Can. J Fish Aquat. Sci. 49:2406-2411.

Johnson DE. 1998. Discriminant Analysis. In: Lorretta, P. editor. Applied Multivariate Methods for Data Analysts. Pacific Grove, CA: Brooks/Cole Publishing Company.

Josson A, Winquist F, Schnurer J, Sundgren H, Lundstrom I. 1997. Electronic nose for microbial quality classification of grains. Int. J Food Microbiol. 35(2):187-193.

Korel F, Luzuriaga DA, Balaban Mö. 2001a. Quality evaluation of raw and cooked catfish (*Ictalurus punctatus*) using electronic nose and machine vision. J Aquat. Food Prod. Technol. 10(1):3-18.

Korel F, Luzuriaga DA, Balaban MÖ. 2001b. Objective quality assessment of raw tilapia (*Oreochromis niloticus*) fillets using electronic nose and machine. J Food Sci. 66(7):1018-1024.

Luzuriaga DA, Balaban MÖ. 1999a. Evaluation of the odor of decomposition in raw and cooked shrimp: correlation of electronic nose readings, odor sensory evaluation and ammonia levels. In: Hurst WJ, editor. Electronic Noses and Sensor Array Based Systems. Lancaster, PA: Technomic Publishing Co. p 177-184.

Luzuriaga DA, Balaban MÖ. 1999b. Electronic nose odor evaluation of salmon fillets stored at different temperatures. In: Hurst WJ, editor. Electronic Noses and Sensor Array Based Systems. Lancaster, PA: Technomic Publishing Co. p 162-169.

Maul F, Sargent SA, Balaban MÖ, Baldwin, EA, Huber DJ, Sims CA. 1998. Aroma volatile profiles from ripe tomatoes are influenced by physiological maturity at harvest: An application for electronic nose technology. J Amer. Soc. Hort. Sci. 123(6):1094-1101.

Meilgaard M, Civille GV, Carr BT. 1991. Factors influencing sensory verdicts. In: Sensory Evaluation Techniques. 2nd ed. Boca Raton, FL: CRC Press, Inc.

Newman DJ, Lazuriaga DA, Balaban Mö. 1999. Odor and microbiological evaluation of raw tuna via correlation of sensory and electronic nose. In: Hurst WJ, editor. Electron Noses and Sensory Array Based Systems Design and Applications. Lancaster, PA: Technomic Publishing Co., Inc. p 170-176.

National Marine Fisheries Service 2004. U.S Aquaculture Production: Oysters. Fisheries Statistics and Economics Division. Available from http://www.msstate.edu/dept/crec/aquaoysters.html. Accessed on July, 4, 2004.

Pearce TC, Schiffman SS, Nagle HT, Cardner JW. 2003. Handbook of Machine Olfaction. Electronic Nose Technology. KGaA, Weinheim: WILEY-VCH Verlag GmbH & Co.

Rogers G, Berg R, Rice TJ. 1995. Comparison of fresh vs. decomposed fish products. In: Hillis NH, editor. Atlanta, GA: Food and Drug Administration: Chemistry Branch. p 9.

Russell, P. 1995. Sensory analysis. Milk Ind Int. 97(5):11-12.

Tokuşoğlu Ö, Balaban MÖ. 2004. Correlation of odor and color profiles of oysters *(Crassostrea Virginica)* with electronic nose and color machine vision. J Shellfish Res. 23(1): 143-148.

Turhan M, Balaban Mö, Turhan KN, Luzuriaga DA. 1998. Potential use of the electronic nose technique for detection of meat adulteration: separation of pork-beef mixtures. Fleischwirschaft Int. 6:26-28

Wood PC. 1976. Guide to shellfish hygiene. WHO Offset Publication no. 31. WHO, Geneva.

Table 7.1 Varying sensory attributes of an oyster [a]

	1	2	3	4
Odor	Hay/crisp	Strong seaweedy	Spoiled with slight sour smell	Sour and putrid smell
Appearance	Cream white	Light tan/beige	Dark tan/beige	Yellow/light brown
Texture	Firm and elastic	Soft and less elastic	Slightly mushy	Mushy

a: Table 7.1 ranks sensory attributes using adjective descriptors to characterize overall quality. Column one represents adjective descriptors of a high quality oyster. Quality decreases as the columns increase from one to four.

Table 7.2 Sensory score of oysters stored at 4 °C and 7 °C

Day	4 °C			7 °C		
	Odor	Appearance	Texture	Odor	Appearance	Texture
1	5.7 [a]	8.1 [a]	6.7 [a]	5.5 [a]	7.5 [a]	6.9 [a]
3	5.6 [a]	8.3 [a]	7.1 [a]	6.4 [a]	9.0 [b]	7.7 [a]
7	7.6 [b]	10 [b]	7.0 [a]	7.3 [b]	9.9 [b]	7.2 [a]
10	7.9 [b]	9.5 [b]	8.8 [b]	9.3 [c]	11.5 [c]	9.4 [b]

Note: Sensory scores indicated by same superscript showed no significant difference ($p<0.1$) within each column.

Table 7.3 Correct verification rates for each training set using Cyranose320 and VOCcheck electronic noses

Electronic nose and Storage temperature	Correct Verification Rate (%)
Cyranose320 (4°C)	96
Cyranose320 (7°C)	91
VOCcheck (4°C)	18
VOCcheck (7°C)	35

Fig. 7.1 Electronic nose devices for oyster odor analysis

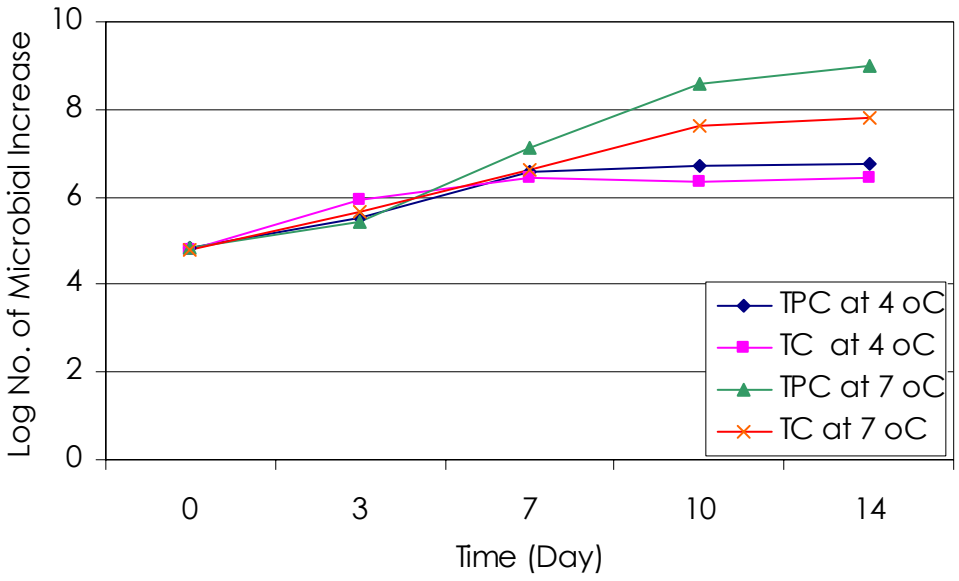
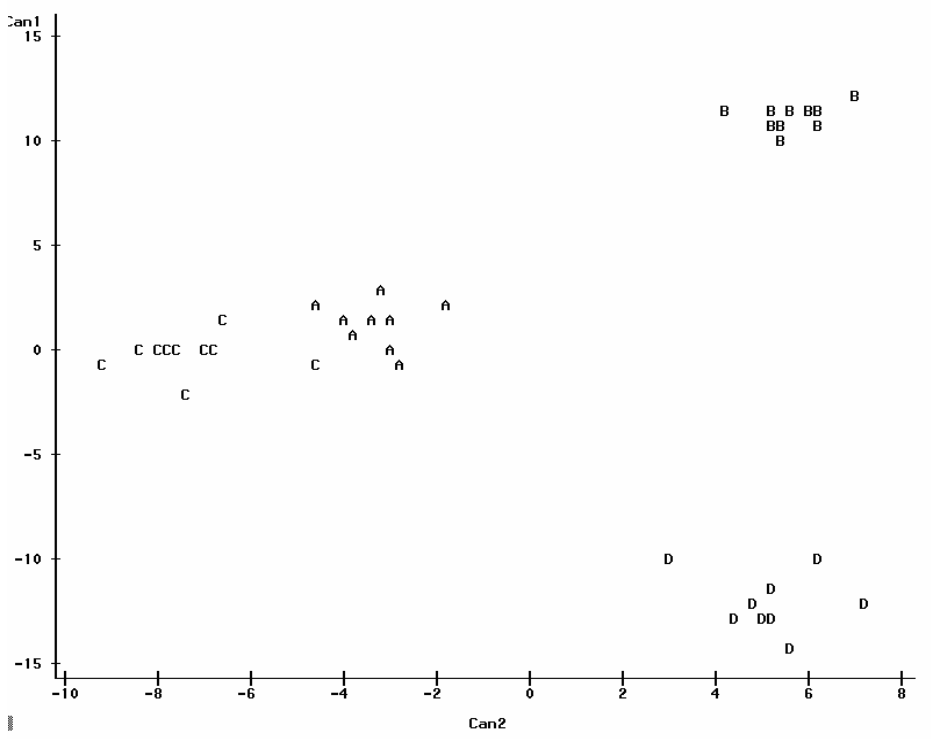
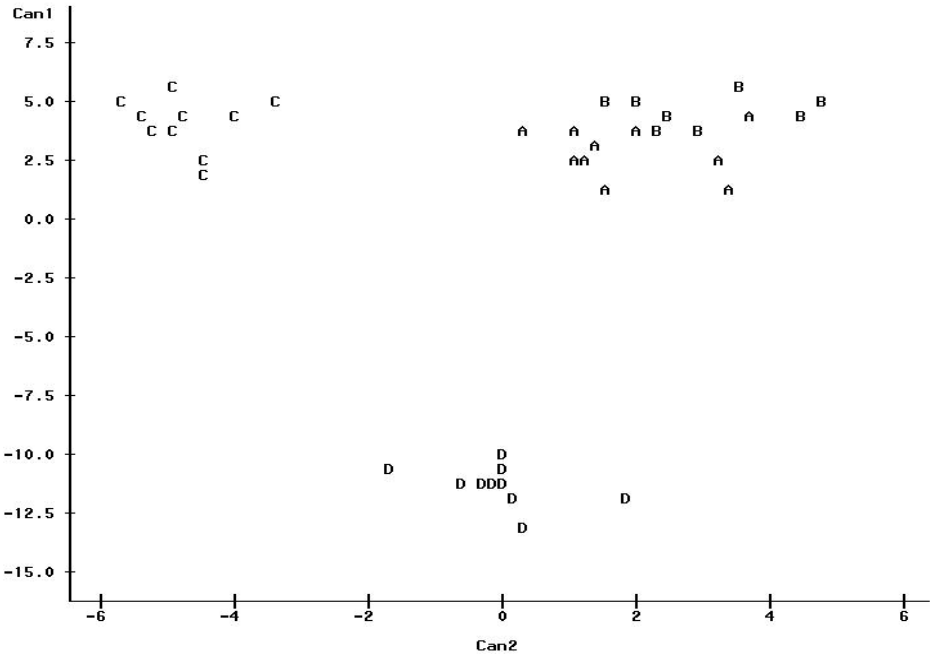


Fig. 7.2: Change of bacteria number on oysters stored at 4 °C and 7 °C

TPC: Total plate counts; TC: Total coliforms.

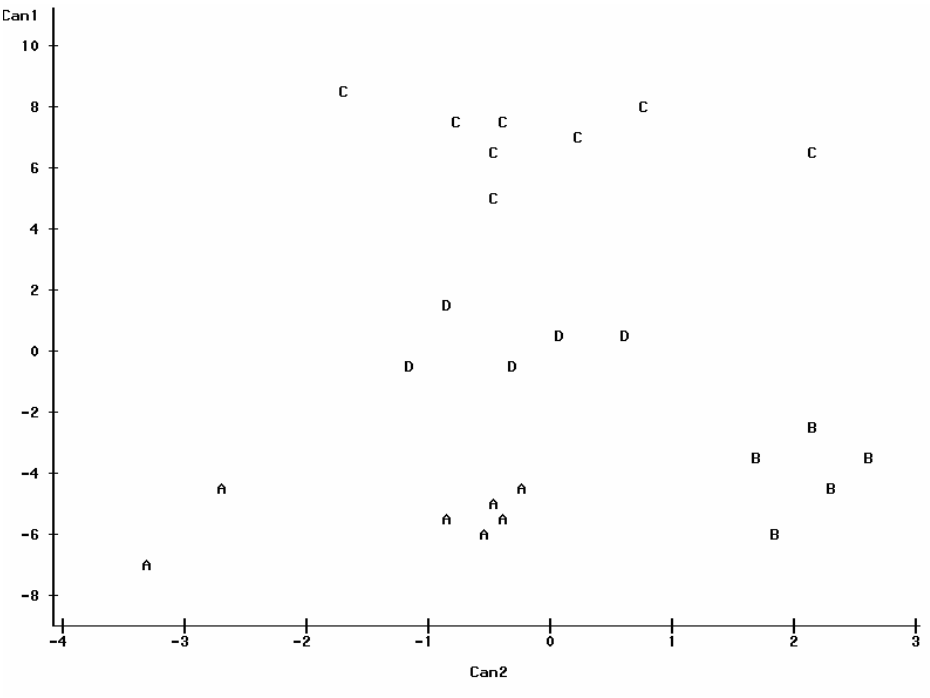


(a)

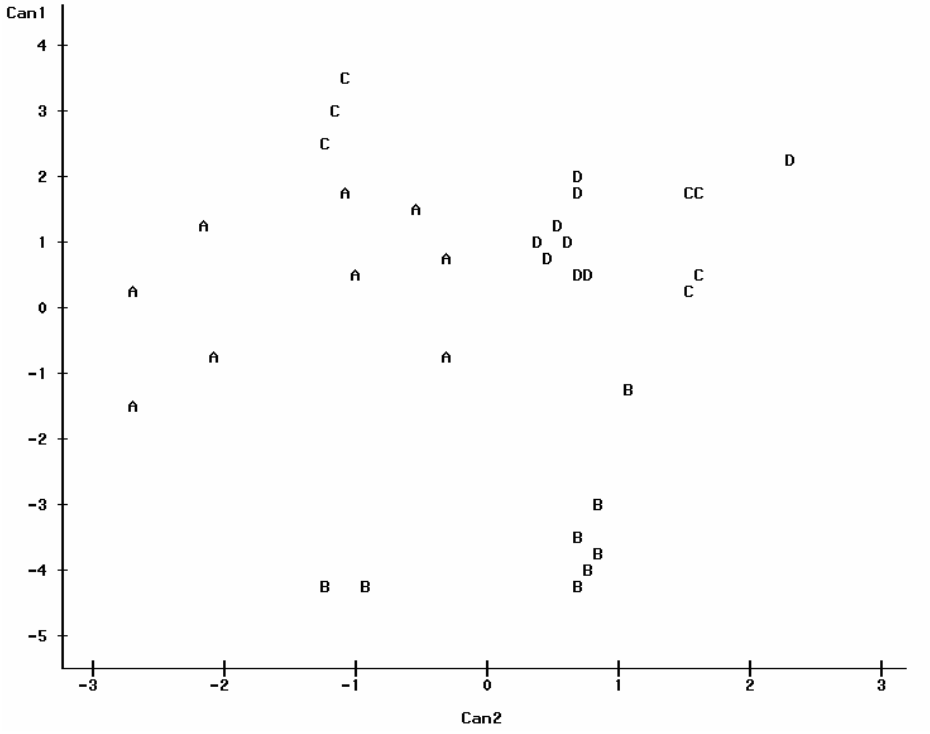


(b)

Fig. 7.3: CDA (Canonical Discriminant Analysis) plot of storage data from Cyranose320 electronic nose, where: A – day 0, B – day 3, C – day 7, D – day 10. (a) 4°C storage; (b) 7°C storage

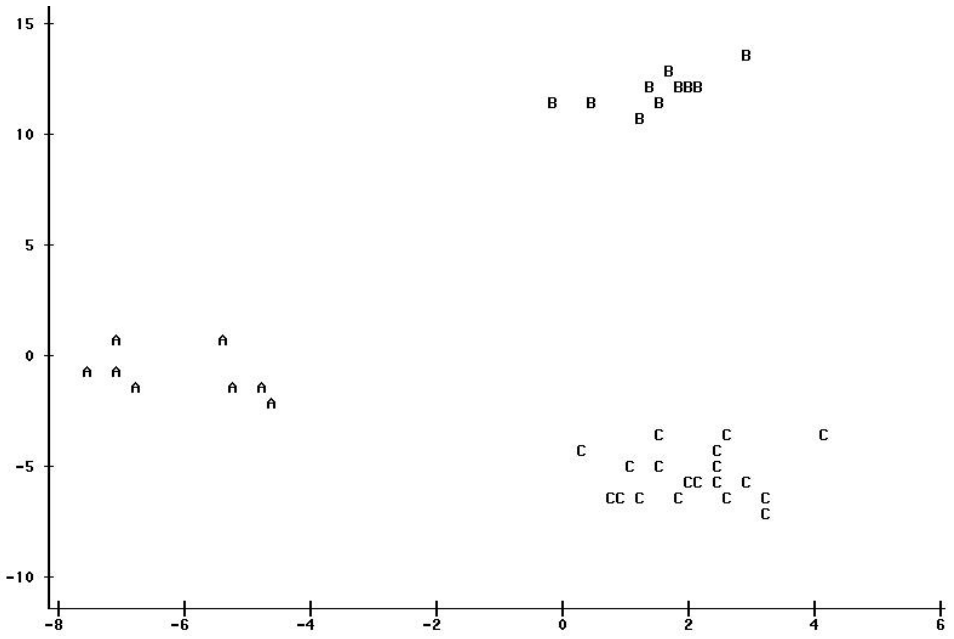


(a)

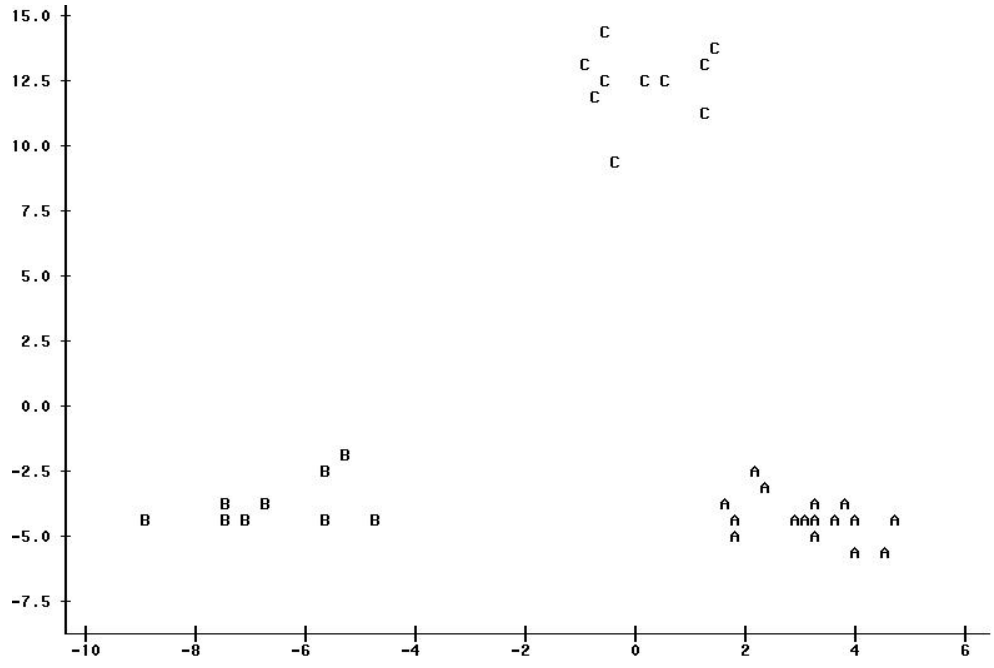


(b)

Fig. 7.4: CDA plot of storage data from VOCcheck electronic nose, where: A – day 0, B – day 3, C – day 7, D – day 10. (a) 4°C storage; (b) 7°C storage



(a)



(b)

Fig. 7.5: Correlation of the Cyranose320 readings with microbial load in oysters stored at 4 °C and 7 °C, where (a) 4 °C storage, (b) 7 °C storage. A-Total plate counts of equal or less than $10x10^5$ CFU/g; B-Total plate counts between $10x10^5$ to $10x10^6$ CFU/g; C-Total plate counts greater than $10x10^7$ CFU/g

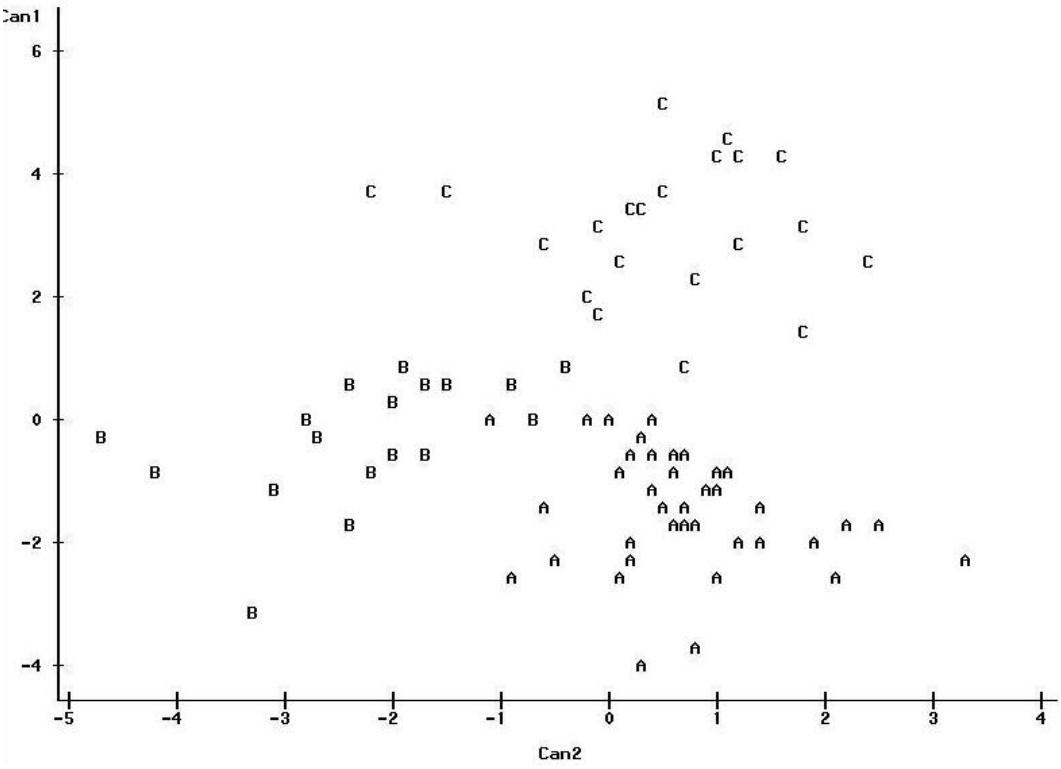


Fig. 7.6: CDA plot correlating Cyranose320 readings with odor sensory scores of oysters stored at 7° C, where A – Sensory score class I, B-Sensory score class II, C- Sensory score class III

CHAPTER 8

Quality Assessment of Oysters by High Pressure Processing and Microwave Processing

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ABSTRACT

The color, odor and texture of live oysters processed by high pressure and microwave were determined. The color was measured using a digital image processing system. The odor of oysters was evaluated by a hand-held electronic nose system. The system examined the odor profile of oysters and generated a unique smell print based on multiple statistical analysis results. The texture of oyster meat was quantified using universal testing machine. The peak force and total energy were measured by allowing a knife edge blade to cut through the maximum cross-section area of the oyster meat. Mechanical property change was detectable for oysters exposed to pressure of 345 MPa for 3 min and 379 MPa for 1.5 min. Deterioration of the quality was distinct for oyster meats undergone 60 °C or above by thermal processing. The critical thermal processing condition was identified to be at 55 °C for 2 min. Microwave processing has shown as a technique to replace the conventional thermal processing to reduce potential bacterial hazard but still retain the quality of the oyster product.

Keywords: Quality, oysters, high pressure processing, microwave, thermal processing

INTRODUCTION

Seafood quality is characterized by distinctive odor, flavor, appearance, and meat texture. Oyster quality is notably affected by rapid deterioration that is unique to seafood. Meanwhile, the quality of oysters can be easily altered by processing techniques such as heating, freezing, high pressure processing and irradiation. Consumer demand for high quality oysters with fresh characteristics requires a minimum amount of processing effort

and time. Thus, it is essential to determine the threshold processing conditions for applied processing technologies under specified microbial safety. Current techniques to assess the quality of oysters largely rely on sensory evaluation panels (Du and others 2002). Such a method of evaluation is subjective, imprecise, and results are difficult to quantify. In addition, the sensory panel is expensive to train and maintain (Pearce and others 2003). Therefore, an accurate instrumental assessment of shellfish quality must be acknowledged.

Color is the most important sensory factor that can be attributed to the quality of the product (Francis 1994). An off-color can significantly alter the acceptance of the product by a consumer. Instrumentally, color can be measured using a colorimeter, a spectrophotometer, or a color machine vision system (Giese 2003). Based on point-by-point measurement on the surface of the object, the colorimeter was more appropriate to determine uniformly distributed color scheme. Spectrophotometers were most applicable to the measurement of a liquid where the amount of light transmitted can be determined (Giese 2003). Comparatively, color machine vision allowed for a large area of sampling, which in most instances means the entire sample can be analyzed. Meanwhile, a color machine vision system can be inexpensively developed through the use of a lighting system, a digital camera, and a computer with Adobe Photoshop software (Papadakis and others 2000). Tokuşoğlu and his co-workers (2004) unitized a computer vision system to create color profiles. This system was reported to be successful in separating the contributing color elements that governed the color of oysters of varying ages.

Raw shellfish have little odor and presents a flavor similar to that of fruit or seaweed. These odors and flavors are due to the action of unsaturated alcohols and aldehydes containing less than 10 carbon atoms (Shahidi and Botta 1994). The typical

aroma could be altered by endogenous hydrolytic reactions or by enzymatic and non-enzymatic oxidation during the processing. Typically, GC and GC-MS were the most common used instruments to detect the odor change of seafood products. Although some of successful applications were reported in examining cod and trout (Milo and Grosch 1995), fish oil enriched milk (Venkateshwarlu and others 2004), prawn (Chinivasagam and others 1998), GC or GC-MS is an expensive technology that required long analyzing time and experienced personnel to operate (Bartlett and others 1997).

An alternative to GC is the electronic nose. Electronic noses are composed of an array of chemical sensors, each tuned to a specific range of odorant molecules (Bartlett and others 1997). Much research work has been conducted on employing the electronic nose to detect aroma of seafood products over varying storage conditions, such as raw yellow-fin tuna by Newman and others (1999) and Du and others (2001), raw and cooked shrimp by Luzuriaga and Balaban (1999a), salmon fillets by Luzuriage and Balaban (1999b), tilapia fillets and catfish fillets by Korel and others (2001a, 2001b), and oysters by Tokuşoğlu and others (2004).

Texture of raw seafood can be measured mechanically using food testing equipment like a universal testing machine. It provides quantified information and is easy to perform compared to subjective human sensory evaluation (Alasalvar and others 2001). Among the commonly used techniques, the shearing force and compression methods are the most often recommended for measuring the hardness/firmness of seafood products, which is the primary interest of the seafood industries (Sigurgisladottir and others 1999). Many attempts have been made to correlate the texture properties with sensory evaluations (Barroso and others 1998; Zapata and Price 1992). Chamberlain and others (1993)

modified the Warner Bratzler device and found a sound correlation between the texture properties and the fish species. Sigurgisladottir and his co-workers (1999) reported that the shear force method based on cutting with a knife edge blade was more sensitive than the puncture methods in differentiating texture properties such as hardness and was recommended for use. According to Jonsson and others (2001), the Warner-Bratzler device was a more reliable method to measure the texture properties of raw salmon fillet compared to Kramer cell method. Unfortunately, data on texture property measurement of oysters are not available.

The objective of this study was to evaluate the color, aroma and texture change of oysters by high pressure and thermal treatment, and to determine the threshold processing conditions.

MATERIALS AND METHODS

High Pressure Processing

Fresh oysters used in this study were obtained from Bevans's Oyster Inc., Virginia. They were kept in 4 °C refrigerators before testing. Three to four oysters (approximately 500g) were placed in a polypropylene plastic bag. Water (18-20 °C) was added to emerge the oysters. The oysters were sealed and then double bagged by a vacuum sealer (MultiVac, Model D-87787, Sepp Haggenmüller Gmbh & Co. KG, Germany). Duplicated bags were prepared for each selected high pressure processing condition. Oysters were exposed to pressure level of 276/40, 310/45, 345/50, 379/55 MPa/kpsi for a minimum of 1s to a maximum of 6 min (QFP 35L-600, Flow International, Co., Washington, USA). The combination the pressure and time was pre-determined based on the work conducted by

(Koo and others 2002), at which the inoculated clinical stains of *Vibrio vulnificus* (MO624) and *Vibrio parahaemolyticus* (O3:K6 TX2103) can be eliminated effectively. Experiments were duplicated.

The operating parameters of the high pressure processing are presented in Table 8.1. The pressure profile was recorded. By integrating the pressure profile with time, an accumulated effect of the pressure including the pressure come-up time can be determined.

Thermal Processing

Seven shucked oysters (approximately 70 g) were placed into a food storage bag. It was dipped into a constant temperature circulator (Haake A82, Model 000-7069, Germany, temperature stability of ±0.5°C), which has been adjust to maintain temperatures of 45 °C, 50 °C, 55 °C, 60 °C or 65 °C. Handheld digital thermometers (Mode HH23, Omega Engineering, Inc., Stamford, CT) were utilized to monitor the temperature increase of the oyster meat by inserting the tips of the temperature probes into the center of selected oysters. Once the desired temperature was reached, the oyster meat was allowed to stay in the water bath for 2 min before they were placed in ice water. Experiments were duplicated. In order to investigate the effect of holding time on the sensory attributes of oysters, further experiments were conducted by keeping oysters at 55 °C for 2 min, 4 min, 6 min, 8 min and 10 min. Immediate cooling in ice water was followed.

The quality of the oysters by microwave processing was also evaluated. Depending on the size and weight of each oyster, 7-8 oysters (approximately 1000 g) were kept in a microwavable glass container. In order to get more uniformed heating, 1000 g water (20°C) was added to the container. They were heated by a microwave oven (Sharp,

1000w/R-21HT, Japan). The power level and heating time were determined by a preliminary study, which can bring a final temperature between 55 and 60 °C. The detailed processing conditions were 30% power level for 13.9 min, 50% power level for 8.3 min, 70% power level for 6 min and 100% power level for 4.2 min. The temperature profile was recorded employing three fiber optic probes (FISO Technologies, Inc., Quebec, Canada). After microwave treatment, oysters were immediately placed in an ice-bath to cool down. Experiment was conducted in duplicate.

Texture Measurement

The texture of processed oysters was measured instrumentally using a Warner Bratzler device, mounted on an Instron Universal Testing Machine (MTS, USA). The "V" shaped blade was replaced with a knife edge blade and was allowed to cut through oyster meat at a speed of 100 mm/min along the maximum cross-section area (Appendix A.6). The peak force and total energy were measured. The benefit of applying the knife edge blade was to reduce the amount of sample and to avoid the effect of the size and the location of oyster abduct muscle on the measurement. In order to obtain an accurate measurement of cross-sectional area at the cutting point, a camera (CCD video camera, Cosmicar Television Lenses, Japan) was set up at the same height as the platform, where oysters had been placed for the texture measurement. A digital picture of the side view of the oyster tissue was taken and grabbed into a computer. The picture was opened in an image processing software, Image Pro (Image-Pro® plus, version 4.1, Media Cybernetics, USA), and the specific tool was utilized to provide the precise measurement of the area.

Color Measurement

The color value of the oyster samples was obtained using procedures described by Papadakis and others (2000). A digital picture of each processed oyster was taken using a digital camera (Fuji Film, model 2600Z, Japan), with the flash off. The camera was mounted on a tripod with the lens facing down towards the oyster sample, which was placed on a black metal plate approximately 0.31 m from the camera lens. The utilized lighting system consisted of four CIE source D65 lamps, 1.2 m long, mounted on four stands. The lamps were located at 0.31 m above, and at an angle of 45° to the oyster sample plane. This angle will facilitate capturing the diffuse reflection responsible for the color, which appeared to be at 45° from the incident light (Francis and Clydesdale 1975).

Excess juices were removed from the oyster samples with paper towels to prevent unnecessary light reflection. The camera was zoomed in a standard distance and the lens was focused to capture the oyster sample and its label. Each image was opened in Adobe® Photoshop® 7.0. Via use of the Magnetic Lasso Tool, each oyster sample was isolated from the black background for analysis. The Histogram function was used to find the mean lightness (L^*), a^*, and b^* value.

Electronic Nose Analysis

Electronic Nose analysis utilized individually sealed 8 oz Gerber® baby food jars for headspace analysis. A hole was punched into the lid top to allow an entry point for the insertion of the sniffing needle of the electronic nose. A small piece of sticky foam was used to cover and seal the hole. Foam pieces were produced by Darice, Inc. and bought in sheets of 0.22 m by 0.28 m, 2 mm thick, acid free with pre-stick backs. The oyster

samples in their respective jars were kept in 25 °C water bath for 30 minutes. This incubation time allowed for the volatile compounds of the oysters to concentrate into the headspace of the jar. Once the volatiles had time to equilibrate in the headspace of the jar, an electronic nose (Cyronose320, Cyrano Sciences, Inc., Pasadena, CA, USA), equipped with 32 conducting polymer sensors, was employed for headspace analysis.

The testing method used to train the Cyranose320 was pre-established from a preliminary study applying response surface analysis. The method used specific sensors and preset times for purging the vessel and drawing the sample. The Cyranose320 data was collected in a hood to ensure an equal baseline purge for all samples. The chamber was purged for 15 seconds to establish a baseline. Sensor responses were read for 10 seconds. After the sample was drawn, the chamber was re-purged for 25 seconds. The process was repeated for each sample. When all samples were tested, the data was cross-validated and saved for further statistical analysis.

Statistical Analysis

Analysis of Variance (ANOV) was used to analyze the data for color and texture measurement. Principle Component Analysis and Canonical Discriminate Function Analysis were used to cluster the data from E-nose response, using statistical software SAS (SAS Institute, Inc., NC, USA).

RESULTS AND DISCUSSION

High Pressure Processing

The pressure increasing profiles to the targeted pressure level of 276, 310, 345, 379 and 552 MPa are presented in Fig. 8.1. This figure also indicated the time required to reach those designated pressures. Second order polynomial relationship can describe the relationship between the pressure elevation and time. The regression coefficients are presented in Table 8.2.

Texture Evaluation

The peak force and total energy required to cut through oysters are presented in Table 8.3. Statistically, no difference ($P<0.05$) was found between the hardness of oysters exposed to pressure up to 276 MPa for 6 min and 310 MPa for 4.5 min. Significant increase in hardness ($P<0.05$) was detectable after HPP processing for 3 min at 345 MPa and for 1.5 min at 379 MPa, indicated by the peak force value. However, there was no significant change indicated by total energy required under tested HPP process conditions. It seemed that the mechanical properties of the oyster meat did not demonstrate any correlation with regard to the integrated effect of pressure. Pressure level showed more dominate effect than the time factor.

Comparatively, the hardness of oysters reduced significantly by thermal processing when temperature reached 60 °C. Both measured peak force and total energy values confirmed this. These results agreed with the published data on the texture change of the seafood products undergoing thermal treatment (Shie and Park 1999; Jaczynski and Park 2003). However, some reported oysters maintained physical quality after heating at 75 °C

for 8 min, where the processing condition was much severe than employed in this study (Chai 1991). The reason behind the opposite mechanical behavior of oyster meat treated with heat and high pressure was not clear. It could be related to the mechanical properties of biomacromolecules, such as proteins and polysaccharides, under pressure. It was observed that the texture of the pressure-induced coagulum was quite different from the coagulum induced by temperature (Heremans and others 1999). Pressure-induced denaturation of proteins occurs mainly because proteins are flexible and compressible. The larger the hydrated state of a protein, the larger is the contribution of void spaces to partial specific volume, and the more unstable the protein will be when pressurized. Fibrous proteins, which are relatively rich in oyster tissues, are mostly lack of void spaces, and hence they are more stable to hydrostatic pressure than globular proteins (Fennema 1996).

However, no significant difference ($P<0.05$) was found between the hardness of oysters treated at 60 °C and 65 °C, indicating that the mechanical properties of oyster meat changed dramatically at certain point. This observation matches with the protein denaturation theory, which states that when a protein solution is gradually heated above a critical temperature, it undergoes a sharp transition from the native state to the denatured state. The temperature at the transition midpoint, where the concentration ratio of native and denatured states is 1, is defined as denaturation temperature (Td) (Scope 1994). This may partially explain the sharp change of the mechanical properties of oysters during the thermal processing.

Based on the information that dramatic change was not observed on oysters undergoing 55 °C for 2 min, but at 60 °C instead, the following experiment was conducted

to study the effect of time on the mechanical properties. The results from Fig. 8.2 showed a significant reduction of peak force and total energy was found for oysters treated at 55 °C for 4 min. Therefore, the threshold thermal processing condition was determined at 55 °C for 2 min. This result was reasonable by the theoretical diagram of the percentage protein remaining undenatured at certain temperature for 10 min (Scope 1994).

The hardness of oysters processed by microwave at different time and power level combinations did not show any significant difference ($P<0.05$) between the treated samples and the controlled samples (20 °C). This was because the selected combination conditions, which provided nearly the same microwave energy input, resulting in a final temperature of the heated oyster samples between 55 to 60 °C and the incubation time was about 2 min. No other influence other than thermal effect was observed responsible for the mechanical property changes of oysters.

Color Evaluation

A distinct correlation (R square 0.94) between the color of HPP processed oysters and the accumulated pressure effect was observed (Fig. 8.3). The results displayed that as pressure and time increased, the whiteness (L^*) increased significantly from 73.3 to 78.5 ($P<0.05$). Meanwhile, a^* values and b^* values did not present any general trend with regard to the increased accumulative effect of pressure.

The color of oyster meat after thermal treatment showed that the L^* value increased from 75.0 to 77.8 when temperature increased from 45 °C to 65 °C. Compared to raw samples, significant value ($P<0.05$) in whiteness was observed when processing

temperature was above 50 °C. Statistically, increase of the temperature did not result in a further increase of L^* values.

These results were confirmed by oyster samples treated with microwave radiation. The statistical results of color measurement showed that, compared to the controls, the whiteness (L^*) of the oyster increased significantly ($P<0.05$). However, no difference was found between the L^* values of oysters treated with microwave of various time and power level combinations. Similarly, for a^* and b^* values, there were no significant difference ($P<0.05$) between the thermally treated oyster samples and un-treated ones. Compared to colorimetry, the applied color measurement method provided more representative information on the color profile by capturing the overall color in the entire surface of the oyster sample.

Aroma Analysis

Evaluation of odor of high pressure processed oysters using the electronic nose system (Cyranose 320) is presented in Fig. 8.4. Distinct clusters were identified between raw oyster samples and oysters undergone high pressure processing at 310, 345 and 379 MPa (100% separation). Moderate separation was observed between oysters treated with 276 MPa and control samples (95% separation). Apparently, the electronic nose did not detect any differences in the aroma for oysters treated with various times under each designated pressure level except for oyster samples exposed to 276 MPa and 310 MPa for 0 min. They were slightly isolated (58% and 75% separation, respectively) from the rest of the pressure treated group. An interesting observation was that similar signal or smell patterns were recognized for oysters having been treated with pressure of 276 MPa, 310

MPa and 345 MPa. However, smell pattern changed significantly at 379 MPa. The underlining mechanism was not clear. Studies on HPP induced aroma changes were not yet discovered. In fact, high pressure processing was believed to have nearly no impact on the covalent bonds (Tauscher 1998, 1999), which is the primary construction of low molecular weight molecules controlling odor.

The results of oyster aroma change by heat are demonstrated in Fig. 8.5. It illustrated that mild heat treatment at 45, 50 and 55 °C for 2 min did not significantly alter the aroma of oysters compared to the raw samples ($P<0.05$). However, distinct separation of clusters was noticed for oysters treated at 60 °C and 65 °C. The results also showed that, the aroma profile of oysters altered significantly when heating time exceeded 4 min at 55 °C, displayed by well separated groups of aroma values in Canonical Discriminant Analysis plots.

The electronic nose readings of oyster samples heated by microwave showed that the odor of the oysters with and without microwave radiation were basically classified into three groups: the control and samples exposed to 70% and 100% microwave radiation together as one cluster, and two individual cluster representing aroma values treated with 30% and 50% microwave power level, respectively. It indicated that oysters processed by higher power levesl produced closer odor profiles compare to raw samples. This may be related to the time which oysters have been exposed to thermal and electromagnetic field.

CONCLUSIONS

High pressure processing of oysters demonstrated the advantage of improved product quality. When applied under certain pressure level, basically, it did not jeopardize

the texture of the processed oysters. Whitening of oysters was actually a positive contribution in favor of consumer's acceptability for oysters. The recommended processing conditions were 345 MPa for less than 3 min and 379 MPa for less than 1.5 min.

Comparatively, the quality of oysters was much easy to be altered by thermal processing. Heating over 60 °C can cause a soften tissue and severely changed aroma profile. The critical processing condition was determined to be at 55 °C for 2 min incubation time. Increased whiteness was also observed. Microwave processing can be considered as an alternative technology to replace traditional thermal processing as long as proper operating conditions were selected. However, employment of this technology required intensive care. Not only the final temperature after heating need to be specifically controlled, but also the distribution of the temperature within the product was required to ensure a uniform heating.

High pressure induced product texture change was detectable. Observation on the aroma change caused by high pressure processing was an interesting phenomenon. In-depth research work needs to be conducted to study the dominating kinetics.

REFERENCES

Alasalvar C, Taylor KDA, Öksüz A, Garthwaite T, Alexis MN, Grigorakis, K. 2001. Freshness assessment of cultured sea bream (*Sparus aurata*) by chemical, physical and sensory methods. Food Chem. 72(1):33-40.

Barroso M, Careche M, Borderias AJ. 1998. Quality control of frozen fish using rheological techniques. Trends Food Sci. Technol. 9:223-229.

Bartlett PN, Elliott JM, Gardner JW. 1997. Electronic noses and their application in the food industry. Food Technol. 51(12):44-48.

Chai T, Liang KT, Pace J, Schlimme DV. 1991. Effect of heat processing on quality of pasteurized oysters. J Food Sci. 56(5):1292-1294.

Chamberlain AI, Kow F, Balasubramaniam E. 1993. Instrumental methods for measuring texture of fish. Food Australia. 45:439-443.

Chinivasagam HN, Bremner HA, Wood AF, Nottingham SM. 1998. Volatile components associated with bacterial spoilage of tropical prawns. Int. J. Food Microbiol. 42(1/2):45-55.

Du WX, Kim J, Cornell JA, Huang TS, Marshall MR, Wei CI. 2001. Microbiological, sensory and electronic nose evaluation of yellow fin tuna under various storage conditions. J. Food Protect. 64(12):2027-2036.

Du WX, Lin CM, Huang TS, Kim J, Marshall MR, Wei CI. 2002. Potential application of the electronic nose for quality assessment of salmon fillets under various storage conditions. J Food Sci. 67(1):307-313.

Fennema OR. 1996. Food Chemistry. 3rd ed. New York, NY: Marcel Dekker, Inc.

Francis FJ. 1994. Color analysis. In: Nielsen SS, editor. Food Analysis. Aspen Publishers, Inc. p 599.

Francis FJ, Clydesdale FM. 1975. Food Colorimetry: Theory and Applications. Westport, Conn: Avi Publishing.

Giese J. 2003. Color measurement in foods. Food Technol. 57(12):48.

Heremans K, Meersman F, Rubens P, Smeller L, Snauwaert J, Vermeulen G. 1999. A comparison between pressure and temperature effects on food constituents. In:

Oliveira FAR, Oliveira JC, editors. Processing Foods: Quality Optimization and Processing Assessment. Boca Raton, FL: CRC Press LLC.

Jaczynski J, Park JW. 2003. Pysicochemical properties of surimi seafood as affected by electron beam and heat. J food Sci. 68(5):1626-1630.

Jonsson A, Sigurgisladottir S, Hafsteinsson H, Kristbergsson K. 2001. Textural properties of raw Atlantic salmon (*Slmo salar*) fillets measured by different methods in comparison to expressible moisture. Aquacult. Nutr. 7(2):81-89.

Koo J, Hu X, Jahncke ML, Mallikarjunan P. 2002. Effect of high hydrostatic pressure on *Vibrio vulnificus* and *Vibrio parahaemolyticus* in pure cultures and whole eastern oysters [abstract]. In: IFT Annual Meeting Book of Abstracts; 2002 June 15-19; Anaheim, CA. Chicago, III.: Institute of Food Technologies.

Korel F, Luzuriaga DA, Balaban Mö. 2001a. Quality evaluation of raw and cooked catfish (*Ictalurus punctatus*) using electronic nose and machine vision. J Aquat. Food Prod. Technol. 10(1):3-18.

Korel F, Luzuriaga DA, Balaban MÖ. 2001b. Objective quality assessment of raw tilapia (*Oreochromis niloticus*) fillets using electronic nose and machine. J Food Sci. 66(7):1018-1024.

Luzuriaga DA, Balaban MÖ. 1999a. Evaluation of the odor of decomposition in raw and cooked shrimp: correlation of electronic nose readings, odor sensory evaluation and ammonia levels. In: Hurst WJ, editor. Electronic Noses and Sensor Array Based Systems. Lancaster, PA: Technomic Publishing Co. p 177-184.

Luzuriaga DA, Balaban MÖ. 1999b. Electronic nose odor evaluation of salmon fillets stored at different temperatures. In: Hurst WJ, editor. Electronic Noses and Sensor Array Based Systems. Lancaster, PA: Technomic Publishing Co. p 162-169.

Milo C, Grosch W. 1995. Detection of odor defects in boiled cod and trout by gas chromatography-olfactometry of headspace samples. J Agric Food Chem. 43(2): 456-462.

Newman DJ, Lazuriaga DA, Balaban Mö. 1999. Odor and microbiological evaluation of raw tuna via correlation of sensory and electronic nose. In: Hurst WJ, editor. Electron Noses and Sensory Array Based Systems Design and Applications. Lancaster, PA: Technomic Publishing Co., Inc. p 170-176.

Papadakis SE, Abdul-Malek S, Kamdem RE, Yam KL. 2000. A versatile and inexpensive technique for measuring color of foods. Food Technol. 54(12):48-51.

Pearce TC, Schiffman SS, Nagle HT, Cardner JW. 2003. Handbook of Machine Olfaction. Electronic Nose Technology. KGaA, Weinheim: WILEY-VCH Verlag GmbH & Co.

Scopes RK. 1994. Protein Purification: Principles and Practice. 3rd ed. Springer-Verlag New York, Inc. p. 95-101.

Shahidi F, Botta JR. 1994. Seafoods: Chemistry, Processing Technology and Quality. London, UK: Chapman & Hall, Blackie Academic & Professional.

Shie JS, Park JW. 1999. Physical characteristics of surimi seafood as affected by thermal processing conditions. J Food Sci. 64(2):287-290.

Sigurgisladottir S, Hafsteinsson H, Jonsson A, Lie ∅, Nortvedt R, Thomassen M, Torrissen O. 1999. Textural properties of raw salmon fillets as related to sampling method. J Food Sci. 64:99-104.

Tauscher BK. 1998. Effect of high pressure treatment to nutritive substances and natural pigments. In: Fresh Novel Foods by High Pressure. VTT Symposium 186. Technical research Centre of Finland, Helsinki, Finland.

Tokuşoğlu Ö, Balaban MÖ. 2004. Correlation of odor and color profiles of oysters *(Crassostrea Virginica)* with electronic nose and color machine vision. J Shellfish Res. 23(1): 143-148.

Venkateshwarlu G, Let MB, Meyer AS, Jacobsen C. 2004. Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. J Agric Food Chem. 52(2):311-317.

Zapata JF, Price JF. 1982. Evaluation of texture and water holding capacity in cooked minced fish. J Food Proc. Pres. 6:189-195.

Table 8.1: Operating parameters of high pressure processing of oysters

Pressure (MPa/kpsi)	Pressure come-up time (s)	Product temp.(°C)	Vessel temp. (°C)	Tank temp. under pressure (°C)	Adiabatic increase (°C)
276/40	68	21	23	28	5
310/45	75	21	23	29	6
345/50	80	21	22	30	8
379/55	88	21	21	30	9

Table 8.2: Correlation coefficients of second order polynomial relationships between pressure increase and time for high pressure unit processing whole oysters

Pressure (MPa)	Quadratic	Linear	Constant	R^2
276	0.0196	2.635	-5.6823	0.9986
310	0.0075	3.567	-9.4504	0.9945
345	0.0064	3.588	-13.780	0.9935
379	0.0124	3.319	-9.7061	0.9970
552	0.0158	2.808	-13.448	0.9988

Table 8.3: Texture measurement of oysters by high pressure processing

HPP Process (MPa-min)	Pressure-time (GPa·s)	Peak force/Area (kPa)	Stdev	Total energy /Area (kJ/m^2)	Stdev
control	0	21.39	2.42	59.04	7.06
276-0	8.35	21.87	4.23	60.81	7.92
276-2	41.47	22.83	3.86	60.41	8.74
276-4	74.59	23.04	3.30	61.43	8.00
276-6	107.7	24.01	3.77	59.96	7.86
control	0	21.39	2.42	59.04	7.06
310-0	11.02	22.52	5.31	60.88	7.96
310-1.5	38.92	23.77	3.46	60.29	8.13
310-3	66.82	23.74	4.98	60.00	7.39
310-4.5	94.72	24.74	4.67	61.13	7.85
control	0	21.39	2.42	59.04	7.06
345-0	13.65	22.78	4.42	59.25	7.08
345-1.5	34.35	23.81	3.26	60.77	7.21
345-3	55.05	25.20 *	2.84	63.67	8.52
345-4.5	75.75	25.68 *	3.21	64.14	7.62
control	0	21.39	2.42	59.04	7.06
379-0	14.85	23.07	3.96	61.04	6.91
379-0.5	26.22	23.96	5.05	63.32	7.56
379-1	37.59	24.37	4.68	62.79	8.37
379-1.5	48.96	26.15 *	3.57	61.10	5.00

Note: * indicate the significance ($P<0.05$) compared to control.

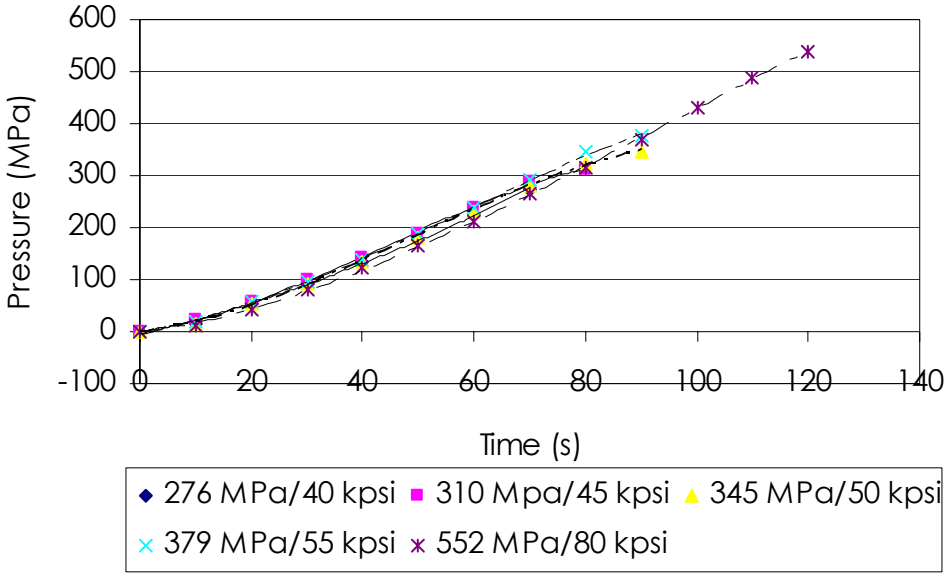


Fig. 8.1: Pressure profile of high pressure unit

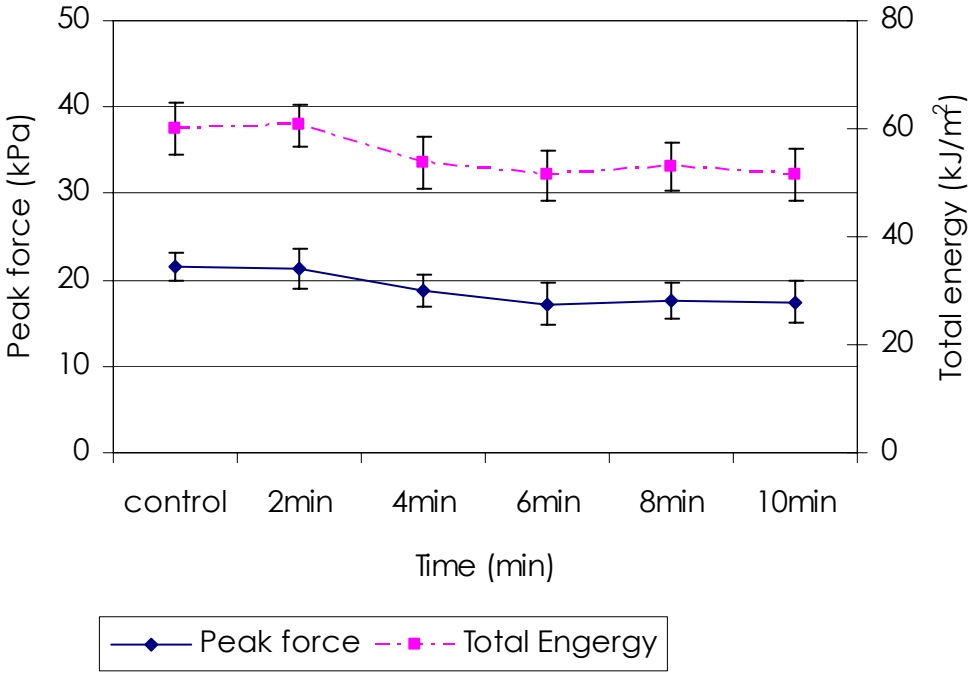


Fig. 8.2: The hardness of oyster meat heated at 55 °C for 10 min

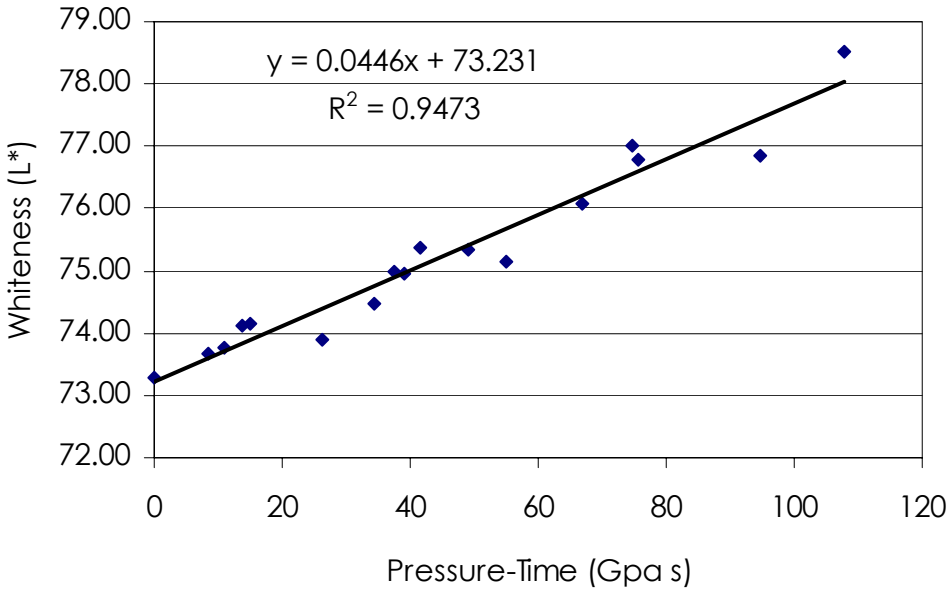


Fig. 8.3: Correlation between the whiteness (L^*) of oyster meat and pressure effect

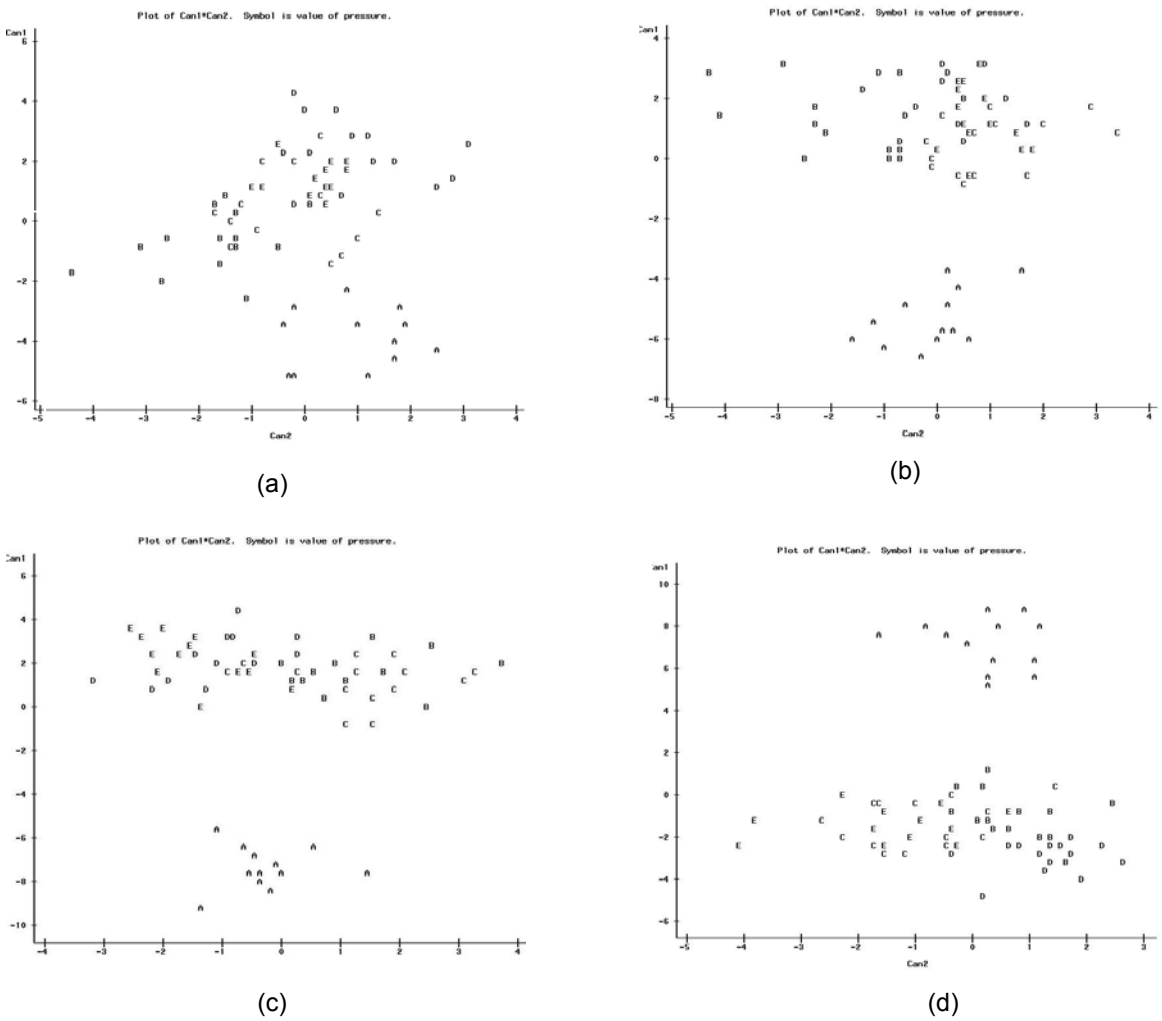
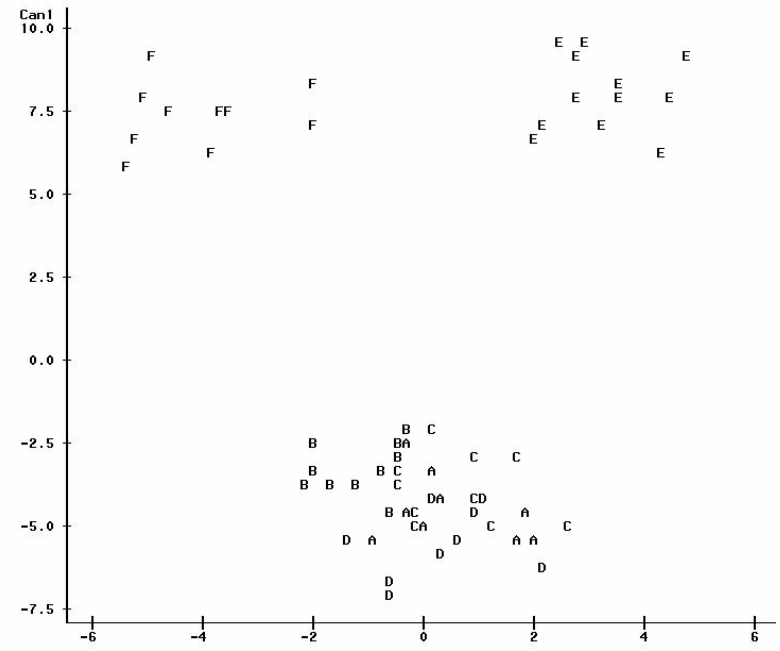


Fig. 8.4: Canonical plots of electronic nose analysis on oyster aroma by HPP, where (a)276 MPa (40kpsi): A-control, B-0 min, C-2 min, D-4 min, E-6 min.

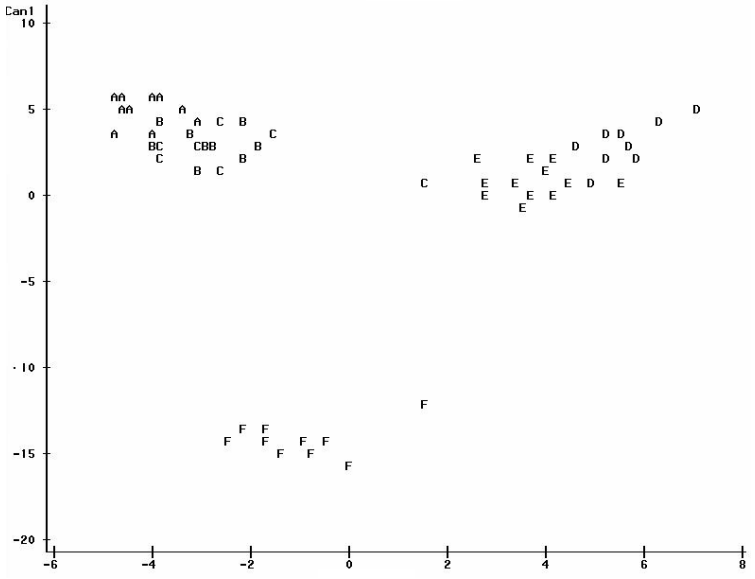
(b)310 MPa (45kpsi): A-control, B-0 min, C-1.5 min, D-3 min, E-4.5 min.

(c)345 MPa (50kpsi): A-control, B-0 min, C-1 min, D-2 min, E-3 min.

(d)379 MPa (55kpsi): A-control, B-0 min, C-0.5 min, D-1 min, E-1.5 min.



(a)



(b)

Fig. 8.5: Canonical plots of electronic nose analysis on oyster aroma by heat. (a) Heat by water bath for 2 min, where A-control; B-45 °C; C-50 °C; D-55 °C; E-60 °C; F-65 °C. (b) Heat by water bath at 55 °C for various times, where A-control; B-2 min; C-4 min; D-6 min; E-8 min; F-10 min

RESULTS SUMMARY

1. Critical processing conditions for high pressure inactivation of *Vibrio* spp. inoculated in oysters was determined to be 345 MPa for less than 3 min and 379 MPa for less than 1.5 min. Under those conditions, 6 log reductions can occur on targeted clinical strain of *V. parahaemolyticus*, which is believed to be the most resistant *Vibrios* discovered so far, without sacrificing the sensory quality of the oyster product.

2. The maximum dosage level to process oyster products using gamma irradiation was 1 kGy, above which unpleasant odor and change in color would be noticeable. Low dose γ-irradiation was proved to be effective in controlling *Vibrios*, resulting in products with comparable quality to raw oysters.

3. As most seafood products, oyster meat was sensitive to heat. Heating at 55 °C for 2 min was identified to be the threshold thermal processing condition. Distinct quality deterioration was observed when oyster meat was heated above 60 °C. When applying microwave radiation as a supplement to conventional thermal processing, extensive care is required to monitor the final temperature of the heated product and to ensure a uniform heating by properly choosing microwave operation procedures. The selection of microwave power supply and heating time needs to consider factors such as the quantity, the size, the shape and the chemical and physical properties of food. In general, high power level and short time combination provided a desirable product quality as well as bacterial reduction.

4. The developed mathematical model was capable of calculating temperature distribution of microwave processed seafood products with an accuracy of ± 8 °C. By changing the corresponding parameters, the model can be used not only to monitor the heat

transfer process in microwave oven, but also to extend its application in computing heat transfer in dielectric heating of 915 MHz, which is the generally applied frequency in food industry utilizing microwave energy. Meanwhile, the obtained information can be further processed to get more useful scientific values, such as to calculate the lethality of the bacteria or enzyme presented in food system or the nutrient loss, affected by temperature.

5. The developed instrumental methods to quantify oyster quality were proved to be feasible and effective. The use of an electronic nose has a great potential for quality evaluation purpose, indicated by having a sound correlation with microbial data and sensory evaluation score. Detection of off-odor of oysters using electronic nose opens a new door for seafood quality control.

RECOMMENDATIONS FOR FUTURE WORK

1. Implementation of differential form of first order reaction rate equation combined with Arrhenius equation to describe the kinetics of non-thermal inactivation needs to be critically considered. The accuracy of the inactivation parameter is largely depended on the coupling effect of pressure and temperature in pressurizing and depressurizing process. The underlining mechanisms need to be further investigated.
2. The developed mathematical model needs to be modified to get more accurate estimation of product temperature distribution in microwave heating. The improvement can be made in the following aspects:

   (1) Consider the heat absorption by oyster shells

   (2) Consider the multi void space effect of live oysters heated in a microwave oven

   (3) Consider the coupling of electromagnetic and thermal effect of microwave processing
3. The approach of utilizing mathematical models to calculate the lethality of microwave heating collectively needs to be validated using other target bacteria in various food systems.
4. The mechanisms of pressure induced changes on macro and micro level molecules needs to be studied. For example, the effect of pressure on protein denaturation, starch gelatinization, covalent bond transformation. Doing so, the pressure induced quality changes can have specific explanation and the governing mechanisms can be discovered.
5. A well-trained sensory panel is a must to get accurate human perception of the quality evaluation on oysters. It is desirable to conduct the sensory training in seashore area

where the recruited sensory panelists are familiar with oyster sensory characteristics and are more willing to participate in the evaluation process.

APPENDICES

APPENDIX A

Table A.1 High pressure processing of *V.vulnificus* in pure culture (PBS, pH=7.4)

Pressure (MPa)				207			
HPP time (min)	Initial	0	2	3	4	6	9
Log reduction	7.80	7.05	4.70	4.45	2.92	1.78	0
Pressure (MPa)				241			
HPP time (min)	Initial	0	1	2	3		
Log reduction	7.80	6.68	4.44	2.00	0		
Pressure (MPa)				276			
HPP time (min)	Initial	0	1	2			
Log reduction	7.80	5.51	1.76	0			
Pressure (MPa)				310			
HPP time (min)	Initial	0	1				
Log reduction	7.80	4.02	0				

Table A.2 High pressure processing of *V.parahaemolyticus* in pure culture (PBS, pH=7.4)

Pressure (MPa)				207			
HPP time (min)	Initial	0	4	8	12	16	20
Log reduction	7.82	7.83	5.66	3.47	2.57	2.11	0
Pressure (MPa)				241			
HPP time (min)	Initial	0	2	4	6	8	10
Log reduction	7.82	7.74	4.86	2.82	2.33	1.63	0
Pressure (MPa)				276			
HPP time (min)	Initial	0	1	2	3	4	
Log reduction	7.82	5.68	3.77	2.46	2.14	0	
Pressure (MPa)				310			
HPP time (min)	Initial	0	1	2	3		
Log reduction	7.82	5.46	2.38	1.48	0		
Pressure (MPa)				345			
HPP time (min)	Initial	0	1				
Log reduction	7.82	3.79	0				
Pressure (MPa)				379			
HPP time (min)	Initial	0	1				
Log reduction	7.82	2.61	0				

Table A.3 High pressure processing of *V.parahaemolyticus* prepared in raw oysters

Pressure (MPa)				276				
HPP time (min)	Initial	0	1	2	3	4	5	6
Log reduction	6.20	4.59	4.27	3.75	3.25	2.83	3.28	2.98
Pressure (MPa)				310				
HPP time (min)	Initial	0	1	2	3	4	5	
Log reduction	6.20	3.83	3.63	3.41	1.60	2.51	2.61	
Pressure (MPa)				345				
HPP time (min)	Initial	0	1	2				
Log reduction	6.20	3.25	1.74	2.29				
Pressure (MPa)				379				
HPP time (min)	Initial	0	1					
Log reduction	6.20	2.70	0					
Pressure (MPa)				586				
HPP time (min)	Initial	0						
Log reduction	6.20	0						

Table A.4 Inactivation (log reductions) of *V.vulnificus* (*V.v.*) and *V.parahaemolyticus* (O3:K6) (*V.p.*) in pure cultures (PBS, pH=7.4) and in inoculated raw oysters by gamma irradiation

Dosage (kGy)	0	0.5	1.0	1.5	2.0
V.v. pure culture	7.25	2.50	1.75	0	0
V.p. pure culture	7.25	4.67	1.67	0.67	0
V.v. pure culture	7.37	3.63	0	0	-
V.p. in oysters	4.04	3.04	2.38	0	-

Table A.5 Cyranose 320 operating conditions

	Time (s)	Speed
Baseline purge	15	Medium
1st sample draw	10	Medium
2nd sample draw	0	-
Snout removal	0	Low
1st sample gas purge	10	High
1st air intake purge	10	High
2nd air intake purge	0	High
2nd air intake purge	5	High
	Conditions	
Digital filtering	On	
Substrate heat	49 °C	
Algorithm	Canonical	
Preprocessing	Auto-scaling	
Normalization	Yes	
In-active sensors	S5, S6, S23, S28, S29, S31	

Table A.6 Universal Testing Machine (Instron) operating conditions

Initial speed (mm/min)	100
Secondary speed (mm/min)	102
% strain limit	100
% deformation limit	200
Load limit (N)	200
Extension limit (mm)	508
Break sensitivity (%)	60
Measurement parameters	Peak load, Elongation at peak load, Energy to peak load, Modulus

APPENDIX B

B.1 DESIRE Program

Computation of the Temperature Distribution in Microwave Heated Fish Gel

```
210   -- Microwave Heating of Fish Gel
220   -------------------------------
230   DT=0.01 |  R=20 |  dr=R/5 |  Z=50 |  dz=Z/10 |  TMAX=60
240   NN=11 |  scale=200
242   w=1/15 |  mod=0.4 |  x=1
245   qi=1000/(3.1415926*(R/1000)^2*(Z/1000))
246   qi=qi*0.5
247   lam=122.37
250   -- Heat Transfer Parameters
260   Pro=0.05 | Carb=0.02 | Water=0.93 | T=20
270   k=(0.179+1.2*1e-3*T-2.72*1e-6*T^2)*Pro
280   k=k+(0.201+1.39*1e-3*T-4.33*1e-6*T^2)*Carb
290   k=k+(0.57+1.76*1e-3*T-6.7*1e-6*T^2)*Water
295   k=1.1*k
300   rho=(1330-0.518*T)*Pro+(1599-0.315*T)*Carb
310   rho=rho+(997.18-3.13*1e-3*T-3.75*1e-3*T^2)*Water
315   rho=0.9*rho
320   cp=(2.01+1.21*1e-3*T-1.31*1e-6*T^2)*Pro
330   cp=cp+(1.55+1.96*1e-3*T-5.94*1e-6*T^2)*Carb
340   cp=(cp+(4.72+9.09*1e-5*T+5.47*1e-6*T^2)*Water)*1000
345   cp=0.9*cp
350   a1=k/rho/cp*1e+6/(dr*dr) |  a2=k/rho/cp*1e+6/(dz*dz)
360   dc=-0.0012*T^2-0.1565*T+59.996
365   dc=1.1*dc
370   dlf=0.0033*T^2-0.2153*T+16.992
375   dlf=0.9*dlf
380    pd=(lam/(2*3.1416*(2*dc)^0.5))/(((1+(dlf/dc)^2)^0.5-1)^0.5)
382   Tinf=22 | Ti=7.5
385   -- h=1.3196*(Tinf-22)^0.04
388   h1=0.001 | h2=25
390   MACRO bound(Tb)
400     BiZ=h1*dz/k | Bir=h2*dr/k
410     Tsz=(BiZ*Tinf+2*Tb)/(2+BiZ) |  Tsr=(Bir*Tinf+2*Tb)/(2+Bir)
420     end
440   -- Set initial conditions
450   T11=Ti | T21=Ti |  T31=Ti |  T41=Ti |  T51=Ti
470   T12=Ti   |  T22=Ti |  T32=Ti |  T42=Ti |  T52=Ti
490   T13=Ti   |  T23=Ti |  T33=Ti |  T43=Ti |  T53=Ti
510   T14=Ti   |  T24=Ti |  T34=Ti |  T44=Ti |  T54=Ti
530   T15=Ti   |  T25=Ti |  T35=Ti |  T45=Ti |  T55=Ti
```

```
  531   T16=Ti  | T26=Ti | T36=Ti | T46=Ti | T56=Ti
  532   T17=Ti  | T27=Ti | T37=Ti | T47=Ti | T57=Ti
  533   T18=Ti  | T28=Ti | T38=Ti | T48=Ti | T58=Ti
  534   T19=Ti  | T29=Ti | T39=Ti | T49=Ti | T59=Ti
  536   T110=Ti | T210=Ti | T310=Ti | T410=Ti | T510=Ti
  550    Tsz=22 | Tsr=22
  560    -- Create a data file
  570    -- connect 'if2.dat' as output 1
  580    drun  | -- display 2 |
  590    -- disconnect 1
  600    -- connect 'con' as output 1
  610    DYNAMIC
  620    ---------------------------
  630    ---- Heat Transfer
  632    p=sgn(p-x)
  633    d/dt x=w*p
  634    y=p*x
  635    q0=qi*(1-sgn(y-mod))
  640    invoke bound(T11)
  650    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-19/2*dz)/pd))/(rho*cp)
  660    d/dt T11=a1*4/3*(T21-T11)+a2*4/3*(3*T11-T12-2*Tsz)+q
  670    invoke bound(T21)
  680    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-19/2*dz)/pd))/(rho*cp)
  690    d/dt T21=a1*((T11-2*T21+T31)+1/3*(T11-T31))+a2*4/3*(3*T21-T22-2*Tsz)+q
  700    invoke bound(T31)
  710    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-19/2*dz)/pd))/(rho*cp)
  720    d/dt T31=a1*((T21-2*T31+T41)+1/5*(T21-T41))+a2*4/3*(3*T31-T32-2*Tsz)+q
  725    invoke bound(T41)
  730    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-19/2*dz)/pd))/(rho*cp)
  740    d/dt T41=a1*((T31-2*T41+T51)+1/7*(T31-T51))+a2*4/3*(3*T41-T42-2*Tsz)+q
  750    invoke bound(T51)
  760    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-19/2*dz)/pd))/(rho*cp)
  770    d/dt T51=a1*(4/3*(T41-3*T51+2*Tsr)+2/9*2/3*(T41-Tsr))+a2*4/3*(3*T51-T52-
2*Tsz)+q
  780    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-17/2*dz)/pd))/(rho*cp)
  790    d/dt T12=a1*4/3*(T22-T12)+a2*(T11-2*T12+T13)+q
  800    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-17/2*dz)/pd))/(rho*cp)
  810    d/dt T22=a1*((T12-2*T22+T32)+1/3*(T12-T32))+a2*(T21-2*T22+T23)+q
  820    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-17/2*dz)/pd))/(rho*cp)
  830    d/dt T32=a1*((T22-2*T32+T42)+1/5*(T22-T42))+a2*(T31-2*T32+T33)+q
  840    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-17/2*dz)/pd))/(rho*cp)
  850    d/dt T42=a1*((T52-2*T42+T32)+1/7*(T52-T32))+a2*(T41-2*T42+T43)+q
  860    invoke bound(T52)
  870    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-17/2*dz)/pd))/(rho*cp)
  880    d/dt T52=a1*(4/3*(T42-3*T52+2*Tsr)+2/9*2/3*(T42-Tsr))+a2*(T51-
2*T52+T53)+q
```

```
 890    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-15/2*dz)/pd))/(rho*cp)
 900    d/dt T13=a1*4/3*(T23-T13)+a2*(T12-2*T13+T14)+q
 910    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-15/2*dz)/pd))/(rho*cp)
 920    d/dt T23=a1*((T13-2*T23+T33)+1/3*(T13-T33))+a2*(T22-2*T23+T24)+q
 930    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-15/2*dz)/pd))/(rho*cp)
 940    d/dt T33=a1*((T23-2*T33+T43)+1/5*(T23-T43))+a2*(T32-2*T33+T34)+q
 950    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-15/2*dz)/pd))/(rho*cp)
 960    d/dt T43=a1*((T33-2*T43+T53)+1/7*(T33-T53))+a2*(T42-2*T43+T44)+q
 970    invoke bound(T53)
 980    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-15/2*dz)/pd))/(rho*cp)
 990    d/dt T53=a1*(4/3*(T43-3*T53+2*Tsr)+2/9*2/3*(T43-Tsr))+a2*(T52-
2*T53+T54)+q
1000    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-13/2*dz)/pd))/(rho*cp)
1010    d/dt T14=a1*4/3*(T24-T14)+a2*(T13-2*T14+T15)+q
1020    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-13/2*dz)/pd))/(rho*cp)
1030    d/dt T24=a1*((T14-2*T24+T34)+1/3*(T14-T34))+a2*(T23-2*T24+T25)+q
1040    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-13/2*dz)/pd))/(rho*cp)
1050    d/dt T34=a1*((T24-2*T34+T44)+1/5*(T24-T44))+a2*(T33-2*T34+T35)+q
1060    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-13/2*dz)/pd))/(rho*cp)
1070    d/dt T44=a1*((T34-2*T44+T54)+1/7*(T34-T54))+a2*(T43-2*T44+T45)+q
1075    invoke bound(T54)
1080    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-13/2*dz)/pd))/(rho*cp)
1090    d/dt T54=a1*(4/3*(T44-3*T54+2*Tsr)+2/9*2/3*(T44-Tsr))+a2*(T53-
2*T54+T55)+q
1100    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-11/2*dz)/pd))/(rho*cp)
1110    d/dt T15=a1*4/3*(T25-T15)+a2*(T14-2*T15+T16)+q
1120    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-11/2*dz)/pd))/(rho*cp)
1130    d/dt T25=a1*((T15-2*T25+T35)+1/3*(T15-T35))+a2*(T24-2*T25+T26)+q
1140    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-11/2*dz)/pd))/(rho*cp)
1150    d/dt T35=a1*((T25-2*T35+T45)+1/5*(T25-T45))+a2*(T34-2*T35+T36)+q
1160    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-11/2*dz)/pd))/(rho*cp)
1170    d/dt T45=a1*((T35-2*T45+T55)+1/7*(T35-T55))+a2*(T44-2*T45+T46)+q
1175    invoke bound(T55)
1180    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-11/2*dz)/pd))/(rho*cp)
1190    d/dt T55=a1*(4/3*(T45-3*T55+2*Tsr)+2/9*2/3*(T45-Tsr))+a2*(T54-
2*T55+T56)+q
1200    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-9/2*dz)/pd))/(rho*cp)
1210    d/dt T16=a1*4/3*(T26-T16)+a2*(T15-2*T16+T17)+q
1220    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-9/2*dz)/pd))/(rho*cp)
1230    d/dt T26=a1*((T16-2*T26+T36)+1/3*(T16-T36))+a2*(T25-2*T26+T27)+q
1240    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-9/2*dz)/pd))/(rho*cp)
1250    d/dt T36=a1*((T26-2*T36+T46)+1/5*(T26-T46))+a2*(T35-2*T36+T37)+q
1260    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-9/2*dz)/pd))/(rho*cp)
1270    d/dt T46=a1*((T36-2*T46+T56)+1/7*(T36-T56))+a2*(T45-2*T46+T47)+q
1275    invoke bound(T56)
1280    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-9/2*dz)/pd))/(rho*cp)
```

```
 1290    d/dt T56=a1*(4/3*(T46-3*T56+2*Tsr)+2/9*2/3*(T46-Tsr))+a2*(T55-
2*T56+T57)+q
 1300    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-7/2*dz)/pd))/(rho*cp)
 1310    d/dt T17=a1*4/3*(T27-T17)+a2*(T16-2*T17+T18)+q
 1320    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-7/2*dz)/pd))/(rho*cp)
 1330    d/dt T27=a1*((T17-2*T27+T37)+1/3*(T17-T37))+a2*(T26-2*T27+T28)+q
 1340    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-7/2*dz)/pd))/(rho*cp)
 1350    d/dt T37=a1*((T27-2*T37+T47)+1/5*(T27-T47))+a2*(T36-2*T37+T38)+q
 1360    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-7/2*dz)/pd))/(rho*cp)
 1370    d/dt T47=a1*((T37-2*T47+T57)+1/7*(T37-T57))+a2*(T46-2*T47+T48)+q
 1375    invoke bound(T57)
 1380    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-7/2*dz)/pd))/(rho*cp)
 1390    d/dt T57=a1*(4/3*(T47-3*T57+2*Tsr)+2/9*2/3*(T47-Tsr))+a2*(T56-
2*T57+T58)+q
 1400    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-5/2*dz)/pd))/(rho*cp)
 1410    d/dt T18=a1*4/3*(T28-T18)+a2*(T17-2*T18+T19)+q
 1420    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-5/2*dz)/pd))/(rho*cp)
 1430    d/dt T28=a1*((T18-2*T28+T38)+1/3*(T18-T38))+a2*(T27-2*T28+T29)+q
 1440    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-5/2*dz)/pd))/(rho*cp)
 1450    d/dt T38=a1*((T28-2*T38+T48)+1/5*(T28-T48))+a2*(T37-2*T38+T39)+q
 1460    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-5/2*dz)/pd))/(rho*cp)
 1470    d/dt T48=a1*((T38-2*T48+T58)+1/7*(T38-T58))+a2*(T47-2*T48+T49)+q
 1475    invoke bound(T58)
 1480    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-5/2*dz)/pd))/(rho*cp)
 1490    d/dt T58=a1*(4/3*(T48-3*T58+2*Tsr)+2/9*2/3*(T48-Tsr))+a2*(T57-
2*T58+T59)+q
 1500    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-3/2*dz)/pd))/(rho*cp)
 1510    d/dt T19=a1*4/3*(T29-T19)+a2*(T18-2*T19+T110)+q
 1520    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-3/2*dz)/pd))/(rho*cp)
 1530    d/dt T29=a1*((T19-2*T29+T39)+1/3*(T19-T39))+a2*(T28-2*T29+T210)+q
 1540    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-3/2*dz)/pd))/(rho*cp)
 1550    d/dt T39=a1*((T29-2*T39+T49)+1/5*(T29-T49))+a2*(T38-2*T39+T310)+q
 1560    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-3/2*dz)/pd))/(rho*cp)
 1570    d/dt T49=a1*((T39-2*T49+T59)+1/7*(T39-T59))+a2*(T48-2*T49+T410)+q
 1575    invoke bound(T59)
 1580    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-3/2*dz)/pd))/(rho*cp)
 1590    d/dt T59=a1*(4/3*(T49-3*T59+2*Tsr)+2/9*2/3*(T49-Tsr))+a2*(T58-
2*T59+T510)+q
 1595    invoke bound(T110)
 1600    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-1/2*dz)/pd))/(rho*cp)
 1610    d/dt T110=a1*4/3*(T210-T110)+a2*4/3*(T19-3*T110+2*Tsr)+q
 1615    invoke bound(T210)
 1620    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-1/2*dz)/pd))/(rho*cp)
 1630    d/dt T210=a1*((T110-2*T210+T310)+1/3*(T110-T310))+a2*4/3*(T29-
3*T210+2*Tsr)+q
 1640    invoke bound(T310)
```

```
 1650    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-1/2*dz)/pd))/(rho*cp)
 1660    d/dt T310=a1*((T210-2*T310+T410)+1/5*(T210-T410))+a2*4/3*(T39-
3*T310+2*Tsr)+q
 1665    invoke bound(T410)
 1670    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-1/2*dz)/pd))/(rho*cp)
 1680    d/dt T410=a1*((T310-2*T410+T510)+1/7*(T310-T510))+a2*4/3*(T49-
3*T410+2*Tsr)+q
 1685    invoke bound(T510)
 1690    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-1/2*dz)/pd))/(rho*cp)
 1700    d/dt T510=a1*(4/3*(T410-3*T510+2*Tsr)+2/9*2/3*(T410-Tsr))+a2*4/3*(T59-
3*T510+2*Tsr)+q
 1720    type Tsr, Tsz, T14, T52, T510
```

B.2 DESIRE Program

Computation of the Temperature Distribution of Microwave Heated Oysters

```
210   -- Microwave Heating of Oysters
220   ------------------------------
230   DT=0.01 |  R=95 |  dr=R/5 | t=0 | TMAX=620
232   Z=67 | dz=Z/10
240   NN=101 |  scale=200
242   w=1/15 |  mod=0 |  x=1
245   qi=1000/(3.1415926*(R/1000)^2*(Z/1000))
246   qi=qi*0.5
247   lam=122.37
250   -- Heat Transfer Parameters
260   Pro=0.08 |  Carb=0.03 |  Water=0.86 |  T=20
270   k=(0.179+1.2*1e-3*T-2.72*1e-6*T^2)*Pro
280   k=k+(0.201+1.39*1e-3*T-4.33*1e-6*T^2)*Carb
290   k=k+(0.57+1.76*1e-3*T-6.7*1e-6*T^2)*Water
300   rho=(1330-0.518*T)*Pro+(1599-0.315*T)*Carb
310   rho=rho+(997.18-3.13*1e-3*T-3.75*1e-3*T^2)*Water
320   cp=(2.01+1.21*1e-3*T-1.31*1e-6*T^2)*Pro
330   cp=cp+(1.55+1.96*1e-3*T-5.94*1e-6*T^2)*Carb
340   cp=(cp+(4.72+9.09*1e-5*T+5.47*1e-6*T^2)*Water)*1000
350   a1=k/rho/cp*1e+6/(dr*dr) |  a2=k/rho/cp*1e+6/(dz*dz)
360   dc=-0.0012*T^2-0.1565*T+59.996
365   -- dc=1.1*dc
370   dlf=0.0033*T^2-0.2153*T+16.992
375   -- dlf=0.9*dlf
380   pd=(lam/(2*3.1416*(2*dc)^0.5))/(((1+(dlf/dc)^2)^0.5-1)^0.5)
382   Tinf=20 |  Ti=20
385   -- h=1.3196*(Tinf-22)^0.04
388   h1=0.001 |  h2=25
390   MACRO bound(Tb)
400     BiZ=h1*dz/k |  Bir=h2*dr/k
410     Tsz=(BiZ*Tinf+2*Tb)/(2+BiZ) |  Tsr=(Bir*Tinf+2*Tb)/(2+Bir)
420     end
440   -- set initial conditions
450   T11=Ti |  T21=Ti |  T31=Ti |  T41=Ti |  T51=Ti
470   T12=Ti   |  T22=Ti |  T32=Ti |  T42=Ti |  T52=Ti
490   T13=Ti   |  T23=Ti |  T33=Ti |  T43=Ti |  T53=Ti
510   T14=Ti   |  T24=Ti |  T34=Ti |  T44=Ti |  T54=Ti
530   T15=Ti   |  T25=Ti |  T35=Ti |  T45=Ti |  T55=Ti
531   T16=Ti   |  T26=Ti |  T36=Ti |  T46=Ti |  T56=Ti
532   T17=Ti   |  T27=Ti |  T37=Ti |  T47=Ti |  T57=Ti
533   T18=Ti   |  T28=Ti |  T38=Ti |  T48=Ti |  T58=Ti
534   T19=Ti   |  T29=Ti |  T39=Ti |  T49=Ti |  T59=Ti
```

```
 536   T110=Ti  |  T210=Ti |  T310=Ti |  T410=Ti |  T510=Ti
 550    Tsz=20 |  Tsr=20
 560    -- Create a data file
 570    connect 'R5-P50-3.dat' as output 1
 580    drun  |  -- display 2 |
 590    disconnect 1
 600    connect 'con' as output 1
 610    DYNAMIC
 620    ---------------------------
 630    ---- Heat Transfer
 632    p=sgn(p-x)
 633    d/dt x=w*p
 634    y=p*x
 636   q0=qi*(1-sgn(y-mod))
 638   q0=q0*(1-swtch(t-500))
 640    invoke bound(T11)
 650    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-19/2*dz)/pd))/(rho*cp)
 660    d/dt T11=a1*4/3*(T21-T11)+a2*4/3*(3*T11-T12-2*Tsz)+q
 670    invoke bound(T21)
 680    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-19/2*dz)/pd))/(rho*cp)
 690    d/dt T21=a1*((T11-2*T21+T31)+1/3*(T11-T31))+a2*4/3*(3*T21-T22-2*Tsz)+q
 700    invoke bound(T31)
 710    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-19/2*dz)/pd))/(rho*cp)
 720    d/dt T31=a1*((T21-2*T31+T41)+1/5*(T21-T41))+a2*4/3*(3*T31-T32-2*Tsz)+q
 725    invoke bound(T41)
 730    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-19/2*dz)/pd))/(rho*cp)
 740    d/dt T41=a1*((T31-2*T41+T51)+1/7*(T31-T51))+a2*4/3*(3*T41-T42-2*Tsz)+q
 750    invoke bound(T51)
 760    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-19/2*dz)/pd))/(rho*cp)
 770    d/dt T51=a1*(4/3*(T41-3*T51+2*Tsr)+2/9*2/3*(T41-Tsr))+a2*4/3*(3*T51-T52-
2*Tsz)+q
 780    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-17/2*dz)/pd))/(rho*cp)
 790    d/dt T12=a1*4/3*(T22-T12)+a2*(T11-2*T12+T13)+q
 800    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-17/2*dz)/pd))/(rho*cp)
 810    d/dt T22=a1*((T12-2*T22+T32)+1/3*(T12-T32))+a2*(T21-2*T22+T23)+q
 820    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-17/2*dz)/pd))/(rho*cp)
 830    d/dt T32=a1*((T22-2*T32+T42)+1/5*(T22-T42))+a2*(T31-2*T32+T33)+q
 840    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-17/2*dz)/pd))/(rho*cp)
 850    d/dt T42=a1*((T52-2*T42+T32)+1/7*(T52-T32))+a2*(T41-2*T42+T43)+q
 860    invoke bound(T52)
 870    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-17/2*dz)/pd))/(rho*cp)
 880    d/dt T52=a1*(4/3*(T42-3*T52+2*Tsr)+2/9*2/3*(T42-Tsr))+a2*(T51-
2*T52+T53)+q
 890    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-15/2*dz)/pd))/(rho*cp)
 900    d/dt T13=a1*4/3*(T23-T13)+a2*(T12-2*T13+T14)+q
 910    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-15/2*dz)/pd))/(rho*cp)
```

```
 920    d/dt T23=a1*((T13-2*T23+T33)+1/3*(T13-T33))+a2*(T22-2*T23+T24)+q
 930    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-15/2*dz)/pd))/(rho*cp)
 940    d/dt T33=a1*((T23-2*T33+T43)+1/5*(T23-T43))+a2*(T32-2*T33+T34)+q
 950    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-15/2*dz)/pd))/(rho*cp)
 960    d/dt T43=a1*((T33-2*T43+T53)+1/7*(T33-T53))+a2*(T42-2*T43+T44)+q
 970    invoke bound(T53)
 980    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-15/2*dz)/pd))/(rho*cp)
 990    d/dt T53=a1*(4/3*(T43-3*T53+2*Tsr)+2/9*2/3*(T43-Tsr))+a2*(T52-
2*T53+T54)+q
1000    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-13/2*dz)/pd))/(rho*cp)
1010    d/dt T14=a1*4/3*(T24-T14)+a2*(T13-2*T14+T15)+q
1020    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-13/2*dz)/pd))/(rho*cp)
1030    d/dt T24=a1*((T14-2*T24+T34)+1/3*(T14-T34))+a2*(T23-2*T24+T25)+q
1040    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-13/2*dz)/pd))/(rho*cp)
1050    d/dt T34=a1*((T24-2*T34+T44)+1/5*(T24-T44))+a2*(T33-2*T34+T35)+q
1060    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-13/2*dz)/pd))/(rho*cp)
1070    d/dt T44=a1*((T34-2*T44+T54)+1/7*(T34-T54))+a2*(T43-2*T44+T45)+q
1075    invoke bound(T54)
1080    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-13/2*dz)/pd))/(rho*cp)
1090    d/dt T54=a1*(4/3*(T44-3*T54+2*Tsr)+2/9*2/3*(T44-Tsr))+a2*(T53-
2*T54+T55)+q
1100    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-11/2*dz)/pd))/(rho*cp)
1110    d/dt T15=a1*4/3*(T25-T15)+a2*(T14-2*T15+T16)+q
1120    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-11/2*dz)/pd))/(rho*cp)
1130    d/dt T25=a1*((T15-2*T25+T35)+1/3*(T15-T35))+a2*(T24-2*T25+T26)+q
1140    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-11/2*dz)/pd))/(rho*cp)
1150    d/dt T35=a1*((T25-2*T35+T45)+1/5*(T25-T45))+a2*(T34-2*T35+T36)+q
1160    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-11/2*dz)/pd))/(rho*cp)
1170    d/dt T45=a1*((T35-2*T45+T55)+1/7*(T35-T55))+a2*(T44-2*T45+T46)+q
1175    invoke bound(T55)
1180    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-11/2*dz)/pd))/(rho*cp)
1190    d/dt T55=a1*(4/3*(T45-3*T55+2*Tsr)+2/9*2/3*(T45-Tsr))+a2*(T54-
2*T55+T56)+q
1200    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-9/2*dz)/pd))/(rho*cp)
1210    d/dt T16=a1*4/3*(T26-T16)+a2*(T15-2*T16+T17)+q
1220    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-9/2*dz)/pd))/(rho*cp)
1230    d/dt T26=a1*((T16-2*T26+T36)+1/3*(T16-T36))+a2*(T25-2*T26+T27)+q
1240    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-9/2*dz)/pd))/(rho*cp)
1250    d/dt T36=a1*((T26-2*T36+T46)+1/5*(T26-T46))+a2*(T35-2*T36+T37)+q
1260    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-9/2*dz)/pd))/(rho*cp)
1270    d/dt T46=a1*((T36-2*T46+T56)+1/7*(T36-T56))+a2*(T45-2*T46+T47)+q
1275    invoke bound(T56)
1280    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-9/2*dz)/pd))/(rho*cp)
1290    d/dt T56=a1*(4/3*(T46-3*T56+2*Tsr)+2/9*2/3*(T46-Tsr))+a2*(T55-2*T56_
+T57)+q
1300    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-7/2*dz)/pd))/(rho*cp)
```

```
 1310    d/dt T17=a1*4/3*(T27-T17)+a2*(T16-2*T17+T18)+q
 1320    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-7/2*dz)/pd))/(rho*cp)
 1330    d/dt T27=a1*((T17-2*T27+T37)+1/3*(T17-T37))+a2*(T26-2*T27+T28)+q
 1340    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-7/2*dz)/pd))/(rho*cp)
 1350    d/dt T37=a1*((T27-2*T37+T47)+1/5*(T27-T47))+a2*(T36-2*T37+T38)+q
 1360    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-7/2*dz)/pd))/(rho*cp)
 1370    d/dt T47=a1*((T37-2*T47+T57)+1/7*(T37-T57))+a2*(T46-2*T47+T48)+q
 1375    invoke bound(T57)
 1380    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-7/2*dz)/pd))/(rho*cp)
 1390    d/dt T57=a1*(4/3*(T47-3*T57+2*Tsr)+2/9*2/3*(T47-Tsr))+a2*(T56-
2*T57+T58)+q
 1400    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-5/2*dz)/pd))/(rho*cp)
 1410    d/dt T18=a1*4/3*(T28-T18)+a2*(T17-2*T18+T19)+q
 1420    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-5/2*dz)/pd))/(rho*cp)
 1430    d/dt T28=a1*((T18-2*T28+T38)+1/3*(T18-T38))+a2*(T27-2*T28+T29)+q
 1440    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-5/2*dz)/pd))/(rho*cp)
 1450    d/dt T38=a1*((T28-2*T38+T48)+1/5*(T28-T48))+a2*(T37-2*T38+T39)+q
 1460    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-5/2*dz)/pd))/(rho*cp)
 1470    d/dt T48=a1*((T38-2*T48+T58)+1/7*(T38-T58))+a2*(T47-2*T48+T49)+q
 1475    invoke bound(T58)
 1480    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-5/2*dz)/pd))/(rho*cp)
 1490    d/dt T58=a1*(4/3*(T48-3*T58+2*Tsr)+2/9*2/3*(T48-Tsr))+a2*(T57-
2*T58+T59)+q
 1500    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-3/2*dz)/pd))/(rho*cp)
 1510    d/dt T19=a1*4/3*(T29-T19)+a2*(T18-2*T19+T110)+q
 1520    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-3/2*dz)/pd))/(rho*cp)
 1530    d/dt T29=a1*((T19-2*T29+T39)+1/3*(T19-T39))+a2*(T28-2*T29+T210)+q
 1540    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-3/2*dz)/pd))/(rho*cp)
 1550    d/dt T39=a1*((T29-2*T39+T49)+1/5*(T29-T49))+a2*(T38-2*T39+T310)+q
 1560    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-3/2*dz)/pd))/(rho*cp)
 1570    d/dt T49=a1*((T39-2*T49+T59)+1/7*(T39-T59))+a2*(T48-2*T49+T410)+q
 1575    invoke bound(T59)
 1580    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-3/2*dz)/pd))/(rho*cp)
 1590    d/dt T59=a1*(4/3*(T49-3*T59+2*Tsr)+2/9*2/3*(T49-Tsr))+a2*(T58-
2*T59+T510)+q
 1595    invoke bound(T110)
 1600    q=q0*(exp(-(R-1/2*dr)/pd)+exp(-(Z-1/2*dz)/pd))/(rho*cp)
 1610    d/dt T110=a1*4/3*(T210-T110)+a2*4/3*(T19-3*T110+2*Tsr)+q
 1615    invoke bound(T210)
 1620    q=q0*(exp(-(R-3/2*dr)/pd)+exp(-(Z-1/2*dz)/pd))/(rho*cp)
 1630    d/dt T210=a1*((T110-2*T210+T310)+1/3*(T110-T310))+a2*4/3*(T29-
3*T210+2*Tsr)+q
 1640    invoke bound(T310)
 1650    q=q0*(exp(-(R-5/2*dr)/pd)+exp(-(Z-1/2*dz)/pd))/(rho*cp)
 1660    d/dt T310=a1*((T210-2*T310+T410)+1/5*(T210-T410))+a2*4/3*(T39-
3*T310+2*Tsr)+q
```

```
 1665    invoke bound(T410)
 1670    q=q0*(exp(-(R-7/2*dr)/pd)+exp(-(Z-1/2*dz)/pd))/(rho*cp)
 1680    d/dt T410=a1*((T310-2*T410+T510)+1/7*(T310-T510))+a2*4/3*(T49-
3*T410+2*Tsr)+q
 1685    invoke bound(T510)
 1690    q=q0*(exp(-(R-9/2*dr)/pd)+exp(-(Z-1/2*dz)/pd))/(rho*cp)
 1700    d/dt T510=a1*(4/3*(T410-3*T510+2*Tsr)+2/9*2/3*(T410-Tsr))+a2*4/3*(T59-
3*T510+2*Tsr)+q
 1720    type T13, T23, T33, T43, T53
```

APPENDIX C

C.1 Worksheet for Quantitative Descriptive Analysis (QDA)

Date_____ WORKSHEET: 4° C Test Code_____

Post this sheet in the area where trays are prepared. Code scorecard ahead of time. Label serving containers ahead of time.

Type of samples: Raw Oysters
Type of test: QDA

Sample identification	Code	Assigned Random numbers
Day 1	H	831, 749, 873,
Day 3	V	313, 999, 717
Day 7	N	785, 457, 143
Day 10	O	647, 205, 484

Code serving containers as follows:

Panelist #	Order of Presentation	3-digit code
1	VOHN	313 647 831 785
	HVON	749 999 205 457
	NHOV	143 873 484 717
2	VHNO	313 749 143 647
	OVHN	205 717 831 785
	NOVH	457 484 999 873
3	OHNV	205 873 785 999
	OVHN	484 717 831 457
	VHON	313 749 647 143
4	OHNV	484 749 457 313
	HVON	873 999 647 143
	NVOH	785 717 205 831
5	NHVO	143 873 999 205
	HONV	749 647 457 313
	VNHO	717 785 831 484
6	VHON	999 749 647 143

	HOVN	873 205 313 457
	HNVO	831 785 717 484
7	VOHN	831 143 999 647
	HOVN	749 484 313 785
	HNOV	873 457 205 717
8	NVOH	457 717 647 873
	OHVN	484 831 999 785
	OHVN	205 749 313 143
9	VHON	313 831 205 785
	NOHV	457 484 873 717
	NHOV	143 749 647 999
10	VNHO	717 457 749 484
	ONVH	647 785 999 831
	HNVO	873 143 313 205
11	ONHV	647 785 873 717
	ONHV	205 143 749 313
	VNOH	999 457 484 831
12	OVNH	205 717 143 831
	HVNO	749 313 785 484
	HVNO	873 999 457 647

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1. Place stickers with panelist's number on tray.
2. Select plates 'E', 'H', 'V', 'N', or 'O' from those previously coded and place on tray from left to right.
3. Write codes selected on panelist's scorecard.
4. Serve samples.
5. Receive filled-in scorecard and note on it the order of presentation used.

Worksheet for Quantitative Descriptive Analysis (QDA)

Date_____ WORKSHEET: 7° C Test Code_____

Post this sheet in the area where trays are prepared. Code scorecard ahead of time. Label serving containers ahead of time.

Type of samples: Raw Oysters
Type of test: QDA

Sample identification	Code	Assigned Random numbers
Day 1	H	831, 749, 873,
Day 3	V	313, 999, 717
Day 7	N	785, 457, 143
Day 10	O	647, 205,484

Code serving containers as follows:

Panelist #	Order of Presentation	3-digit code
1	VHON	999 749 647 143
	HOVN	873 205 313 457
	HNVO	831 785 717 484
2	VOHN	831 143 999 647
	HOVN	749 484 313 785
	HNOV	873 457 205 717
3	NVOH	457 717 647 873
	OHVN	484 831 999 785
	OHVN	205 749 313 143
4	VHON	313 831 205 785
	NOHV	457 484 873 717
	NHOV	143 749 647 999
5	VNHO	717 457 749 484
	ONVH	647 785 999 831
	HNVO	873 143 313 205
6	ONHV	647 785 873 717
	ONHV	205 143 749 313
	VNOH	999 457 484 831

7	OVNH	205 717 143 831
	HVNO	749 313 785 484
	HVNO	873 999 457 647
8	VOHN	313 647 831 785
	HVON	749 999 205 457
	NHOV	143 873 484 717
9	VHNO	313 749 143 647
	OVHN	205 717 831 785
	NOVH	457 484 999 873
10	OHNV	205 873 785 999
	OVHN	484 717 831 457
	VHON	313 749 647 143
11	OHNV	484 749 457 313
	HVON	873 999 647 143
	NVOH	785 717 205 831
12	NHVO	143 873 999 205
	HONV	749 647 457 313
	VNHO	717 785 831 484

1. Place stickers with panelist’s number on tray.
2. Select plates ‘E’, 'H', 'V', 'N', or 'O' from those previously coded and place on tray from left to right.
3. Write codes selected on panelist’s scorecard.
4. Serve samples.
5. Receive filled-in scorecard and note on it the order of presentation used.

C. 2 Quantitative Descriptive Analysis (QDA) Scorecard

Judge Number_____

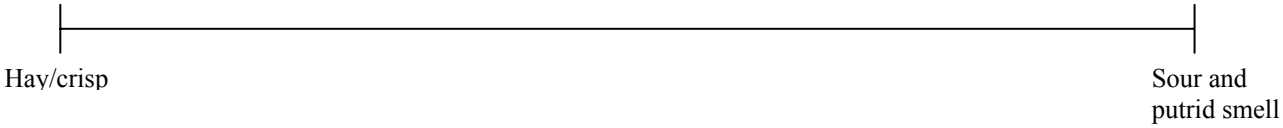
Product: Raw Oysters

There are five samples for you to evaluate. Sniff the samples in the order indicated and place a vertical line across the odor horizontal line at the point that best describes the odor of the sample. Take the sample close to your nose and open the sample container slightly. Sniff the samples for **1-2 seconds** only and immediately close the cover. **DO NOT TASTE THE SAMPLES.** Visually inspect the appearance of the samples and place a vertical line across the appearance horizontal line that best indicates the color of the sample. Finally, assess the texture of the sample by touching the sample with your hand. Use the hand sanitizer provided to cleanse your hands after touching each sample. Indicate the appropriate texture of the oysters by placing a vertical line across the texture horizontal line. Please sniff the coffee provided and rinse your fingers in lemon juice between each sample. Please do not talk to one another during the test.

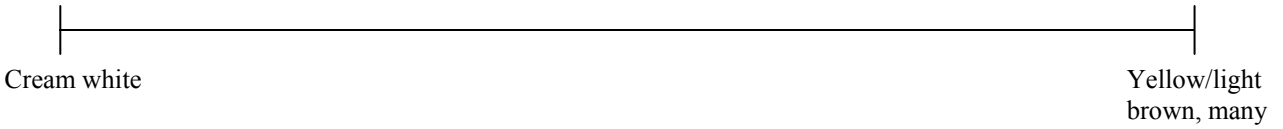
Set 1

Code: _____

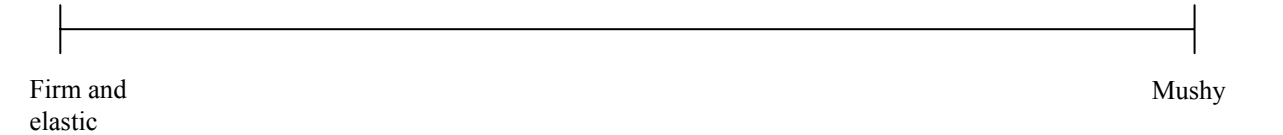
Odor



Appearance



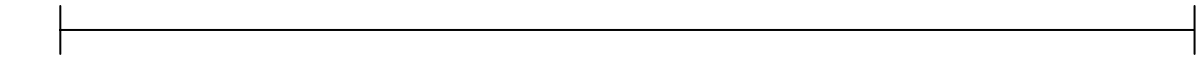
Texture



Code: _____

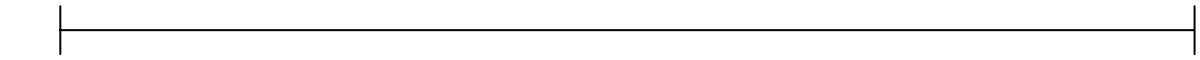
Odor

Appearance



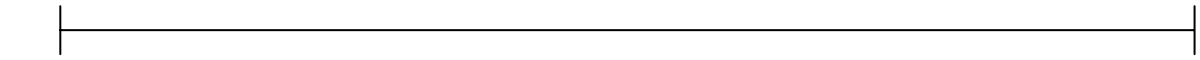
Cream white — Yellow/light brown, many striations

Texture



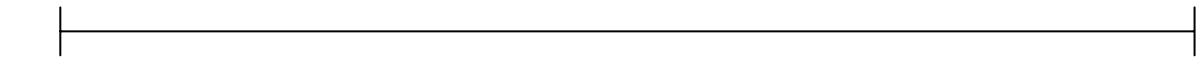
Firm and elastic — Mushy

Odor



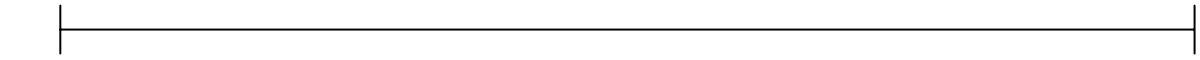
Hay/crisp — Sour and putrid smell

Appearance



Cream white — Yellow/light brown, many striations

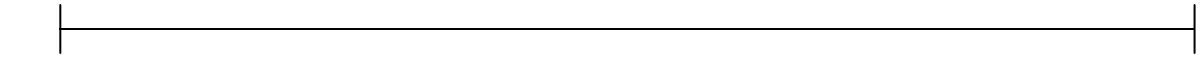
Texture



Firm and elastic — Mushy

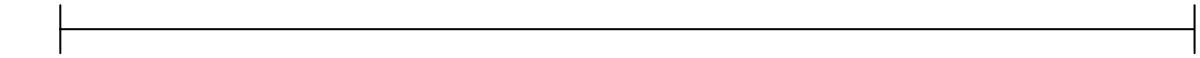
Code: _____

Odor



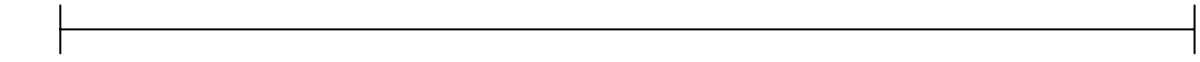
Hay/crisp — Sour and putrid smell

Appearance



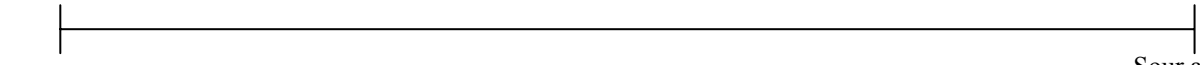
Cream white — Yellow/light brown, many striations

Texture



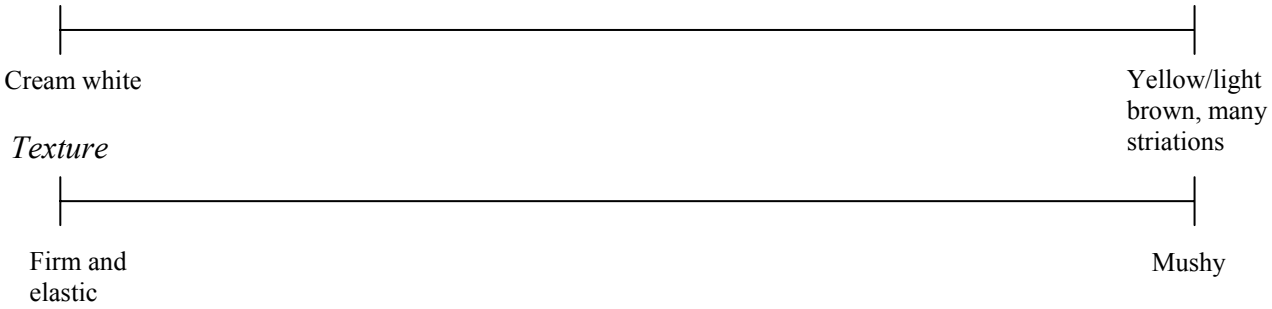
Firm and elastic — Mushy

Odor



Hay/crisp — Sour and putrid smell

Appearance


Cream white
Yellow/light brown, many striations
Texture
Firm and elastic
Mushy

VITA

The author, Xiaopei Hu, was graduated with a Bachelor of Engineering degree in Food Science & Engineering in China Agricultural University in 1996. She continued to pursue a Master of Engineering degree in the same university. In 1999, she received "Royal King's Scholarship for Education of Asia" from Thai. government and started her Master of Engineering degree in Agricultural Engineering in Asian Institute of Technology in Thailand. After graduation, she worked as a Research Associate for the Department of Agriculture Aquaculture System Engineering for 4 months in AIT. In 2001, she began pursuing Doctor of Philosophy degree in Biological Systems Engineering Department at Virginia Polytechnic Institute and State University.