`

PROCESS OPTIMIZATION FOR MICROBIAL SAFETY AND SENSORY EVALUATION OF HIGH PRESSURE PROCESSED HARD CLAMS (*MERCENARIA MERCENARIA*)

By

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ABSTRACT OF THESIS

Process Optimization for Microbial Safety and Sensory Evaluation of High Pressure Processed Hard Clams (*Mercenaria mercenaria*)

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Hard clams (*Mercenaria mercenaria*) are the most valuable commercially harvested clams in the United States. Among the various market grades of hard clams, littleneck and topneck are often eaten raw. Although most of the hard clams are harvested from approved waters under the guidelines of National Shellfish Sanitation Program, there are hard clams which are harvested from restricted waters under special permit. These restricted waters often have high levels of fecal coliforms (14-88 MPN). Clams harvested from these waters must undergo post harvest treatment either by relaying to clean waters or through depuration before entering the market. These treatments are time consuming and are not always effective in purging the bacteria and viruses. Thus, an alternate post harvest technology would benefit both the fishermen and the clam processors in terms of increased landings by accessing the special restricted areas and also by having a premium safe product in the market.

High pressure processing (HPP) has been proven to be successful for reducing bacteria and viruses in oysters along with the retention of raw flavor. We investigated the effect of HPP on microbial safety of raw littleneck clams from restricted waters. Since the profitability of the process also depends on the preferences of consumers, we

evaluated the impact of HPP process on the consumer acceptance of hard clams from approved waters.

Littleneck hard clams from special restricted water were high pressure processed following a Response Surface Methodology (RSM) experimental design to optimize the pressure and hold time and then were evaluated microbiologically. The log reduction of the total bacterial count due to HPP was found to be primarily a function of pressure. Clams from approved waters were used for sensory evaluation. These clams were high pressure processed at 310 MPa for 3 minutes. A consumer panel sensory evaluation of these clams showed that consumers (n=60) equally preferred the HPP and the unprocessed clams. Consumers preferring the HPP clam (n=28) liked plumpness and saltiness of this sample more, whereas consumers preferring the unprocessed clam (n=32) liked the saltiness and chewiness of the unprocessed sample more. Thus, it was a taste driven preference.

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Shalaka Narwankar

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1. INTRODUCTION

1.1 Introduction to Shellfish

Shellfish come under a broad category of mollusc species which have the distinct characteristics of having an outer shell. These molluscs are further divided into three classes:

- Bivalves (two shells) - examples are clams, scallops, mussels, and oysters.
- Univalves (single shell) - examples are abalone and conch.
- Cephalopods - examples are squid, octopus, and cuttlefish.

Shellfish filter large volumes of water and trap particulate matter and dissolved substances suspended in the water as a source of food. Consequently, if the water in which they are grown is polluted, then the shellfish may concentrate microbes or chemicals which may be injurious to humans. Because shellfish are often consumed raw or (slightly cooked) and whole, (including their gastrointestinal tract), they are generally classified as a high-risk food group by health authorities worldwide (Dore, 1991).

The majority of all seafood-related illnesses in the United States are associated with consumption of bivalve molluscan shellfish (Potasman et al., 2002). Therefore, the shellfish are under constant official scrutiny to ensure public health. There are two major issues. First is the raw consumption wherein the whole animal is eaten including the gut and viscera where most of the microorganisms are concentrated. As molluscs filter feed, they can accumulate pathogenic bacteria and viruses in their intestinal tract and gills. Some of these microorganisms are naturally present in the estuarine environment. However, the filter feeding mechanism increases the concentration of these microorganisms in the shellfish meat to levels that are harmful for human consumption. Second issue is the estuarine water itself as these waters are the receiving waters for both

treated and untreated human and animal waste. Thus any microorganism excreted in the fecal material can potentially find its way in the bivalve molluscs.

1.1.1 Biological contamination found in shellfish

Bacteria:

Bacteria found in the shellfish are mostly gram negative rods. *Salmonella, Campylobacter, Shigella, Escherichia,* and *Vibrio cholerae O1* usually enter the shellfish through contaminated waters. There are other bacteria like *Vibrio cholerae Non-O1, Vibrio parahaemolyticus*, and *Vibrio vulnificus* that are naturally present in non-contaminated estuarine waters, which also enter the shellfish due to filter feeding and get concentrated in their gut. These bacteria can be pathogenic to humans if consumed in large numbers. *Vibrio* spp. has been known to cause food-borne illness due to raw shellfish consumption in the summer months because of its high prevalence in the estuaries in summer (Oliver and Kaper, 1997).

Viruses:

Viral agents have been a major cause of shellfish-borne illnesses. Most of the viruses are heat resistant and can survive in the shellfish for a long time. Since they may not be concentrated just in the gut of the animal, they are difficult to eliminate by depuration. Hepatitis A (HAV) and Norovirus outbreaks have been associated with raw shellfish consumption (Dore, 1991).

Toxins:

Red Tide, Paralytic Shellfish Poison (PSP), Neurotoxic Shellfish Poison, Diarrhetic Shellfish Poison, and Amnesiac Shellfish Poison are examples of toxins found

in shellfish. These are the toxins produced by certain natural planktons consumed by the shellfish. These toxins are not harmful to the shellfish but are extremely dangerous to humans. Monitoring these toxins in the harvest water is important as they are not destroyed by heating or freezing the shellfish (Dore, 1991).

1.1.2 Regulation

The National Shellfish Sanitation Program (NSSP) is a joint effort by the Food and Drug Administration (FDA), shellfish industry, and the states producing shellfish. It ensures that shellfish reaching the consumers is safe to consume. The program covers mussels, clams, oysters, and scallops harvested or sold in the United States. Each harvest area is reviewed annually and completely surveyed every three years. The NSSP standard for the average concentration of fecal coliforms in shellfish growing waters is less than 14 fecal coliforms in 100 ml (MPN). The details of the calculation for the most probable number (MPN) are given in Cochran (1950). Note that this is specified for harvest water and not the shellfish harvested from these waters.

Based on the surveys by NSSP, the growing waters are generally classified as:

- **Approved**- Shellfish may be harvested and sold for human consumption (fecal coliforms: <14 MPN).
- **Conditionally Approved**- For areas which are usually clean but are known to suffer from predictable periods of contamination. In that case they are closed for harvesting.
- **Restricted or Special Restricted**- These areas suffer a limited degree of pollution, but shellfish may be taken from these waters (Fecal coliforms: 14-88 MPN) and then subjected to post harvest treatment such as relaying or depuration. Please refer to the next section for more explanation on this.

- **Prohibited**- These areas are permanently closed to shellfish harvesting because of its high levels of pollution with sewage or with marine biotoxins (Fecal coliforms: >88 MPN).

There are other types of classification of harvest wasters which are mentioned in the 2005 NSSP Guide for the Control of Molluscan Shellfish based on the number of surveys and the status of the harvesting waters.

1.1.3 US market for shellfish

Apart from Japan, the United States is the most important market for most molluscan shellfish. The shellfish of economic importance are oysters, clams, and scallops. Clams are mostly eaten raw on the East coast. Because clams, mussels, and oysters are eaten either raw or lightly cooked, there are strict controls on imported products. At present US imports clams, oysters, and mussels from Australia, Canada, Chile, England, Iceland, Korea, Mexico, and New Zealand.

1.1.4 Shellfish storage and transportation

Fourteen days is the maximum duration of storage under refrigerated conditions that the FDA recommends in order to ensure good quality of the shellfish. Also, under the interstate shipping regulation, shippers must ensure that their product is cooled and stored at 7.2°C (45°F) or less. Shellfish should not be left in melting ice water because they cannot survive in fresh water since their gills are not designed to draw oxygen from water that lacks salt (Brenton et al., 2001).

1.1.5 Post harvest treatments to reduce the bacterial load in molluscs shellfish

Molluscs shellfish harvested from special restricted waters must undergo post harvest treatment before reaching the market. Following are the two post harvest treatments currently employed.

Relaying: In this method, the shellfish is placed in approved waters for a designated period of time (at least 4 weeks in New Jersey) to purge themselves of any pollutants. **Figure 1** shows the relaying treatment. After confirming through microbiological tests that the shellfish is wholesome, it may be sold. The salinity (minimum of 12 parts per thousand) and temperature of the relaying water needs to be controlled in order to ensure that the shellfish are alive.

Depuration: In this method, sanitized (UV treated) water is passed over the shellfish in closed tanks for a period of time long enough to ensure that bacteria are eliminated from their guts. The time for depuration is usually 24 to 48 hours. The molluscs are sampled at 0, 24, and 48 hours for fecal coliforms. Two indices of performance, the geometric mean and the 90^{th} percentile of the fecal coliform count measured as MPN/100 grams, have been developed to describe the effectiveness of the depuration process. Critical limits for these parameters have been established empirically for each shellfish species. For hard clams, a geometric mean of 20 MPN/100 grams and a 90^{th} percentile of 70 MPN/100 grams have been adopted (NSSP Guide for the Control of Molluscan Shellfish, 2003). The system is shown in **Figure 2.**

The above mentioned post harvest treatments cannot reliably remove viruses. Also, human pathogens like *Vibrio vulnificus* are difficult to depurate as they can tightly attach themselves to the meat tissue of the shellfish (Croonenberghs, 2000).

Figure 1: Relaying as a post harvest treatment

(archive.southcoasttoday.com/.../a01lo388.htm)

Figure 2: Depuration tanks as a post harvest treatment (www.kiluea.net/Shellfish.htm)

1.1.6 Prior research in the preservation of molluscs shellfish

There has been a continual effort by the shellfish industry and cooperative research programs to find ways and means to keep the shellfish safe for raw consumption. Even though thermal processing such as steaming, boiling, or flame broiling inactivates most of the bacteria in the shellfish, it also destroys the raw flavor

which is savored by many people. Thus, thermal processing is not a suitable technology for the preservation of shellfish which are meant for raw consumption. Low temperatures in the range of 3-7°C has been one of the preservation technologies that have shown lower *Vibrio* count in hard clams (Brenton et al., 2001). However, there are certain types of bacteria (pseudomonad) present in the shellfish that can survive at these low temperatures. Storage temperatures above 20°C have shown to cause an increase in *Vibrio* count in raw shell stock oysters (Lorca et al., 2001). An irradiation dose of 2.7 kGy had shown promising application for the inactivation of fecal coliforms (Harewood et al., 1994) in hard clams (*Mercenaria mercenaria*). However, an irradiation dose of more than 0.5 kGy is lethal to the clams. Thus, irradiation is not a practical method for the preservation of shellfish, particularly raw shellfish. Alternating low amperage electric current has been used to inactivate *Vibrio parahaemolyticus* in seawater (Park et al., 2004) but no study has been reported on inactivation using this technology in the molluscs, per se. There are some patented technologies adopted by companies like AmeriPure® that promote "treated" raw oysters that are in-shell or packed meat. These oysters had a good response from the retailers (Red Lobster® chain of restaurants) and consumers (Degner et al., 1994).

Thus, we need a preservation technology that would inactivate the pathogenic microorganisms, extend the shelf life, and still maintain the raw flavor of the shellfish. High pressure processing has the potential to attain these goals and hence its application has been studied in this research for raw hard clams. The next few sections will give background on clams and high pressure processing.

1.2 Introduction to clams

Clams are bivalve filter-feeding molluscs. They are benthic creatures which mean that they live near the shore. They burrow themselves into the sea bed or in the mud. This is a defense mechanism to stay away from the predators and it allows the clam to relax their adductor muscles and remain closed.

In New Jersey, clams are harvested either by hand digging. Hand digging is a traditional method and is still prevalent along the East coast.

1.2.1 Anatomy of a clam

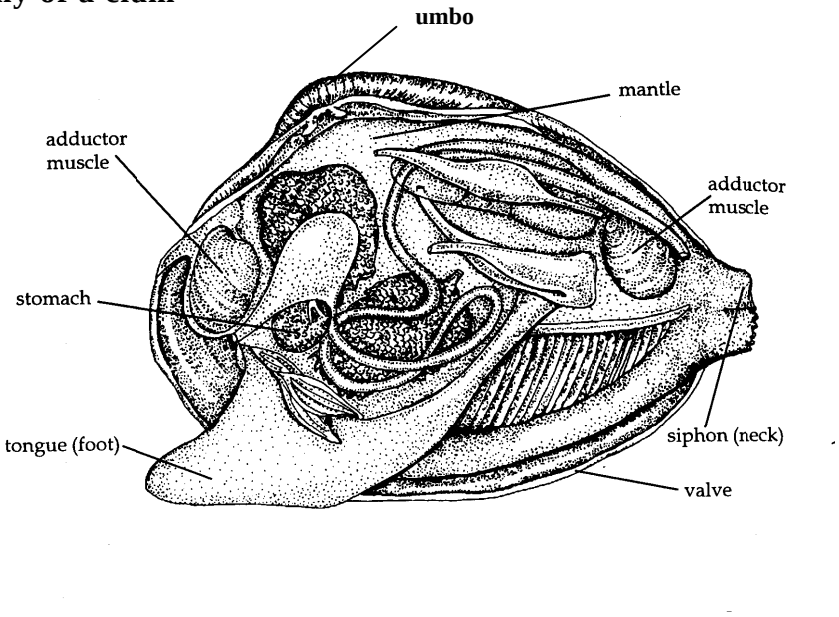


Figure 3: Anatomy of a clam

Figure 3 shows the anatomy of a clam. The **siphon** or the neck is a long tube through which the clam inhales and exhales water. The siphon has two inner tubes, an inhalant and an exhalant. The **foot** or tongue is used as a digging tool to burrow itself in the sand. **Mantle** is the meaty body part. **Stomach** is the digestive system. **Umbo** is the part of the shell above the hinged ligament and it joins the clam shell together. To protect

the soft tissues of the clam, the valves of the shell are held together by two **adductor muscles**. Unlike other bivalve molluscs like oysters, scallops, and mussels; clams have two adductor muscles while the others have just one adductor muscle that joins the two shells.

1.2.2 Types of clams

Based on the size, shapes, utility, and harvest area the clams are classified as follows.

a. Surf clam (*Spisula solidissima*):

It is also called as a skimmer, hen clam, sea clam, or bar clam (**Figure 4(a)**). Commercially these clams are harvested in the ocean off the coast of New Jersey with depths varying from near shore to about 55 meters. The maximum shell length is 152 mm (~6”). These are not sold live or whole. The mantle is used for producing clam strips and clam steaks.

b. Ocean quahog (*Arctica islandica*):

It is also called as a mahogany clam, mahogany quahog, ocean clam, or black clam (**Figure 4(b)**). These are found in depths ranging from 4-256 meters in the ocean on the Atlantic coast. The maximum shell length is 60 mm (2.3”). The meat is strongly flavored but tough and dark in color. This species provides a cheaper substitute for surf clam products.

c. Butter clam (*Saxidomas giganteus*):

It is found from Sitka, Alaska, to San Francisco Bay, California (**Figure 4 (c)**). The maximum shell length for this type of clam is 127 mm (5”). They retain the PSP (Paralytic Shellfish Poisoning) toxin longer than any other species. Although they are

large clams with hard shell, they are quite delicate and must be handled swiftly once they are out of the water.

d. Razor clam (*Siliqua patula*):

It is found from the Aleutian Islands to Pismo Beach, California (**Figure 4 (d)**). These clams occur on broad open beaches at a depth of 6 meters. The maximum shell length for this type of clam is 180 mm (7”). The shell is brittle and the clams are difficult to store out of water. The meat is minced and used in appropriate dishes.

e. Soft clam (*Mya arenaria*):

It is also called as Ipswich clam, belly clam, fryer, steamer, gaper, or squirt clam (**Figure 4 (e)**). This species lives in shallow estuarine water from the Arctic southward to North Carolina. It is most favored for steaming, breading, and frying in the northeast region. The maximum shell length for this type of clam is 150 mm (6”). It has an egg shaped shell and is thin and brittle. The long siphon or neck cannot be entirely enclosed by the shell. The end of the siphon is covered in a thin black skin which is removed after steaming the clam and before eating.

f. Geoduck clam (*Panope abrupta*):

These clams are found from Alaska to Southern California (**Figure 4 (f)**). This species has the ability to burrow itself four feet into the mud. It is the largest clam found in North America. Maximum shell length is 229 mm (9”). The siphon is 4-7 time longer than the shell. The mantle is used in sushi preparations and is a highly priced item in Japan.

(a)

(b)

(c)

(d)

(e)

(f)

(g)

Figure 4: Types of clams

(a) Surf clam (b) Ocean quahog (c) Butter clam (d) Razor clam (e) Soft clam (f) Geoduck clam (g) Hard clam

g. Hard clam (*Mercenaria mercenaria*):

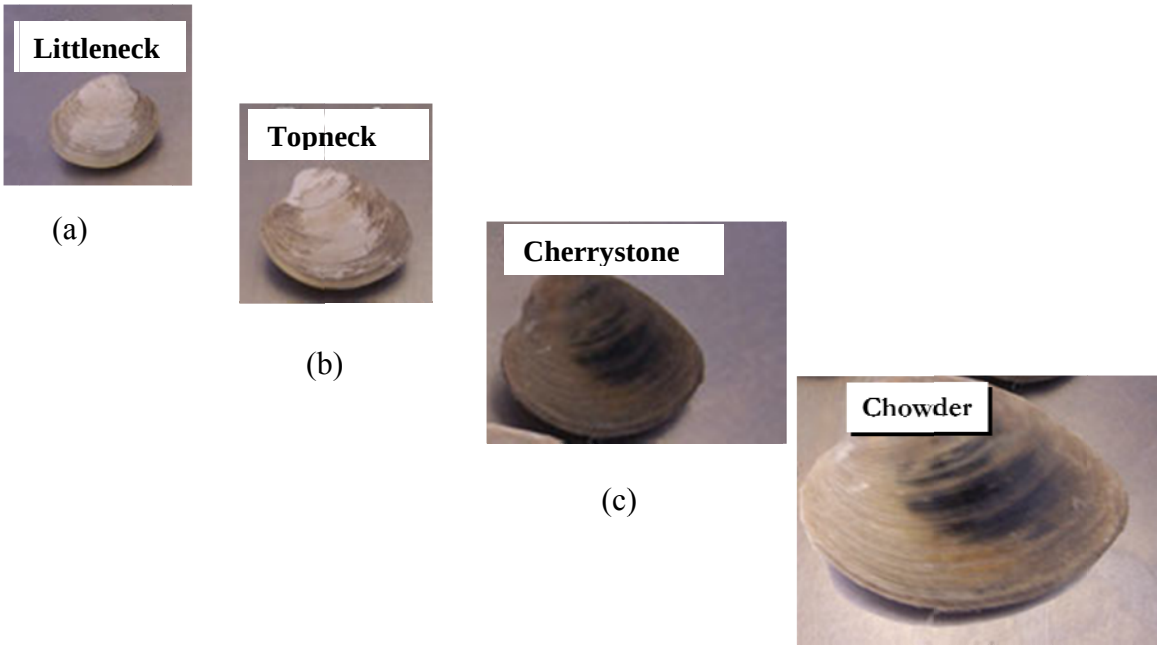
It is also called hard shell clam, bay quahog, or chowder hog (**Figure 4 (g)**). They are primarily classified as the Northern Quahog (*Mercenaria mercenaria*) and the Southern Quahog (*Mercenaria capechiensis*). Amongst the Northern Quahog there is a

further classification based on their size as littleneck, topneck, cherrystone, and chowder as shown in **Figure 5.** The maximum shell length for this type of clam is 135 mm (5.3”). The major harvest area for the hard shell clams is from Massachusetts southward to Florida in coastal estuaries. Smaller Quahogs are mostly eaten as raw whereas the larger ones are often chopped or minced.

This species has a wide range of tolerance for temperature and salinity. Hard clams are typically found in estuaries where the salinities are above 12 parts per thousand. Adult clams can survive temperatures between -6°C (21.2°F) and 45°C (113°F), however they become dormant below 5°C water temperature. Despite their protective shells, clams die rapidly if held at warm temperatures i.e. above 45°C.

1.3 Commercial Importance of Hard Clams

Quahogs are the highest valued bivalves in the United States as observed in **Table 1**. They are mainly sold as fresh and alive. The littleneck clams are eaten raw whereas the larger varieties are steamed. Live clams are sold by bushel which is a volume measure of eight gallons. Clams with cracked or broken shell are unsafe to eat. Larger clams are processed into chopped clams, clam juice, clam strips, breaded clams, and stuffed clams.



(a) (b) (c) (d)

Figure 5: Size gradation in Hard Clams

(a) Littleneck (2-2.25”), (b) Topneck (2.25-3”), (c) Cherrystone (3- 3.25”), (d) Chowder (3.25-4”) (Dore, 1991).

Table 1: Comparison of hard clams with other species

Source: Fisheries of United States, 1999, US Dept. of Commerce, NOAA

1995 Landings of the Major United States Bivalve Species		
Species	**Pounds**	**Dollars**
Hard clams	7,200,000	41,800,000
Ocean quahogs	39,900,000	18,400,000
Soft clams	2,800,000	12,200,000
Surf clams	54,000,000	29,200,000

(a) (b) (c)

(d)

Figure 6: Hard clam products in the market/restaurants

(a) Fettuccine with clams (b) Steamed clams (c) Raw clams on half shell (d) Clam chowder (Google images)

The prime market for the hard clams is live in the shell. The product served is either fresh on the half shell or steamed. Frozen in-shell clams are also available which are served over pasta. Some of the products that are available in the restaurants and markets are shown in **Figure 6**.

Nationally, the harvest of hard clams either from the wilds or from aquaculture is nearly 5,000 metric tons of meat valued at about $46,000,000 annually (National Marine Fishery Service data, 2002). Despite this volume and scale, scientific efforts and popular literature on hard clams has been overshadowed by that on the oysters (*Crassostrea virginica*).

The need for a processing technology that gives a safe and flavorful raw clam is currently hindering the value addition to this commercially important species. High pressure processing has been gaining popularity in this regard in the shellfish processing industry because of the recent research on high pressure processing of oysters has been successful in enhancing its shelf life (He et al., 2002). Companies like Motivatit Seafoods in Houma, Louisiana are already selling high pressure processed oysters under the brand name "Gold Band Oysters®" as a premium product.

1.4 High Pressure Processing

High Pressure Processing (HPP) is a novel non-thermal food processing technology that subjects liquid or solid foods, with or without packaging, to pressures between 50 and 1000 MPa (megapascal). An example of a pressure as high as 1000 MPa is that this pressure value is approximately equivalent to two 5000 kg elephants balancing on a 1 cm^2 area as shown in **Figure 7**. Structural changes in food, inactivation of microorganisms and enzymes, and denaturation or alteration of functionality of proteins are some of the benefits of this technology.

The first use of HPP in food was reported in 1899. Bert Hite of the Agriculture Research Station in Morganstown, West Virginia, designed and constructed a high pressure unit to pasteurize milk at pressures of 450 MPa and reported that such high pressures could improve the keeping quality of milk.

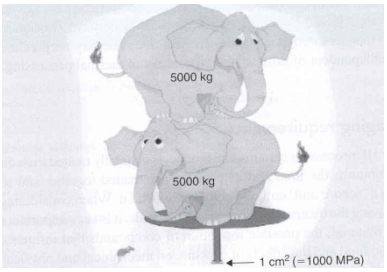


Figure 7: Weight equivalence of 1000 MPa pressure

Source: www.relayresearch.ie

During HPP, the product to be treated is kept in a pressure vessel. The vessel is then filled with a liquid which acts as a pressure transmitting medium. In most cases, water is used as the pressure transmitting medium. The pressurization is done either by using high pressure pump or a piston. Once the desired pressure is reached, the pump or piston is stopped and pressure is maintained without further energy input. After the required hold time has elapsed, the system is depressurized, vessel is opened and the product is unloaded. During pressurization, there is a temperature rise which depends on the pressure transmitting medium. For example, a 3°C increase for every 100 MPa increase in pressure for water as the pressure transmitting medium. This increase in temperature is due to the compression work. However, the food cools down to below its original temperature after decompression.

1.4.1 Effect of high pressure processing on microorganisms

The effect of high pressure on the microorganisms depends on the magnitude of applied pressure, duration of compression, come-up time (CUT-time required to reach desired pressure), process temperature, pH, water activity, salt concentration in the product, number and type of microorganisms, and the state of cell growth.

Amongst the pathogenic microorganisms, *Yersinia enterocolitica* is shown to be the most sensitive as it can be reduced by 5 log at 275 MPa and 15 min, whereas cells of *Salmonella typhimurium, Listeria monocytogenes, Salmonella enteritidis, E. coli O157:H7*, and *Staphylococcus aureus* required 350, 375, 450, 700, and 700 MPa, respectively, for 15 min, to achieve the same reduction (Patterson et al., 1995). In general, gram positive bacteria are more resistant to pressure as compared to the gram negative ones. Yeasts and molds are very sensitive to high pressure. Bacterial spores are most resistant to high pressure as they can survive at pressures higher than 1000 MPa at room temperature. Thus, high pressure along with high temperature is used for their inactivation (Hayakawa, 1994). The sensitivity of microorganisms to high pressure also depends on their growth phase. For examples, cells are more sensitive in the exponential phase than in the stationary phase.

The effect observed in a buffered solution cannot be extrapolated to a real food system because of the interaction effect of the components of the food. Some constituents of food can actually act as baroprotectants. For example, milk and cream protect the microorganisms against the action of high pressure as compared to the destructive action of high pressure on the same microorganism in model systems (Welti-Chanes et al., 2005). At low a_w of the medium, on the application of pressure, the cells experience partial dehydration. In general, high concentrations of salt, sugar, glycerol, and other constituents have a protective effect on the cells against high pressure. Low pH of the medium not only favors microbial inactivation during high pressure processing, but also prevents the growth of the cells that are sub lethally damaged due to high pressure (Smelt, 1998).

Certain combinations of antimicrobials, low temperature, ultrasound, and irradiation have been attempted to study if there is any synergistic effect on microbial inactivation. But the scope of commercializing such a combination of technologies depends on the ultimate objective and the economics.

1.4.2 Mechanism of microbial inactivation

The increased permeability of the cell membrane due to high pressure is one of the factors responsible for inactivation. This increase in permeability is due to the denaturation of proteins in the cell membrane at high pressures. Other mechanisms are modification of the cell nucleus, release of intracellular materials to extracellular spaces, inhibition of the ATPase, crystallization of the phospholipids in membranes, and reduction in a_w which causes partial dehydration of the cell. Changes in the cell morphology and motility have been observed for *E.coli* and protozoa (Hoover et al., 1989).

1.4.3 Effect of high pressure processing on myosystems

From the appearance point of view, there have been studies showing drastic change in the meat color of pork and beef muscles due to high pressure. Pressures up to 400-500 MPa at 10°C caused the minced beef to turn brown and gave it a cooked appearance (Carlez et al., 1995). They showed that packaging the meat under vacuum with an oxygen scavenger partly protects the meat color. Poultry meat also goes through change in color, but not as drastic as mammalian meat. Chicken breast muscle lightens slightly in color after pressurization at 500 MPa for 10 minutes (Yoshioka et al., 1992). High pressure processed chicken and fish fillet showed an opacity of the tissue which can

be attributed to protein denaturation (Hoover et al., 1989). In contrast to mammalian meat, the molluscs' meat seems to retain the fresh appearance after high pressure. High pressure processed oyster meat becomes more plump and has a pleasant appearance (Lopez-Caballero et al., 2000).

1.4.4 Recent research in HPP and shellfish

Researchers and the shellfish industry are very keen to explore HPP technology because of its potential to inactivate bacterial pathogens and also due to its ability to shuck the molluscan shellfish due to pressure. *Vibrio* spp. has been effectively inactivated in medium as well as in oysters at 230-586 MPa for 0-20 minutes with up to 6 log reductions (Koo et al., 2002). He et al. (2001) studied extension in shelf life of raw oysters using HPP. Kingsley et al (2005) studied the inactivation of Hepatitis A virus in a buffered medium and in oysters. In oysters, the pressure alone was responsible for the inactivation (3 log reduction at 300-400 MPa for 1 minute) of HAV whereas in pure culture medium a synergistic effect of pressure and high temperature was observed. Chen et al. (2005) studied the effect of dissolved NaCl and sucrose on the inactivation of Feline Calci Virus (FCV) in aqueous suspensions. They observed that the dissolved NaCl and sucrose have a protective effect for FCV against pressure. Pressure of 250 MPa at 20°C for 1 to 5 minutes caused a reduction of FCV up to 5 log. Kingsley et al (2007) studied the inactivation of Murine Norovirus (MNV-1) in aqueous suspension and observed that low temperature (5°C) along with high pressure is effective in causing a reduction of 4.05 log PFU with pressures ranging from 350-450 MPa (50-65 kpsi) for 5 minutes. **Table 3, 4, and 5** gives a succinct summary of the recent work done in the field of HPP for the

inactivation of microorganisms categorized as vegetative bacteria, spore-forming bacteria, and viruses.

Sensory studies on high pressure processed oysters have been reported by Nell et al. (2006) wherein they found that high pressure processed oysters were liked by the consumer and its sensory quality was unaffected by high pressure.

1.4.5 Commercial products

During the past five years, a number of companies in the U.S. have commercialized products using the HPP technology. Motivatit Seafood of Houma, Louisiana, was the first to apply this technology to raw oysters. A side benefit was that the oyster gets auto-shucked during the process, thus providing labor saving benefits. **Figure 8** shows the commercial products available worldwide.

Figure 8: Commercial HPP products in the market worldwide

Table 2 gives the list of companies currently using high pressure processing to process their products:

Table 2: Companies using high pressure processing (Source: Hogan et al., 2000)

Products	Manufacturer	Country
Jams, Fruit sauces, Yogurt, & jelly	Meida-Ya	Japan
Mandarin Juice	Wakayama Food Industries	Japan
Tropical fruits	Nishin Oil Mills	Japan
Beef	Fuji Ciku Mutterham	Japan
Guacamole, salsa dips, ready meals and fruit juices	Avomex	USA
Fruit & vegetable juices	Odwalla	USA
Hummus	Hannah International	USA
Ham	Hormel Foods	USA
Processed Poultry products	Purdue Farms	USA
Oysters	Motivatit Seafoods	USA
Oysters	Goose Point Oysters	USA
Oysters	Joey Oysters	USA
Orange juice	Ultifruit	France
Fruit juices	Pampryl	France
Apple Juice	Frubaca	Portugal
Sliced ham & tapas	Espuna	Spain
Fruit juices & smoothies	Orchard House	UK

Table 3: Brief summary of recent work in inactivation of vegetative bacteria using HPP (Source: Patterson , 2005)

Vegetative bacteria	Substrate	Treatment	log unit reduction	Comments
Campylobacter jejuni	Pork slurry	300MPa/10min/25°C	6	-
Salmonella Seftenberg 775W	Strained baby food	340MPa/10min/23°C	<2	-
Escherichia coli O157:H7 NCTC 12079	UHT milk Poultry meat	600MPa/15min/20°C	<2 3	Pressure-resistant strain
Staphylococcus aureus	UHT milk Poultry meat	600MPa/15min/20°C	2 3	
Listeria monocytogenes	UHT milk Poultry meat	375MPa/15min/20°C	<1 2	Most resistant of three strains studied
Vibrio parahaemolyticus O3:K6	Oysters	300MPa/3min/ 10°C	5	Most resistant of 10 strains studied
Pseudomonas fluorescens	Ewe's milk	450MPa/10min/10°C	4	-

Table 4: Brief summary of recent work in inactivation of spore forming bacteria using HPP (Source: Patterson, 2005)

Spore-forming bacteria	**Substrate**	**Treatment Conditions**	**log unit reduction**	**Comments**
C. botulinum type E	Sorensen phosphate buffer (pH 7)	827 MPa/5min/50°C	5	-
C. sporogenes	Chicken breast	680 MPa/20min/80°C	2	
B.stearothermophilus	Water	600MPa/5min/70°CX 6cycles	5	-
C. sporogenes, *B. subtilis,* *B. stearothermophilus*	Meat emulsion	621MPa/5min/98°C	>5 >9 >10	Method relies on adiabatic heating

Table 5: Brief summary of recent work in inactivation of viruses using HPP (Source: Patterson , 2005)

Viruses	**Substrate**	**Treatment Conditions**	**Inactivation (logPFU units)**	**Comments**
HIV-1	Culture medium	550MPa/10min/25°C	4	-
MNV-1	Culture medium Oyster tissue	450MPa/5min/20°C 400MPa/5min/5°C	6.85 4.05	Better reduction at lower temp.
Foot & Mouth Disease virus	Culture medium	250MPa/60min/-15°C/1M urea	>4	Novel method of viral vaccine production
Feline calcivirus	Tissue culture medium	275MPa/5min/21°C	7	-
Hepatitis A	Tissue culture Sea water Strawberry puree Sliced green onions Oysters	450MPa/5min/21°C 450MPa/5min/21°C 375MPa/5min/25°C 375MPa/5min/25°C 400MPa/1min/8.7-10.3°C	>6 <2 4.32 4.75 >3	HHP may cause organoleptic alterations
Poliovirus	Tissue culture	450MPa/5min/21°C	No reduction	-

1.5 Hypotheses

Based on the literature review, we made the following hypotheses.

1. High pressure processing can be used as a post harvest technology and as an alternative to depuration for hard clams from special restricted waters. High pressure can reduce the bacterial count in these clams by significant amount.
2. High pressure processed hard clams from approved waters will be accepted and liked by the consumer as an alternative to raw (unprocessed) hard clams.

Justification: Hard clam is a commercially important species along the East coast and most of them are harvested from special restricted waters. These clams (from special restricted waters) cannot go into the market for direct consumption as per the regulation. The post harvest treatments currently applied, viz. relaying and depuration, take long time and are not very effective for certain bacteria and viruses. High pressure processing has the potential to inactivate the bacterial population with little or no effect on the organoleptic quality. Thus, the raw flavor of the clams is preserved along with attaining bacterial inactivation.

However, the consumer needs to be aware and educated about this technology. There is a common notion amongst the raw clam eaters that if the shell is already open, the clams are dead and are no longer safe to consume.

1.6 Rationale

In order to use the new technology as an application, process optimization needs to be done to find the processing conditions which are most practical in terms of safety and cost. Although, high pressure processing has been proven to reduce the bacterial and viral load in pure cultures and in whole oysters, for it to be considered as a depuration tool for clams, it needs to reduce the bacterial load of clams from special restricted waters which have higher initial load of bacteria.

1.7 Specific Objectives

Specific objectives of this research were:

- To use statistical design of experiments approach for process optimization, namely for pressure and hold time as the two factors, in order to recommend a process to the shellfish industry that gives adequate microbial reduction, without significantly increasing the cost and decreasing the quality.
- To study the consumer acceptance of high pressure processed clams, as compared to unprocessed raw clam by conducting a sensory evaluation study with a “liking” scale for the desired attributes.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Hard clams

Littleneck clams for bacterial inactivation studies were procured from JT White Depuration Plant (Highlands, NJ). These clams were harvested from waters that are classified as special restricted (fecal coliforms is 14 to 88 MPN (most probable number)/100 ml). Littleneck clams for sensory evaluation were harvested from approved waters (fecal coliforms below 14 MPN) and were procured from McCarthy Wholesale Clams (Manahawkin, NJ).

2.1.2 Media

1. Butterfield buffer solution was prepared by adding 1.25 ml of butterfield buffer concentrate to 1 liter of distilled water. A two liter buffer solution in bottle and 9 ml in test tubes were prepared for serial dilution. The test tubes and the bottle were closed with screw caps and autoclaved at 121°C (250°F) for 20 minutes. Buffer preparation was and should be done a day before the bacterial analysis.

The buffer concentrate was prepared by adding 34 g KH_2PO_4 to 500 ml distilled water. After adjusting the pH to 7.2 with 1 N NaOH, the volume was brought to 1 liter with distilled water. The concentrate was autoclaved at 121°C for 20 minutes and then stored in a refrigerator.

2. Plate count agar is the media for the bacteria to grow. It is prepared by adding 23 grams of Standard Plate Count Agar (Difco™) to 1 liter distilled water and then autoclaving in a 2 liter glass bottle. The agar should be autoclaved at 121°C (250°F) for

20 minutes. The agar solidifies if kept below 45°C. So care should be taken in order to maintain the agar in the liquefied form during the microbial experiments.

2.1.3 Processing equipment

A 10 liter processing unit (Elmhurst Research, Inc., Albany, NY) (**Figure 9**) was used to process clams at high pressure. Details of the unit are shown in **Figure 10**. This unit is capable of reaching a maximum working pressure of 690 MPa (100,000 psi) in less than 3 min. The maximum depressurization time is 10 seconds. The internal bore diameter of the vessel is 127 mm, its length is 800 mm, and the wall thickness is 145 mm. The vessel can be tilted and the top closure can be removed pneumatically for loading an unloading the samples. This vessel can accommodate up to 100-125 littleneck clams.

Filtered tap water was used as a pressure transmitting fluid. A 20 HP pump pressurized the water from 0.1 MPa (=14.7 psi=1atm) to a desired pressure. Temperature of the water inside the high-pressure vessel was measured using three thermocouples (type K) that were placed near the top, at the center of the vessel, and near the bottom of the vessel. The operation of the high pressure process is controlled using a table top PLC unit. The data on pressure, temperature and time are logged using LabVIEW 7® (National Instruments, Austin, TX) software on a computer.

Figure 9: High Pressure unit at Rutgers University

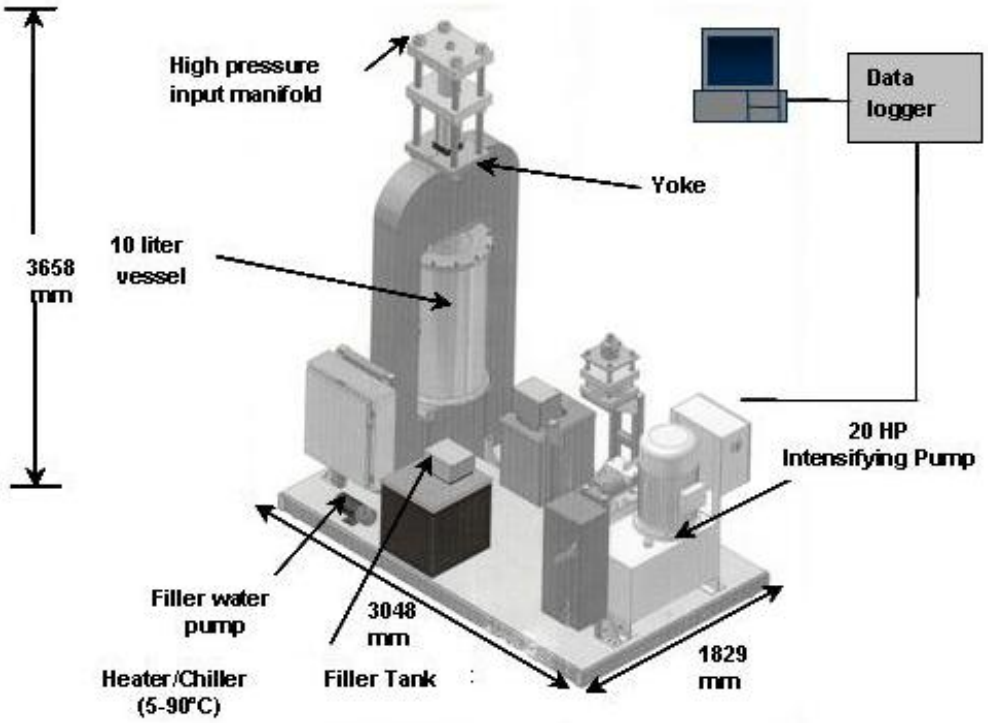


Figure 10: Details of the High Pressure unit at Rutgers University

2.2 Methods

2.2.1 Central Composite Design of experiments

A Central Composite Design (CCD) is a response surface design wherein a series of sequential experiments using independent variables leads to an optimum response (Montgomery, 2005). It is another version of a general 2^k factorial design where k denotes the number of factors and the number 2 denotes two levels (high or low) of the factors. The difference between a 2^k factorial design and CCD is that this design (CCD) includes the central point. This is advantageous in order to verify the response near the central condition. Also, the CCD takes into account the changes in the response due to interaction of the factors and the quadratic effects. The model of the response would be of the form:

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{i<j} \sum \beta_{ij} x_i x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \varepsilon \qquad (1)$$

Where y denotes the response, β_0 is the constant term, and x is the independent variable. Subscript i and j denotes the two factors and k is the levels of the two factors. ε is the random error.

Following are the details of the central composite design used in our research. Design and Analysis of Experiments by Montgomery (2005) was used as the standard reference.

q= number of factors; in our case; q=2

$F = 2^q = 2^2 = 4$; where F is the number of center points

Total number of runs= $2^k + 2q + F = 2^2 + 2*2 + 4 = 12$

The distance α as shown in **Figure 11** is the axial distance from the center of the design grid. It is this parameter that imparts rotatability to the design so that the points are equidistant in all directions. Since RSM is used for optimization and the location of the optimum is unknown prior to running the experiment, it makes sense to use a design that provides equal precision of estimation in all directions. The value α can be calculated as follows:

$\alpha = F^{1/4} = 4^{1/4} = 1.414$

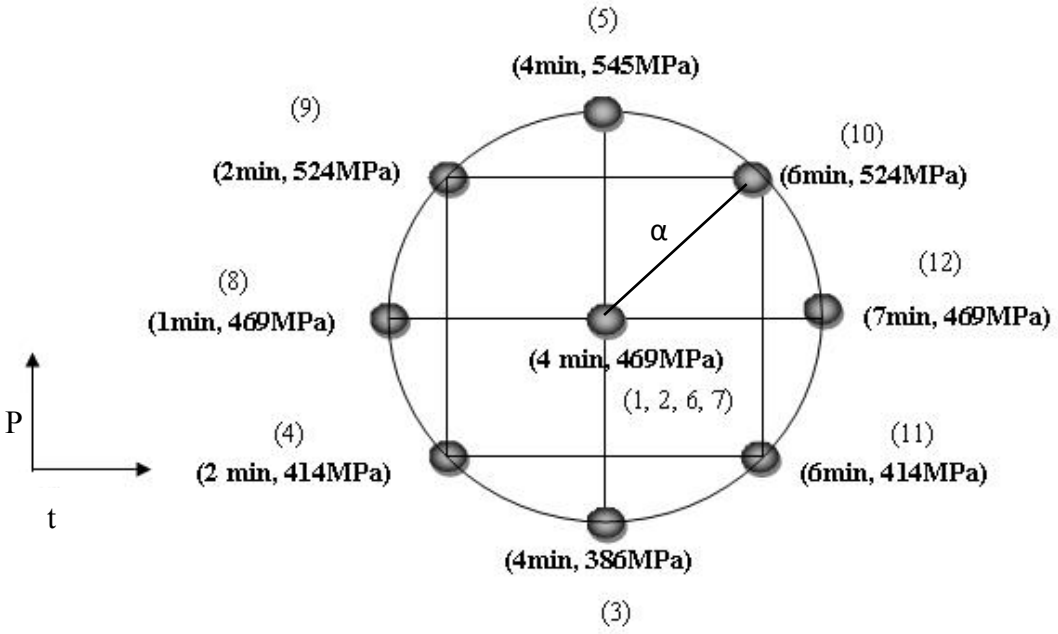


Figure 11: Grid for the Central Composite Design

The advantage of using a central composite design over a one factor at a time approach is that the number of runs is drastically reduced. For a one factor at a time

design, 25 (5 X 5) experimental runs are required for 5 levels of each factor (pressure and time). Also, with at least one repetition, the number of runs would have increased to 50. A central composite design reduces the number of runs to 12 along with more information about the main effects (effects due to just pressure or time) and the interaction effects (effects due to both pressure and time).

The pressure range selected was 386 to 545 MPa (56 to 79 kpsi) and the process hold time range was 1 to 7 minutes. The central condition was repeated four times to get better precision.

Table 6 gives the codes of the factors and **Table 7** gives the sequence in which experiments were carried out. The sequence of experimental runs was randomized. The response studied was log reduction of bacterial load using total plate count which is described in details in the section 2.2.2.

Table 6: Codes of pressure (in MPa) and time (in minutes) parameters

Code	**-α**	**-1**	**0**	**1**	**α**
Pressure (MPa)	386	414	469	524	545
Time (min)	1	2	4	6	7

Table 7: Sequence of run in CCD of experiments

Run #	**Coded Factors**		**Original Factors**	
	Pressure code	**Time code**	**Pressure (MPa)**	**Time (min)**
1	0	0	469	4
2	0	0	469	4
3	-α	0	386	4
4	-1	-1	414	2
5	α	0	545	4
6	0	0	469	4
7	0	0	469	4
8	0	-α	469	1
9	1	-1	524	2
10	1	1	524	6
11	-1	1	414	6
12	0	α	469	7

The design grid was created and analyzed using Minitab 15® statistical (Minitab Inc., Harrisburg, PA, U.S.A.).

Once the clams were brought into Rutgers Food Science facility, they were cleaned under tap water and brushed to remove any surface mud. The clams were individually banded with rubber bands in such a way that they do not open by

themselves. This is required to avoid the falling of meat out of the shells as the shells auto-shucks due to high pressure. The banded clams were then filled in net bags. The bags were put in the vessel followed by filling of the vessel with filtered tap water. The high pressure pump, which starts the pressurization, was then switched on. After the desired hold time, the vessel was depressurized and the clams were unloaded by tilting the vessel and draining out the water.

2.2.2 Microbial Analysis

1. Spread plating: It is a common technique used in the industry for microbial analysis of food. It counts the mixture of bacterial colony forming units (CFU) in a given food sample. In our research, no specific bacterial species was targeted. The detection limit of this technique is 2 log CFU/g.

Protocol:

1. Label the petri dishes (100 mm dia. X 15 mm) and the double compartment filter bags. The petri dishes and filter bags were bought from Fisher Scientific™. Pipette out about 20 ml of the autoclaved (121°C and 20 minutes) plate count agar in the petri dishes suing a 25 ml sterile pipette and cool the dish till the agar solidifies.

2. Brush the shell of a clam with a clean brush thoroughly using distilled water.

3. Shuck the clam carefully near a flame into a clean beaker. The beaker should be previously rinsed with 95% alcohol. The knife should be sterilized by rinsing with 95% alcohol followed by heating on a flame before shucking each clam.

4. Tare the weighing balance and weigh out the clams in the filter bag using a sterile spoon (Fisher Scientific™). Add butterfield buffer solution from the bottle using a clean measuring cylinder in the same compartment of the filter bag which contains the sample

to make 1/10th dilution. The 1/10th dilution can be made by multiplying the weight of the clam by 9 and adding that much volume of butterfield buffer using the measuring cylinder.

5. Stomacher the mixture for 2 minutes in Stomacher Lab-Blender 400® for homogenizing the sample.

6. Make serial dilutions by inoculating 1 ml of the stomacher sample into the 9 ml butterfield buffer tubes. Mix the contents in the tube thoroughly using Fisher Vortex (Genie 2™) every time the serial dilutions are made.

7. Inoculate 0.1 ml of each dilution on petri dishes containing the previously cooled agar. Spread the inoculant using a sterile glass spreader near a flame.

8. After evenly spreading the sample, close the lid of the petri dish. Invert the dish and stack it in the plate rack (The plates are inverted to avoid the water upon condensation of the hot liquefied agar from coming in contact with the sample).

9. Place the rack in the Isotemp® incubator (Fisher Scientific™) at 37 ± 0.5°C for 24 ± 3 hrs.

10. Count colonies grown on the plates using QUEBEC® Darkfield Colony counter on the next day using colony counter and report the results.

2. Pour plating: It is a practical and common technique in microbiology laboratories to enumerate the living organism in a given food sample. The detection limit of this technique is 1 log CFU/g. The advantage of pour plating is that it can detect both aerobic and anaerobic microorganisms as the bacterial colonies grow in the agar rather than on the surface. Also the sample size is higher (1 ml) as compared to spread plate (0.1 ml) so the detection is magnified.

Protocol:

1. Label the petri dishes (100 mm dia. X 15 mm) and the filter bags.

2. Autoclave plate count agar media an hour before starting the experiment. After autoclaving maintain the agar at 45°C (113°F) using a hot plate.

3. Brush the shell of a clam with a clean brush thoroughly using distilled water.

4. Shuck the clam carefully near a flame into a clean beaker. The beaker should be previously rinsed with 95% alcohol. The knife should be sterilized by rinsing with 95% alcohol followed by heating on a flame before shucking each clam.

5. Tare the weighing balance and weigh out the clams in the filter bag using a sterile spoon (Fisher Scientific™). Add butterfield buffer solution from the 2 L bottle using a clean measuring cylinder in the same compartment of the filter bag which contains the sample to make $1/10^{th}$ dilution. The $1/10^{th}$ dilution can be made by multiplying the weight of the clam by 9 and adding that much volume of butterfield buffer using the measuring cylinder.

6. Stomacher the mixture for 2 minutes in Stomacher Lab-Blender 400® for homogenizing the sample.

7. Make serial dilutions by inoculating 1 ml of the stomacher sample into the 9 ml butterfield buffer tubes. Mix the contents in the tube thoroughly using Fisher Vortex (Genie 2™) every time the serial dilutions are made.

8. Inoculate 1 ml of each dilution on petri dishes. For maximum precision, do the inoculation in triplicates.

9. Pour about 20 ml of the agar media in the petri dish using a 25 ml pipette.

10. Swirl the plate slowly so that the inoculant is sufficiently dispersed.

11. Once the agar is cooled, invert the plates and arrange them in the plate rack (The plates are inverted to avoid the water upon condensation from coming in contact with the sample)

12. Place the rack in the Isotemp® (Fisher Scientific™) incubator at 37± 0.5°C for 48± 3 hrs.

13. Count colonies grown on the plates the next day using QUEBEC® Darkfield Colony counter and report the results.

2.2.3 Sensory Evaluation

Since high pressure processing is still an emerging technology in the consumer market, not many people know about this technology and its applications. A consumer study would help in knowing if the consumer did perceive a difference in taste or appearance between a HPP and an unprocessed clam and whether they would prefer a HPP clam over a regular raw clam on a half shell.

In order to conduct the sensory evaluation study, one hundred and fifty littleneck hard clams were transported to the Rutgers facility on ice from Manahawkin, NJ. All clams were washed and brushed under tap water. The clams were divided into two batches of 75 each. Each clam in the batch for HPP was clasped with a rubber band, and put into a netted bag. The bag was loaded in the high pressure vessel and processed at 310 MPa for 3 minutes. The other 75 clams were hand shucked by an experienced shucker (Gef Flimlin) immediately before the sensory study. The processed and unprocessed (hand shucked) clams were used for sensory evaluation on the same day. Sixty subjects from both within and outside the Rutgers University community participated in the sensory evaluation. Prior to conducting such a study, an approval from

the Institutional Review Board of Rutgers University (**Appendix A**) was sought and every possible step was taken to avoid any subject who is vulnerable to allergies, from participating in the study. Subjects were screened beforehand using a questionnaire (**Appendix B**). The subjects were seated in individual booths in the Sensory Evaluation Laboratory (Room # 211) at the Department of Food Science, SEBS. They were presented with a fact sheet (**Appendix D**) informing them of their rights to withdraw from the study at any point of time during the evaluation. They were not informed about the high pressure technology in order to not create any bias in the study. The sensory liking scale ballot (**Appendix C)** was uploaded in the FIZZ™ software installed in the computer in each individual booth. The clam samples were identified by a 3-digit code and were presented on a bed of ice (approx. 4°C) in plastic odorless disposable soufflé cups as shown in **Figure 12**. The placing of the cups on the serving plate was random. A cocktail sauce (Heinz™ seafood cocktail sauce) was also provided with the clams as the raw clams are usually eaten with some kind of condiment (lemon, chili, or cocktail sauce). The subjects were asked to judge the samples based on its appearance and taste. The questionnaire (**Appendix C**) was presented on the computer screen using FIZZ™ software (Biosystemes, Couternon, France).

Figure 12: Sample presented for sensory evaluation

Sample to the left is unprocessed raw clam and sample to the right is a high pressure processed clam. The top container has Heinz™ cocktail sauce.

3. RESULTS AND DISCUSSION

3.1 Preliminary experiments

Before arriving at the final central composite design with 12 experimental conditions, some preliminary experiments were conducted to determine the pressure range for the design and also for developing and fine tuning microbiology method. The conditions for preliminary experiments were chosen based on the literature review of HPP and shellfish.

3.1.1 Shelf life study of market clams

Littleneck clams were bought from A & P store (North Brunswick, NJ) for this study. The clams were banded and high pressure processed at 310 MPa (45 kpsi) for 3 minutes. This was followed by microbial analysis on day 0, 7, and 14. The clams were stored in a refrigerator at 4°C. The microbial analysis has been described in section 2.2.2. The analysis was done in duplicates, which is a standard accepted practice in food microbiology.

Results with spread plating showed that there was no growth above the detection limit (2 log CFU/g) in the high pressure processed market clams for up to 14 days (**Figure 13**). When the pour plate technique for bacterial enumeration was adopted for the same experiment, the growth in the high pressure processed clam above the detection limit of pour plating i.e., 1 log CFU/g, was detected after 7 days (**Figure 14**). However, after 7 days, the control clams usually die (apparent by their opened shell) and are accompanied by putrid odor.

Thus, high pressure processed market clams when processed at 310 MPa for 3 minutes were microbiologically safe for a maximum of 7 days.

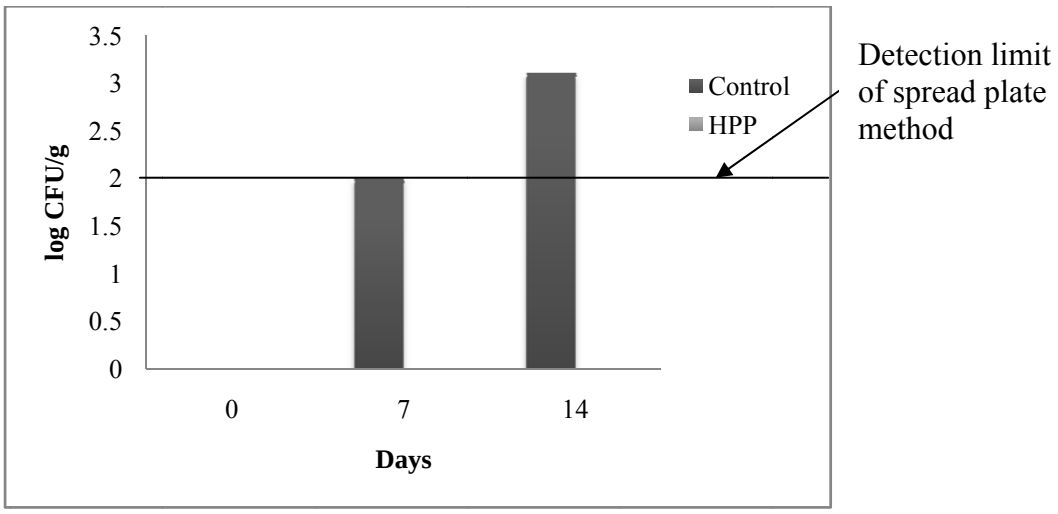


Figure 13: Storage study of littleneck market clams using spread plate technique Control: - No processing; HPP: - High pressure processed at 310 MPa and 3 minutes at 25°C

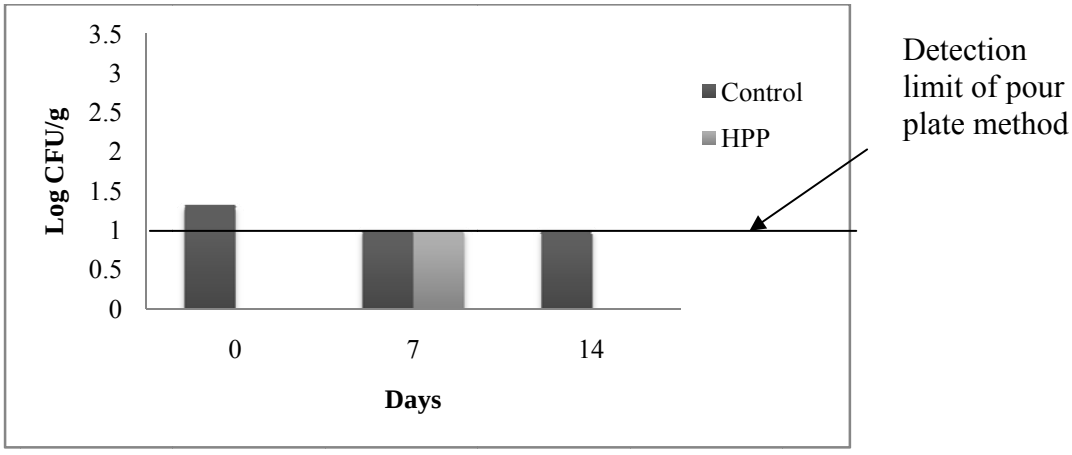


Figure 14: Storage study of littleneck market clams using pour plating. Control: No processing; HPP: - high pressure processed at 310 MPa and 3 minutes at 25°C

3.1.2 Effect of salt on enumeration of bacterial colonies

Brenton et al. (2001) had found that an addition of 2% NaCl in the growth media increases enumeration of the bacterial counts in hard clams. The rationale was that most of the microflora in shellfish are halophilic (salt loving), e.g., *Vibrio vulnificus*. These bacteria may not grow in media that do not have the required level of salinity.

To find if the salinity of the growth media has any effect on the enumeration, we conducted a study with hard clams from special restricted waters. 3% NaCl (Sigma Aldrich™ Inc., 99+ % A.C.S. reagent) was added to the plate count agar and then autoclaved (121°C for 20 minutes). The pour plate technique as described in section 2.2.2 was used.

It was found that the addition of 2% salt did not affect enumeration as shown in **Figure 15**. A plausible reason could be that there were low or undetectable numbers of halophilic bacteria initially in the clams. In the subsequent analysis we did not add salt in the agar.

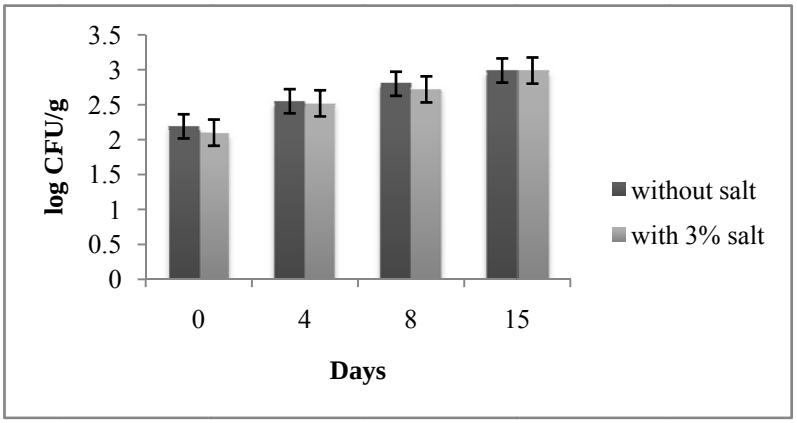


Figure 15: Effect of salt in the media on enumeration of bacterial colonies for unprocessed raw clams from special restricted water. 3% NaCl was added in the plate count agar.

3.1.3 Initial Central Composite Design Experiments

Experiments based on the central composite design (**Table 8**) with pressures ranging from 255-407 MPa (40-56 kpsi) and pressure hold time of 1-7 minutes were carried out for littleneck hard clams from special restricted waters. This range was chosen because the typical pressures used in the oyster processing were 207-310 MPa and the pressure hold time were1-5 minutes. These conditions have been proven to reduce the bacterial load in oysters by 2-3 log (He et al., 2001). However, when the experiments were carried out using these conditions for clams, we obtained less than 1 log reduction in the clams from special restricted waters.

We hypothesized that the low reduction might be due to the following:

1. Size of clams (littleneck, topneck, cherrystone, chowder) - The inactivation might be a function of the shell size.
2. Protective effect of the shell - The shell might be acting as a barrier to the pressure against the inactivation of bacteria.
3. Initial bacterial count - The initial load of bacteria in clams that we were working with was 3 log CFU/g. Our hypothesis was that the log reduction is a function of initial bacterial count, i.e., lower the initial count; lower the log reduction due to pressure. This is sometimes referred to as a tailing or non-linear behavior.

It was decided to prove or disapprove the above hypotheses by carrying out experiments designed for that purpose.

Table 8: Experimental conditions and results of the initial central composite design

Run #	**Coded factors**		**Original Factors**		**Log reduction**
	Pressure code	**Time Code**	**Pressure (MPa)**	**Time (min)**	
1	-1	-1	276	2	0.5
2	1	-1	386	2	0.1
3	-1	1	276	6	0.2
4	1	1	386	6	0.8
5	0	0	331	4	0.2
6	0	0	331	4	0.5
7	$-\alpha$	0	255	4	0.2
8	α	0	407	4	0.3
9	0	$-\alpha$	331	1	0.1
10	0	α	331	7	0.1
11	0	0	331	4	0.2
12	0	0	331	4	0.7

3.1.4 Proof of concept experiments

The following series of experiments were carried out to understand the reasons for low inactivation in the initial central composite design. The bacterial analysis of the experiments discussed below was done using pour plate technique because this enumeration technique has lower detection limit compared to spread plating.

3.1.4.1 Effect of size of clam on inactivation

Experiments were carried out to study the effect of size gradation in clams (viz. littleneck (2”), topneck (2.5”), cherrystone (3”), and chowder (3.5”)) on inactivation due to pressure. Five clams of each type/size were high pressure processed at 414 MPa (60 kpsi) for 8 minutes and total plate count was measured using pour plate technique immediately after processing. This pressure and time parameters were used because they were the extreme conditions compared to the ones reported in the literature on HPP of shellfish, i.e., 207-310 MPa and 1-5 minutes.

We conducted a two- way ANOVA test (using Minitab 15 statistical software) on the effect of type and high pressure treatment on inactivation as shown in **Table 9**. We found that the type/size of clam did affect the inactivation by high pressure. This could be because as the clams grow older their adductor muscles may be becoming weaker and thus allowing the pressure to break open the shell and inactivate bacteria. This is just a conjecture at this time. The other rationale is that there could pressure variation inside the clam meat, thus causing difference in inactivation due to pressure. This needs to be verified in future research. Although **Figure 16** shows a statistically significant log reduction in the littleneck, topneck, and chowder clam, this log reduction (less than 0.5) is not microbiological significant.

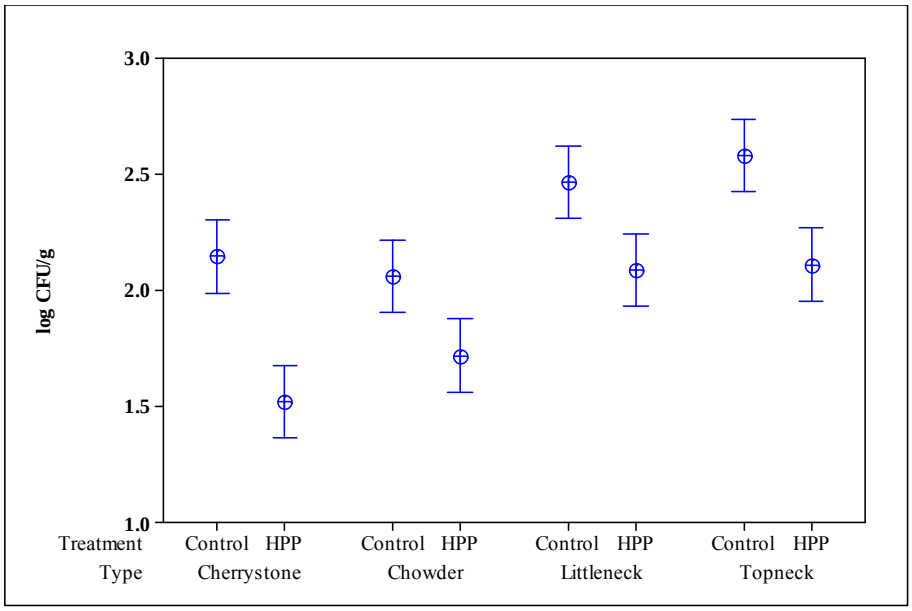


Figure 16: Effect of clam type/size on bacterial inactivation (HPP conditions: 414 MPa and 8 minutes) (Minitab 15®)

Table 9: Two-way ANOVA for the effect of clam size and treatment (HPP) on inactivation

Source	**DF**	**SS**	**MS**	**F**	***p***
Type	3	1.24797	0.41599	5.67	**0.008**
Treatment	1	1.24257	1.24257	16.94	**0.001**
Interaction	3	0.07258	0.02419	0.33	0.804
Error	16	1.17393	0.07337		
Total	23				

3.1.4.2 Effect of shell on inactivation:

In order to find out whether the shell of a clam has a protective effect, littleneck clams in the shell (banded but not vacuum packed), half shelled (vacuum packed), shucked (vacuum packed), and control (unprocessed) clams were processed at 414 MPa (60 kpsi) for 8 minutes and total plate count was measured using pour plate technique immediately after processing. There was no significant difference in inactivation (p>0.05) for clams that were in the shell, on a half shell, and shucked (**Figure 17**). A two-way ANOVA (**Table 10**) shows that there is no significant difference between half shell, no shell, and in the shell clams and also between HPP treatment and control (unprocessed). This proves that the shell in littleneck clams did not have a protective effect on inactivation due to high pressure. However, we observed considerable log reduction (1.5-2 log) in clams at these conditions compared to the initial CCD experiments. This led us to verify if the initial bacterial count has an effect on the inactivation due to pressure.

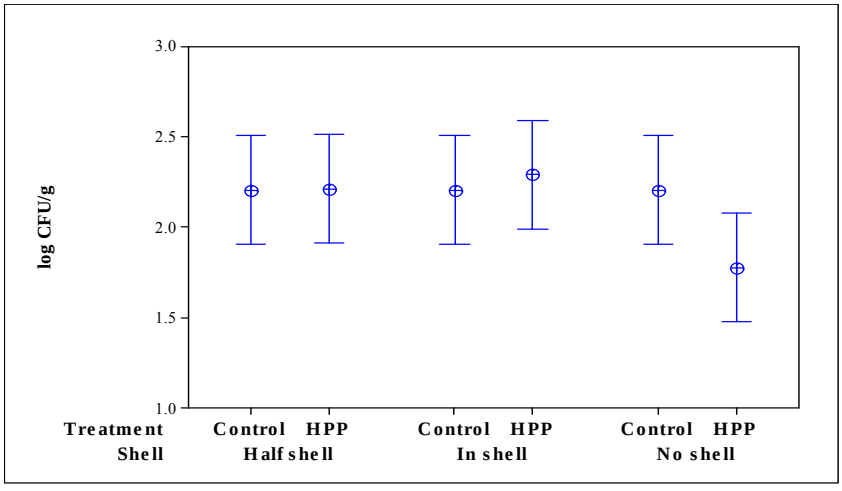


Figure 17: Effect of shell on inactivation (HPP conditions: 414 MPa and 8 minutes) (Minitab 15®)

Table 10: Two-way ANOVA of the effect of shell and treatment on inactivation

Source	**DF**	**SS**	**MS**	**F**	***p***
Treatment	1	0.05600	0.056003	0.98	0.343
Shell	2	0.23034	0.115169	2.01	0.177
Interaction	2	0.23034	0.115169	2.01	0.177
Error	12	0.68805	0.057337		
Total	17	1.20473			

3.1.4.3 Effect of initial count on inactivation:

In the CCD experiments, the initial load was not considered as a control factor. The clams were not inoculated with bacteria because our goal was to simulate the real life conditions. In order to measure the effect of the initial load, the clams harvested from special restricted waters were stored at 5°C and high pressure processed (5 clams) on day 0, 4, 8, 16, and 23. Microbial analysis of the high pressure processed and control (unprocessed) clams was done immediately after processing using pour plating (see section 2.2.2).

Inactivation due to high pressure at 310 MPa and 3 minutes was found to be a function of the initial bacterial load as shown in **Figure 18**. The 95% confidence interval

(CI) and prediction interval (PI) are shown with dotted lines. The adjusted R^2 accounts for the number of variables and the sample size (Triola, 1997).

We found that higher the initial load, higher was the inactivation. The regression equation for HPP inactivation was found to be as follows:

$$\log(\frac{N_0}{N}) = 0.698 + 0.0002 \times N_0 \quad (R^2 = 0.933) \ldots\ldots (2)$$

We conducted the same study at higher pressures (510 MPa and 3 minutes) and found a similar trend (**Figure 19**). The regression equation for HPP inactivation at 510 MPa and 3 minutes is as follows:

$$\log(\frac{N_0}{N}) = 1.063 + 0.00064 \times N_0 \quad (R^2 = 0.965) \ldots\ldots (3)$$

where:

N_o= initial bacterial count (control)

N= bacterial count after high pressure processing (310 MPa and 3 minutes)

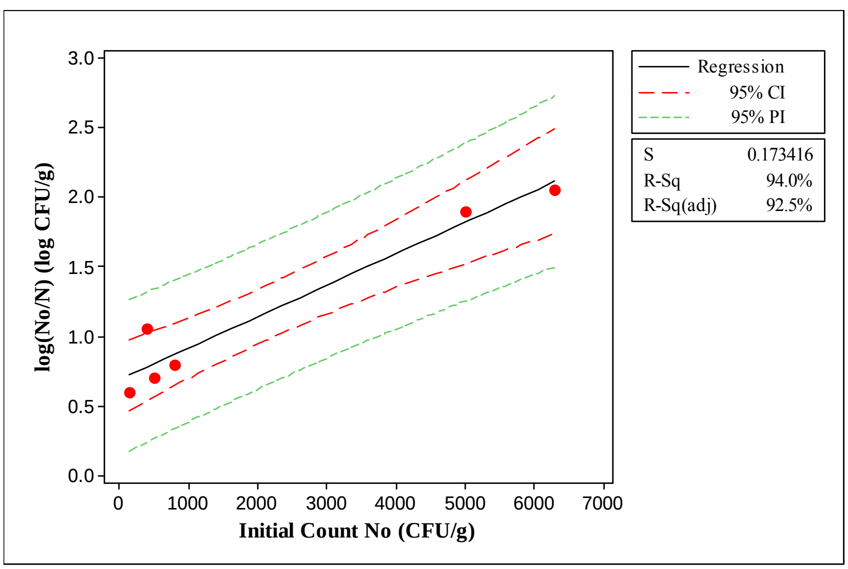


Figure 18: Effect of initial count on inactivation due to HPP (310 MPa and 3 minutes)

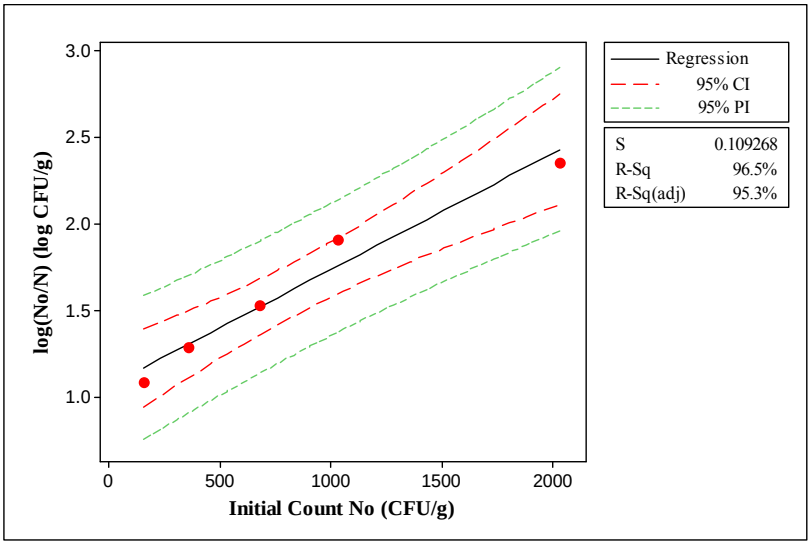


Figure 19: Effect of initial count on inactivation due to HPP. (HPP conditions: 510 MPa, 3 min)

3.2 Optimization using Response Surface Methodology (RSM)

Optimization of the pressure and time parameters was repeated using the central composite design, but this time at higher pressures as very low log reduction was observed for lower pressure ranges (see section 3.1.3). The range of pressure and time were 386 MPa to 544 MPa and 1 to 7 minutes. The design is explained in detail in section 2.2.1.

The design was analyzed using Minitab 15® statistical software. The statistical significance of all the terms including linear, square, and interaction terms for pressure and time, are shown in **Table 11.** The parameter *p* in the table is the p-value indicates the probability that the estimated coefficients of the terms in the predictive equation are a chance occurrence. The standard error (S.E.) of the coefficients of the terms in the equation is also given in this table. Table 10 gives the analysis of variance (ANOVA) for

the model. The final predictive equation for bacterial inactivation (log (No/N)) is given below, where P in the equation is the process pressure in MPa, t is process hold time in minutes, No is the initial load of bacteria in CFU/g, N is the bacterial count in CFU/g at time t.

$$\log(\frac{N_0}{N}) = 20.98 - 0.091 \times P - 0.586 \times t + 0.0001 \times P^2 + 0.0171 \times t^2 + 0.001 \times P \times t \quad \text{…… (4)}$$

The above model could explain 97.58% (R^2) of the variance observed. By further analyzing the response surface design by selecting just the significant terms, we found that the linear and square terms of pressure alone were statistically significant ($p<0.05$). The interaction effects between pressure and time were not significant. The equation after taking just the significant terms into account is as follows.

$$\log(\frac{N_0}{N}) = 17.8 - 0.083 \times P + 0.0001 \times P^2 \quad \text{………………………………………..….. (5)}$$

The R^2 for this model is 93.3%. The initial bacterial load in the raw clams before processing was 3.2 log CFU/g. The contours of log reduction as a function of pressure and time are shown in **Figure 20**. The contour lines indicate that the log reduction is more sensitive to pressure than time. It is a pressure driven inactivation.

Table 11: Regression model for the CCD (The standard errors were calculated based on the repetitions (4 times) at the center point)

Term	**Coefficient**	**S.E. coefficient**	**T**	***p***
Constant	20.981	3.472	6.043	**0.001**
Pressure (MPa)	-0.091	0.0143	-6.376	**0.001**
Time (minutes)	-0.586	0.262	-2.232	0.067
Pressure*Pressure	0.0001	0.00002	6.873	**0.000**
Time*Time	0.0171	0.01137	1.500	0.184
Pressure*Time	0.001	0.00052	2.004	0.092

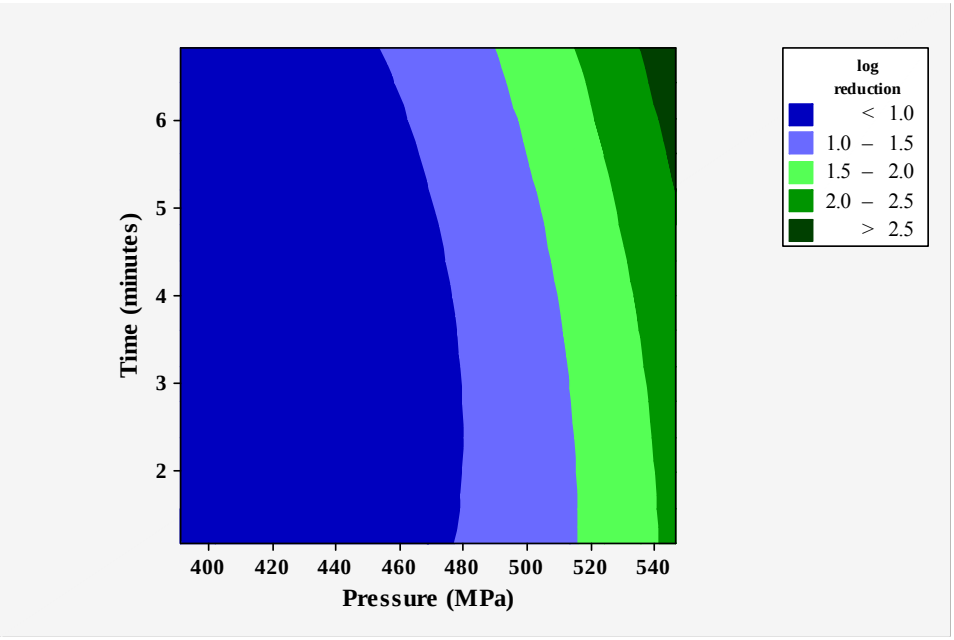


Figure 20: Contours of log reduction (log (N_O/N)) as a function of pressure and time

Table 12: Analysis of variance (ANOVA) for the CCD model

Source	DF†	SS	Adj. SS *	Adj. MS *	F	*p*
Regression	5	3.210	3.210	0.642	48.47	**0.000**
Linear	2	2.531	0.5497	0.2748	20.75	**0.002**
Square	2	0.626	0.6259	0.3129	23.63	**0.001**
Interaction	1	0.0532	0.0532	0.0532	4.02	0.092
Residual error	6	0.0794	0.0794	0.01324		
Lack of fit	3	0.0607	0.0607	0.02024	3.24	0.180
Pure error	3	0.0187	0.01874	0.006248		
Total	11	3.2896				

* Adjusted Sum of Square (Adj. SS) and Mean Sum of Square (Adj.MS) account for the number of factors (or size) of the model
† Degree of freedom

3.3 Comparison of steaming with high pressure processing

Since steaming of clams is commonly done before consumption, the bacterial inactivation due to high pressure was compared with that due to steaming process for clams. The clams for steaming were banded and steamed over a stainless steel perforated steamer in a closed vessel for 15 minutes as shown in **Figure 21**. High pressure processing of banded clams was done at 510 MPa (74 kpsi) for 3 minutes. These processing conditions were chosen because the clams used for this study were from special restricted waters and we had found from our CCD that pressures higher than 480 MPa were required for more than 1 log reduction of bacterial count in these clams. Pour plate technique was

used for bacterial enumeration in clams. Three clams from each batch (HPP and steamed) were analyzed and the plating was done in triplicates in order to get the standard deviation and standard error. **Figure 22** shows that there was no significant difference in log reduction (p>0.05) between steaming and high pressure processing the clams. In both cases, reduction of about 1.5 log was observed.

(a) (b)

Figure 21: Steaming of littleneck clams

(a):- Banded littleneck hard clams; (b):- steaming of these clams in a closed vessel.

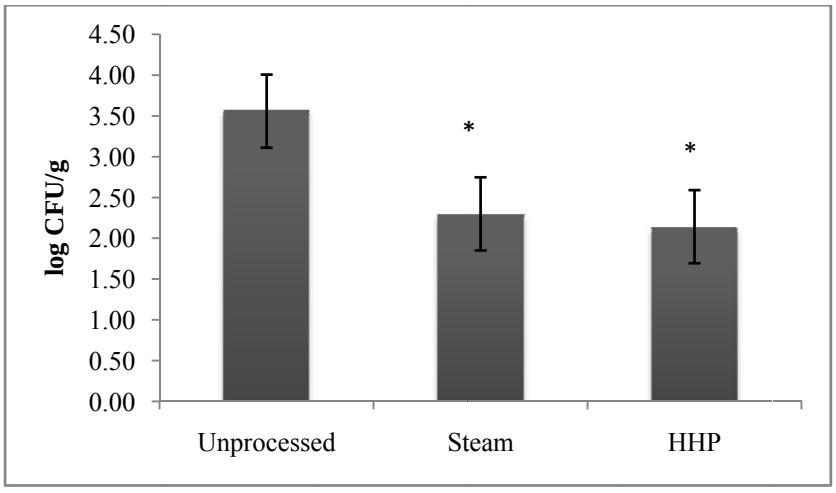


Figure 22: Comparison of steaming with HPP (HPP was done at 510 MPa and 3 minutes. Steaming was done for 15 minutes)

3.4 Effect of pressure cycling on bacterial inactivation

It has been shown that short pressure pulses are more effective than a single continuous pressure cycle for the inactivation of spores (Hayakawa et al., 1994). The rationale is that the first cycle induces germination of spores into vegetative cells and the subsequent cycles inactivate the germinated cells. A preliminary study was conducted wherein the clams from special restricted waters were pressurized at 414 MPa in 2 cycles of 4 minutes each, with a 2 minutes time interval between the 2 cycles. The inactivation of this process was compared to a single high pressure run at 414 MPa and 8 minutes. The microbial analysis was done immediately after the high pressure runs.

We found that the log reduction due to two pressure cycles was almost twice as compared to a single run (**Figure 23**) for the same dwell time of high pressure.

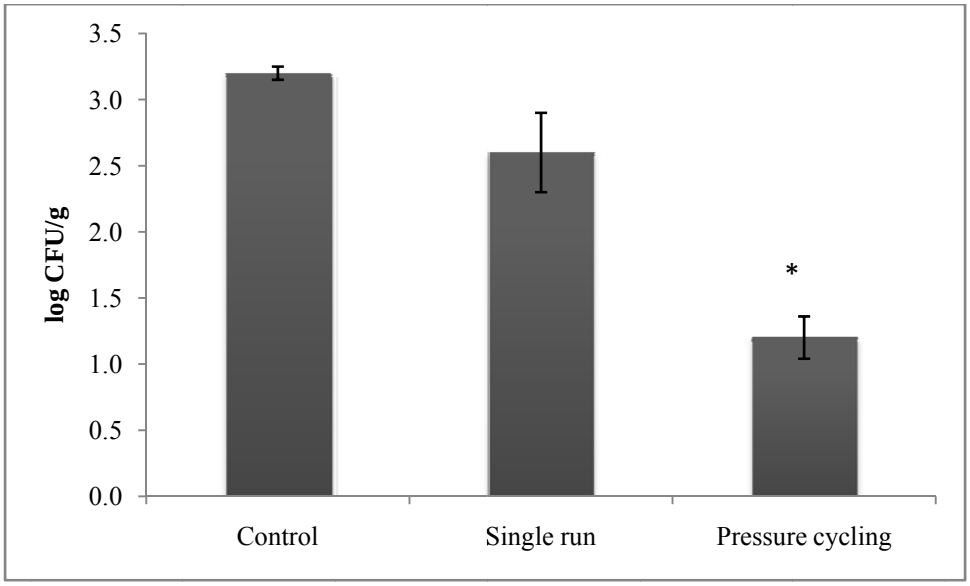


Figure 23: Effect of pressure cycling on inactivation

Pressure cycling: 2 consecutive cycles at 414 MPa for 4 minutes each.

Single run: 1 cycle at 414 MPa for 8 minutes.

3.5 Sensory Evaluation of Hard Clams

The rationale behind a sensory evaluation study was to find out the consumer preference for a HPP clam vs. an unprocessed clam. Unlike steaming, high pressure processing is not a household word. So we wanted to find out if the consumers would accept a processed clam as that would govern the profitability of the high pressure processed product.

After collecting the data from the subjects by the method which is described in detail in section 2.2.3, the liking was converted into a 15 point hedonic scale using the FIZZ™ software. The data were then analyzed in Microsoft Excel™. From the liking ratings of the 60 subjects who tasted the clams during the sensory evaluation, approximately 50% of them preferred the HPP clam ($\chi^2(1, N=60) = 0.266$ $p>0.05$) i.e., 28 subjects preferred the HPP clam whereas 32 subjects preferred the regular clam. **Figure 24** shows the average liking score of 60 subjects. We divided the data from 60 subjects into two parts viz., subjects who preferred the HPP clam and those who preferred the unprocessed clam. This was done in order to look closely at the liking attributes that were significantly different for the two clams. From the appearance point of view, subjects who preferred the HPP clam significantly liked the plump appearance ($p<0.05$) of this clam as compared to the unprocessed clam. The taste attributes preferred in the HPP clam by this subset of subjects were saltiness ($p<0.05$), firmness ($p<0.05$), and chewiness ($p<0.05$) as shown in **Figure 25**. Thus, appearance may be playing a role in the preference for the HPP clam as compared to the unprocessed clam. However, there could be a "halo effect" involved. This would mean that the subjects who preferred the HPP clam significantly liked the plumpness of this clam and hence the rest of the attributes

too. The same effect holds true for the 32 subjects who preferred the unprocessed clam. These subjects (**Figure 26**) liked the aroma of the unprocessed clam significantly and this might have affected their rating for the other attributes. More details of the "halo effect can be found in standard sensory evaluation books (Lawless and Heymann, 1998). Another conjecture is that since this subset of subjects preferred the unprocessed clam over HPP clam, the HPP clam does taste different. Also, for these subjects the liking score for appearance was not significantly different ($p>0.05$), but it was the taste driven preference, especially saltiness ($p<0.05$), chewiness ($p<0.05$), and firmness ($p<0.05$). In other words, they preferred the saltiness, chewiness, and firmness of the unprocessed clam.

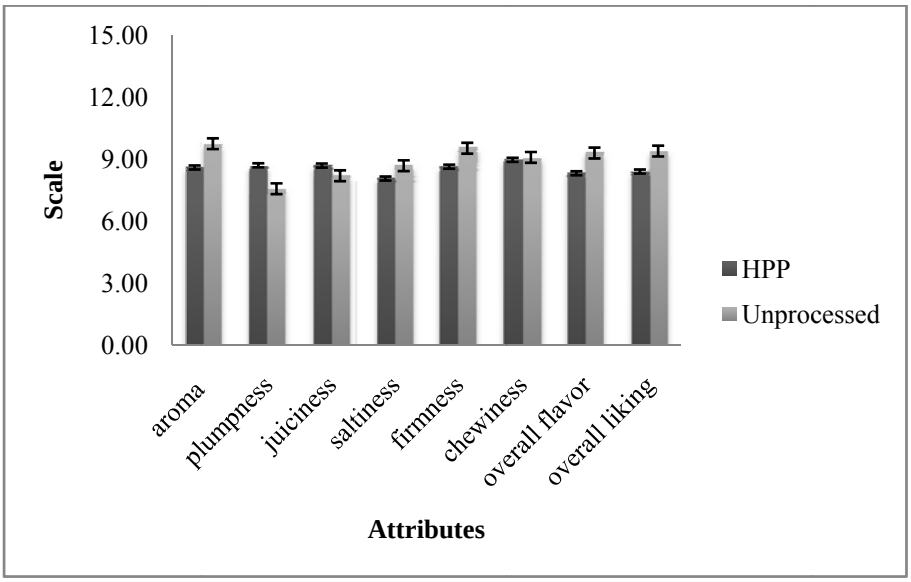


Figure 24: Attribute rating of the consumer panel study (n=60)

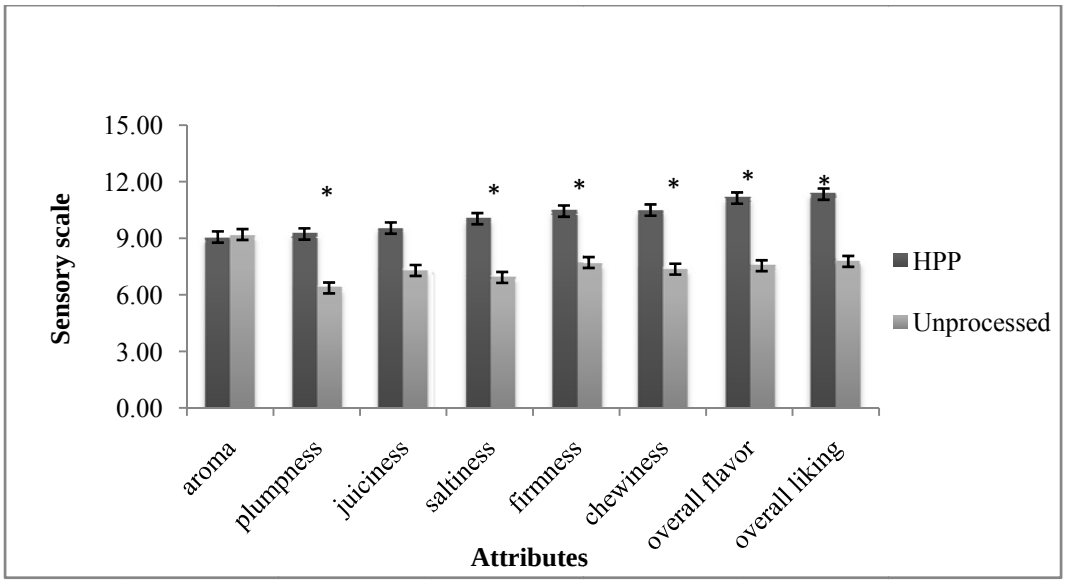


Figure 25: Attribute rating of the consumer who preferred HPP clam (n=28)

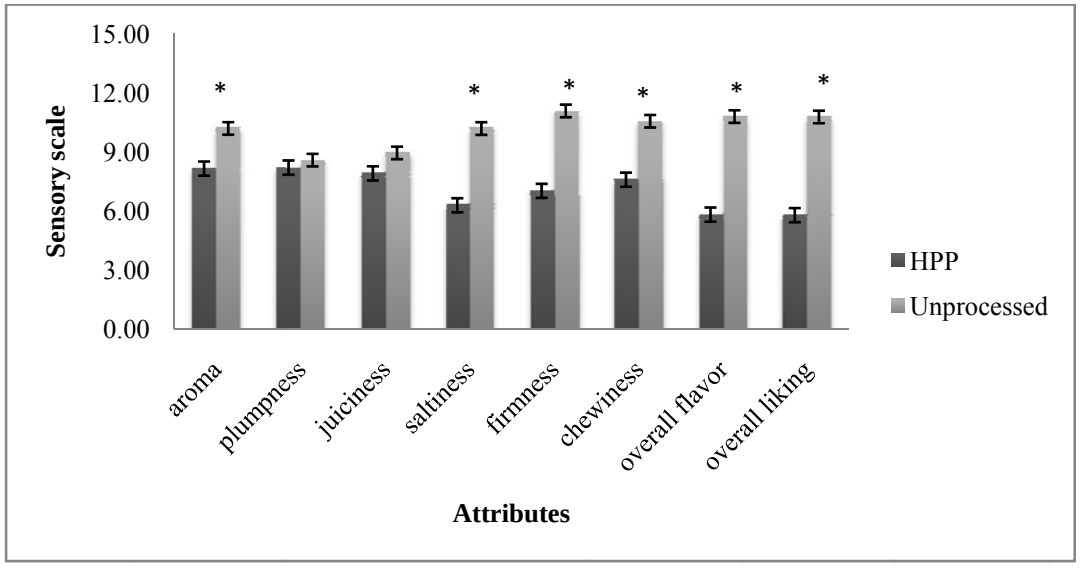


Figure 26: Attribute rating of the consumer who preferred unprocessed clam (n=32)

("*" indicates significant difference i.e., $p<0.05$)

In order to study the preference as a function of age, gender, and the frequency of consumption, we plotted these parameters (**Figure 27, 28, 29**).

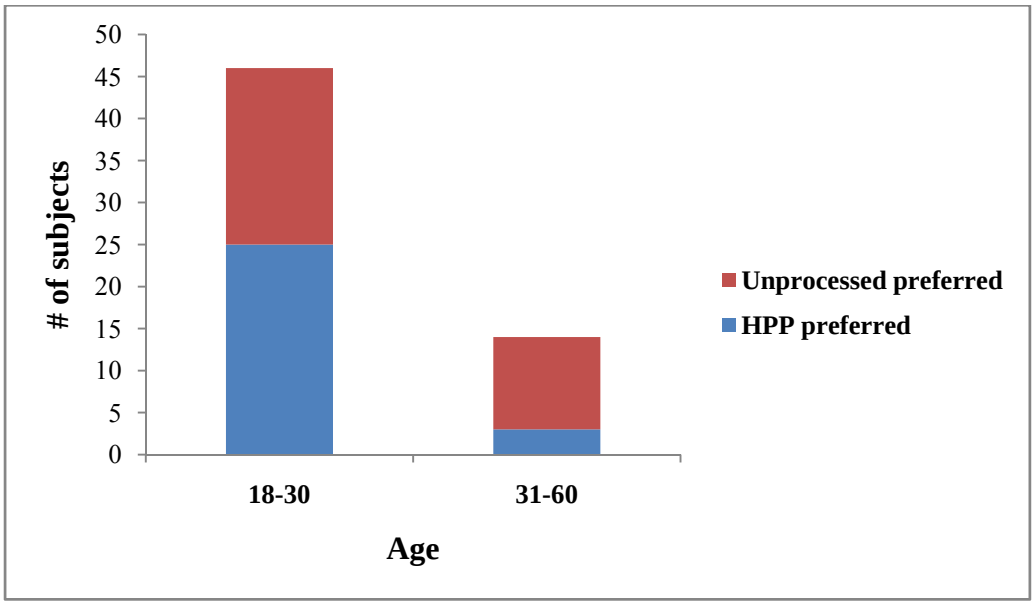


Figure 27: Preference as a function of age of the subjects

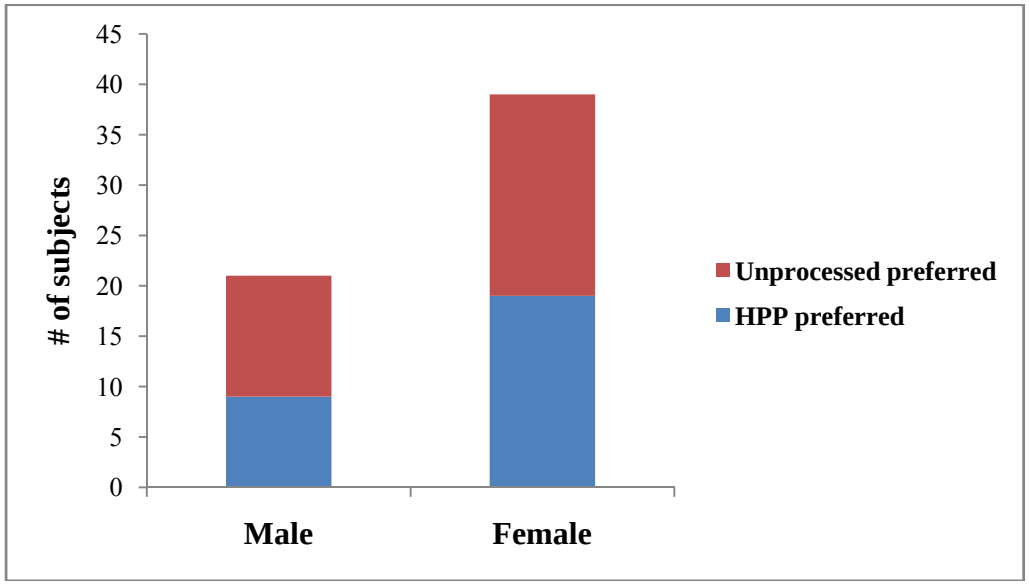


Figure 28: Preference as a function of gender of the subjects

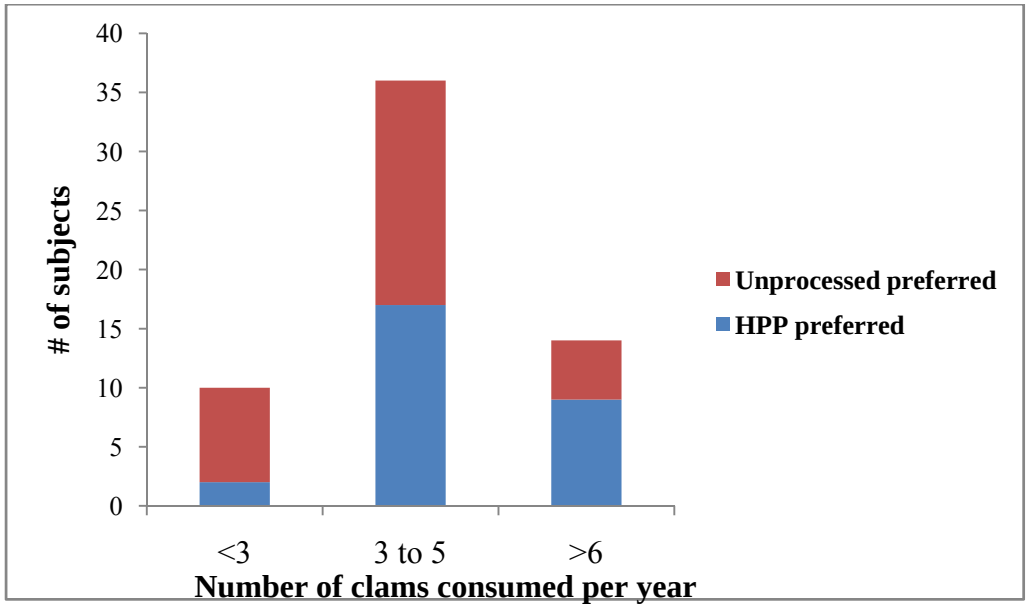


Figure 29: Preference as a function of consumption pattern of the subjects

The figures show that the preference for either of the clam was evenly split amongst the groups. Even though there were more subjects in the age group of 18-30 year old, there is almost equal preference in this age group for both the clams. We cannot say the same thing for the age group of 30-60 because the population is too small to infer. Amongst the males versus females, more females participated in the study (almost double than the males) and here too there is equal preference. In the consumption pattern study, there were more subjects with an average consumption of 3-5 clams/year and these subjects too were evenly divided.

3.6 Effect of pressure on the meat color and appearance

It was observed that HPP did affect the color of the clam meat. The texture was also different as evident by the plumpness of the meat. Also, the meat looked more gelatinous and plump. We found that the appearance was a function of pressure. **Figure**

30 shows the difference in appearance observed after processing at different pressures. More studies need to be done to quantify this difference in appearance.

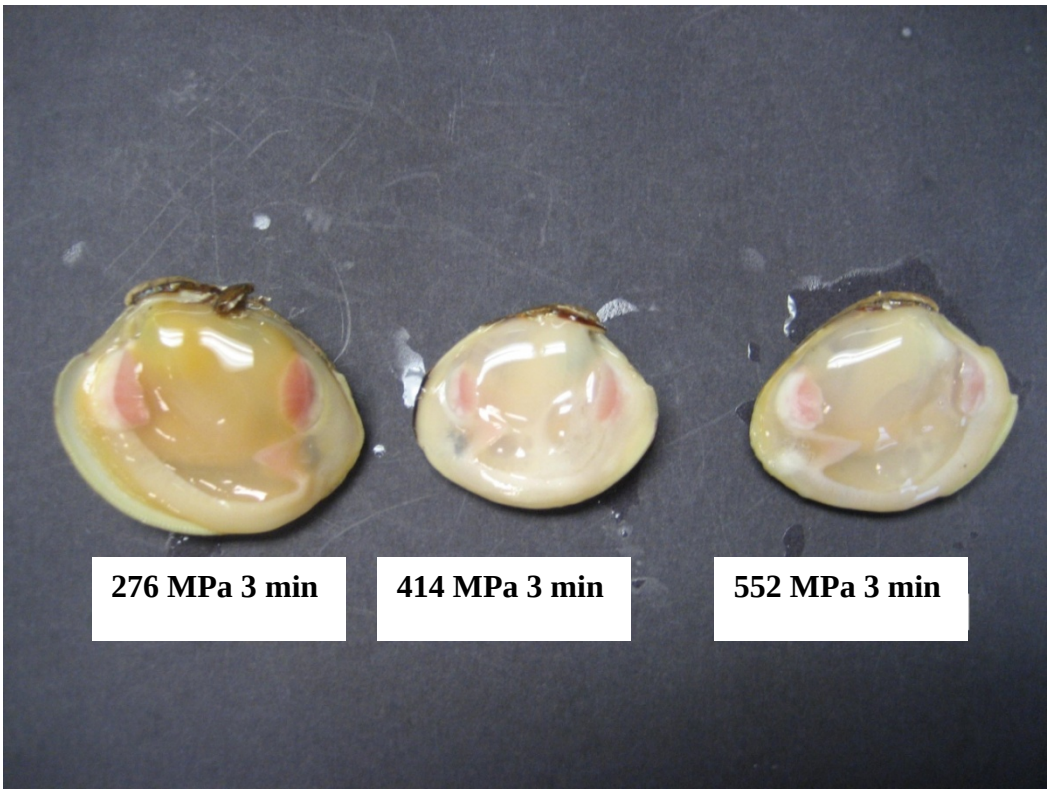


Figure 30: Change in color of clam meat due to HPP

4. CONCLUSIONS

The following conclusions could be drawn from this research:

1. The preliminary microbial experiments with high pressure processed littleneck clams were useful in method development wherein we found that the pour plate technique was better for enumeration of bacterial colony forming units in the clams as compared to the spread plating because of its lower detection limit. This is because pour plating accounts for both aerobic and anaerobic bacteria that may be growing in the clams.
2. Addition of 3% NaCl to the growth media did not enhance the enumeration of bacterial colonies.
3. The initial central composite design of experiments with pressure range of 255 to 407 MPa and dwell time range of 1 to 7 minutes gave less than 1 log reduction.
4. Experiments were conducted to understand the low inactivation achieved in the initial central composite design experiments for the clams and we found that the littleneck type of clams were more resistant to pressure as compared to the larger varieties viz., cherrystone and chowder clams.
5. The shell of the clams did not have a protective effect against inactivation due to pressure.
6. The log reduction due to high pressure was a function of initial bacterial load in the clams. Higher the initial bacterial count, higher was the inactivation observed due to pressure. This indicates the tailing effect which has been observed during

HPP for microbial survival curves as the surviving number of microbes approach <1000 CFU/g (FDA-CFSAN, 2000).

7. The final central composite design of experiments conducted in the pressure range of 386-545 MPa and time range of 1-7 minutes showed that pressures above 480 MPa gave more than 1 log reduction in clams from special restricted waters containing the natural microflora. The inactivation was primarily a function of process pressure. However, minimum process time of 1 minute was required. The response surface methodology approach gave a good predictive model (R^2=93.3%) for pressure and time optimization for the desired bacterial reduction.
8. There was no significant difference in the log reduction (p>0.05) between steaming (15 minutes at about 100°C) and high pressure processing at 510 MPa and 3 minutes. Both the processes gave up to 1.5 log reduction for littleneck clams from special restricted waters.
9. Preliminary experiments using pressure cycling showed that it was more effective for inactivation of bacteria than a single cycle process of the same duration.
10. Sensory study using a consumer panel showed that high pressure processed clams and unprocessed clams were liked equally by the consumers.
11. On further dividing the subjects based on their preference for either of the clams, a “halo effect” was observed wherein the subjects preferring the HPP clam significantly liked the plumpness and hence all the other attributes, whereas the subjects preferring the unprocessed clam significantly liked the aroma and hence the other attributes.

12. As the processing pressure increased, the clams looked lighter in color. More studies need to be done in terms of the changes in meat color and texture as a function of process pressure for hard clams.

The overall conclusion from this research is that high pressure processing could be used as a post harvest technology for raw hard clams from special restricted waters. High pressure processed clams would be liked and accepted as a value-added product in the market as indicated by our sensory evaluation study with hard clams from approved waters. The consumers and retailers would be benefited with a safe premium quality product. The restaurants would not have to hire an experienced shucker for serving clams on the half shell as these clams are already shucked due to pressure.

5. FUTURE WORK

Based on the work done so far, the following future work is suggested:

5.1 Effect of pressure cycling on bacterial inactivation

In our preliminary studies, it was found that pressure cycling was more effective than a single cycle of pressure. More work needs to be done to study the microbial inactivation at different number of pressure cycles, its effect on the meat quality, and its economic viability.

5.2 Effect of HPP on viruses, vibrios, and spores

Specific bacterial and viral species in hard clams need to be targeted to claim that the HPP hard clams from restricted waters are completely safe.

5.3 Change in color of meat at higher pressures

We found a visible change in the appearance of meat due to HPP. The meat looked lighter at high pressures (**Figure 27**). However, quantification of such change needs to be done using colorimetric techniques.

5.4 Quantification of change in texture/ chewiness

Our sensory evaluation showed that subjects perceived a marked change in the appearance. They described the HPP clam as plumper and gelatinous than the unprocessed clam. Instrumental analysis needs to be done to quantify these changes and correlate them with sensory data.

5.5 Quantification of change in saltiness

Taste change was realized during the sensory evaluation. More work needs to be done to confirm the change in taste attributes using an expert panel.

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APPENDIX A

RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY
INSTITUTIONAL REVIEW BOARD FOR THE PROTECTION
OF HUMAN SUBJECTS IN RESEARCH (IRB)
REQUEST FOR EXEMPTION FROM FULL IRB REVIEW

1	Does this activity involve research*? **Note:** Program evaluation may not meet the definition of research; see definitions in Section B of the instructions. **Note:** Student investigators must review the special criteria for students listed in section D of the instructions.	_X__ Yes	___ No - **STOP** - submission to the IRB is not required.
2	Do the individuals that will participate in this activity meet the definition of human subjects*? **Note:** For an individual to be considered a human subject, data ABOUT them must be collected.	_X__ Yes	___ No - **STOP** - submission to the IRB is not required.
Note: IRB review is ONLY required if an activity involves BOTH research AND human subjects. If you have answered "Yes" to questions 1 AND 2, proceed with completion of this form.			

Title of Project: Improving the safety of hard clams (*Mercenaria mercenaria)* through post harvest treatment with high hydrostatic pressure processing (HHP)
Name of Principal Investigator: Dr. Beverly Tepper
Mr.____ Ms.__X__ (*Please check one)*
Department: Food Science
Mailing Address: 65 Dudley Road, New Brunswick, NJ 08901.
Phone:732-932-9611 ext. 221 Fax: 732-932-6776 **Email**: tepper@aesop.rutgers.edu

Check one: ___X__ Faculty _____ Staff _____ Student*
Please note that undergraduate student investigators may not be named as the principal investigator on protocols and must instead name their faculty advisor.

Name of Undergraduate Investigator (*if applicable):*

***Check here [ ]** if Student is to receive a copy of Official Notices from the IRB.*

Department:

Mailing Address:

Phone: Fax: Email:

Completion of the **Human Subjects Certification Program (HSCP)** is required for the principal investigator, all key personnel, and the faculty advisor (if the principal investigator is a graduate student or is acting on behalf of an undergraduate student). Although HSCP certification is recommended prior to filling out this form, the form will be accepted for review with a certification status of *pending* for any named individual who has not yet completed the program. List below **all key individuals, including the principal investigator and any co-investigators**, who are responsible for the design or conduct of the study, their email address, and date of successful completion of HSCP (or *pending* status). If the Principal Investigator is a student, both student and advisor must be certified. An asterisk (*) should follow the advisor's name, for identification purposes.

Name	Email address	Date of HSCP certification
Mukund V. Karwe Professor, Dept. of Food Science	karwe@aesop.rutgers.edu	October 4, 2002
Beverly Tepper Professor, Dept. of Food Science	tepper@aesop.rutgers.edu	October 13, 2000
Shalaka Narwankar Graduate Student	shalakan@eden.rutgers.edu	February 19, 2007
Yvonne Koelliker Graduate Student	koelliker@aesop.rutgers.edu	July 25, 2005

Funding Status: __X___ Funded _____Funding Request Submitted

_____
Not Funded

If the project is funded, or an application for funding has been submitted, indicate the name of the funding agency or organization: **Unites States Department of Agriculture (USDA)**

Does any member of the research team have a financial interest in the research or its products or in the study sponsor? ___Yes _X__No If yes, please describe. (University policies regarding Conflict of Interest should be reviewed at <http://orsp.rutgers.edu/policies/ >) Attach additional sheets if necessary.

Instructions: *Answer each of the following questions, in order, (unless specifically directed otherwise) as they relate to the research project you would like to initiate. The comments immediately following each response will assist you in determining whether exemption is appropriate for this protocol. Continue until you reach "**STOP**". Relevant*

definitions and clarifications are contained in Section B of the instructions. Failure to comply with directions will result in a return of the Request for Exemption and a delay in the review process.

3	Does the activity present more than minimal risk* to subjects?	___ Yes - **STOP** - the protocol is not eligible for exemption. Stop here on this checklist. You must complete an IRB application for full review.	__X_ No
4	Does the research involve prisoners*, fetuses, pregnant women, human *in vitro* fertilization, deception or incomplete disclosure?	___ Yes - **STOP** - the protocol is not eligible for exemption. Stop here on this checklist. You must complete an IRB application for full review.	_X__ No

EDUCATIONAL ENVIRONMENTS

5	Will the research be conducted in established or commonly accepted educational settings, involving normal educational practices, such as (i) research on regular and special education instructional strategies, or (ii) research on the effectiveness of, or the comparison among, instructional techniques, curricula, or classroom management methods?	___ Yes- Exemption #1 may be applicable.	_X__ No - Exemption #1 does not apply.

TESTS, SURVEYS, INTERVIEWS, OBSERVATION OF BEHAVIOR

6	Will the research involve the use of educational tests (cognitive, diagnostic, aptitude, achievement), survey procedures, interview procedures or observation of public behavior?	_X_ Yes - Exemption #2 may apply.	___ No - Exemption #2 does not apply. <u>Go to question 10.</u>

* See definition in Section B of the instructions.

7	Is information that is obtained recorded in such a manner that: • subjects can be identified, directly or through identifiers linked to the subjects; <u>AND</u> • any disclosure of the human subjects' responses outside the research could reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects' financial standing, employability, or reputation? •	__X_ Yes - the protocol is not eligible for exemption under Category #2. Proceed to question #9 to determine whether exemption category #3 may apply.	___ No - If subjects can be identified, Exemption #2 may apply only if their responses, if disclosed, would not be harmful to them.
8	For projects that involve the use of educational tests, survey procedures, interview procedures, or observation of public behavior, will minors be involved in this project, other than as subjects in public observation of activities in which the investigator does not participate? (If minors will ONLY be asked questions about standard educational practices in an accepted educational setting, " NO" is the appropriate response. In this situation, Category 1 is appropriate.	___ Yes - **STOP** - the protocol is not eligible for exemption. Stop here on this checklist. You must complete an IRB application for full or expedited review.	_X__ No - Exemption #2 may be applicable.

9	Will the research: • involve the use of educational tests (cognitive, diagnostic, aptitude, achievement), survey procedures, interview procedures or observation of public behavior that is not exempt under exemption #2 (see questions 6 through 8 above); AND • (i) the human subjects are elected or appointed public officials or candidates for public office; OR • (ii) federal statute(s) require(s) without exception that the confidentiality of the personally identifiable information will be maintained throughout the research and thereafter?	___ Yes - Exemption #3 may be applicable.	_X__ No - Exemption 3 does not apply.

USE OF EXISTING DATA

10	Will the research involve the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available OR if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects?	___ Yes - Exemption #4 may apply.	_X__ No Exemption #4 does not apply. Go to question 12.
11	Are the records involved those of Rutgers students?	___ Yes - **STOP** - the protocol is not eligible for exemption. Stop here on this checklist. You must complete an IRB application for full or expedited review.	___ No Exemption #4 may be applicable.

RESEARCH OF FEDERAL GOVERNMENT BENEFIT/SERVICE PROGRAMS

12	Is the research or demonstration project conducted by or subject to the approval of Federal department or agency heads, and designed to study, evaluate, or otherwise examine: (i) public benefit or service programs; (ii) procedures for obtaining benefits or services under those programs; (iii) possible changes in or alternatives to those programs or procedures; or (iv) possible changes in methods or levels of payment for benefits or services under those programs?	• ___ Yes - Exemption #5 may be applicable.	__X_ No Exemption #5 does not apply.

FOOD TESTING

13	Do the activities involve taste and food quality evaluation and consumer acceptance studies wherein, (i) wholesome foods without additives are consumed or (ii) a food is consumed that contains a food ingredient at or below the level and for a use found to be safe, or agricultural chemical or environmental contaminant at or below the level found to be safe, by the Food and Drug Administration or approved by the Environmental Protection Agency or the Food Safety and Inspection Service of the U.S. Department of Agriculture?	_X__ Yes - Exemption #6 may be applicable.	___ No Exemption #6 does not apply.

14	Do ALL procedures of the proposed research activity fall into one or more of the exemption categories described in questions #5 through 13?	__X_ Yes - All procedures fall into one or more of the categories described above. The protocol is eligible for exemption under the category(ies) indicated.	___ No - **STOP** - There are other procedures that do not fall into these category descriptions. The protocol is not eligible for exemption.

Statement of principal investigator:
I certify that I have answered each of the questions on the checklist accurately as they pertain to the research described in the attached protocol entitled:

_____
_____

I understand that ALL of the procedures for research on human subjects described in the protocol must fall within one or more of the exemptions categories described within this checklist in order for the project to qualify for exemption from full IRB review.

_____ _____
Name (printed) of principal investigator Signature of principal investigator

Statement of faculty advisor for student investigator:
As faculty advisor/course instructor/dissertation Chair for the student named above, I certify that I am familiar with Rutgers University policies and federal regulations as they apply to research involving human subjects. I have advised and/or assisted the student in the preparation of this application and have reviewed it for completeness and accuracy. I endorse the study and certify that it fulfills all the guidelines and requirements for IRB review.

_____ _____
Name (printed) of advisor (*for graduate research*) Signature of advisor Date
Advisor's telephone number: Advisor's email address:
In order to perform a substantive review of the protocol, and to ensure that exemption is appropriate, a complete research protocol (narrative description of the project), consent documents and study instruments are required. **If study instruments, consent forms or assent forms have not yet been developed, please supply sample documents.** The final versions must be submitted to the IRB for review and approval prior to implementation.

APPENDIX B

Screening Form
Sensory Evaluation - Clams on the Half-Shell

Interviewer: please prompt for all questions.
A positive response to question # 5 or # 6 automatically disqualifies the subject from participating

1. Age:

2. Gender:

3. How often do you eat clams on the half-shell?

   ☐ <3 times/yr ☐ 3-5 times/yr ☐ ≥ 6 times/yr

4. Do you have asthma? Yes ☐ No ☐

5. a. Do you have any food allergies? Yes ☐ No ☐

   If yes, explain: _____

   If yes,

   b. Do you have an allergy to seafood? ☐ Yes ☐ No

6. Have you ever had any of the following reactions to seafood:

   ☐ Hives or a rash

   ☐ Trouble breathing

   ☐ Swelling of the mouth or throat

   ☐ Gastrointestinal symptoms

APPENDIX C

Rutgers Food Science Department
Sensory Evaluation – CLAMS ON THE HALF-SHELL

Subject #_____

Instructions:

You will receive two samples to evaluate. Please evaluate the sample that matches the sample number that appears below. Rinse your mouth thoroughly with water before you begin and between the samples.
Draw a mark on each line for your answers.
Smell the sample and write your response on page 1. Then taste the sample and fill page 2

Sample #_____

1. AROMA

|----------|

LIKE EXTEREMELY DISLIKE EXTREMELY

2. APPEARANCE - PLUMPNESS

|----------|

LIKE EXTEREMELY DISLIKE EXTREMELY

3. APPEARANCE - JUICINESS

|----------|

LIKE EXTEREMELY DISLIKE EXTREMELY

4. SALTY TASTE

|----------|

LIKE EXTEREMELY DISLIKE EXTREMELY

5. FIRMNESS

|----------|

LIKE EXTEREMELY DISLIKE EXTREMELY

6. CHEWINESS

|----------|

LIKE EXTEREMELY DISLIKE EXTREMELY

7. OVERALL FLAVOR

|----------|

DISLIKE EXTREMELY LIKE EXTREMELY

8. OVERALL LIKING

|----------|

DISLIKE EXTREMELY LIKE EXTREMELY

9. How often do you eat clams on the half-shell? Please circle….

<3 times/yr 3-5 times/yr ≥ 6 times/yr

10. What kind of condiment do you usually use when you eat clams on the half-shell?

☐ Plain ☐lemon juice ☐ hot sauce ☐ cocktail sauce ☐other:———

11. Which one would you prefer?

☐High pressure processed clam on a half-shell

☐ Raw unprocessed clam on a half-shell

Comments:

APPENDIX D

Fact Sheet

SENSORY EVALUATION OF FOOD AND PERSONAL CARE PRODUCTS

Principal Investigator: Beverly J. Tepper, Ph.D.
Sensory Evaluation Laboratory Room 211
Department of Food Science
Rutgers University
(732) 932-9611 ext. 221

Thank you for participating in this study. In today's session you will be asked to taste and evaluate raw clams. You will also complete a brief questionnaire on your clam eating preferences and clam eating habits.

If you are allergic to shellfish, please alert the server as you will be unable to participate in the test.

All items used during the study are either commercially available products or that have been processed using approved procedures. None of the activities you will be participating in pose any foreseeable risks.

At the completion of your participation will receive a payment of $15.00 .

Your participation in this study is completely voluntary and you have the right to withdraw at any time without penalty. The information collected in this experiment will be collected anonymously - your personal identity cannot be linked with your responses.

Rutgers University has made no general provision for financial compensation or medical treatment for any physical injury resulting from this research. Questions about this research can be directed to the Principal Investigator at the number listed above or the Rutgers University Office of Research and Sponsored Programs at (732) 932-0150 ext. 2104.