EFFECT OF HIGH HYDROSTATIC PRESSURE PROCESSING (HHPP) ON *ESCHERICHIA COLI* O157:H7 CONTAMINATED GROUND BEEF

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ABSTRACT OF THE THESIS

Effect of High Hydrostatic Pressure Processing (HHPP) on *Escherichia Coli* O157:H7 Contaminated Ground

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Escherichia coli O157:H7 contaminated ground beef has been linked to major food recalls in recent years, creating the need for improved processing methods. High hydrostatic pressure processing (HHPP) is a reliable non-thermal processing method used to process foods such as oysters, meats, and juices to improve microbiological safety while retaining quality and organoleptic properties. The application of HHPP with pressure cycling to boost the effectiveness of pressure to inactivate *E. coli* O157:H7 in ground beef was the focus of this research.

The effect of the combination of pressure (300-600 MPa), holding time (6-60 min) and temperature (7-45 $^{\circ}$C) on inactivating *E. coli* JM109 and *E. coli* O157:H7 (ATCC 43895) in ground beef was

investigated. Change in the color of ground beef was also determined. Compared to holding time, pressure and temperature had a significant effect on the color of ground beef. Experiments were conducted using single strains and a 6-strain cocktail from 25 °C to 45 °C, at 400 MPa and at pre-determined pressure cycles totaling a holding time of 15 minutes. The reduction for *E. coli* O157:H7 population increased from 3 to 5 logs as the number of cycles was increased from 1 to 5. The fate of surviving cells, post-processing and during frozen storage, was also determined by plating on selective and non-selective media. It was found that HHPP caused substantial sub-lethal injury leading to further inactivation during frozen storage.

The effect of HHPP on the color of ground beef was investigated. Process temperature, as compared to pressure or time, has the most impact on the color change immediately after processing. Higher process temperature (45 °C) resulted in an undesirable color change. Refrigerated or frozen storage had minimal impact on the color of HHPP ground beef.

These results suggest that HHPP has the potential to decrease levels significantly or eliminate *E. coli* O157:H7 from ground beef; especially when pressure cycling is applied. A 5 log reduction in *E. coli* O157:H7

in ground beef was achieved at 400 MPa at 25 °C with 5 pressure cycles with total time at high pressure of 15 min. Frozen storage at -20 °C had an additional negative effect on survival of *E. coli* O157:H7.

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	Degree Celsius
E. coli	*Escherichia coli*
FDA	Food and Drug Administration
FIFO	First-In/First-Out
HHPP	High hydrostatic pressure processing
HUS	hemolytic-uremic syndrome
lb	Pound (weight)
lbs	Pounds (weight)
µL	Microliters
mL	Milliliters
min	Minute
MPa	Mega Pascal
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
US	United States
USDA	U.S. Department of Agriculture
Oz.	Ounce

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I. Introduction

I.1. Beef

Pork, poultry, and beef are the top three most widely consumed meats in the world, accounting for about 38%, 30% and 25% of the meat production worldwide, respectively (Raloff, 2003). Beef consumption in the U.S., Brazil, and the People's Republic of China is greater than any of the other countries in the world (USDA, 2009).

The meat derived from domestic cattle, including cows, bulls, heifers, and steers, is referred to as beef. Beef is the principle meat in some countries, including Pakistan and Afghanistan. However, in other countries, it is treated as a taboo food for some particular religions, including Hindus and Jains in India culture (Timesofindia, 2011).

I.2. History of Ground beef

Although Americans consume a lot of ground beef each year, ground beef was not created in the United States. The Mongolian and Turkic tribes according to accepted theory are the ones that thought of shredding the low quality meat to make it more digestible, which became ground beef. (http://www.gourmetsleuth.com/Articles/Meats-Poultry-Fish-Seafood-652/ground-beef.aspx, 05/30/2012)

I.3. Ground beef

Ground beef is a minced meat food, which is usually made up of various grades of meats from different parts of cows that is finely chopped by a meat grinder. However, mostly, these portions are the lower-grade types of meat, thus less tender and less popular portions beef are used to manufacture ground beef, which is listed in many recipes including hamburgers and cottage pie.

I.4. Ground Beef Consumption in the U.S.

Beef consumption is huge in the US; roughly 42% of beef is consumed in the form of ground beef. According to the USDA, on a per capita basis Americans consume about 67 lbs. of ground beef per year. With respect to the ground beef market, restaurants and other commercial retailers make up only a small portion; households are the largest customers. The trend may be explained by the economical price of ground beef products compared to other beef products, and the myriad of ways to prepare it, including hamburger, sandwiches, casseroles, and so on. (http://www.gourmetsleuth.com/Articles/Meats-Poultry-Fish-Seafood-652/ground-beef.aspx, 05/30/2012)

A few facts about U.S. beef:

- 2011 Cattle inventory: 92,582,400 (USDA NASS)
- Economic impact: $44 billion in farm gate receipts (USDA NASS)
- 2011 beef exports: $4.08 billion, 2.35 billion pounds (USMEF)
- Top export markets: Mexico, Canada, Japan, Hong Kong and Taiwan

- 33.5 million head of cattle harvested under USDA inspection; 26 billion pounds of beef harvested under USDA inspection
- Per capital spending on beef in 2009: $261.90 (47.8 percent of per capital spending on all meat)

(http://www.beefusa.org/beefindustrystatistics.aspx, 05/30/2012)

I.5. Types of ground beef

According to the USDA standard, ground beef should contain less than 30% fat, and more than 70% lean. Only products defined and sold as “hamburger” can add fat. Additionally, the USDA allows ground beef products to have added seasonings, but definitely no water, fillers, phosphates, extenders, or binders.

I.5.1. Standard Grades of Ground Beef

Table 1: Standard grades of ground beef

Cut	Ground Beef	Ground Chuck	Ground Sirloin	Ground Round
% of Lean	70% lean	80% lean	85% lean	90% lean
% Of Fat	30% fat	20% fat	15% fat	10% fat
Calories per ounce*	77	76	73	65

I.5.2. Other grades

In addition to standard grading there are some new varieties on the market

- ***Grass Fed Beef***

Grass fed means that for the lifetime of the animal it consumed a diet of forage consisting of grasses, legumes, and Brassica. Animals cannot be fed grain or grain byproducts. However, mineral and vitamin supplementation are allowed.

- ***Organic Beef***

Animals must be fed 100% organic feeds that contain no animal parts. Additionally the animals must be allowed access to the outdoors, sun, grass and pasture.

- ***Kobe / Wagyu Beef***

Kobe beef grown in the U.S. is referred to as Wagyu beef. The typical ground beef is “chuck” which offers enough fat to be moist with good flavor. This is the most expensive ground beef available and can be found in some specialty markets as well as online.

(http://www.gourmetsleuth.com/Articles/Meats-Poultry-Fish-Seafood-652/ground-beef.aspx, 05/30/2012)

I.6. Ground beef manufacturing process

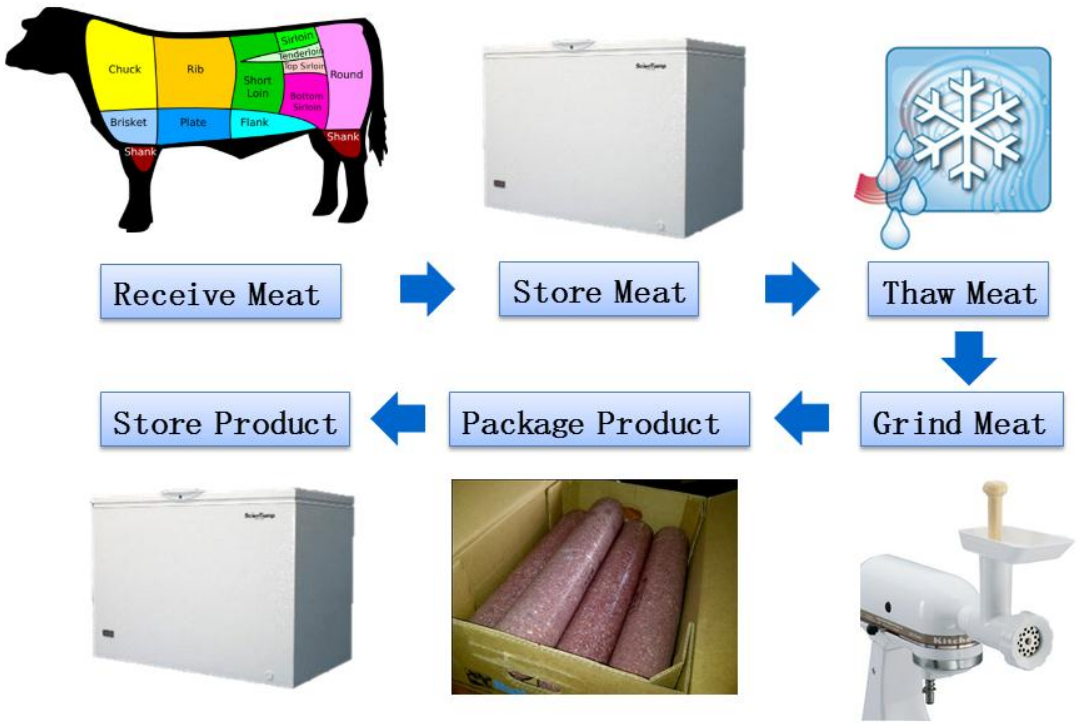


Figure 1: Ground beef manufacture process.

The process for manufacturing ground beef consists of the following (**Figure 1**): receiving, storing, thawing, and grinding meat, packaging product, storing, and finally followed by loading and shipping.

I.6.1. Receiving Meat

Incoming meats, as well as the trucks, containers and carriers of raw materials, are evaluated to ensure they meet the plant-established purchase specifications and transporting meat requirements.

I.6.2. Non-Meat Items

All non-meat items, such as packaging materials, seasonings/spices, etc. need to meet the plant-established specifications, and maintain the integrity of the items after the company accepts, stores, handles and uses the non-meat items.

I.6.3. Storage of Raw Materials

A First-In/First-Out (FIFO) basis is recommended for using the raw materials. In the meantime, a plant specified product rotation/inventory control schedule is also a good way for storage of raw materials which should be kept at temperatures that maintain the quality, unless tempering or thawing is required prior to use. A package should contain an in-plant tracking system that maintains integrity throughout the storage period.

I.6.4. Tempering/Thawing of Frozen Materials

The traceable package integrity is also important in this procedure. The tempering/ thawing should be done under an adequately monitored and documented time/temperature controlled manner prior to use.

I.6.5. Grinding/Processing

The temperature should be maintained and documented throughout this processing procedure including weighing, mixing, blending, coarse and final grinds, forming, packaging, and labeling. Traceable package integrity should be carried out during grinding, as well as steps to prevent species cross-contamination. Sensory, physical, chemical, and microbiological evaluations also need to be in place to ensure proper end product characteristics.

I.6.6. Storage of Finished Product

A FIFO or a plant specified product rotation/inventory control schedule should be maintained for finished products. The product should be stored at plant-designated time/temperatures, as well as it should contain the traceable system to ensure package integrity.

I.6.7. Loading and Shipping

In order to prevent any product deterioration causing by temperature abuse or improper handling practices, all finished products should be properly handled on the loading docks and during transportation, while all the transportation equipment should be passed the inspection and met the transportation requirement.

For intended use, recall or market withdrawal purposes, it is recommended all the finished products are identified or coded.

(http://extension.psu.edu/food-safety/educators/food-safety-lessons-for-middle-school-students/food-safety-in-food-processing-and-manufacturing/FSLssn11-2-10-05.pdf, 06/20/2012),(http://www.haccpalliance.org/sub/food-safety/Nmagmp2.pdf, 6/20/2012)

I.7. *E. coli* O157:H7

Escherichia coli are present in the intestines of animals and humans. It is a type of fecal coliform bacteria. There are hundreds of serotypes of *E. coli*. Most of the serotypes of *E. coli* are harmless. One serotype, known as *E. coli* O157:H7 produces harmful toxin and is the key reason causing food borne and water borne illness (www.epa.gov/safewater/ecoli.html, 2002). The letters and numbers in the name of the bacterium indicate the specific markers found on the surface and distinguish them from each other.

E. coli infection can be either foodborne or waterborne. One can become infected by eating inadequately cooked contaminated meat, drinking unpasteurized milk or juice, sprouts, lettuce, and salami, or swimming-in sewage contaminated water or drinking inadequately chlorinated water.

(www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacoli_g.html, 2003).

I.8. Outbreaks of *E. coli* O157:H7 in ground beef

In the United States, in Sept. 2007, over 21.7 million pounds of frozen ground beef patties were recalled by USDA because of contamination with *E. coli* O157:H7. Cases occurred in 8 states, 21 patients (64%) were hospitalized, and 2 developed a type of kidney failure called hemolytic-uremic syndrome (HUS).

In July 2008, a multistate outbreak of *E. coli* O157:H7 foodborne illness was reported. One patient developed HUS out of 49 cases. Over 5.3 million pounds of ground beef made by Kroger/Nebraska Beef Ltd was recalled.

In 2009, from June to Nov., there were two recalls of *E. coli* O157:H7 contaminated ground beef, which infected people in 14 states. Seven of the 36 cases developed HUS.

It is estimated that *E. coli* O157:H7 contaminated ground beef causes 62,000 illnesses, 1,800 hospitalizations, and 50 deaths in the United States every year.

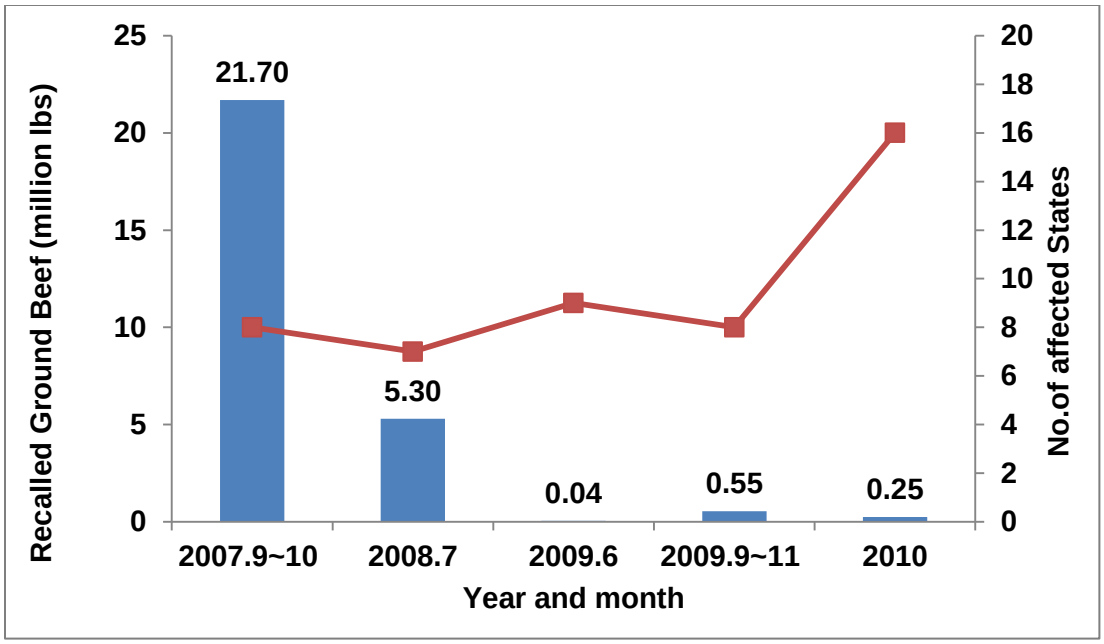


Figure 2: Recent outbreak of *E. coli* O157:H7 contaminated ground beef.

(CDC 2007; CDC 2008; CDC 2009; CDC2010)

I.9. *E. coli* O157:H7 survival in ground beef

The contamination and survival of *E. coli* O157:H7 in ground beef can be explained by many reasons.

First of all, the farming and slaughterhouse practices need to be improved, especially production practices and post-slaughter sanitizing measures, in order to reduce the presence of bacteria in cattle and on the carcass. In this case, it is possible to diminish the chances of beef products becoming a common source of outbreaks of *E. coli* O157:H7. (Ebel et al., 2004; Koohmaraie et al., 2007).

Secondly, cross-contamination during processing also contributes to contamination and survival of *E. coli* O157:H7, especially during grinding of beef. At this stage, the ground beef will make contact with equipment surfaces in the mixing, blending, cutting and forming processes (Erikson and Doyle, 2007). If the equipment is not properly cleaned and sanitized, then the entire lot of the ground beef would be considered contaminated.

Meanwhile, the ground beef itself provides an ideal medium for the growth of microorganisms, such as *E. coli* O157:H7, since beef is nutrient-rich with complex composition (Hugas et al., 2002).

Insufficient thermal treatment of ground beef allows pathogens such as *E. coli* O157:H7 to survive. This is very common in a consumer's home in the US, resulting in numerous cases of *E. coli* O157:H7 infection (Rhee et al., 2003).

Thus, an alternative effective processing method is needed to improve the microbial safety of ground beef, and HHPP is the one method that will be discussed in this thesis.

II. Background

II.1. High Hydrostatic Pressure Processing (HHPP)

High Hydrostatic Pressure Processing (HHPP) is a food processing method that statically treats a food product at or above 100 MPa by means of a liquid transmitter, in order to achieve microbial inactivation or consumer-desired qualities (Juneja and Sofos, 2002). The pressure that foods may be subjected to goes up to 1000 MPa (145,000 psi).

HHPP provides safer, higher quality and nutritious product, retains sensory quality and higher consumer acceptance than both conventional (e.g. thermally processed foods) and other non-thermal technologies (Doona and Feeherry, 2007). The main commercial advantage of high pressure processing is that packaged products, whether processed or raw, can be treated by HHPP. High pressure acts instantaneously and uniformly throughout the food product, regardless of the size, shape, and food composition (for most foods, especially those that are homogeneous and do not contain any inclusions such as bones), with minimum loss of food quality. It retains the freshness, quality, flavor, color, and nutritional properties of foods, denatures enzymes, extends shelf life, inactivates/kills microbes, reduces the need for preservatives and eliminates post-process contamination.

Different composition and size of the product could influence differently HHPP treatment (Yağız et al., 2007; Yağız et al., 2009). Based on the processing conditions, potential detrimental changes, in appearance, texture and chemical

parameters, such as pH, in HHPP products might occur. Therefore a judicious selection of treatment parameters, time, pressure and cycles, can minimize the undesirable changes (Erkan et al., 2010a,b).

The HHPP works by disrupting the structure of secondary- or tertiary-bonded molecules, but covalently bonded molecules are generally not affected (Hoover etc., 1993; Mertens, 1993a, b), resulting in the denaturation of large protein molecules. Many components responsible for sensory and nutritional quality such as color, flavor components and vitamins, remain unaffected (Mertens, 1993a, b).

During HHHP, a food product is placed in a pressure vessel, submerged in a pressure-transmitting medium which could be water, castor oil, silicon oil, sodium benzoate (aqueous), ethanol or glycol. By using a piston to compress the medium or pumping more medium into the vessel, the pressure in the vessel is increased. If water is used as the transmitting media, the temperature of water goes up by about 3°C per 100 MPa increase because of adiabatic compression. The pump is turned off once the desired pressure is reached, and pressure is held for a desired period of time. Depressurization then occurs and the product removed. These three stages are shown in **Figure 3.**

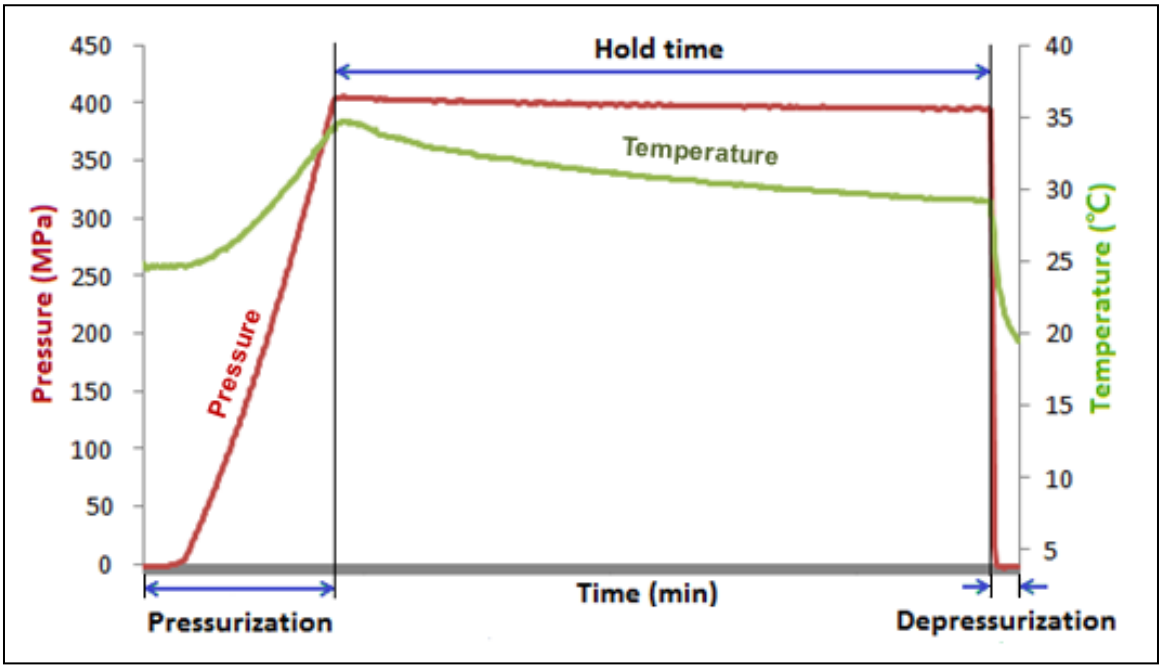


Figure 3: Pressure vs. time and temperature vs. time data for ground beef during HHPP at 400 MPa (hold pressure).

The three stages are:

1. Pressurization: Pressure goes up to the desired level, while temperature increases as well due to the adiabatic compression heating

2. Hold Time: Time period for the pressure to be held at a desired value; temperature goes down a little due to heat loss to thick wall of vessel

3. Depressurization: Pressure goes down rapidly to ambient pressure and temperature goes down and ends up lower than initial temperature.

II.2. Commercial application of HHPP

The very first high pressure-processed products were strawberry, apple, and kiwi jams, which were launched in the Japanese food market in 1990 (Knorr, 1993). America followed the wave with high pressure treated guacamole, and other avocado based products. In 1997, the first industrial production of guacamole in North America was manufactured by Avomex, currently known as Fresherized Foods (Balasubramaniam et al., 2008). Over the past ten years, there has been a constant increase in the use of high-pressure technology. A dramatic rise in the number and variety of meat and meat products, as well as other food products, treated by high pressure technology occurred as commercial scale HHPP food production developed (Garriaga and Aymerich, 2009). By 2010, 158 industrial HHPP installations were in use worldwide for commercial scale food production. Worldwide growth in the food industry for use of high-pressure processing technology is shown in **Figure 4** (www.nchyperbaric.com, 05/31/2012). The top 20 HHPP foods include meats and ready-to-eat meats, seafood, fruits, vegetables, juices, smoothies, jams and jellies (**Figure 5**) (www.hiperbaric.com, 05/31/2012). Among meat processors, Hormel Foods, Kraft Foods, Perdue, Foster Farms, and Wellshire Farms have successfully utilized HHPP technology for a variety of minimally processed meat products (Balasubramaniam et al., 2008).

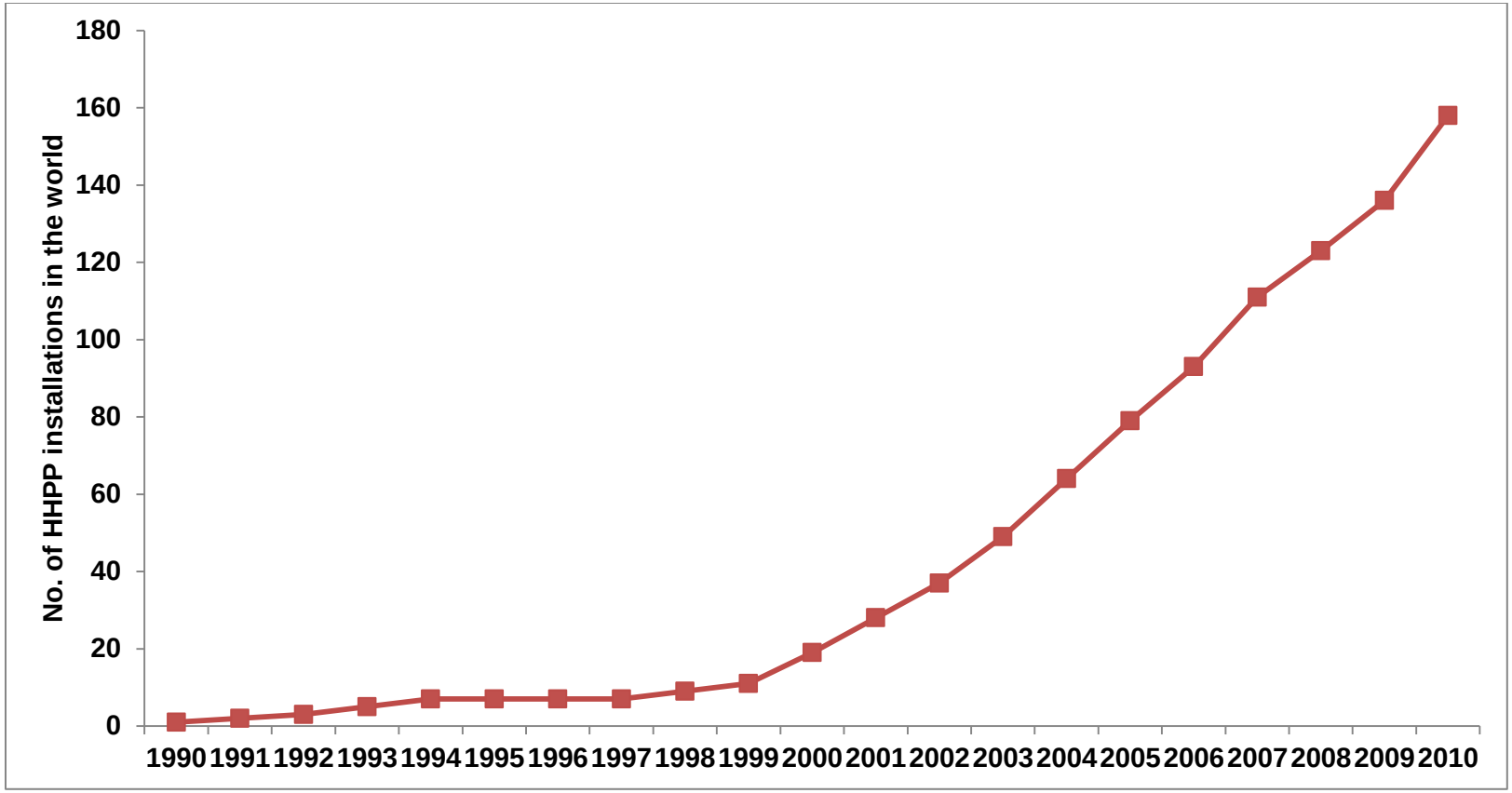


Figure 4: The world growth of the food industry use of high-pressure processing technology.

(www.nchyperbaric.com, 05/31/2012)

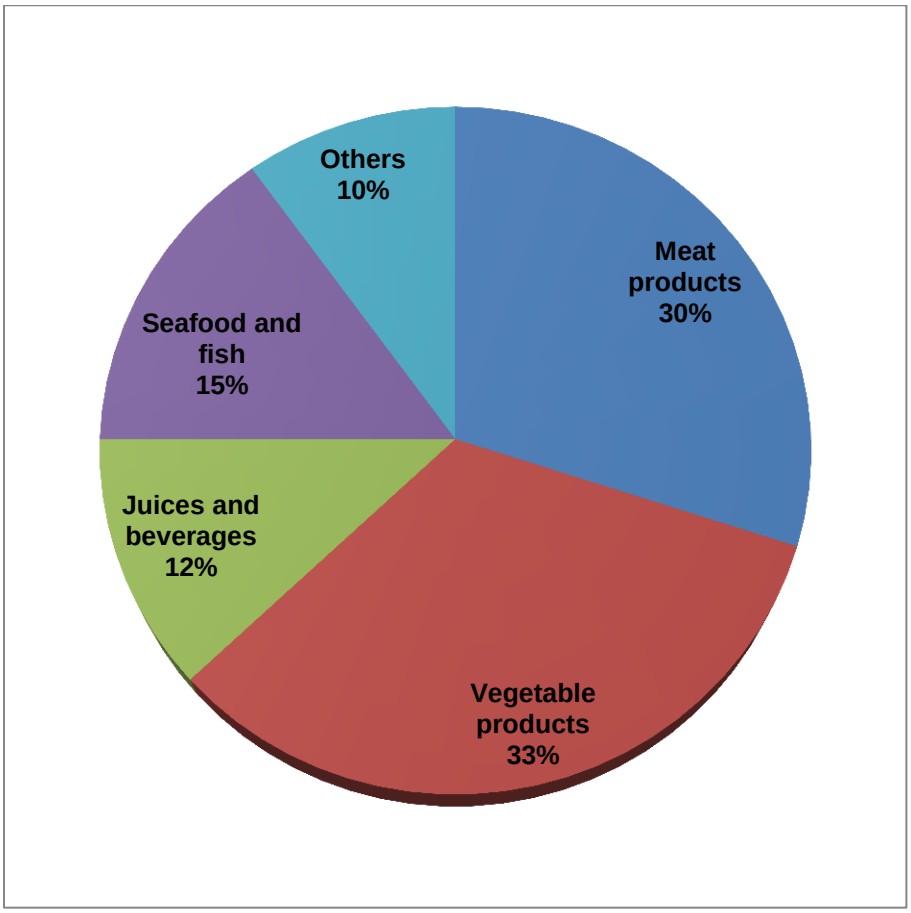


Figure 5: HHPP products. (www.hiperbaric.com, 05/31/2012)

II.3. Microbial inactivation by HHPP

The fact that high pressure treatment could prevent souring of milk, suggesting that high pressure inactivates microorganisms and thus preserves food, was first discovered by Bert H. Hite in 1899. (Hendrickx and Knorr, 2002). However, it wasn't until early 1980s that high pressure technology was thoroughly investigated in food and biological system (Knorr, 1993).

It is still not well understood what the mechanism(s) of microbial inactivation associated with HHPP are. As far as we know, morphological changes, cell membrane perturbation, biochemical changes, and genetic changes, caused by HHPP compression inactivate microbial cells (Mañas and Mackey 2004).

Also, the temperature of the pressure media increases due to the compression heating, which likely has a negative effect on the survival of microbial cells (Hendrickx and Knorr, 2002). The denaturation of proteins in the cell membrane at high pressures results in an increased permeability of the cell membrane, which is one of the factors responsible for inactivation (Smelt, 1998; Kato, 1999).

II.4. Studies conducted on *E. coli* O157:H7 and HHPP in buffer and in foods

Most vegetative bacteria, molds, and yeasts can be inactivated by high pressure. However, *E. coli* O157:H7 is less sensitive to HHPP compared to *Yersinia enterocolitica*, yeasts and molds. Thus, higher pressures are required to inactivate *E. coli* O157:H7 (Patterson et al., 1995).

The pressure sensitivity of vegetative bacteria is influenced by the composition of the food matrix, as well as general intrinsic food properties such as pH and water activity. Chen and Hoover (2003b) indicated that when *Y. enterocolitica* is treated at 350–450 MPa, at 22 °C for 10 min, whole UHT milk showed a strong baroprotective effect compare to phosphate buffer, with 3.5-4.5 log units less inactivation. Hugas et al. (2002) compared cooked ham homogenized with water (3:1) with phosphate buffer after 500 MPa at 40 °C for 10 min treatment. They demonstrated that in that particular food matrix, the inactivation of *Carnobacterium piscicola* LMG2739, *Enterococcus faecium* CTC492, *Lactobacillus sakei* CTC494 and CTC746, *Leuconostoc carnosum* CTC747, *L. innocua* CTC1014, *Pediococcus acidilactici* F, *Staphylococcus carnosus* LTH2102, and *E. coli* CTC1007 and CTC1023 was 1.12–3.46 log CFU lower. Additionally, for *E. coli* CTC1018 there was no significant inactivation compared with buffer with food matrix. Patterson (2005) compared UHT milk to poultry meat and determined that, under 600 MPa at 20 °C for 15 min, there was a 3 log reduction for *E. coli* O157:H7 NCTC 12079 in poultry meat, but less than 2 log in UHT milk.

On the other hand, the fat content might also have some effect on the inactivation of microorganisms, however, contradictory information is found in the literature. Styles et al (1991) and Garcia-Graells et al (1999) found a baroprotective effect of fat on inactivation of vegetative bacteria that increased with fat content, while other researchers reported opposite results (Garcia-Risco et al., 1998; Gervilla et al., 2000). Gervilla et al. (2000) for example, found that microorganisms, such as *E. coli, Pseudomonas fluorescens, L. innocua, S. aureus* and *Lactobacillus helveticus*, are more resistant in ovine milk than buffer, but fat content did not affect barotolerance.

Temperature is another key factor that has a strong impact on the inactivation of vegetative bacteria. It is well known and documented that elevated temperature (above 30 °C) promotes pressure inactivation of microorganisms (Patterson and Kilpatrick, 1998), but for low temperature (below 20 °C), it is less clear. For example, *E. coli* and *S. aureus* showed lower resistance at 25 °C than at 4 °C in ewe's milk, while *P. fluorescens, L. helveticus* and *L. innocua* showed the opposite effect (Trujillo et al., 2002).

II.5. Hypothesis

The application of HHPP with pressure cycling and pressure-temperature combination treatment will boost the effectiveness of pressure to inactivate *E. coli* O157:H7 in ground beef.

II.6. Rationale

- High hydrostatic pressure processing (HHPP) is a reliable non-thermal processing method used to process foods such as oysters, meats and juices to improve microbiological safety while retaining quality and organoleptic properties.
- It has been demonstrated that pressure cycling is more effective than application of steady pressure.
- HHPP has been showed to inactivate gram positive and gram negative bacteria in liquid and semi-solid foods.
- This research investigates the potential of pressure cycling to inactivate a pathogenic *E. coli* O157:H7 cocktail in inoculated ground beef.

II.7. Overall Objective

The objective of this research was to investigate the effectiveness of HHPP, specifically, the application of steady high pressure and pressure cycling to inactivate 6 single strains and a cocktail of pathogenic *E. coli* O157:H7 (obtained from ground beef related outbreaks) in ground beef.

II.8. Specific objectives

- To study the effect of HHPP with varying pressure-time conditions on inactivation of *E. coli* O157:H7 in inoculated ground beef.
- To study the effect of HHPP and heat with varying temperature-pressure-time conditions on inactivation of *E. coli* O157:H7 in inoculated ground beef.
- To study the effect of pressure cycling on inactivation of *E. coli* O157:H7 in inoculated ground beef.

III. Materials and Methods

III.1. Materials

III.1.1. Ground beef

Ground beef with 80 % lean 20 % fat was purchased from local supermarkets (A&P in North Brunswick, NJ, USA) one day before the experiments. Since the ground beef was used in the experiments within a day of purchase, the unopened packs of fresh ground beef were stored in the refrigerator at 4 $^{\circ}$C. Once opened, the ground beef was placed into separate packages and any remaining portion, not used, was discarded.

III.1.2. *E. coli* strains

One non-pathogenic and six pathogenic strains of *E. coli* O157: H7 were obtained from Dr. Karl Matthews from the Department of Food Science at Rutgers University, and were stored in glycerol at -85˚C. These strains were obtained from ground beef outbreaks and used as inoculum. The strains were:

- *E. coli* JM109
- *E. coli* O157:H7 (86-24)
- *E. coli* O157:H7 (WM98A06026)
- *E. coli* O157:H7 (C7927)
- *E. coli* O157:H7 (F4546)
- *E. coli* O157:H7 (SEA13B88)
- *E. coli* O157:H7 (ATCC 43895)

III.1.3. Media for culturing and enumeration of *Salmonella*

The following media were used in the study: Peptone water (0.1%), (DifcoTM, Benkitson and Dickson, MD, USA); Tryptic soy broth (TSB, Soybean - Casein Digest Medium) powder (Difco™); Tryptic Soy Agar (TSA, Soybean – Casein Digest Agar) powder (Difco™); Rainbow agar (Biolog, CA, USA); MacConkey Agar, (Acumedia, MD, USA).

III.1.4. High Pressure Processing Equipment

Figure 6 shows the high hydrostatic pressure processing unit (Elmhurst, Inc., Albany, NY) in the Rutgers Food Science department, which comprises a 10 liter stainless steel high pressure vessel with a 20 HP (horse power) high pressure intensifier pump to reach a maximum pressure at 690 MPa (100,000 psi) within 3 min or less. The depressurization takes 10 seconds, maximum. This unit does not have an internal heating or cooling devise, but can be heated or cooled with an external heating/cooling tank. It can be operated between 5 $^{\circ}$C to 90 $^{\circ}$C, while holding the pressure for one hour. This unit also has the capability to run pressure cycles manually within the temperature range.



Figure 6: Rutgers 10 liter High Hydrostatic Pressure Processing Unit located at CAFT Building (63 Dudley Road, New Brunswick, NJ) basement.

Figure 7 shows the setup of the HHPP unit. The 10 L stainless steel cylinder has an external length of 1090 mm, external diameter of 445 mm, internal bore diameter of 127 mm, internal length of 823 mm, and a wall thickness of 142 mm. When the unit is not in use, the high pressure vessel remains in a horizontal position. It is only made vertical during an experiment. At each run, the ground beef samples were loaded into the pressure vessel when it was in a horizontal position, the top closure inserted, the vessel then made vertical, and filled with water at a predetermined temperature. If a temperature-pressure combined treatment was needed, the vessel was filled with water one day before and pre-heated or pre-chilled overnight by using an external heating devise to warm or chill the vessel. The desired pressures in kpsi and hold time in minutes were set by using a PLC control panel. Three thermocouples (type K) located inside the vessel, near the top, center, and bottom of the vessel recorded the temperature of water inside the vessel during the HHPP. The data for pressure, temperature as well as time were logged on a computer by using LabVIEW 7 ® (National Instruments, Austin, TX) software.

After the process finished, the vessel was made horizontal, followed by removing the top closure, dumping the water and unloading the sample.

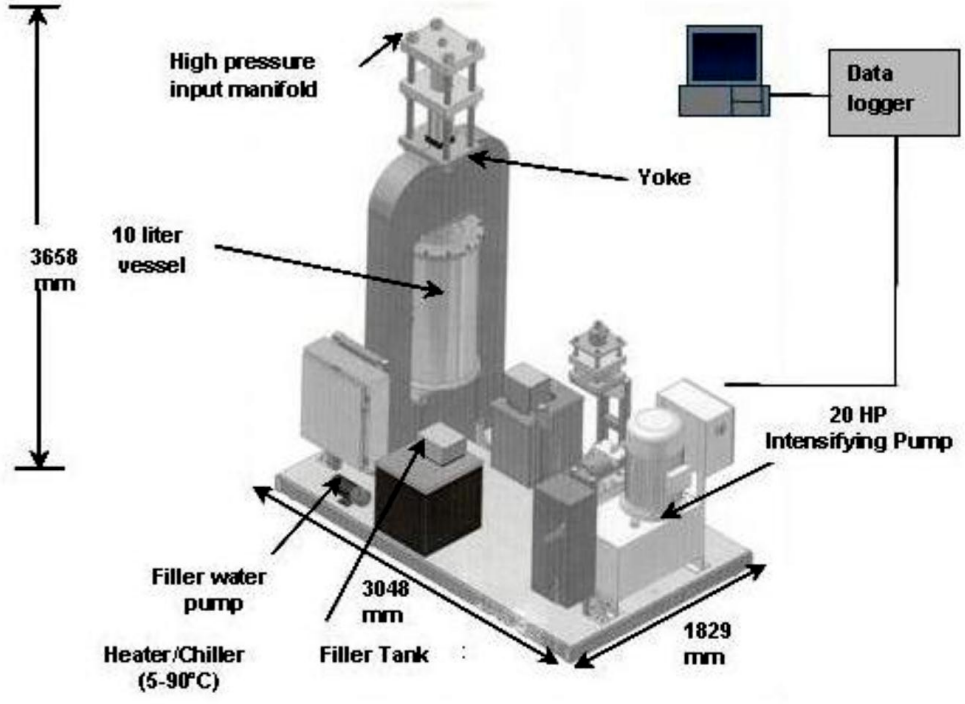


Figure 7: Detailed setup of the HHPP unit at Rutgers University.

III.2. Methods

III.2.1. Bacterial cultures and inoculum preparation

The non-pathogenic strain, *Escherichia coli* JM109, and pathogenic strains of *Escherichia coli* O157:H7 (86-24), *E. coli* O157:H7 (WM98A06026), *E. coli* O157:H7 (C7927), *E. coli* O157:H7 (F4546), *E. coli* O157:H7 (SEA13B88) and *E. coli* O157:H7 (ATCC 43895) were stored at – 85 ˚C in a freezer. Each culture was inoculated into 10 mL of tryptic soy broth (BD, Sparks, MD) in a 15 mL conical centrifuge tube (Fisher Scientific, Pittsburgh, PA), vortexed, and incubated at 37 ˚C for 18 to 24 h. Then, the 10 mL of overnight culture of each strain was centrifuged at 4500 rpm for 4 min at 4 ˚C. The supernatant of each tube was decanted and 10 mL of peptone water was added to each tube, vortexed, 1 mL of the culture media of each strain was transferred to a single 15 mL conical centrifuge tube (Fisher Scientific), and the mixture was vortexed to produce a cocktail of six *E. coli* O157:H7 strains.

III.2.2. Preparation of inoculum and inoculated ground beef

Packs of 80 % lean ground beef were purchased from a local grocery store. To inoculate, the purchased ground beef was removed from the original package and 2 g portions dispensed into individual 7 oz. Whirl-Pak* Sterile Filter Bags (Nasco, WI, USA), and 1 µL of inoculum (each strain or the cocktail) was added per gram of the sample. The inoculated ground beef was either Stomached or massaged by hand to distribute the inoculums evenly throughout the sample.

Then each sample bag was repacked into two heat-sealable pouches that were cut out, and vacuum packed using a FoodSaver® vacuum sealer (Sunbeam Products, Inc., Boca Raton, FL) to prevent the pouches from bursting due to air pockets during high pressure processing. This procedure was repeated before each HHPP.

III.2.3. Experiment Design

The flow chart for the experiment design is shown in **Figure 8**. Ground beef purchased from the local grocery store was repacked into several individual sterile bags as discussed in III.2.2. These were inoculated either with the single strains or the cocktail; others were kept as a control without inoculation.

For the control samples, the organoleptic quality parameters and microbial count of the samples were determined. If the sample was microbiologically negative after 24-hour enrichment, then the sample was considered negative. However, most samples were positive, which meant that the ground beef samples had bacterial populations that were lower than the detectable limit of the direct plate count. Those samples would go through HHPP without inoculation, followed by another microbial measurement. The results for this 24-hour enrichment always came out as negative, which the load of the micro-organisms in the ground beef itself would not interfere with the final results for the HHPP samples.

For the inoculated samples, 2-3 samples were not HHPP and processed to determine the initial *E. coli* O157:H7 population. The remaining samples (1-2

samples for each serotype and their cocktails for each HHPP run), were subjected to HHPP, followed by the microbial and chemical quality analyses. All the conditions were repeated 2-3 times. And each time, the whole sample was used to determine change in target microbe population or color measurement.

The range of the pressure, time, temperature, and number of cycles were as follows: pressure from 300 MPa to 600 MPa, time period at high pressure from 6 min to 60 min, initial temperature from 7 $^{\circ}$C to 55 $^{\circ}$C, pressure cycles from 1 to 5 cycles such that total time at high pressure was the same.

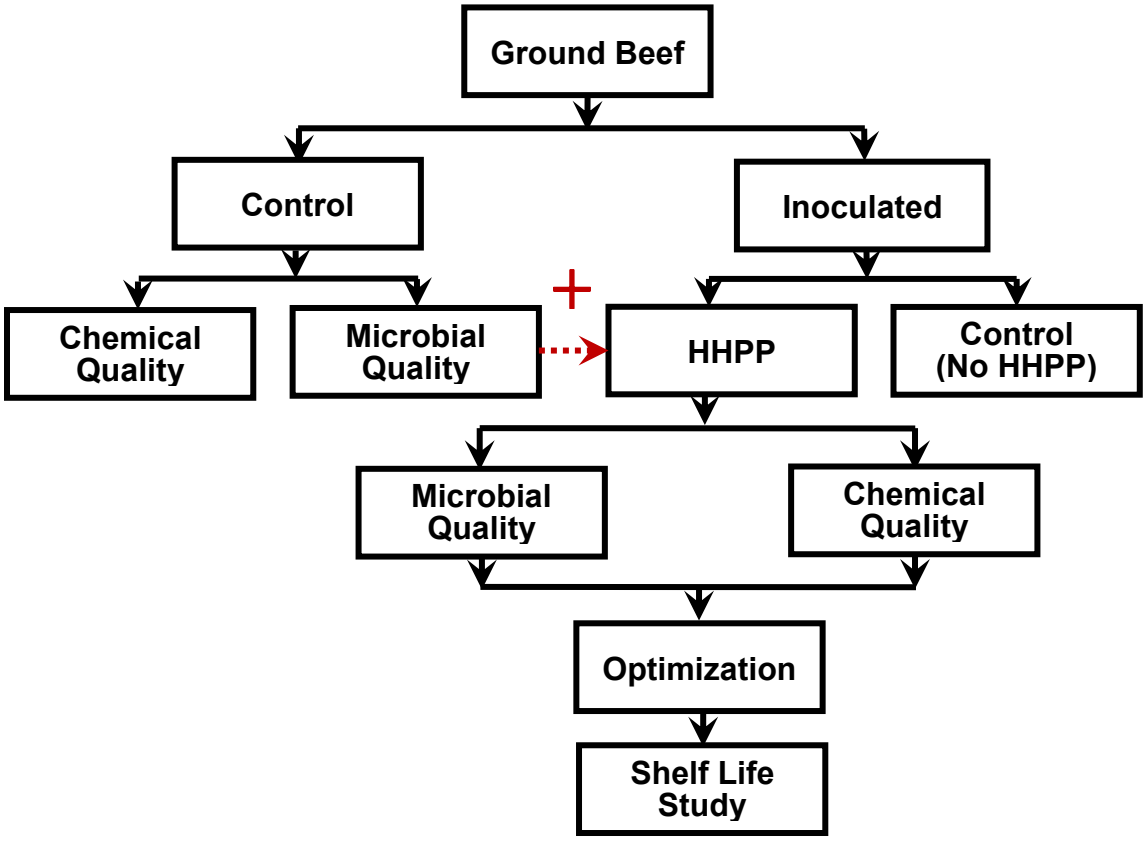


Figure 8: Experiment Design.

Inoculated ground beef samples for each experimental condition were high pressure processed in the 10-liter HHPP vessel (Elmhurst Research, Inc., Albany,

NY). During an HHPP run, the sample underwent pressurization, hold time at the desired pressure, and depressurization. Pressurization time varied between 1 to 2 min depending upon the desired final pressure. Depressurization occurred in less than 10 s. The initial temperature of the water inside the vessel varied between 22 to 25 ˚C. It would increase to a maximum of 35 ˚C during pressurization due to adiabatic compression heating, then drop by a few degrees during the hold time due to the heat loss to the vessel wall, and then drop rapidly to a few degrees below the initial temperature after depressurization.

III.2.4. Color analysis

For each treatment, the color measurements were carried out on three different ground beef samples. Four areas of each sample were chosen to measure the color values using the CIELAB color system. L* (lightness, range from 0 to 100),

a* (from green to red, range from -120 to 120) and b* (from blue to yellow, range from -120 to 120) values were measured with the help of a colorimeter (Konica Minolta CR410, Osaka, Japan). The instrument was calibrated each time with a white D65 standard disc (Y =94.7, x = 0.3156 and y = 0.3319). Averages and standard deviations of L*, a* and b* values were calculated as the total color differences.

Accroding to the American meat science association guidelines (Hunt,1991) for color measurements on ground beef, total color difference (ΔE), whiteness index (WI), saturation index (SI), hue angle (H), and browning index (BI) are used to describe the color change as compared to the control sample. These values are calculated using measured L*, a* and b* values (Homco-Ryan, 2001) as follows and used to where subscript “o” indicates the color reading of control sample used as the reference and a larger ΔE indicates greater color change from the reference sample (Saricoban, 2010).

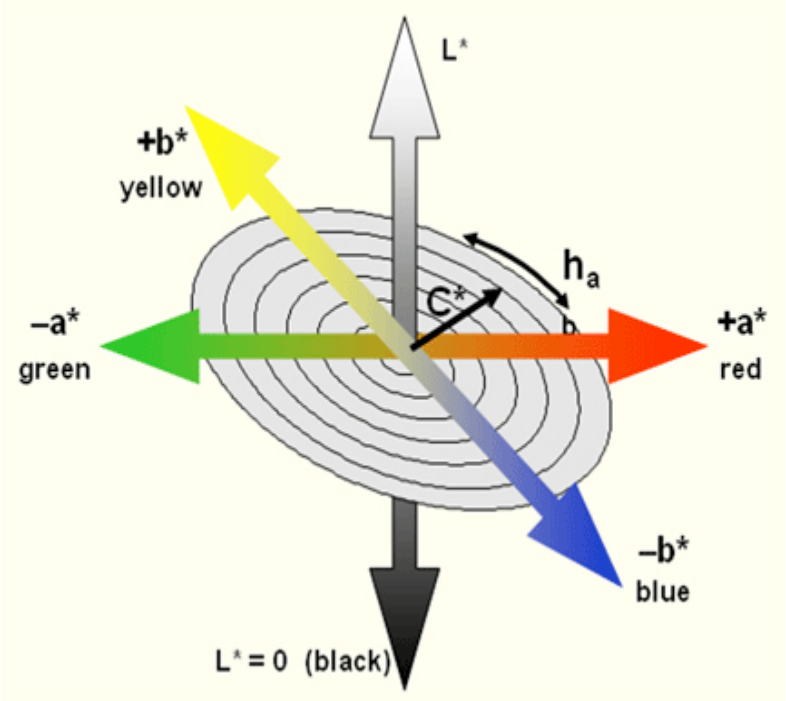


Figure 9. CIELAB color space.

- $\Delta a^* = a^*_{sample} - a^*_{standard}$
- $\Delta b^* = b^*_{sample} - b^*_{standard}$
- $\Delta E = \sqrt{(a^* - a_0^*)^2 + (b^* - b_0^*)^2 + (c^* - c_0^*)^2}$
- $SI = \sqrt{a^{*2} + b^{*2}}$
- $WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$
- $h = \arctan(\frac{b^*}{a^*})$
- $BI = \frac{100(\frac{a^* + 1.75L^*}{5.646L^* + a^* - 3.012b^*} - 0.31)}{0.172}$

III.2.5. Shelf life study

A shelf life study was conducted using samples treated under optimum parameters of 400 MPa for 5 cycles with total pressure holding time 15 min and initial temperature of 25 ˚C. The pressure, temperature, pressure cycling, and time conditions for the samples chosen for shelf life study were based on the microbiological and chemical quality of the inoculated ground beef.

During the shelf life study, samples were maintained at 4 ˚C and -20 ˚C immediately after HHPP. From Day 0 (right after HHPP) to Day 5 (five days after HHPP), samples were obtained each day from both storage temperatures, and microbial analysis conducted (total plant count method). When determining change in bacterial population an entire sample was processed. In brief, one milliliter peptone water was added per 1 g ground beef sample, mixed well by hand then, 1 mL liquid was transferred from the sample bag to a centrifuge tube. A 100 µl volume was plated both on TSA and Rainbow agar, after centrifuging. Agar Plates were incubated at 37 ˚C for 24 h, colonies were counted.

IV. Results and Discussion

IV.1. Preliminary Experiments

JM109 was used in initial experiments to develop processing methodologies. **Figure 10** shows the effect of HHPP at 600 MPa for 20 min on JM109 levels in ground beef at Ti =25 ˚C (temperature reached approximately 35 ˚C after pressurization). The level of JM109 was below the detection limit following treatment. Samples were then subjected to a 24-hour enrichment which demonstrated JM109 was completely inactivated at these extreme conditions.

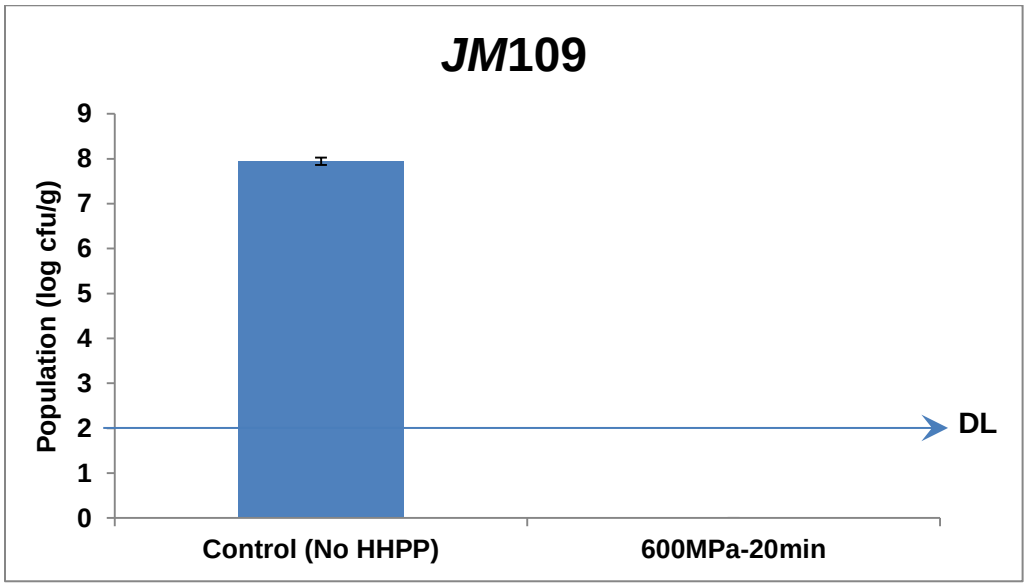


Figure 10: Effect of HHPP at 600 MPa for 20 min on JM109 in ground beef

*** No growth following enrichment.**

Data see Appendix 1.

The results for experiments carried out at less severe conditions, i.e., lower pressure and equivalent or less time are shown in **Figure 11**. Processing for 21 min or 18 min at 400 MPa resulted in all of the JM109 being inactivated. However, when the time was decreased to 15 min, a few surviving JM109 cells remained. When the process time was reduced to 10 min the level of JM109 could be determined using the plate count method.

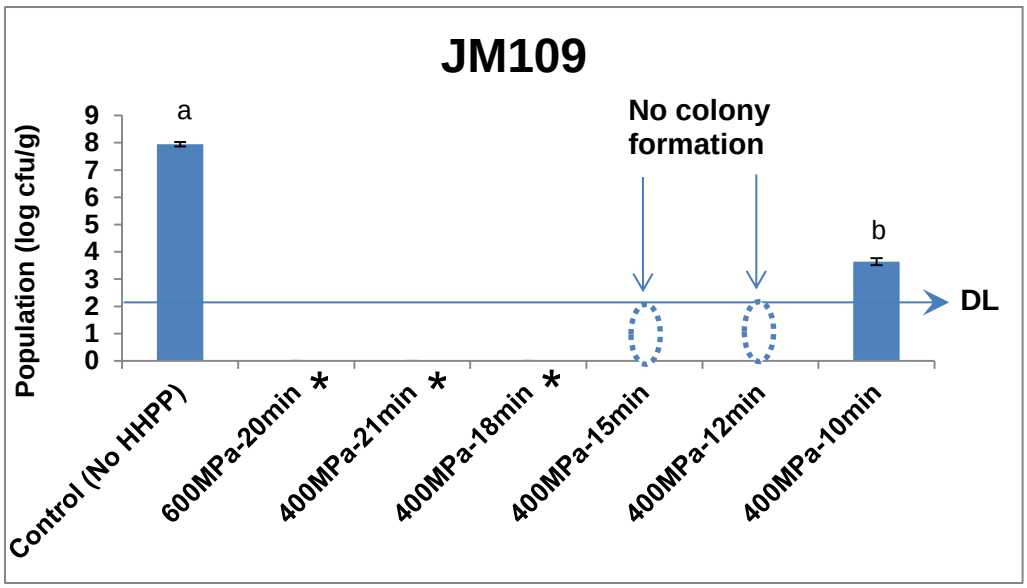


Figure 11: Effect of HHPP on JM109 in ground beef at various pressure-time conditions.

Same lowercase letters indicate results are not significantly different ($p<0.05$).

*** No growth following enrichment.**

Data see Appendix 2.

Three-hundred MPa was also applied to ground beef inoculated with JM109. However, it required 1 hour to achieve a 5.30 log reduction of the JM109 population.

Based on these experimental results the optimal processing parameters were set at 400 MPa for 15 to 18 min at Ti=25 ˚C. Thus, further experiments were conducted at 400 MPa 15 min with some modifications for temperature, time and number of pressure cycles as required, based on the appearance and microbial quality.

IV.2. Effect of HHPP on *E. coli* O157:H7 inoculated ground beef

Fresh ground beef was purchased and inoculated with six strains of *E. coli* O157:H7, and a cocktail of the strains. A 2-3 log reduction in the level of *E. coli* O157:H7 was achieved at 400 MPa for 15 min in those seven samples (samples inoculated with one of the 6 single strains or their cocktail) (**Figure 12**). The SEA 13B88 and ATCC43895 strains showed a greater resistance to the HHPP compared to the other strains and the cocktail. Results of preliminary studies suggested that the JM109 strain is not a representative surrogate of the six strains of *E. coli* O157:H7. The *JM*109 isolate is more sensitive to HHPP as evidenced by four log greater reduction at 400 MPa 15min. The high sensitivity of JM109 compared to pathogenic *E. coli* O157:H7 strains may be associated with its long-term use as a laboratory strain used in molecular biology studies. *E. coli* O157:H7 strains in the host or the environment would encounter an array of adverse conditions that it must overcome to survive.

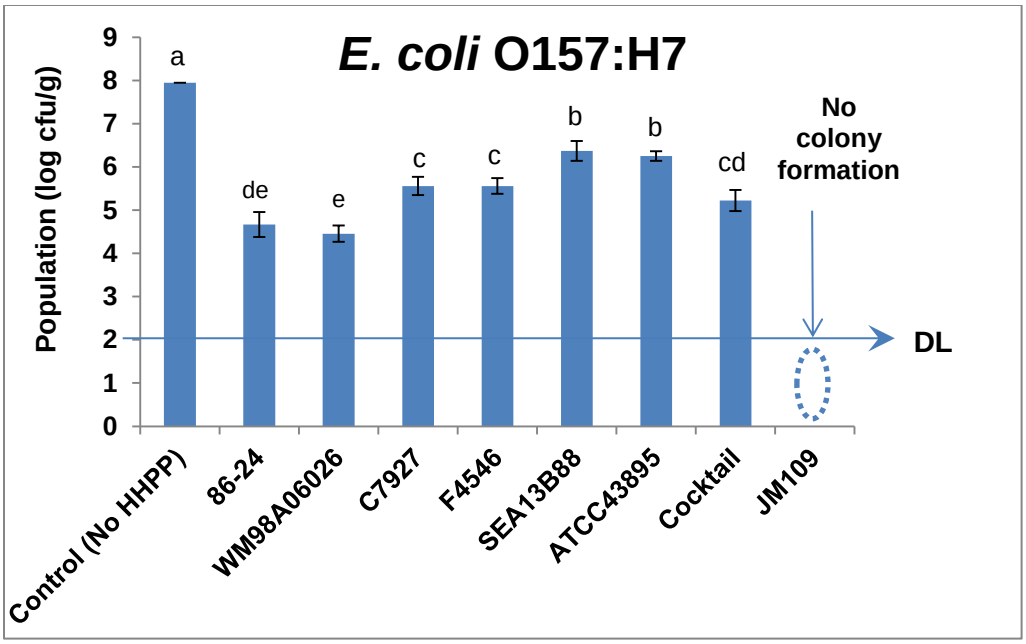


Figure 12: Effect of HHPP at 400 MPa for 15 min at 25 °C on *E. coli* O157:H7 in ground beef.

Same lowercase letters indicate results are not significantly different ($p<0.05$).

Data see Appendix 3.

IV.3. Effect of the combination of pressure-temperature treatment on *E. coli* O157:H7 inoculated ground beef

HHPP experiments were conducted at 400 MPa 15 min with varying initial temperature: 25 °C, 35 °C, and 45 °C (temperature reached approximately 35, 45, and 55 °C after pressurization) (**Figure 13**). Greater log reduction was obtained at higher temperatures. For strains 86-24 and WM98A06026, populations were not reduced significantly at 25 °C and 35 °C, but there was a significant reduction when the temperature was increased to 45 °C. For the other 4 strains of *E. coli* O157:H7 and the cocktail, the results were all significant when the temperature was increased from 2°C 5 to 35 °C and 35 °C to 45 °C. A minimum one log reduction in the population was achieved for all the strains and their cocktail when the temperature was increased from 25 °C to 45 °C, specifically, a 3-log reduction for SEA 13B88, and a 2-log reduction for C7827, F4546, ATCC43895, and the cocktail.

Since the D Values at 125 °F (51.67 °C) for ground beef (10% fat and 30 % fat) are 78.2 min and 115.5 min (Line, 1991), the D Value for the 20% fat ground beef is greater than 78.2 min but less than 115.5 min at 51.67 °C. Therefore, the inactivation of *E. coli* O157:H7 shown above was enhanced by the synergy of the combined temperature-pressure treatment, but not because of the heat alone.

Experiments using HHPP below room temperature, and especially at low temperature were also conducted. However, processing under those conditions failed to adequately inactivate *E. coli* O157:H7 in ground beef. Only a one log reduction in the activation of *E. coli* O157:H7 was achieved at 400MPa, 7 °C for

15 min. This may be associated with metabolic and phenotypic characteristics of the cell, decreased metabolic activity and decreased membrane fluidity which can protect the cell from deleterious actions. Membrane structure and composition will change with respect to temperature. The membrane becomes increasingly viscous with decreasing membrane fluidity as temperature decreases. Especially, at certain temperature, the membrane might undergo a phase change to a gel phase when biological function is lost. Thus, the cell may be more resistance to the HHPP at low temperature compared to the relatively high temperature.

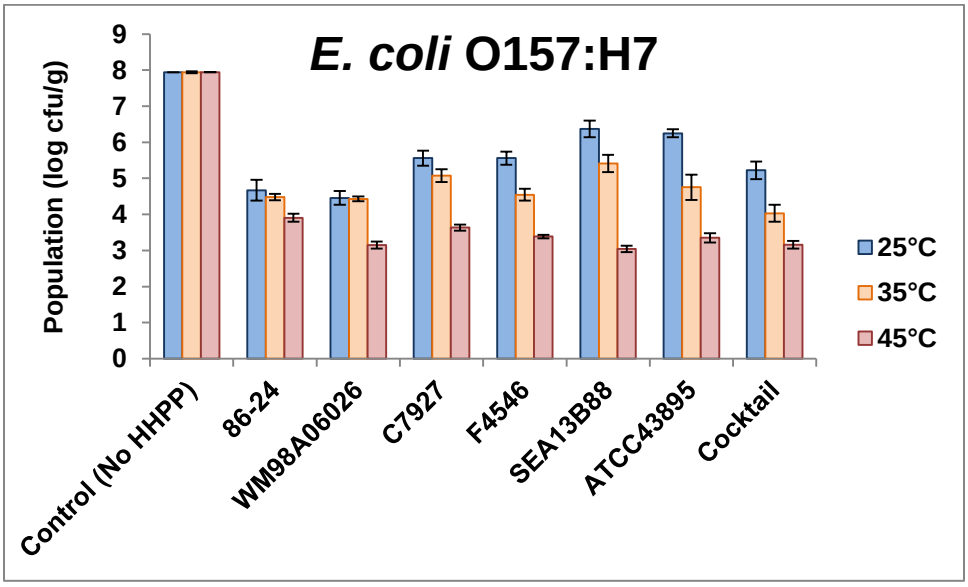


Figure 13: Effect of HHPP at 400 MPa for 15 min at 25 °C, 35 °C, and 45 °C on individual *E. coli* O157:H7 strains and their cocktail in ground beef.

Data see Appendix 4.

IV.4. Effect of the combination pressure cycling-pressure treatment on *E. coli* O157:H7 inoculated ground beef

Pressure cycles were introduced to the HHPP method. Each cycle consisted of raising the pressure from 0.1 MPa to high pressure, keeping it there for a period of time such that the total time at high pressure remained the same in all experiments. Three and five cycles were applied with the total time at 400 MPa fixed at 15 min. Actual data for P Vs. time and T Vs. time for 1,3, 5 cycles is showed in **Figure 14, 15, and 16**. The processing time only counts the pressure holding time. The pressurization time and depressurization time was not taken into account. However, even the pressurization time and depressurization time were counted, it was unable to achieve the same log reduction by HHPP alone as the one by pressure cycling.

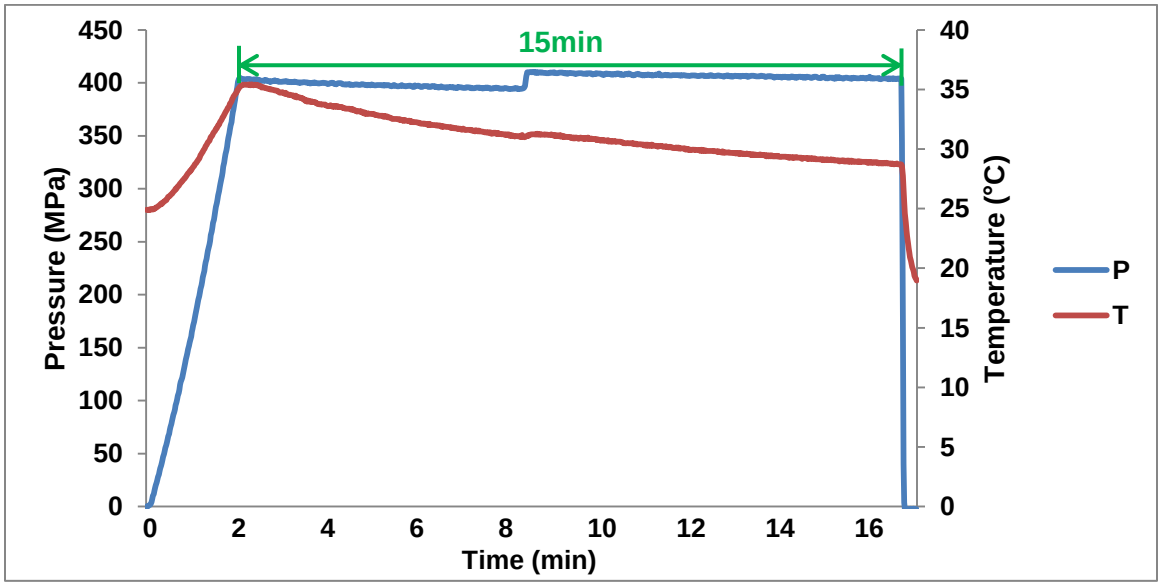


Figure 14: Pressure vs. time and temperature vs. time data for ground beef during HHPP at 400 MPa, 25 °C for 15 min.

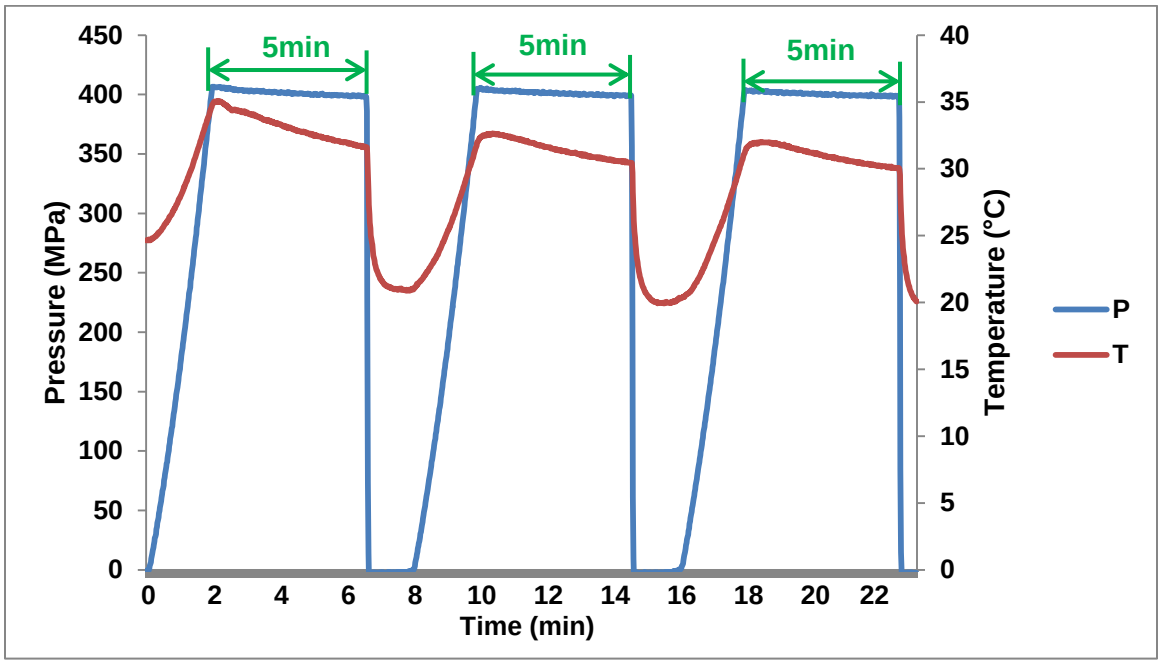


Figure 15: Pressure vs. time and temperature vs. time data for ground beef during HHPP at 400 MPa, 25 °C, for 3 cycles with total pressure holding time 15 min.

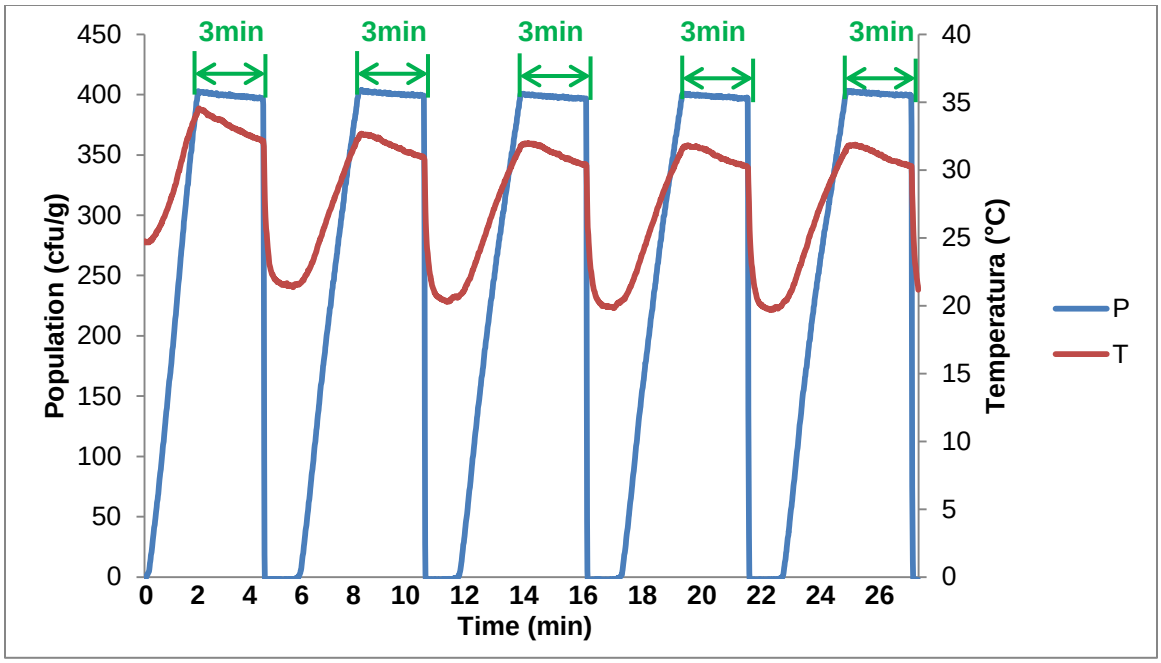


Figure 16: Pressure vs. time and temperature vs. time data for ground beef during HHPP at 400 MPa, 25 ˚C, for 5 cycles with total pressure holding time 15 min.

Results demonstrate that when the pressure cycles were increased from one to five, a greater reduction in the population of *E. coli* O157:H7 occurred (**Figure 17**).

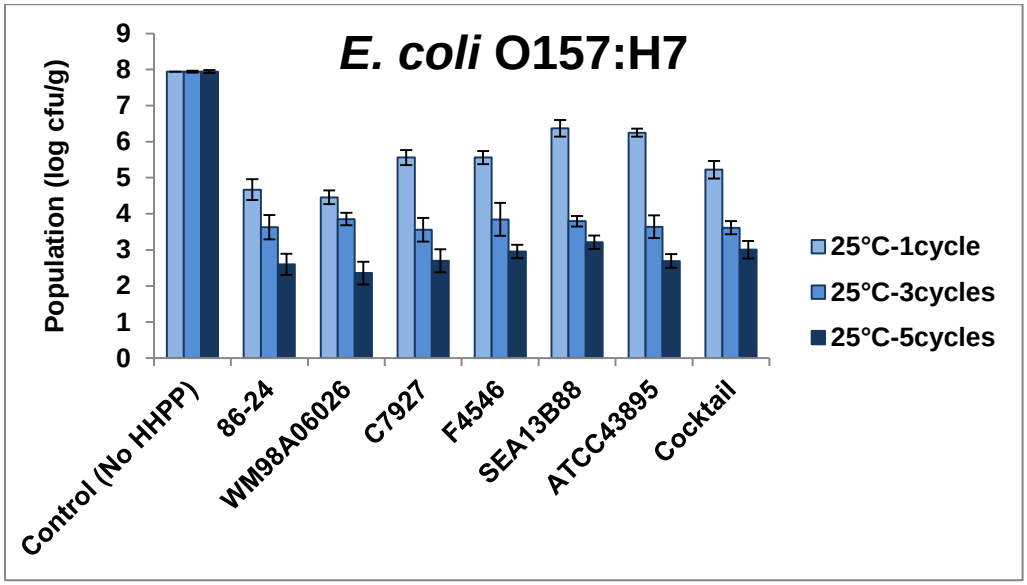


Figure 17: Effect of high pressure cycling at 400 MPa at 25 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on *E. coli* O157:H7 in ground beef.

The reduction in population of *E. coli* O157:H7 for each strain and their cocktail are significant at all conditions ($p<0.05$).

Data see Appendix 5.

For 86-24 strain, there was a one log greater reduction when the pressure cycles were increased from 1 to 3 and from 3 to 5.

For WM98A06026 strain, greater inactivated occurred when the pressure cycles were increased from three to five, than one to three.

For C7927, F4546, SEA 13B88, ATCC43895 strains and the cocktail, reduction in population increased from 1 to 3 logs as the number of cycles were increased. Specifically, there was more than a two log reduction for C7927, F4546, and the cocktail. For SEA 13B88 and ATCC43895, a 3 log difference was achieved between the steady pressure processing and high pressure cycling processing.

IV.5. Effect of temperature on pressure cycling treatment of *E. coli* O157:H7 inoculated ground beef

For these experiments the high pressure vessel was heated overnight to a desired temperature. **Figure 18** and **Figure 19** show the effect of pressure cycling at 35 °C and 45 °C, respectively.

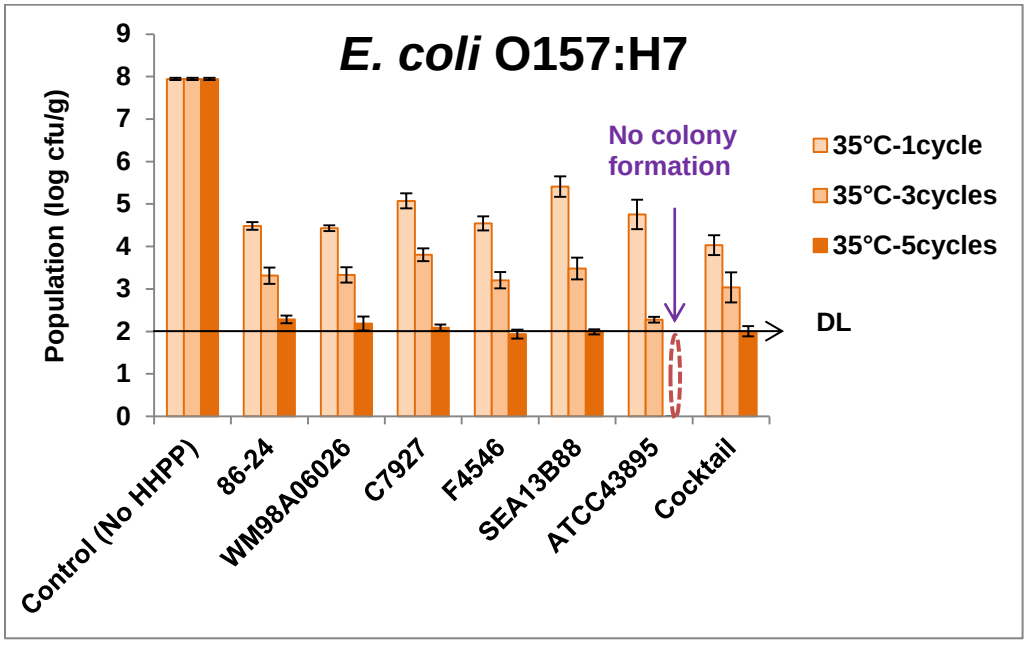


Figure 18: Effect of high pressure cycling at 400 MPa at 35 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on *E. coli* O157:H7 in ground beef.

The reduction in population of *E. coli* O157:H7 for each strain and their cocktail are significant at all conditions ($p<0.05$).

Data see Appendix 6.

At 35 °C, as shown in **Figure 18**, there was greater than a one log decrease when the pressure cycles were increased from one cycle to three cycles and from three cycles to five cycles. Specifically, for ATCC43895 strain, more than a two log reduction was achieved when the three pressure cycles were applied; moreover, there was no colony formation when the sample was treated at 400 MPa at 35 °C for 5 cycles with the total pressure holding time of 15 min. However, samples were positive after a 24-hour enrichment.

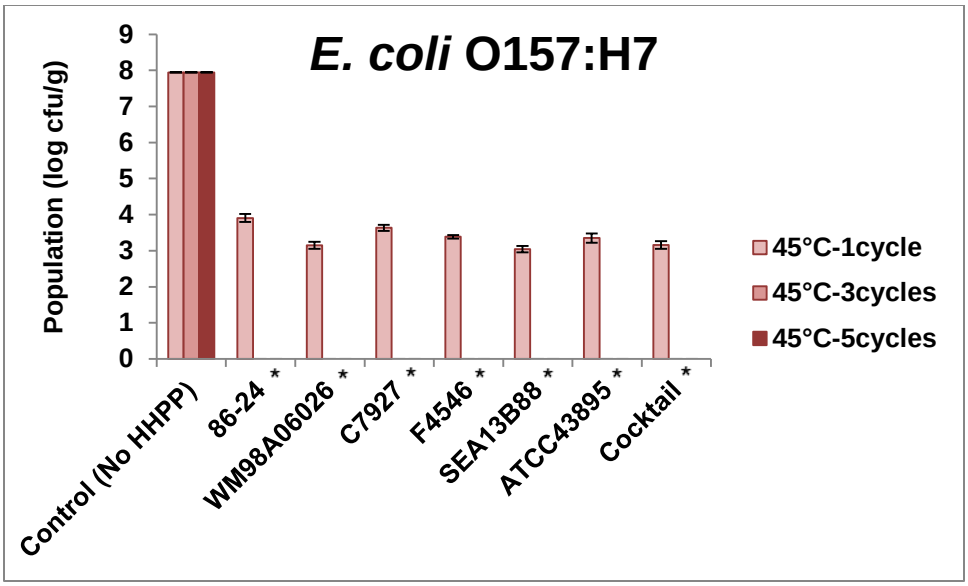


Figure 19: Effect of high pressure cycling at 400 MPa at 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on *E. coli* O157:H7 in ground beef.

The reduction in population of *E. coli* O157:H7 for each strain and their cocktail are significant at all conditions ($p<0.05$).

*** No growth following enrichment.**

Data see Appendix 7.

At 45 °C, as shown in **Figure 19**, there was no colony formation after processing at both three pressure cycles and five pressure cycles under 400 MPa for a total pressure holding time of 15 min. Samples were subjected to 24-hour enrichment; all samples failed to exhibit growth. In this case, all of the six strains and their

cocktail were inactivated at 400 MPa 45 °C after three pressure cycles, with a total pressure holding time of 15 min at high pressure.

Pressure cycling does not just extend the pressure processing time leading to greater inactivation of microbes. Even including the pressurization and depressurization time, without pressure cycling, steady pressure treatment is unable to achieve the same inactivation with comparable total holding time. Results have shown that oscillatory treatments are more effective than equivalent continuous treatment of comparable total time. This is because a slow ramp rate may induce a stress response and so make the process less effective. However, fast changes in pressure while pressurization, especially at depressurization, may cause cavitation in the cells and spores, which results in physical disruption and contributes to a higher inactivation.

IV.6. Effect of steady pressure and pressure cycling at three different temperatures on the color of processed ground beef

IV.6.1. L*, a* and b* values

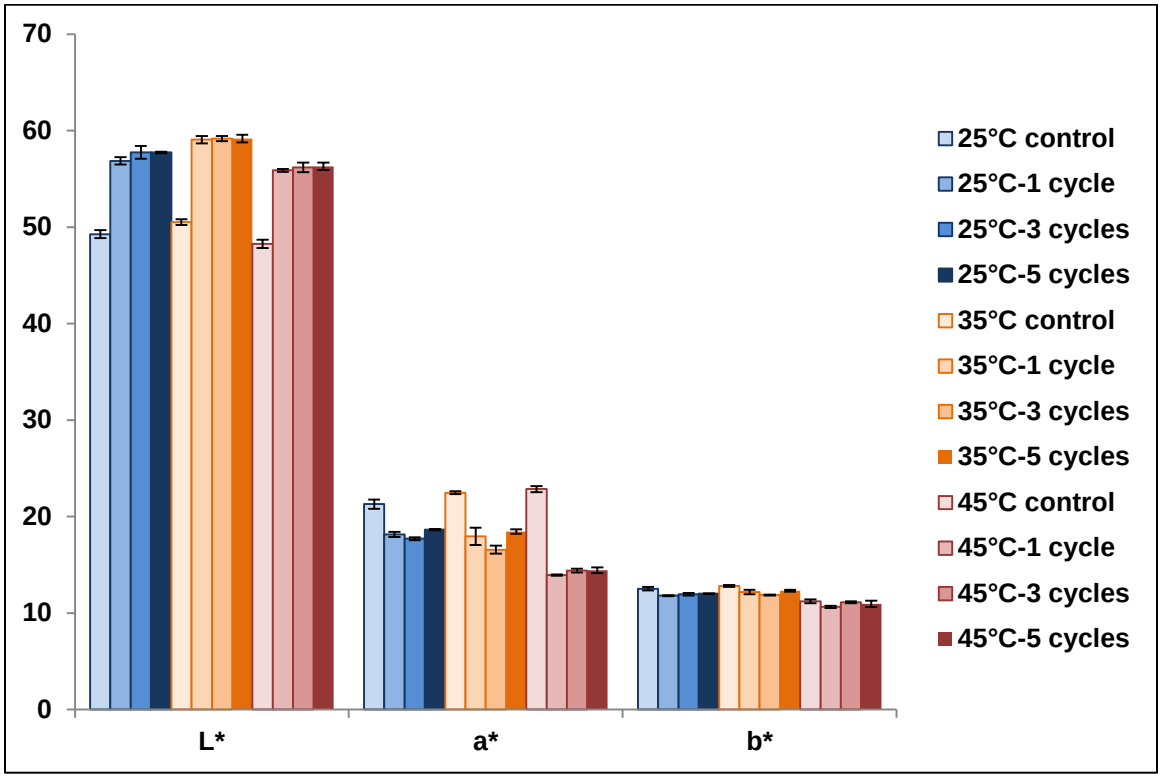


Figure 20: Effect of HHPP, temperature and pressure cycling at 400 MPa at 25 °C, 35 °C, 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on L*, a*, and b* value of ground beef.

Data see Appendix 8.

L*, a* and b* values for the samples treated at 400 MPa at 25 °C, 35 °C, or 45 °C with 1, 3, or 5 pressure cycles with the total pressure holding time of 15 min are showed in **Figure 20**.

The lightness value L*, increased after the HHPP, indicating the ground beef was lighter in color after the processing. However, there was no significant difference ($p<0.05$) between the pressure cycle processed, steady pressure processed ground beef.

For color-opponent dimensions a*, the value went down after the HHPP, indicating the ground beef was darker red after processing. The higher the temperature applied, the greater the change in redness. But, pressure cycling did not have a significant effect ($p<0.05$) on the redness index.

For color-opponent dimensions b*, it did not change significantly ($p<0.05$) before or after the processing, no matter what temperature or pressure cycles were applied.

IV.6.2. ΔL*, Δa*, Δb* value

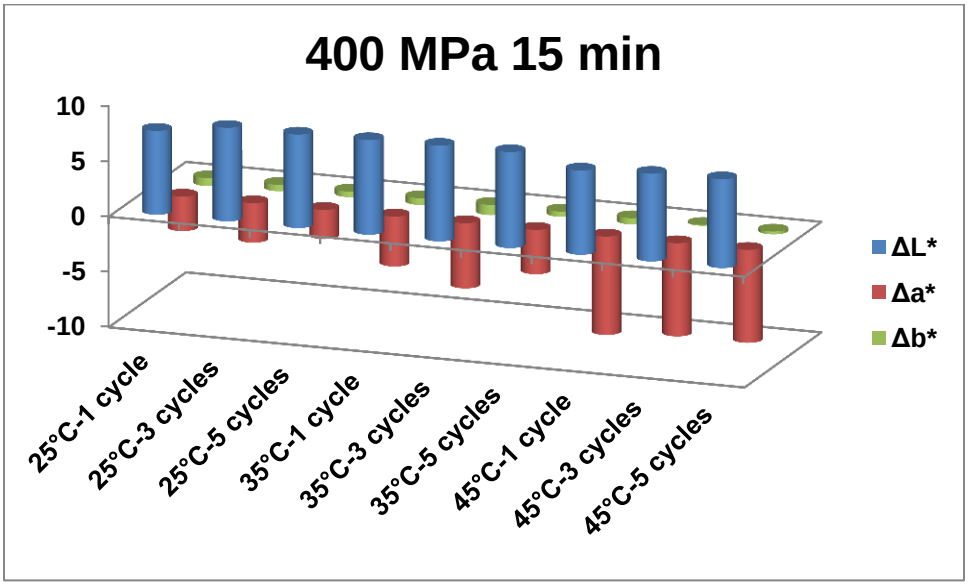


Figure 21: Effect of HHPP, temperature and pressure cycling at 400 MPa at 25 °C, 35 °C, 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on ΔL*, Δa*, and Δb* value of ground beef.

Data see Appendix 9.

Another way of looking at the color changes is to plot changes in L*, a*, and b* values as a results of processing.

Figure 21 shows the ΔL*, Δa* and Δb* values for the samples treated at 400 MPa at 25 °C, 35 °C, or 45 °C with 1, 3, or 5 pressure cycles with the total pressure holding time of 15 min.

For ΔL*, there was no significant difference regardless of pressure cycling or temperature applied with the HHPP.

For Δa^*, the higher the temperature is, the larger the Δa^* value. However, no clear pattern was observed. Collectively, pressure cycling did not have a great effect on the redness index.

For Δb^*, all of the Δb^* values listed here were less than 1, and there was no significant difference (**Figure 21**) among samples processed at different conditions.

IV.6.3. Total color difference ΔE

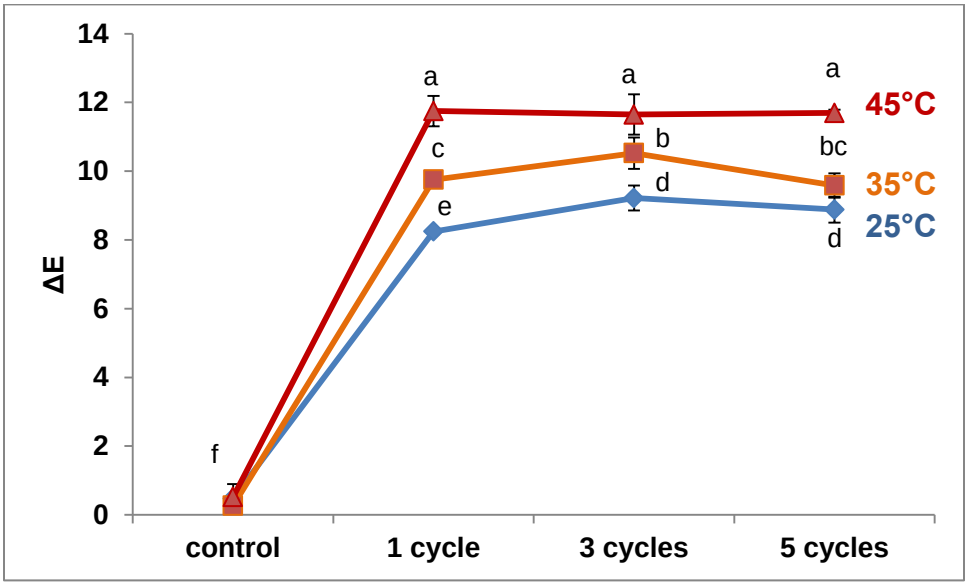


Figure 22: Effect of HHPP, temperature and pressure cycling at 400 MPa at 25 °C, 35 °C, 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on total color difference (ΔE) of ground beef.

Same lowercase letters indicate results are not significantly different ($p<0.05$).

Data see Appendix 10.

The total color difference ΔE indicates that the temperature alone has a significant impact on the total color change (**Figure 22**). At higher temperature (45 °C), the ΔE value was much greater than at 35 °C and 25 °C.

Pressure cycling does not seem to be a key factor influencing color change. There was no significant difference between samples processed at different pressure cycles at the same pressure and temperature.

Overall, the HHPP, as well as temperature, had measurable impact on the color change. Under the same pressure condition, the lower the temperature, the less color changes, regardless of the number of pressure cycling applied.

IV.6.4. Saturation Index SI

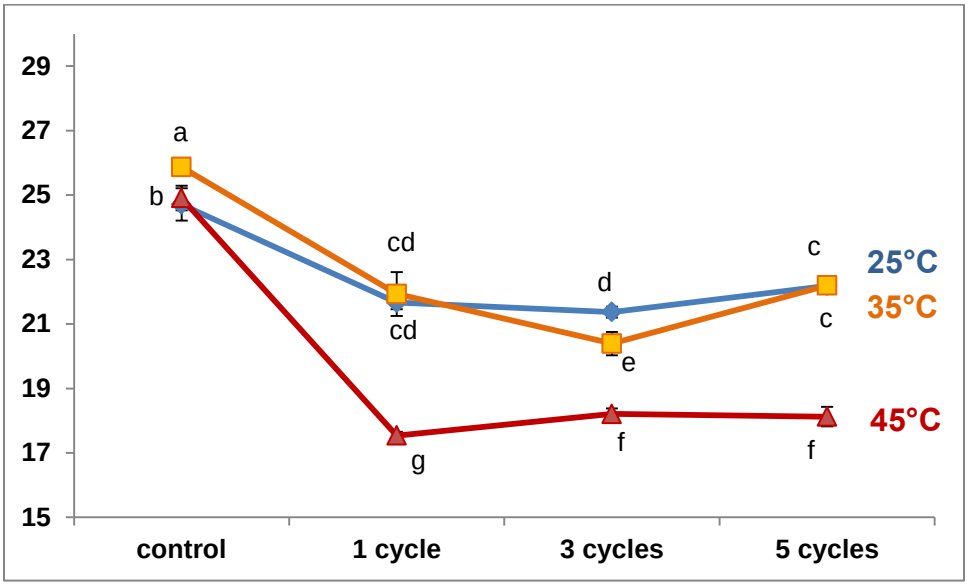


Figure 23: Effect of HHPP, temperature and pressure cycling at 400 MPa at 25 °C, 35 °C, 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on the saturation index SI of ground beef.

Same lowercase letters indicate results are not significantly different ($p<0.05$).

Data see Appendix 11.

The initial saturation index SI of fresh ground beef was around 24-26 in this study (**Figure 23**). After HHPP, the SI value went down. Also, the higher the temperature applied, the greater the difference between the initial and the values of SI. Results show that at 25 °C and 35 °C, SI was around 20. When temperature was increased to 45 °C, the SI value decreased to 18. However,

there was no significant difference between different pressure cycles at 25 °C and 45 °C. At 35 °C, there was a difference between 1 cycle and 3 cycles, and 3 cycles and 5 cycles, but the difference was minimal. This would be expected and that is why several readings were obtained at randomly selected locations.

IV.6.5. Whitening Index WI

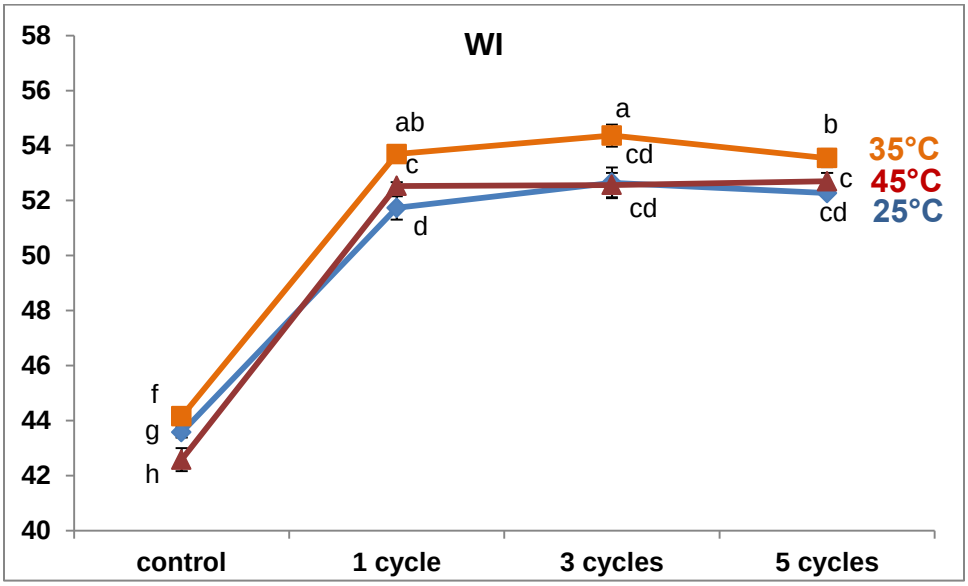


Figure 24: Effect of HHPP, temperature and pressure cycling at 400 MPa at 25 °C, 35 °C, 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on the whitening index WI of ground beef.

Same lowercase letters indicate results are not significantly different ($p<0.05$).

Data see Appendix 12.

WI is the whitening index (**Figure 24**). It is obvious that after HHPP, the WI value increased the result of a loss in redness and a higher lightness value. There was no significant difference between the WI values at 25 °C and 35 °C. However, when the temperature increased to 45 °C, the WI increased significantly.

The pressure cycling does not have a significant influence on the WI value. There was no obvious difference between non-cycling and cycling results.

IV.6.6. Hue angle ΔH

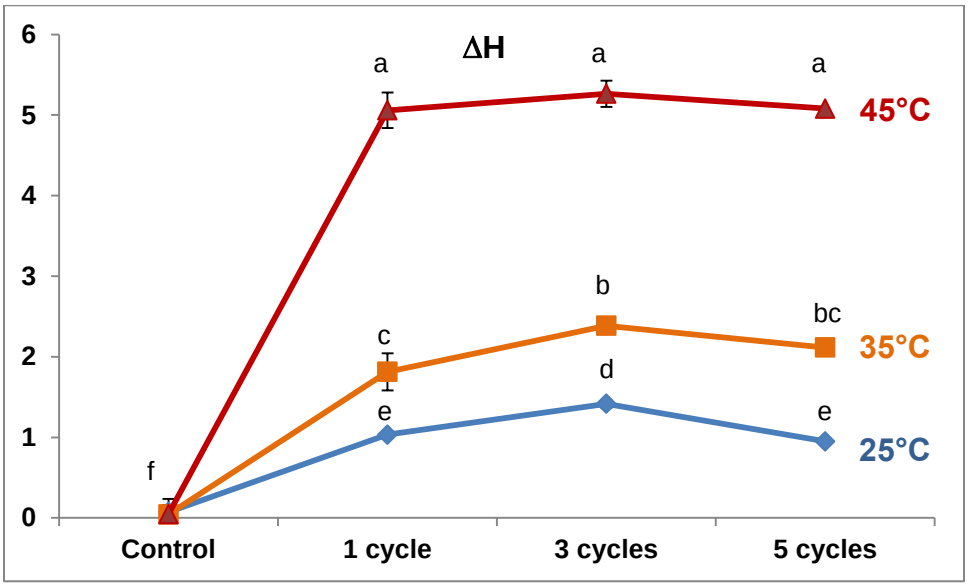


Figure 25: Effect of HHPP, temperature and pressure cycling at 400 MPa at 25 °C, 35 °C, 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on the change in hue angle ΔH of ground beef.

Same lowercase letters indicate results are not significantly different ($p<0.05$).

Data see Appendix 13.

ΔH is the difference between the hue angle of the control sample and the HHPP sample. The ΔH changed significantly when the temperature was raised from 25 °C to 45 °C. Moreover, the difference between the 35 °C and 45 °C was about

three fold greater than the difference between 25 °C and 35 °C. However, the pressure cycling was not a key factor here for the hue angle.

IV.6.7. Browning Index BI

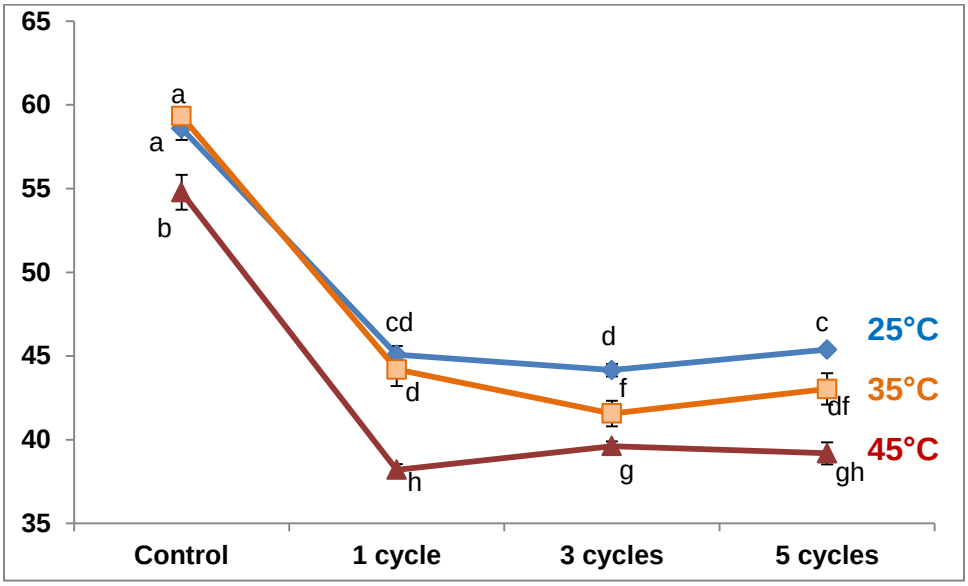


Figure 26: Effect of HHPP, temperature and pressure cycling at 400 MPa at 25 °C, 35 °C, 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on Browning Index BI of ground beef.

Same lowercase letters indicate results are not significantly different ($p<0.05$).

Data see Appendix 14.

Figure 26 shows the Browning Index of the ground beef before and after HHPP. Temperature was an important factor influencing the BI value. There was a significant change in B.I. as temperature increased and decreased. The higher the temperature was, the greater the difference between the control and

processed samples. Additionally, pressure cycling processing with 3 pressure cycles showed a significant impact compare to steady pressure processing.

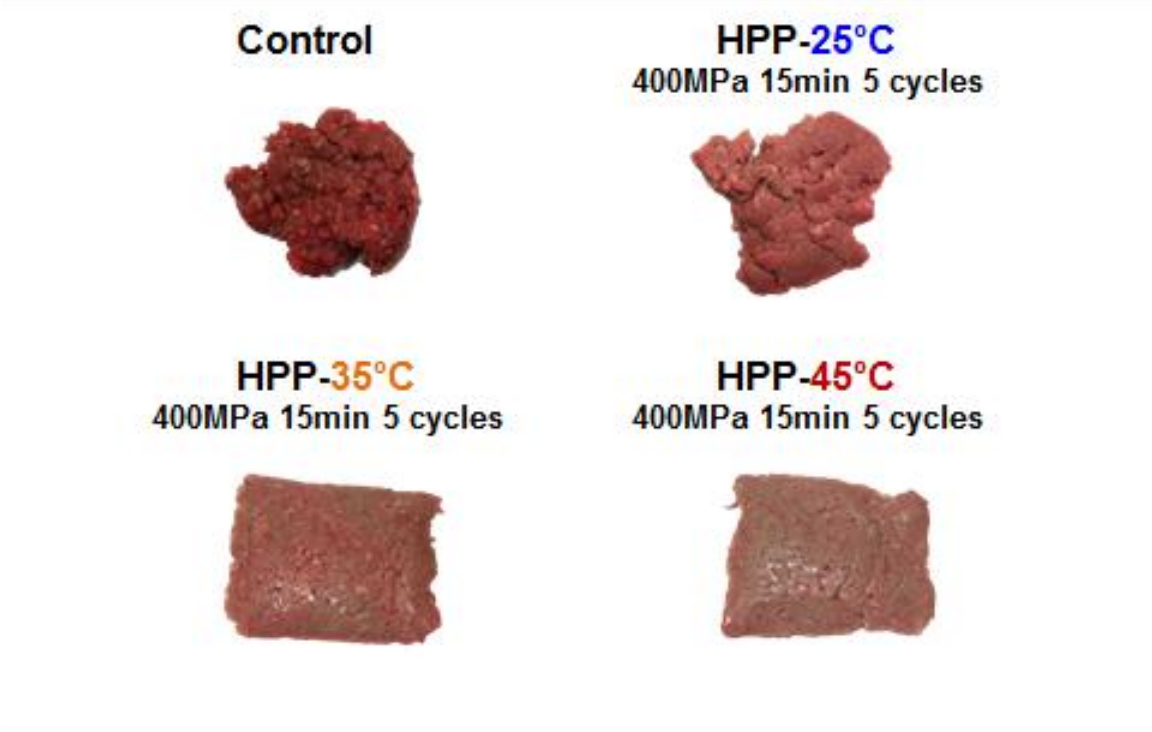


Figure 27. Pictures of ground beef samples before and after HHPP at 400 MPa 15 min 5 cycles. From left to right, up to down, they are control sample, 25 °C processed sample, 35 °C processed sample, and 45 °C processed sample.

IV.7. Effect of cold storage on *E. coli* O157: H7 survival in HHPP processed ground beef

During a five-day shelf life study at 4°C, there was no significant change in the population of *E. coli* O157:H7 for samples plated on the non-selective media-TSA (**Figure 28**). However, for samples plated on the selective media, Rainbow agar, a significant decline in the population of *E. coli* occurred after one-day of storage and then leveled off.

E. coli O157:H7 was unable to recover and grow during cold storage, resulting in a further log reduction.

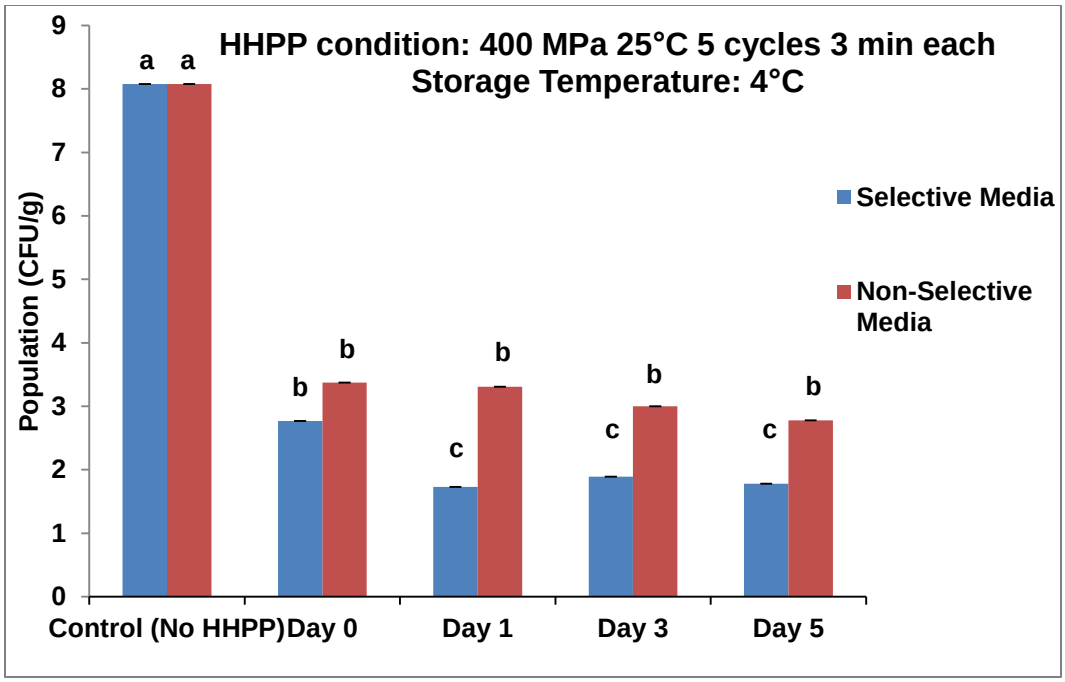


Figure 28: Effect of the effect of cold storage on *E. coli* O157:H7 survival in HHPP processed ground beef.

Same lowercase letters indicate results are not significantly different ($p<0.05$).

Data see Appendix 15.

IV.8. Effect of frozen storage on *E. coli* O157: H7 survival in HHPP processed ground beef

During a five-day shelf life study at -20°C there were significant differences in the population of *E. coli* O157:H7 measured using selective and non-selective media after one-day frozen storage (**Figure 29**). A 0.5 log difference in *E. coli* levels were apparent for samples plated on TSA and Rainbow agar.

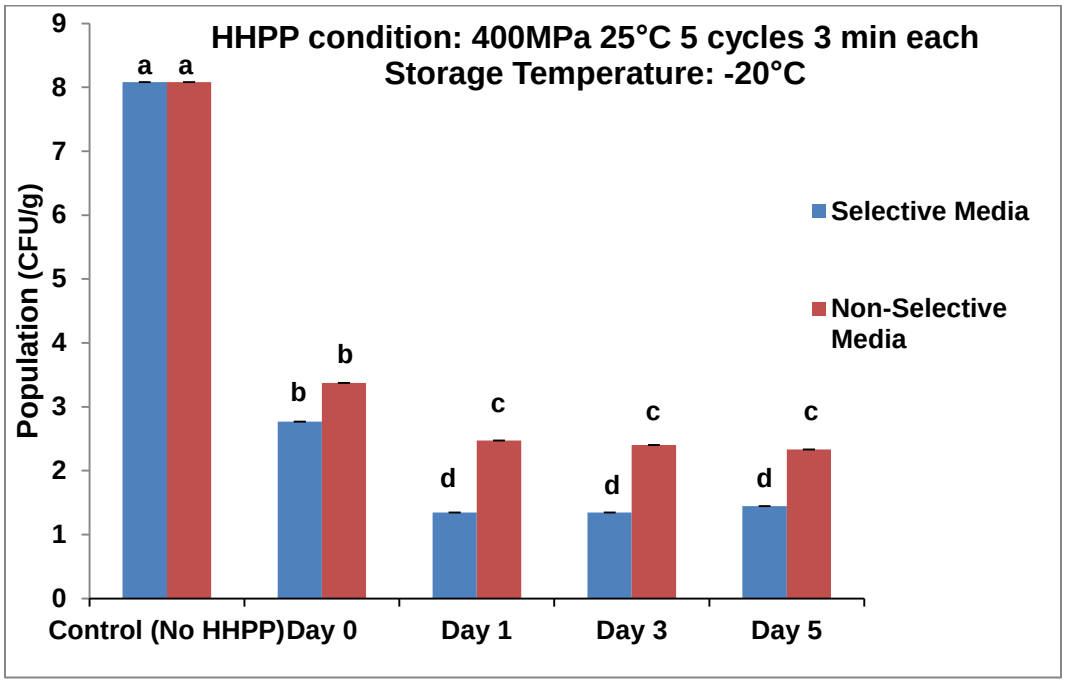


Figure 29: Effect of the effect of frozen storage on *E. coli* O157:H7 survival in HHPP processed ground beef.

Same lowercase letters indicate results are not significantly different ($p<0.05$).

Data see Appendix 16.

IV.9. Comparison of cold and frozen storage impact on *E. coli* O157:H7 survival in HHPP processed ground beef

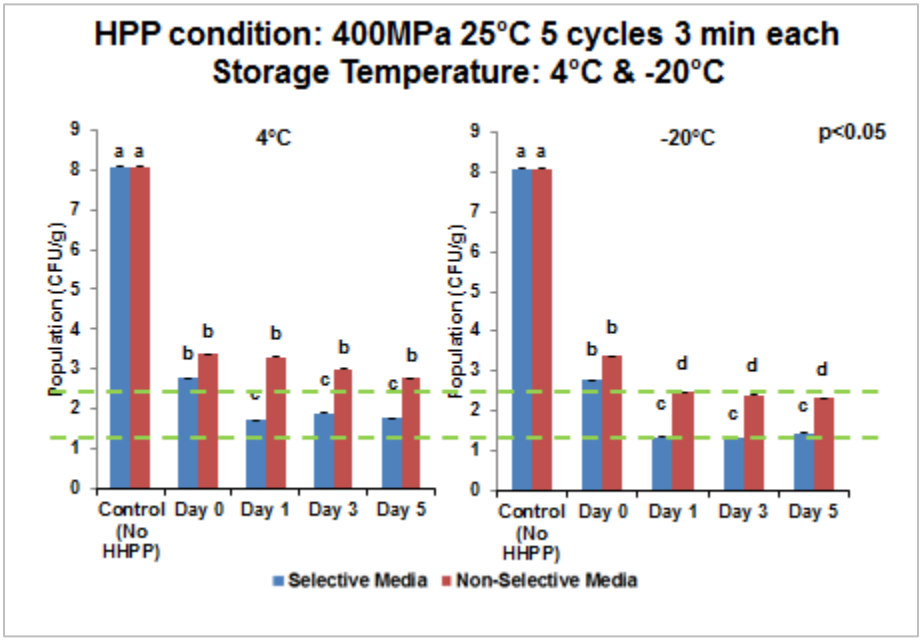


Figure 30: Comparison of cold and frozen storage impact on *E. coli* O157:H7 survival and recovery in HHPP processed ground beef

Same lowercase letters indicate results are not significantly different (p<0.05)

It can be seen from the data presented that compared to the cold storage, freezing had a significantly greater impact on the survival and recovery of *E. coli* O157:H7 (**Figure 30**).

Also, the difference between the *E. coli* O157:H7 population on selective media (Rainbow Agar) and non-selective media (TSA) indicated the number of injured *E. coli* O157:H7 cells after HHPP. Less than one log difference in cell number

occurred on the first day of storage between samples held at 4 °C and -20 °C and plated on selective and non-selective median. But, more than a 1.5 log difference occurred thereafter suggesting that severely injured cells were no longer capable of recovery.

V. Conclusions

This research has expanded our understanding of the effect of high pressure processing, as well as pressure cycling, on inactivation of *E. coli* O157:H7 in ground beef. The hypothesis of this research was proven, that is the application of HHPP with pressure cycling and pressure-temperature combination treatment boost the effectiveness of pressure to inactivate *E. coli* O157:H7 in ground beef.

High hydrostatic pressure processing does show the ability to inactivate *E. coli* O157:H7 in ground beef. Various combinations of HHPP pressure and time were applied to the inoculated ground beef resulting in a minimum of one log greater reduction of *E. coli* O157:H7. The two factors, hold time and maximum pressure, significantly influenced the measured log reduction of *E. coli* O157:H7. Higher pressure and longer period of time will inactive more *E. coli* O157:H7 in ground beef.

Temperature and pressure acted synergistically to inactive *E. coli* O157:H7 in ground beef. The impact on inactivation of *E. coli* O157:H7 in ground beef was much greater, compared to thermal processing or HHPP alone. When ground beef was processed at lower initial temperatures (e.g., 7 ˚C) a lower log reduction of *E. coli* O157:H7 occurred. This reduction can likely be attributed to effect of pressure alone. The results of HHPP treatment of ground beef at higher temperatures (45 ˚C) were comparable to the results obtained at initial room temperature and does enhance inactivation of *E. coli* O157:H7 in ground beef. Although the high temperature and pressure effectively inactivated the *E. coli* the process had a negative impact on ground beef quality.

HHPP treatment has the potential to decrease levels of *E. coli* O157:H7 significantly from ground beef; especially when pressure cycling is applied. A 5 log reduction in *E. coli* O157:H7 in ground beef was achieved at 400 MPa at 25 °C with 5 pressure cycles with total time at high pressure of 15 min. Following HHPP treatment, frozen storage at -20°C had an additional negative effect on survival of *E. coli* O157:H7.

The further study on pressure cycling showed that pressure cycling was more effective than non-cycling for inactivation of *E. coli* O157:H7 in ground beef. The log reduction achieved by pressure cycling was always significantly greater than the non-cycling HHPP. Specifically, a 5 log reduction in *E. coli* O157:H7 in ground beef was achieved at 400MPa at 25 °C with 5 pressure cycles with total time at 400MPa of 15min.

During the 5-day shelf life study, frozen storage at -20 °C had an additional negative effect on survival of *E. coli* O157:H7, compared to storage at 4 °C.

In summary, pressure cycling is possible to achieve greater inactivation of *E. coli* O157:H7 in ground beef and ensure the microbiological safety of ground beef.

VI. Future Work

- The effect of HHPP on *E. coli* O157:H7 survival in ground beef with other fat percentages should be studied. The patterns of *E. coli* O157:H7 inactivation by HHPP in a variety of foods should be compared with ground beef to better understand the characteristics of a food matrix that allow *E. coli* O157:H7 survival.
- The effect of HHPP on the texture of ground beef will also be an interesting factor to study. The texture analysis results, as well as the color data and microbial data, will also have an effect on the selection of the pressure, temperature and time conditions. However, minimum cooking should be achieved before evaluating the texture of high pressure processed ground beef.
- The molecular mechanism behind the survival of *E. coli* O157:H7 in ground beef during HHPP needs to be investigated. Detailed microscopy such as the use of fluorescence microscope and on *E. coli* O157:H7 staining/labeling for inoculated ground beef would be useful to understand the ground beef matrix structure and the possible locations within the ground beef matrix where on *E. coli* O157:H7 survives.
- The mechanism for color change of the ground beef during HHPP should be elucidated. Further study on minimum or eliminate the color change by adding other ingredient or combining other technology will also be attractive to do some research on.

- Lowering the initial temperature below 25 °C should be studied. Since ground beef is generally held at refrigeration temperatures, it would be more reasonable to treat the samples at a relatively low temperature. However, at low temperatures the action of HPP in the inactivation of bacteria may be diminished. However, with the help of the pressure cycling, the effect might be boosted.

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VIII. Appendices

Appendix 1:

Table 2: Effect of HHPP at 600 MPa for 20 min on JM109 in ground beef.

	Population (Log cfu/g)	Standard Error
Control (No HHPP)	7.94	0.08
600MPa-20min	0	0

Appendix 2:

Table 3: Effect of HHPP on JM109 in ground beef at various pressure-time conditions.

	Population (Log cfu/g)	Standard Error
Control (No HHPP)	7.94	0.08
600MPa-20min	0	0
400MPa-21min	0	0
400MPa-18min	0	0
400MPa-15min	0	0
400MPa-12min	0	0
400MPa-10min	3.63	0.13

Appendix 3:

Table 4: Effect of HHPP at 400 MPa for 15 min at 25 °C on *E. coli* O157:H7 in ground beef.

	Population (Log cfu/g)	Standard Error
Control (No HHPP)	7.94	1.09E-15
86-24	4.67	0.29
WM98A06026	4.46	0.19
C7927	5.56	0.21
F4546	5.56	0.18
SEA13B88	6.37	0.23
ATCC43895	6.25	0.11
Cocktail	5.22	0.25
JM109	0	0

Appendix 4:

Table 5: Effect of HHPP at 400 MPa for 15 min at 25 °C, 35 °C, and 45 °C on individual *E. coli* O157:H7 strains and their cocktail in ground beef.

	Population (Log cfu/g)			Standard Error		
	25 °C	**35 °C**	**45 °C**	**25 °C**	**35 °C**	**45 °C**
Control (No HHPP)	7.94	7.94	7.94	0	0.03	0
86-24	4.67	4.48	3.91	0.29	0.09	0.11
WM98A06026	4.46	4.43	3.15	0.19	0.07	0.10
C7927	5.56	5.07	3.63	0.21	0.18	0.09
F4546	5.56	4.54	3.39	0.18	0.16	0.05
SEA13B88	6.37	5.41	3.04	0.23	0.24	0.09
ATCC43895	6.25	4.75	3.35	0.11	0.35	0.13
Cocktail	5.22	4.03	3.16	0.25	0.23	0.11

Appendix 5:

Table 6: Effect of high pressure cycling at 400 MPa at 25 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on *E. coli* O157:H7 in ground beef.

	Population (Log cfu/g)			Standard Error		
	25 °C - 1cycle	25 °C - 3cycles	25 °C - 5cycles	25 °C - 1cycle	25 °C - 3cycles	25 °C - 5cycles
Control (No HHPP)	7.94	7.94	7.94	0	0.03	0.04
86-24	4.67	3.63	2.60	0.29	0.34	0.29
WM98A06026	4.46	3.85	2.36	0.19	0.17	0.31
C7927	5.56	3.56	2.70	0.21	0.33	0.32
F4546	5.56	3.85	2.95	0.18	0.45	0.19
SEA13B88	6.37	3.79	3.21	0.23	0.14	0.19
ATCC43895	6.25	3.64	2.69	0.11	0.31	0.19
Cocktail	5.22	3.61	3.00	0.25	0.18	0.25

Appendix 6:

Table 7: Effect of high pressure cycling at 400 MPa at 35 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on *E. coli* O157:H7 in ground beef.

	Population (Log cfu/g)			Standard Error		
	35 °C - 1cycle	35 °C - 3cycles	35 °C - 5cycles	35 °C - 1cycle	35 °C - 3cycles	35 °C - 5cycles
Control (No HHPP)	7.94	7.94	7.94	0.03	0.03	0.03
86-24	4.48	3.31	2.28	0.09	0.19	0.09
WM98A06026	4.43	3.33	2.19	0.07	0.18	0.16
C7927	5.07	3.81	2.09	0.18	0.15	0.08
F4546	4.54	3.20	1.94	0.16	0.19	0.11
SEA13B88	5.41	3.48	1.99	0.24	0.26	0.06
ATCC43895	4.75	2.27	NA	0.35	0.07	NA
Cocktail	4.03	3.04	2.00	0.23	0.36	0.12

Appendix 7:

Table 8: Effect of high pressure cycling at 400 MPa at 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on *E. coli* O157:H7 in ground beef.

	Population (Log cfu/g)			Standard Error		
	45 °C - 1cycle	45 °C - 3cycles	45 °C - 5cycles	45 °C - 1cycle	45 °C - 3cycles	45 °C - 5cycles
Control (No HHPP)	7.94	7.94	7.94	0	0	0
86-24	3.91	NA	NA	0.11	NA	NA
WM98A06026	3.15	NA	NA	0.10	NA	NA
C7927	3.63	NA	NA	0.09	NA	NA
F4546	3.39	NA	NA	0.05	NA	NA
SEA13B88	3.04	NA	NA	0.09	NA	NA
ATCC43895	3.35	NA	NA	0.13	NA	NA
Cocktail	3.16	NA	NA	0.11	NA	NA

Appendix 8:

Table 9: Effect of HHPP, temperature and pressure cycling at 400 MPa at 25 °C, 35 °C, 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on L*, a*, and b* value of ground beef.

	L*a*b* Values			Standard Error		
	L*	**a***	**b***	**L***	**a***	**b***
25 °C control	49.27	21.29	12.53	0.41	0.47	0.19
25 °C -1 cycle	56.87	18.16	11.81	0.37	0.27	0.05
25 °C -3 cycles	57.74	17.71	11.95	0.67	0.14	0.14
25 °C -5 cycles	57.74	18.65	12.02	0.09	0.07	0.03
35 °C control	50.51	22.48	12.81	0.30	0.15	0.11
35 °C -1 cycle	59.07	17.95	12.20	0.39	0.90	0.24
35 °C -3 cycles	59.17	16.57	11.88	0.27	0.42	0.05
35 °C -5 cycles	59.19	18.46	12.32	0.40	0.25	0.09
45 °C control	48.27	22.85	11.21	0.44	0.33	0.20
45 °C -1 cycle	55.89	13.94	10.64	0.15	0.07	0.11
45 °C -3 cycles	56.20	14.41	11.12	0.50	0.20	0.11
45 °C -5 cycles	56.31	14.44	10.95	0.37	0.31	0.34

Appendix 9:

Table 10: Effect of HHPP, temperature and pressure cycling at 400 MPa at 25 °C, 35 °C, 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on ΔL*, Δa*, and Δb* value of ground beef.

	ΔL*	Δa*	Δb*
25 °C -1 cycle	7.59	-3.13	-0.72
25 °C -3 cycles	8.47	-3.58	-0.58
25 °C -5 cycles	8.46	-2.65	-0.51
35 °C -1 cycle	8.56	-4.52	-0.61
35 °C -3 cycles	8.66	-5.90	-0.93
35 °C -5 cycles	8.68	-4.02	-0.48
45 °C -1 cycle	7.62	-8.91	-0.57
45 °C -3 cycles	7.93	-8.44	-0.09
45 °C -5 cycles	8.04	-8.41	-0.26

Appendix 10:

Table 11: Effect of HHPP, temperature and pressure cycling at 400 MPa at 25 °C, 35 °C, 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on total color difference (ΔE) of ground beef.

	Values			Standard Error		
ΔE	**25 °C**	**35 °C**	**45 °C**	**25 °C**	**35 °C**	**45 °C**
control	0.48	0.27	0.52	0.38	0.18	0.09
1 cycle	8.25	9.76	11.75	0.44	0.19	0.08
3 cycles	9.22	10.53	11.65	0.59	0.45	0.36
5 cycles	8.88	9.58	11.69	0.10	0.36	0.38

Appendix 11:

Table 12: Effect of HHPP, temperature and pressure cycling at 400 MPa at 25 °C, 35 °C, 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on the saturation index SI of ground beef.

	Values			Standard Error		
SI	**25 °C**	**35 °C**	**45 °C**	**25 °C**	**35 °C**	**45 °C**
control	24.71	25.87	24.91	0.50	0.17	0.38
1 cycle	21.66	21.93	17.54	0.20	0.68	0.12
3 cycles	21.37	20.39	18.21	0.17	0.37	0.18
5 cycles	22.18	22.19	18.12	0.08	0.24	0.30

Appendix 12:

Table 13: Effect of HHPP, temperature and pressure cycling at 400 MPa at 25 °C, 35 °C, 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on the whitening index WI of ground beef.

	Values			Standard Error		
WI	**25 °C**	**35 °C**	**45 °C**	**25 °C**	**35 °C**	**45 °C**
control	43.57	44.15	42.58	0.19	0.19	0.42
1 cycle	51.73	53.69	52.53	0.42	0.18	0.14
3 cycles	52.64	54.36	52.56	0.56	0.40	0.44
5 cycles	52.27	53.55	52.70	0.10	0.33	0.31

Appendix 13:

Table 14: Effect of HHPP, temperature and pressure cycling at 400 MPa at 25 °C, 35 °C, 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on the change in hue angle ΔH of ground beef.

	Values			Standard Error		
ΔH	**25 °C**	**35 °C**	**45 °C**	**25 °C**	**35 °C**	**45 °C**
control	0.53	0.52	0.41	0.00	0.00	0.00
1 cycle	0.58	0.59	0.65	0.01	0.01	0.00
3 cycles	0.59	0.62	0.66	0.00	0.01	0.01
5 cycles	0.57	0.59	0.65	0.00	0.00	0.02

Appendix 14:

Table 15: Effect of HHPP, temperature and pressure cycling at 400 MPa at 25 °C, 35 °C, 45 °C, with 1, 3, and 5 pressure cycles with total pressure holding time of 15 min on Browning Index BI of ground beef,

	Values			Standard Error		
BI	25 °C	35 °C	45 °C	25 °C	35 °C	45 °C
control	58.59	59.33	54.77	0.69	0.07	1.04
1 cycle	45.07	44.19	38.20	0.53	0.98	0.34
3 cycles	44.15	41.56	39.61	0.38	0.76	0.29
5 cycles	45.38	43.03	39.18	0.20	0.93	0.65

Appendix 15:

Table 16: Effect of the effect of cold storage on *E. coli* O157:H7 survival in HHPP processed ground beef.

	Population (Log cfu/g)		Standard Error	
4°C	**Selective Media**	**Non-Selective Media**	**Selective Media**	**Non-Selective Media**
Control (No HHPP)	8.08	8.08	0	0.14
Day 0	2.77	3.37	0.29	0.25
Day 1	1.73	3.31	0.43	0.21
Day 3	1.89	3.00	0.09	0.07
Day 5	1.78	2.78	0.23	0.28

Appendix 16:

Table 17: Effect of the effect of frozen storage on *E. coli* O157:H7 survival in HHPP processed ground beef.

	Population (Log cfu/g)		Standard Error	
-20°C	**Selective Media**	**Non-Selective Media**	**Selective Media**	**Non-Selective Media**
Control (No HHPP)	8.08	8.08	0	0.14
Day 0	2.77	3.37	0.29	0.25
Day 1	1.35	2.47	0	0.22
Day 3	1.35	2.40	0	0.25
Day 5	1.45	2.33	0.17	0.19