

High Hydrostatic Pressure Processing Reduces *Salmonella enterica* from Diced and Whole Tomatoes

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ABSTRACT:

Fresh and fresh-cut tomatoes have been associated with numerous outbreaks of salmonellosis in recent years. While the exact routes of contamination are unknown, high pressure processing (HPP) is being evaluated as a post harvest treatment to eliminate *Salmonella enterica* from tomatoes. The objectives of the study were to determine the potential for of HPP to reduce *S. enterica* serovars Newport, Javiana, Braenderup and Anatum (clinical isolates from tomato outbreaks) in tryptic soy broth (TSB) and to determine the effect of HPP to reduce the most pressure resistant *S. enterica* serovar from fresh diced and whole tomatoes. Five ml portions of broth containing 8 log CFU/ml of one of the four serovars (nalidixic acid resistant) were packaged in sterile stomacher bags and subjected to one of three different pressures (350, 450, or 550 MPa) for 120s. Samples were enumerated by surface plating onto tryptic soy agar supplemented with 50 ppm nalidixic acid (TSAN) and incubated at 35°C for 48 hours. The most pressure resistant *S. enterica* serovar evaluated was Braenderup. Subjecting the broth culture to 350, 450 and 550 MPa resulted in a 4.53, 5.74 and 7.09 log reduction in *S. Braenderup*, respectively. Diced tomatoes (150g) and whole red round tomatoes (150g; packaged in 350ml of 1% CaCl₂) were inoculated with *S. Braenderup*, to obtain 6 log CFU/g throughout the sample and subjected to the same pressure treatments as described above. After HPP, diced tomatoes were homogenized for 1 minute and then plated on TSAN. Whole tomatoes were surface sampled, and then homogenized for 1 minute. Surface and homogenate samples were plated on TSAN supplemented with 1% pyruvic acid (TSANP). Significant reductions of *S. Braenderup* concentrations in diced tomatoes ($P < 0.05$) were seen after processing at 350 (0.46 CFU/g), 450 (1.44 log CFU/g), and 550 MPa (3.67 log CFU/g). In whole tomatoes, significant reductions ($P < 0.05$) were also seen at 350 (1.41 log CFU/g), 450 (2.25 log CFU/g) and 550 MPa (3.35 log CFU/g). There were no differences in visual appearance between fresh and HPP diced and whole tomatoes. HPP may be an effective post harvest strategy to reduce low levels of *S. enterica* contamination in diced tomatoes.

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DEDICATION

I dedicate this work to my parents, Jimmy and Leslie Maitland, for unconditionally supporting me through every step of the last 24 years.

Attribution

Several colleagues and coworkers aided in the writing and research behind the chapters of this thesis. A brief description of their background and their contributions are included here.

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Chapter 3: High Hydrostatic Pressure Processing Reduces *Salmonella enterica* from Diced and Whole Tomatoes

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Chapter 1

Introduction and Justification

Over the last two decades, there has been a noticeable increase in the frequency of produce related foodborne illness outbreaks. Between the years 1997 and 2005, the proportion of all foodborne illnesses associated with the consumption of produce more than doubled, from 6% to 13% (Dewaai, 2007). This increase could be based on several factors. The development of a better understanding of the relationship between eating a well balanced diet and lowering the risk of many common diseases has lead Americans to consume fruits and vegetables at a higher rate than ever before. In addition, better outbreak tracking and surveillance systems can now detect similar isolates and trace back outbreaks all the way to the farm. Fruits and vegetables are often consumed raw with little or no post harvest physical or chemical treatment to reduce microbial loads. The absence of a mitigation step increases the potential of contamination reaching the consumer.

Salmonella enterica serovars are one of the most common pathogenic microorganisms associated with fresh and fresh cut produce-related outbreaks. It is estimated that *S. enterica* causes 1.4 million illnesses and 400 deaths annually in the United States (Greene, 2007). Since 1999, there have been 15 outbreaks of salmonellosis linked with the consumption of raw tomatoes (Hill, 2008). Between 2005 and 2006 alone four outbreaks associated with tomatoes led to 459 confirmed infections in over 21 states (CDC, 2007) The mechanism for contamination of the tomato is still unknown, so farmers and researchers are trying to identify these contamination routes in order to find ways to prevent it from occurring. Currently researchers are exploring both pre and post harvest applications to eliminate *S. enterica* contamination.

High Hydrostatic Pressure Processing (HPP) is an alternative food processing procedure developed over a century ago (FDA, 2000). This technology has received a lot of attention in the last two decades as researchers have begun to study the relationship of high pressure and the preservation of solid foods. HPP is a non-thermal process, which acts uniformly throughout the food, preventing any changes to nutritional value, flavor, or color of the foods, and allowing the product to appear fresh while also extending microbiological shelf life (Smelt, 1998). Both liquid and solid foods can be subjected to pressures between 100 and 800 MPa at temperatures ranging from 0°C to 100°C, and depending on food consistency, maintain quality (FDA, 2000). The process is most effective in high moisture content products, especially liquids. High pressure processing may be an effective way to process whole and diced tomatoes while maintaining a high quality fresh market product.

Before addressing the objectives of this study, we analyzed the effect of packing the tomatoes in various solutions (distilled water and 1% solutions of NaCl and CaCl) on the quality (texture, skin, firmness) of whole tomatoes under HPP conditions and parameters. Researchers wanted to ensure that they were packing tomatoes in the best solution. The primary objectives of this study are to determine the effect of HPP (350, 450, and 550 MPa) to inactivate *S. enterica* serovars Newport, Javiana, Braenderup and Anatum (isolated from tomato outbreaks) in tryptic soy broth. Then to use the information gathered to determine the optimal (pressure and time) for application of HPP to whole and diced red round tomatoes to inactivate the most pressure resistant *S. enterica* serovar

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Chapter 2

Literature Review

Salmonella

Salmonella enterica spp. is one of the leading causes of pathogenic bacterial foodborne illness in the United States. In 2005, *S. enterica* serotypes accounted for 45,322 reported illnesses in the United States (CDC, 2007). However, many milder cases are not always reported, the Center for Disease Control and Prevention (CDC) estimates that 2 to 4 million infections occur annually in the United States (CDC, 2008). The annual total cost associated with *S. enterica* is estimated at 3 billion dollars in the United States when taking into account doctor and hospital visits, medication costs, productivity loss, disutility costs, and the cost of premature death (WHO, 2005).

S. enterica is a facultatively anaerobic, gram-negative, rod-shaped bacterium that belong to the family *Enterobacteriaceae* (Montville, 2005). This pathogen has made a significant impact on the food industry and been the focus of extensive research due to its wide range of adaptability. Currently, over 2,600 different serovars have been identified of the subspecies *enterica* (Rhen, 2007). *S. enterica* grows optimally at 37°C and at neutral pH, but can adapt to survive at temperatures ranging from as low as 2°C to as high as 54°C and pH levels ranging from 4.5 to 9.5 (Montville, 2005). This organism's adaptability may reduce the efficacy of many common handling and processing practices.

The intestines of humans and animals provide an optimal environment for *S. enterica* to grow. For this reason, *S. enterica* contamination in food typically originates from fecal contamination either from humans or animals. Contaminated feces can then infiltrate the environment, creating secondary sources of the pathogen including water, soil, factory and kitchen surfaces, and improperly washed hands of food handlers.

Contaminated foods are usually animal in origin, such as meat, fish, or eggs because domestic and wild animals such as birds, cattle, and pigs are typical reservoirs for *S. enterica*. In the last two decades there has been a significant increase in contaminated vegetables (Dewaal, 2007). The exact infectious dose is unknown, but symptoms have occurred from ingestion of as few as 15-20 cells (FDA, 1992). The illness caused by *S. enterica* is a type of gastroenteritis called salmonellosis. Symptoms include nausea, vomiting, abdominal cramps, diarrhea, and fever and will begin between 6 to 48 hours following ingestion of contaminated food. The symptoms are usually self-limiting and even subside within a few days after their onset (CDC, 2006). Occasionally treatment may be required in the form of fluid and electrolyte replacement to minimize dehydration effects (D-Aoust, 1994). Antibiotic treatment is not necessary unless the bacterium has spread from the intestines to the blood stream (D-Aoust, 1994). Antibiotics, however, are becoming less effective as some strains have become resistant (D-Aoust, 1994). Duration and extent of symptoms vary between patients. Children are the most likely to become infected, along with elderly and immuno-compromised individuals. The CDC estimates that nearly 600 individuals a year die from acute salmonellosis (CDC, 2008).

If salmonellosis goes untreated for too long or if a patient's immune system is fairly weak, secondary complications may develop. The most common of these complications is called Reiter's syndrome. About 2% of culture-proven cases develop a triad of arthritis (pain in their joints), conjunctivitis (irritation of the eyes) and urethritis (painful urination) typically 3 weeks after infection (FDA, 2007). This syndrome can last for weeks or months and can eventually lead to chronic arthritis. Antibiotic treatment

seems to have no effect on which patient does or does not develop chronic arthritis.
(CDC, 2008)

Produce Related Outbreaks

There has been a notable increase in the reported cases of fresh produce as a carrier of foodborne pathogens. The number of reported produce related outbreaks doubled between 1973-1987 (2%) and 1987-1991(5%). Additionally, the number of cases of illnesses associated with these outbreaks has more than doubled (Tauxe, 1997). *S. enterica* was reported in documented outbreaks as a highly prevalent bacterial pathogen found in a wide range of produce including: lettuce, watermelon bean and alfalfa sprouts, parsley, cabbage, cantaloupe, and tomatoes (Beuchat, 1995).

While the USDA has recognized the potential for contamination of *Salmonella* in the meat and poultry industry by requiring Hazard Analysis Critical Control Point (HACCP) plans in meat processing facilities, these protocols are not mandatory in the produce industry and may not be as effective. This is largely due in part to the fact that meat is often cooked (a critical control point) during manufacturing or at home before consumption, while produce is often consumed raw.

Factors associated with increased produce-related outbreaks

Americans are becoming increasingly health conscious. Researchers are putting more and more emphasis on discovering how the human body works and how foods that are consumed affect human health. The importance of eating adequate fruits and vegetables for their vitamin and antioxidant qualities has become well known in the last 20 years and the consumption of fresh vegetables and fruits has increased per capita by 33% and 26% respectively (Pollack, 2001). The increased demand for produce has

directly affected the growth of global trade. By importing produce from all over the world, the produce industry ends the supply limits of seasonality. On the other hand, from exotic climates come unfamiliar microflora and potential for lower standards of hygiene quality.

Produce is a particularly good host for microorganisms because it contains adequate water, sugars and other nutrients to improve growth and survival of microorganisms. Additionally, bacteria are most likely to attach and survive better in cracks and crevices found on many produce surfaces because the nutrients are more available there. Examples of these surfaces include the stomata on lettuce leaves and the stem scar on tomatoes.

Produce items are commonly served as a ready-to-eat raw products. The lack of effective physical or chemical treatments during post-harvest processing increases the survival rate of many pathogens. This is why it is important in the produce industry to carefully monitor conditions such as storage and transport temperature as well as hygiene from the farm to the table.

Possible Routes of Contamination: Farm to fork

Field contamination of fresh produce can occur through many different routes. These can include the application of improperly composted manures to the field, the use of non-potable water for irrigation and fertilizer application, and contamination from infected field workers. Additionally, and perhaps most difficult to monitor, contamination can be introduced through infected wildlife (birds, deer, amphibians, rodents, etc.). In 1995, 62 travelers from 21 states were infected with salmonellosis after drinking unpasteurized orange juice from a theme park in Florida. Contamination was

traced back to one farm where the oranges were irrigated with contaminated surface water and often picked off the ground without being washed (Cook, 1998). Mohle-Boetani et al (2001) described a multistate outbreak of *S. enterica* serovar Stanley in 1997 from alfalfa sprouts, where the origin of contamination was traced back to the seed farm that was using un-composted chicken manure and contaminated irrigation water from a canal.

Salmonella species are particularly difficult to control on farmland because of their long term survival in certain high-risk environments. *S. enterica* has been shown to survive at high numbers ($6.70 \log_{10}$ CFU/g) in slightly moist soil for up to 45 days (Guo, 2002) and can even survive for months or years in the soil of flooded croplands (Beuchat, 1997).

At the processing plant level, fresh produce can become contaminated from contaminated food handlers, equipment and/or water. In addition, pathogens present from the field can be amplified due to improper handling and storage conditions. Forty-one cases of *S. enterica* infection in Australia were traced back to a single contaminated wheel of a lettuce shredder (Satfford, 2002). In 1991, more than 400 cases of *S. enterica* infections were traced back to pre-sliced cantaloupe coming from Texas or Mexico due to contaminated slicing equipment and high humidity and temperature during storage (CDC, 1991). Poor storage conditions in holding facilities or transport trucks can also contribute to spreading or amplifying contamination.

In response to the wide variety of contamination factors, Good Agricultural Practices (GAPs) are guidelines that have been developed by state and federal governments as a way to keep all components of fresh produce safe from contamination

while promoting economic viability and social stability. Many universities have joined together to develop the National Good Agricultural Practices Program in order to develop educational materials that will promote the use of these practices on the farm and in the packaging house. Although these guidelines do not carry the power of the law they are strongly suggested and producers can voluntarily agree to audits and certifications through the USDA to enhance the farm or packaging house's credibility.

Consumers are also responsible to properly handle produce in their homes. Li-Cohen et al (2002) performed a study that focused on consumer knowledge of handling fresh fruits and vegetables. Although 81% of participants said they washed their produce at home, the most common method of washing was to run it under tap water (Li-Cohen, 2002). This method would remove visible dirt but not adequately remove microorganisms. The study also found that almost half of the subjects indicated that they did not wash their hands before handling the fresh produce and stored raw meat and poultry products above their produce in the refrigerator: two practices that could lead to cross contamination (Doyle, 2000).

Tomatoes

The health benefits of consuming a diet high in fruits and vegetables have long been common knowledge. Raw tomatoes have been specifically linked with high levels of lycopene, a possible cancer preventative and powerful antioxidant, as well as a large percentage of daily vitamin A and C (IFIC, 2006). For this and other reasons, approximately 5 billion pounds of fresh tomatoes are eaten annually eaten in the United States (CDC, 2004).

Survival of Salmonella on Tomatoes

S. enterica has been isolated from produce during several market surveys. Tomatoes are one of the leading produce items implicated in carriers of *S. enterica* outbreaks. Wash water collected from tomatoes in local retail marketplaces was positive for *Salmonella* in 55% of healthy ripe tomatoes and 64% of soft rotted tomatoes (Wells, 1997).

The desired neutral pH environment of this particular pathogen (between 6.5 and 7.5) would suggest that the slightly acidic conditions of the tomato would limit growth. *S. enterica*, however, have become more tolerant to low pH ranges when exposed to temperatures between 25 and 30°C, which are common tomato storage temperatures (Chung, 1970). In 1991, Asplund et al., determined that *S. enterica* could grow in tomatoes despite their acidic environment because of the main acid found in tomatoes, citric acid, which permits the growth of *S. enterica* even at low levels of pH (3.99 to 4.37). In more recent study, research was conducted to determine if initial acid adaptation of *S. enterica* cells would affect growth and survival after inoculation into tomatoes (Beuchat, 2008). Glucose was added to tryptic soy broth containing naladixic acid to drive the pH of the cells down to 4.75 as opposed to a 7.07 pH level in those cells grown in the absences of glucose (Beuchat, 2008). These two cell cultures were inoculated into both whole and diced tomatoes (Beuchat, 2008). The study found that previous exposure to acid did not consistently or significantly influence the cells ability to grow and survive in either type of tomato. These findings not only back up previous findings that *S. enterica* species grow well in the low pH environment of the tomato, but also emphasize that simply exposing tomatoes to an acidic wash in the packaging houses or at home may not be enough to prevent illness.

Environmental temperature and humidity also play a large role in the survival rate of *S. enterica* in tomatoes. On the surface of tomatoes stored at a temperature of 27°C, the pathogen has been shown to survive up to 14 days (Guo,2002) and for up to 6 days under 100% relative humidity (Rathinasabapathi, 2004). On the surfaces of red and green round tomatoes, relative humidity and temperature were shown to affect the level of *S. enterica* cell attachment to the product. The lowest number of attached *S. enterica* cells occurred at temperatures around 22°C and 75% relative humidity, suggesting that this may be a safer storage environment (Iturriaga, 2003).

The stem scar of the tomato has been found to provide a particularly protective environment for *S. enterica* to grow (Das, 2006) and a direct route for potential internalization of the pathogen. When placed in a contaminated water bath that is 10°C or more degrees colder than the tomato, the colder water enters into the tomato through the stem scar (Zhuang, 1993). If the water is contaminated, then internalization of the pathogen can occur. This internalization renders any attempts at surface sanitation ineffective and presents the need for alternative treatments (Zhuang, 1993).

Outbreaks

Reported tomato-associated *S. enterica* outbreaks have drastically increased. Since 1999 there have been 12 outbreaks. Between 1990-2004, the CDC reports that the combined data from nine outbreaks have represented an approximate 60,000 cases of salmonellosis (Voetsch, 2004). Refer to Table 1 for a complete listing of outbreaks.

A multistate outbreak of *S. Montevideo* in 1993, led to 100 cases of infections in Illinois, Michigan, Minnesota and Wisconsin (Hedberg, 1999). Through laboratory and community based studies, these infections were linked to an earlier outbreak of *S. Javiana*

in the same states during 1990 and both cases were linked to the consumption of tomatoes at restaurants (Hedberg, 1999). A single packinghouse in South Carolina was implicated as the distributor of the infected tomatoes (Hedberg, 1999). Investigators discovered that the likely source of contamination was a cool un-chlorinated water bath used to wash the incoming tomatoes (Hedberg, 1999). These early outbreaks identified the issue that widely distributed produce has the potential to cause large geographically dispersed and seemingly unrelated outbreaks. Instead of the blame falling on restaurant employees and consumers, these cases implied that the source of contamination occurred during farming and packaging (Hedberg, 1999).

Three major *S. enterica* outbreaks occurred in the summer of 2004 that were later associated with eating roma tomatoes. Between June 18th and July 21st 125 confirmed cases of a *S. Braenderup* infection were reported throughout 16 eastern states (CDC, 2004). In July, 429 outbreak-associated salmonellosis cases were identified in 9 northeast states. *Salmonella* serotypes Javiana, Typhimurium, Anatum were identified predominantly responsible. Finally, during the July 4-8 week, seven confirmed cases of *S. Javiana* infections occurred in one Canadian province (CDC, 2004). After comparing data from case-control studies of the patients, the infection was linked back to roma tomatoes eaten at several restaurants. Traceback investigations identified four packinghouses and five farms as possible sources, although no clear source of contamination was found (Gupta, 2007).

Two large multistate tomato related outbreaks emerged in 2005. The first occurred between July and November, resulting in 72 cases of *S. Newport* infections in 16 eastern states (CDC, 2007). Investigations traced the contamination back to two farms

on the eastern shores of Virginia where the same strain of *Salmonella* was isolated from irrigation ponds near the tomato fields (Greene, 2007). From November to December, 82 cases of salmonellosis were linked back to *S. Braenderup* isolates (CDC, 2007). The environmental investigation revealed that multiple reservoirs of animal feces contaminated with *S. enterica* were present in and around drainage ditches leading to the fields (CDC, 2007).

The summer and fall of 2006 also saw two major widespread outbreaks. 115 culture-confirmed cases of *S. Newport* infections were reported in 19 northern and eastern states between July and November of 2006 (CDC, 2007). From September to October, 190 cases of *S. Typhimurium* were reported in 21 states and Canada. Both of these outbreaks are still under investigation and no direct source of contamination has been identified (CDC, 2007).

FDA Tomato Safety Initiative

Food safety programs are in place to help ensure proper handling of produce, as well as to reduce any risk of further contamination. In 1994, a HACCP program was developed and implemented in many packing houses to insure water quality, particularly to monitor proper levels of chlorine, pH and water temperature in wash tanks (Rushing, 1996). The FDA has encouraged the produce industry to instate Good Manufacturing Practices (GMPs) and Good Agricultural Practices (GAPs) for any water used in packaging houses, but since these practices are merely guidelines do not carry the force of the law following them is voluntary.

In response to the increasing frequency of *S. enterica* outbreaks, the government regulatory agencies have focus attention on the tomato industry. On June 12, 2007, the

FDA released a statement announcing the beginning of a Tomato Safety Initiative (FDA, 2007). The ongoing initiative is a collaborative effort between the FDA, state and local health and agriculture departments in Virginia and Florida, as well as several universities and members of the produce industry. In July of 2007, federal and state investigators visited Virginia based tomato farms and packaging facilities to assess their implementation of suggested Good Agricultural Practices (GAPs) and GMPs as well as general food safety. The same investigations were done throughout Florida later in the year to coincide with their growing and harvesting seasons.

The aim of this initiative is to further improve guidance and policy intended to minimize future outbreaks by identifying practices or conditions that may be causing product contamination and fulfilling produce safety research, education, and outreach needs (FDA, 2007).

High Pressure Processing (HPP)

Use of HPP In Foods:

High pressure processing (HPP) or high hydrostatic pressure processing uses water to equally apply pressure on all sides of an object (FDA, 2000). HPP has the potential to be a very effective tool in producing high quality foods with an extended shelf life. HPP can be applied to liquid and solid foods at pressures between 100 and 800 MPa (FDA, 2000). Temperature (ranging from 0°C to above 100°C) and exposure time (ranging from a millisecond to over 20 min) can also be manipulated for optimal results (FDA, 2000). The benefit of HPP over other types of processing is the reaction of food structure to pressure. The application of pressure to foods has little effect on covalent bonds and therefore pressure alone will not have any affect on the food's chemical

structure (FDA, 2000). Most food items can maintain their physical integrity during processing due to the fact that pressure is applied instantaneously and uniformly to the product through water that surrounds the food independent of size or shape (FDA, 2000).

Although this technology has only recently become the subject of extensive research and development, it is not a new concept. As early as 1895, the first reports were made of high hydrostatic pressure possessing the ability to kill microorganisms (FDA, 2000). In 1899, Bert Hite was the first to experiment with the affects of HPP on pathogen reduction in foods (FDA, 2000). He found that the shelf life of raw milk could be extended by 4 days after a pressure treatment of 600 MPa for 1 hour at room temperature (Hite, 1899). Fifteen years later, Hite again found that pressure treated fruits would remain commercially sterile for at least 5 years after being exposed to pressures ranging from 400 to 820 MPa (Hite, 1914). Researchers appeared to have lost interest in high pressure processing as no real significant studies were published for several decades. Recently however, HPP has re-emerged as potentially successful alternative to microbial inactivation by heat or chemicals.

Unlike some novel technologies (i.e. irradiation) HPP is widely accepted by consumers (Nielsen, 2009). A study done in six countries throughout Eastern and Northern Europe was developed to demonstrate consumer attitude towards HPP. Researchers found that overall consumers felt positive about HPP because no preservatives were used in the process so the end result was a natural and fresh product. They felt HPP was environmentally friendly and retained natural texture well. The only negative feelings documented were a fear of increased prices and lack of information available from food producers on this new technology (Nielsen, 2009). The list of food

products that currently use HPP as part of their development includes; fruit jellies and jams, fruit juices, salad dressings, raw oysters, guacamole, ham, and salsa (Douglas, 2002). The list will continue to grow as more research is currently being done on a variety of different foods. Although microbial inactivation may be a significant application of HPP, it can also be used to activate or inactivate enzymes, marinate meats, shuck oysters, and promote ripening in cheeses (Douglas, 2002).

Use of HPP in Tomatoes

Preliminary studies have shown the potential of HPP as an effective treatment to reduce contamination in tomatoes while maintaining fresh characteristics and nutrients. Arroyo et al (1999) discovered that pressurization at 400 MPa caused nearly complete elimination (> 10 CFU /g) of viable aerobic mesophiles and molds and yeasts while mostly maintaining texture and flavor in tomatoes immersed in water (Arroyo, 1999). After 350 MPa the skin of the tomatoes loosened but firmness was maintained (Arroyo, 1999). The effects of HPP on reducing certain acid-tolerant pathogenic and nonpathogenic bacteria in salsa was studied by Raghubeer et al. (2000) *Escherichia coli* O157:H7, enterotoxic *Staphylococcus aureus*, and *Listeria monocytogenes* were inoculated into fresh salsa and then run at 545 MPa for 0.5, 1.0, 1.5, and 2.0 minutes (Raghubeer,2000). All three pathogens were eliminated (<0.3 MPN/g). The inoculated salsas were then stored at 4°C and 21-23°C for two months (Raghubeer, 2000). None of the inoculated pathogens were detected in these treated samples for all treatments throughout the entire storage period (Raghubeer, 2000). Non-pathogenic forms of the three microorganisms (*E.coli*, *Listeria innocua*, *Listeria welshimeri*, and nonenterotoxigenic *S. aureus*) were inoculated together into a tank containing 100 L of

salsa (Raghubeer, 2000). No survivors were detected in any of these samples both following processing and during storage for 2 months.

While these studies have shown that HPP has a desirable effect on reducing microorganisms, Butz et al. (2001) studied the effects of pressure on some of the desirable nutritional characteristics of several vegetables, including tomatoes. An ultra high-pressure treatment was found to have little to no effect on carotenoid and antioxidant capacity and only a medium effect on the antimutagenicity against a cooked food mutagen (Butz, 2001).

High Pressure Resistance of Salmonella

Gram-negative bacteria are less pressure resistant than gram-positive bacteria, but even within the gram-negative strains there appears to be a wide range of pressure sensitivity. Strains of *S. enterica* have shown relatively high levels of pressure resistance in many studies (Garriga, 2005; Ponce, 1999; Chen, 2006; Metrick, 1989).

Garriga et al (2005) compared the effects of starter culture and HPP on the quality of slightly fermented sausages. The sausages were subjected to a treatment of 400 MPa for 10 minutes at 17°C (Garriga, 2005). After 28 days of storage, the non-treated sausages *S. enterica* counts dropped down to below 10 CFU/g due to several hurdles including; slightly acidic conditions, the presence of curing agents, and a_w conditions less than 0.93 (Garriga, 2005). The only factor to totally eradicate *S. enterica* serovars, however, was the pressure treatment at the end of ripening (Garriga, 2005). This study suggests that HPP was necessary to ensure total absence of detectable *S. enterica*.

Ponce et al (1999) studied the effects of different HPP parameters through the inactivation of *S. Enteritidis* inoculated in liquid whole egg. Treatment at 450 MPa at

20°C for 5 minutes reduced bacterial counts by 3.6 log units more than treatment at 350 MPa at the same time and temperature (Ponce, 1999). At a treatment of 350 MPa at 20°C, inactivation of *S. Enteritidis* increased by 0.5 log units just by increasing the treatment time from 5 to 15 minutes (Ponce, 1999). Ponce et al. also discovered that 50°C was the most effective temperature in pressure inactivation compared to -15, 2, and 20°C (Ponce, 1999).

Chen et al (2006) set out to compare the pressure sensitivities of eight different foodborne pathogens (*Vibrio parahaemolyticus*, *Yersinia enterocolitica*, *L. monocytogenes*, *S. Typhimurium*, *S. Enteritidis*, *E. coli* O157:H7, *Staphylococcus aureus*, and *Shigella flexneri*). The pathogens were inoculated into UHT whole milk and then treated at pressure levels ranging from 200 to 690 MPa at 21.5°C for 10 minutes (Chen, 2006). *S. Enteritidis* was found to be less pressure resistant than *S. Typhimurium*, but more resistant than *E. coli* O157:H7, *S. aureus* and *S. flexneri* (Chen, 2006). The critical pressure level for inactivation (a > 0.5-log reduction was defined as the occurrence of inactivation) was 400 MPa for *S. Enteritidis* (Chen, 2006).

Metrick et al. (1989) uncovered a relationship between pressure resistance and heat resistance in *Salmonella* strains. The pressure resistance of a heat resistant strain (Senftenberg 775W) and a heat sensitive strain (Typhimurium) were compared in a neutral buffer solution and chicken baby food (Metrick, 1989). After 10 minutes at 340 MPa the heat resistant strain showed a 4 log decrease in the neutral buffer and a 3 log decrease in the chicken baby food while the heat sensitive strain only showed a 2 log decrease in both mediums (Metrick, 1989).

Table 2.1 Outbreaks associated with *Salmonella enterica* spp. and tomato fruit between 1990 and 2007 in the United States.

Year	Tomato Type	Agent	Source	Number of Cases
1990	Tomato	<i>S. Javiana</i>	SC	176
1993	Tomato	<i>S. Montevideo</i>	SC	100
1998	Tomato	<i>S. Baildon</i>	FL	86
2000	Tomato	<i>S. Thompson</i>	FL or GA	29
2002	Red round	<i>S. Newport</i>	VA	512
2002	Grape	<i>S. Newport</i>	FL or MX	12
2002	Roma	<i>S. Javiana</i>	FL or MX	90
2004	Roma	<i>S. Javiana</i>	FL or GA or SC	471
2004	Roma	<i>S. Braenderup</i>	FL	123
2005	Red round	<i>S. Newport</i>	VA	71
2005	Tomato (salsa)	<i>S. Enteritidis</i>	CA	73
2005	Roma and/or Red round	<i>S. Braenderup</i>	FL	73
2006	Red round	<i>S. Typhimurium</i>	OH	190
2006	Tomato	<i>S. Newport</i>	unknown	107
2007	Red Round	<i>S. Newport</i>	VA	65

**Source: modified from FDA table provided by Dr. John Guzewich, 2008

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Chapter 3: High Hydrostatic Pressure Processing Reduces *Salmonella enterica* from
Diced and Whole Tomatoes

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Abstract:

Fresh and fresh-cut tomatoes have been associated with numerous outbreaks of salmonellosis in recent years. While the exact routes of contamination are unknown, high pressure processing (HPP) is being evaluated as a post harvest treatment to eliminate *Salmonella enterica* from tomatoes. The objectives of the study were to determine the potential for of HPP to reduce *S. enterica* serovars Newport, Javiana, Braenderup and Anatum (clinical isolates from tomato outbreaks) in tryptic soy broth (TSB) and to determine the effect of HPP to reduce the most pressure resistant *S. enterica* serovar from fresh diced and whole tomatoes. Five ml portions of broth containing 8 log CFU/ml of one of the four serovars (nalidixic acid resistant) were packaged in sterile stomacher bags and subjected to one of three different pressures (350, 450, or 550 MPa) for 120s. Samples were enumerated by surface plating onto tryptic soy agar supplemented with 50 ppm nalidixic acid (TSAN) and incubated at 35°C for 48 hours. The most pressure resistant *S. enterica* serovar evaluated was Braenderup. Subjecting the broth culture to 350, 450 and 550 MPa resulted in a 4.53, 5.74 and 7.09 log reduction in *S. Braenderup*, respectively. Diced tomatoes (150g) and whole red round tomatoes (150g; packaged in 350ml of 1% CaCl₂) were inoculated with *S. Braenderup*, to obtain 6 log CFU/g throughout the sample and subjected to the same pressure treatments as described above. After HPP, diced tomatoes were homogenized for 1 minute and then plated on TSAN. Whole tomatoes were surface sampled, and then homogenized for 1 minute. Surface and homogenate samples were plated on TSAN supplemented with 1% pyruvic acid (TSANP). Significant reductions of *S. Braenderup* concentrations in diced tomatoes ($P < 0.05$) were seen after processing at 350 (0.46 CFU/g), 450 (1.44 log CFU/g), and 550 MPa (3.67 log CFU/g). In whole tomatoes, significant reductions ($P < 0.05$) were also seen at 350 (1.41 log CFU/g), 450 (2.25 log CFU/g) and 550 MPa (3.35 log CFU/g). There were no differences in visual appearance between fresh and HPP diced and whole tomatoes. HPP may be an effective post harvest strategy to reduce low levels of *S. enterica* contamination in diced tomatoes.

Introduction:

Salmonella enterica serovars are one of the most common pathogenic microorganisms associated with fresh and fresh cut produce-related outbreaks. *S. enterica* is estimated to cause 1.4 million illnesses and 400 deaths annually in the United States (Greene, 2007). Since 1999, there have been 15 outbreaks of salmonellosis linked with the consumption of raw tomatoes (Guzewich, 2008). Between 2005 and 2006 alone four outbreaks associated with tomatoes led to 459 confirmed infections in over 21 states (CDC, 2007). Exact routes for contamination of the tomato remain unclear, but most incidents of contamination are thought to occur in fields and/or packing houses. While researchers are trying to identify these contamination routes, another approach would be a pre or post harvest mitigation steps that would eliminate *S. enterica* contamination from the tomato.

One method that could be used to reduce or eliminate *S. enterica* contamination in a tomato after it has occurred is the use of high Hydrostatic Pressure Processing (HPP). HPP is an alternative food processing procedure developed over a century ago (FDA, 2000). This technology has received a lot of attention in the last two decades as researchers have begun to study the relationship between high pressure and the preservation of foods. HPP is a non-thermal process, which acts throughout the food, minimizing any changes to nutritional value, flavor, or color, allowing the product to appear fresh while also extending microbiological shelf life (Smelt, 1998). Both liquid and solid foods can be subjected to pressures between 100 and 800 MPa at temperatures ranging from 0°C to 100°C, and depending on food consistency, maintain high quality

(FDA, 2000). HPP may be an effective way to process whole and diced tomatoes while maintaining a high quality fresh market product.

The objectives of this study were to determine the potential for HPP (350, 450, or 550 Mpa) to reduce *S. enterica* serovars Newport, Javiana, Braenderup and Anatum (isolated from tomato outbreaks) in tryptic soy broth. Also, to analyze the effect of packing the tomatoes in various solutions (distilled water, 1% NaCl, and 1% CaCl₂) on the visual quality (texture, skin, firmness) of whole tomatoes under HPP conditions and parameters. Then to use this information to determine the effect of pressure to reduce or eliminate the more pressure resistant *S. enterica* tomato outbreak serovar from whole and diced red round tomatoes.

Materials and Methods

Cultures and Culture Maintenance

Four serovars of *S. enterica* originally clinically isolated from tomato outbreaks were used for this study. These include *S. enterica* serovars Newport, Javiana, Anatum, and Braenderup. Each of the four serovars were received from the Center for Disease Control and Prevention (CDC; Atlanta, GA) culture collection. Cultures were activated in Tryptic Soy Broth (TSB ; Difco, Becton Dickinson, Sparks, Md.) at 35°C and transferred three times in 24-hour intervals and confirmed by subsequent plating on Hektoen Enteric agar (HE; Difco, Becton Dickinson, Sparks, Md.). Colonies considered positive for Salmonella were blue green colonies. One colony was further confirmed using an API 20E Strip (Biomérieux, Durham, NC).

Nalidixic Acid Resistance

S. Newport, *S. Javiana*, *S. Anatum*, and *S. Braenderup* serovars were made nalidixic acid resistant by consecutive 24-hour transfers of isolated colonies on tryptic soy agar) (Acros Organics, Morris Plains, NJ) with increasing concentrations of nalidixic acid (TSAN) until colonies were resistant to a level of 50 µg/ml. Using media supplemented with nalidixic acid suppresses the formation of colonies by background microorganisms naturally present on the tomatoes. Once nalidixic acid resistance was achieved, cultures were stored in a 20% glycerol solution in a -74°C freezer in the Food Science and Technology department at Virginia Tech until use.

Inoculum Preparation:

Cultures of the four nalidixic acid resistant serovars were obtained from the -80°C freezer and activated by serially transferring the culture in TSB over three days and

incubated each time for 24 hours at 35°C. Once the culture was activated, determination of the most pressure resistant serovar was completed. For tomato studies, the most pressure resistant serovar was activated as described above. After the third transfer, cells from a 24-hr culture in TSB were centrifuged at 10,000 x g for 5 minutes using a Fisher Scientific AccuSpin 400 (FisherBrand, Pittsburgh, PA). The cells were washed with sterile 0.1% peptone water and re-suspended in sterile de-ionized water to create an inoculum of 8 log CFU/ml.

Pressure Resistance Determination

99 ml of the test media (TSB) was separately inoculated with 1 ml of activated inoculum (as described above) to produce approximately an 8 log CFU/ml starting inoculum. The broth cultures were then packaged in sterile stomacher bags (FisherBrand Secure T, Pittsburgh, PA) in 5 ml aliquots. Bags were sealed with a 1.25-hp vacuum (Koch UltraVac 250, Kansas City, MO) and double bagged (three bags total) with 10ml disinfectant (120 ppm QUAT) in the outer bag to ensure no contamination of potentially leaked viable cells into the pressure chamber. The broth cultures were then subjected to one of three different pressures (350, 450, or 550 MPa), for a hold time of 120s using a Quintus Food Press QFP 35L-600 (Avure Technologies, Kent, WA). The bags were pressurized under all conditions at a temperature that was consistent with tomato storage temperature (~20°C). After treatment, broth cultures were surface plated onto TSAN and plates were incubated at 35°C for 48 hours. Three bags from each serovar group were run during each pressure treatment. The complete broth experiment was replicated 3 times (n=9).

Determination of optimal packaging solution

In order for tomatoes to maintain a fresh quality during HPP, they must be packaged in a solution. To determine the optimal packaging solution for tomatoes, store bought red ripened tomatoes (Kroger, Blacksburg, VA) were packed in one of three different solutions; distilled water, 1% NaCl, and 1% CaCl₂. The bags were subjected to one of three different pressures (350, 450, or 550 MPa) for 120s. The tomatoes were then weighed and visually analyzed for texture, firmness, and how well the skin was kept intact compared to non-pressurized tomatoes.

Inoculation and Treatment

Whole tomatoes (150 ± 15 g) were obtained from Kroger grocery in Blacksburg, VA. Tomatoes were either diced manually using a Nicer Dicer™ (Genius, Chamblee, GA) to create 1 cm x 1 cm cubes or kept whole. Whole tomatoes at room temperature (24°C) were spot inoculated (at stem scar) with 0.1 ml of inoculum and then placed in a vacuum chamber and subjected to approximately 0.6 MPa for 2 minutes. The pressure was then allowed to equilibrate to atmospheric pressure, and then a vacuum was pulled again. This procedure was repeated 3 times to pull inoculum inside tomatoes. This was done to ensure that the *Salmonella* was internalized into the whole tomatoes.

The vacuum treated tomatoes were then allowed to air dry in a laminar flow-through hood for 30 minutes. Each tomato was placed into a separate sterile stomacher bag, covered with 350 ml of 1% CaCl₂ solution and sealed at a 95% vacuum.

Diced tomatoes were portioned into 150 g samples, inoculated with 0.1 ml of *S. Branderup* per 150 g of product. Each 150g sample was placed into a sterile stomacher bag without additional solution and sealed.

The primary bag holding the tomato sample (whole or diced) was placed into a slightly larger second sterile stomacher bag to prevent possible leakage of contents into the HPP unit. Samples were subjected to one of three different pressures (350, 450, or 550 MPa) for 120s at 20°C ($\pm 7^\circ\text{C}$). Three tomatoes from each treatment group were run during each pressure treatment. The whole procedure was completed 3 times (n=9).

Enumeration of *S. enterica* from tomatoes

Following treatment, the sealed bag containing the post-HPP tomato samples were cut open across the top using sterilized scissors and enumerated according to sample type. For whole tomatoes, the processing solution was disposed of, and 20 ml of sterile 0.1% peptone water was added. The tomato was hand rubbed for 2 minutes and the peptone water surface plated onto TSAN supplemented with 1% pyruvic acid (TSANP) to enumerate *S. enterica* present on the surface of the tomato. Pyruvic acid was added to TSAN to enhance the recovery of injured *Salmonella* from tomatoes (Lang et al, 2004). After rubbing with 0.1% peptone, the whole tomato was transferred to a fresh sterile bag and homogenized for 1 minute. Homogenate was surface plated onto TSANP to enumerate *S. enterica* remaining in or on the tomato.

To analyze HPP diced tomatoes, the bags were homogenized for 2 minutes and surface plated onto TSAN. All plates were incubated for 48 hour at 35°C.

Statistical Analysis

Each experiment was completed three times and each replicate consisted of three culture preparations (n=9). A split plot design was used to model the data.

Log differences between control and treated samples were analyzed using Statistical Analysis System (SAS Institute, Cary, NC). The general linear model

procedure was used to determine the difference of least squared means. A P-value of 0.05 was used.

Results and Discussion

The ability of HPP at pressures of 350, 450, and 550 MPa for 120 seconds at tomato storage temperature ($\sim 20^{\circ}\text{C}$) to reduce *S. enterica* serovars from broth cultures and diced and whole tomatoes was investigated. Throughout this study, *S. enterica* was not detected in any un-inoculated samples of diced or whole tomatoes.

Pressure Resistance Studies

Pressure treatment at 350, 450, or 550 for 120 seconds at 20°C ($\pm 7^{\circ}\text{C}$) resulted in significant reductions of all four serovars in broth culture at initial levels of 8 log CFU/mL ($P < 0.05$). The most pressure sensitive strain was *S. Anatum*, which was almost completely eliminated (log reductions of 7.89, 7.49, and 7.89 CFU/ml) at 350, 450, and 550 MPa respectively (Figure 3.1). This finding was similar to a study done by Perry et al., where *S. Anatum* was found to be one of the two significantly ($P < 0.05$) least pressure resistant out of 18 different *Salmonella* strains that were subjected to 400 and 500 MPa for 60 seconds at 25°C . In the current study, *S. Newport* was only slightly more resistant with log reductions of 6.05, 7.83, and 7.86 CFU/ml, while *S. Javiana* was reduced by log values of 5.07, 6.41, and 7.92 CFU/ml at 350, 450, and 550 MPa respectively (Figure 3.1). The most resistant strain to all three pressures was *S. Braenderup* with log reductions of only 4.53, 5.74, and 7.09 CFU/ml at 350, 450, and 550 MPa respectively. (Figure 3.1)

There have been no previous studies to compare the pressure resistance of these four serovars in a broth culture. Other studies have shown similar results of other strains of *Salmonella* at similar parameters in a broth or liquid culture (Alpas et al, 2000, Chen et al, 2006, Whitney et al, 2007, Styles et al, 1991). Alpas et al. (2000) subjected 2 ml of *S. enteritidis* FDA in TSB to 276 and 345 MPa at 25°C for 5 minutes. The culture was reduced by 2.34 and 4.12 CFU/ ml respectively. Another study done subjected milk inoculated with *S. Enteritidis* to pressures ranging from 350 MPa to 700 MPa at 21.5°C for 10 mins. There was no significant reduction in *S. Enteritidis* populations at 350 MPa, but numbers were reduced by approximately 2 log CFU/ ml at 450 MPa and 6 log CFU/ml at 550 MPa. (Chen, 2006) Whitney et al studied the effects of HPP on *S. Baildon*, another common tomato outbreak related strain in TSB, distilled water, and orange juice. The 5ml samples were subjected to 300 MPa for 2 minutes at 6°C (Whitney, 2007). A 2.40 log CFU/ml, 2.39 log CFU/ml, and 0.36 log CFU/ml reduction of *S. Baildon* was seen in TSB, distilled water, and orange juice respectively. The difference in these numbers and those found in the current study is most likely due to the impact of slightly different liquid mediums. The milk and orange juice may have had protective properties against pressure as seen in a study done by Styles et al where the resistance of *L. monocytogenes* was studied in both UHT milk and a sodium phosphate buffer (Styles, 1991). After pressurization at 350 MPa for 20 minutes, the sodium buffer was reduced by 7 log CFU/ml while the milk was only reduced by a 2 log CFU/ml (Styles, 1991).

Reduction of *S. Braenderup* in diced and whole tomatoes

Once *S. Braenderup* was determined the most pressure resistant of the 4 serovars,

samples of inoculated diced and whole tomatoes were treated with HPP at pressures of 350, 450, or 550 MPa for 120 seconds at 20°C ($\pm 7^\circ\text{C}$). Significant reductions ($P < 0.05$) of *S. Braenderup* concentrations in diced tomatoes were seen after processing at 350 (0.46 CFU/g), 450 (1.44 log CFU/g) and 550 MPa (3.67 log CFU/g) (Figure 3.2).

Preliminary work was done to ensure that this method resulted in internalization of *S. enterica* in the pulp of the tomato. After running control tomatoes inoculated with 8 log CFU/g were subjected to the vacuum process and sampled. Skin levels reached an average of 6.22 log CFU/g and pulp levels reached an average level of 5.44 log CFU/g (data not shown). After pressurizing the whole tomatoes, significant reductions ($P < 0.05$) were seen at 350 (1.41 log CFU/g), 450 (2.25 log CFU/g) and 550 MPa (3.35 log CFU/g) in whole tomatoes. (Figure 3.3)

Although no other studies have been done examining these serovars of *S. enterica* in tomatoes, previous work has been done determining the potential of HPP in reducing microbial populations in tomatoes. (Arroyo, 1999, Raghubeer, 2000) The same levels of pressure used in this current study slightly reduced levels of *S. enterica* populations, were able to eliminate different microorganisms in previous studies. Arroyo et al (1999) discovered that pressurization at 400 MPa caused nearly complete elimination (> 10 CFU /g) of viable aerobic mesophiles as well as yeasts and molds while preserving texture and flavor in whole tomatoes processed in water. The effects of HPP on reducing certain acid-tolerant pathogenic and nonpathogenic bacteria in salsa was studied by Raghubeer et al. *E. coli* O157:H7, *L. monocytogenes*, and enterotoxigenic *S. aureus* were inoculated into fresh salsa and then subjected to 545 MPa for 0.5, 1.0, 1.5, and 2.0 minutes. All three pathogens were eliminated (< 0.3 MPN/g) in the salsa. Non-pathogenic forms of the three

microorganisms (*E.coli*, *L. innocua*, *L.welshimeri*, and nonenterotoxigenic *S. aureus*) were inoculated together into a tank containing 100 L of salsa. No survivors were detected in any of these samples both following processing and during storage for 2 months.

Some work has been done to examine survival and elimination of surface contamination on tomatoes with treatments like irradiation and chlorine washes, but little has been done looking at comparing elimination of internalized vs. surface contamination. This study found that although there was an overall significant reduction of *S. Braenderup* in whole tomatoes, there was no significant difference between skin and pulp reduction values after high pressure processing ($P < 0.05$).

Observations on HPP effects on treated tomatoes physical characteristics

No significant changes were seen in appearance of diced tomatoes, even at the highest application of pressure (550 MPa) (Image 3.1). Before treatments in this study, preliminary work was done to determine the optimal packaging solution to maintain fresh characteristics in the treated whole tomatoes. Samples were pressurized in solutions of distilled water, 1% NaCl, and 1% CaCl₂. At all levels of pressure, the distilled water caused the skin of the tomato to peel and break away from the pulp. There was very little observable difference between the results of the 1% NaCl and 1% CaCl₂. In general, the tomatoes packaged in calcium chloride had very few soft spots, a lower weight gain and kept their skin intact (Table 3.1). The sodium chloride kept the skin intact, but would often allow for bubbling to appear under the skin and the tomatoes packaged in this solution often had soft spots (Image 3.2). Calcium chloride has often been associated in other studies with minimizing softening during processing in both whole and cut

tomatoes and other vegetables. (Magee et al, 2003, Prakash et al, 2007, Izumi et al, 1995). There were no significant changes in the weight of whole tomatoes at all three pressures (Table 3.2) and no visual changes in appearance (Image 3.3).

The results of this study suggest that high pressure processing may be an effective method to maintain the characteristics of fresh diced and whole tomatoes while also reducing common *S. enterica* contamination. Future research may examine the effects of pressure on sensory and enzymatic characteristics of the treated tomatoes. The results of these experiments would determine if any changes to taste or ripening occur after treatment. Also an experiment run with a lower starting inoculum and limit of detection may be helpful in determining real world application by more closely representing field conditions.

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Figure 3.1 Population survival (log CFU/ml) of four *Salmonella enterica* serovars (tomato outbreak isolates) in broth culture pressurized at 350, 450, or 550 MPa for 120 seconds at 20°C.

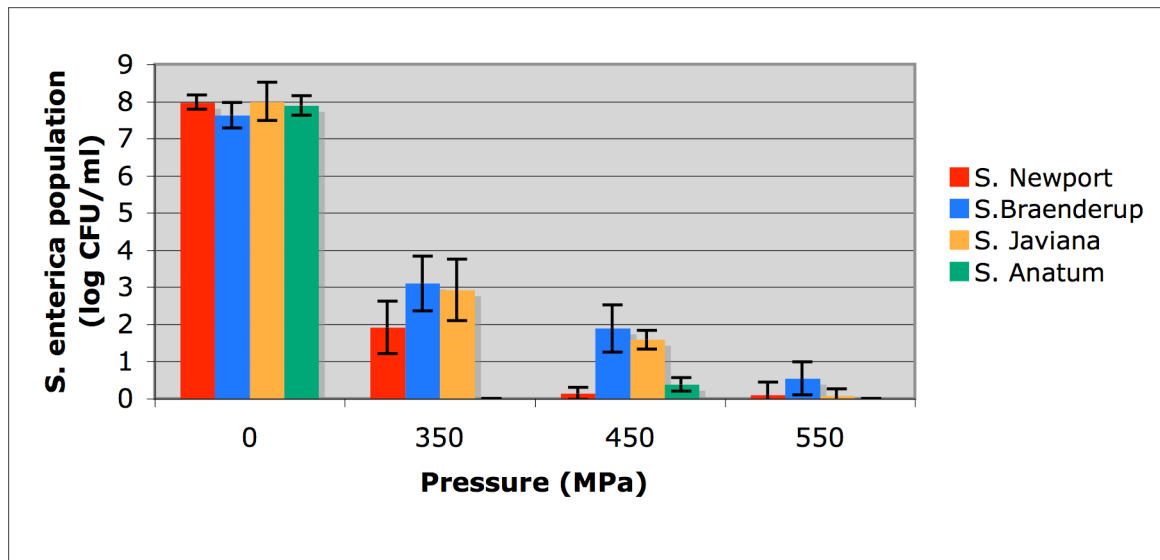
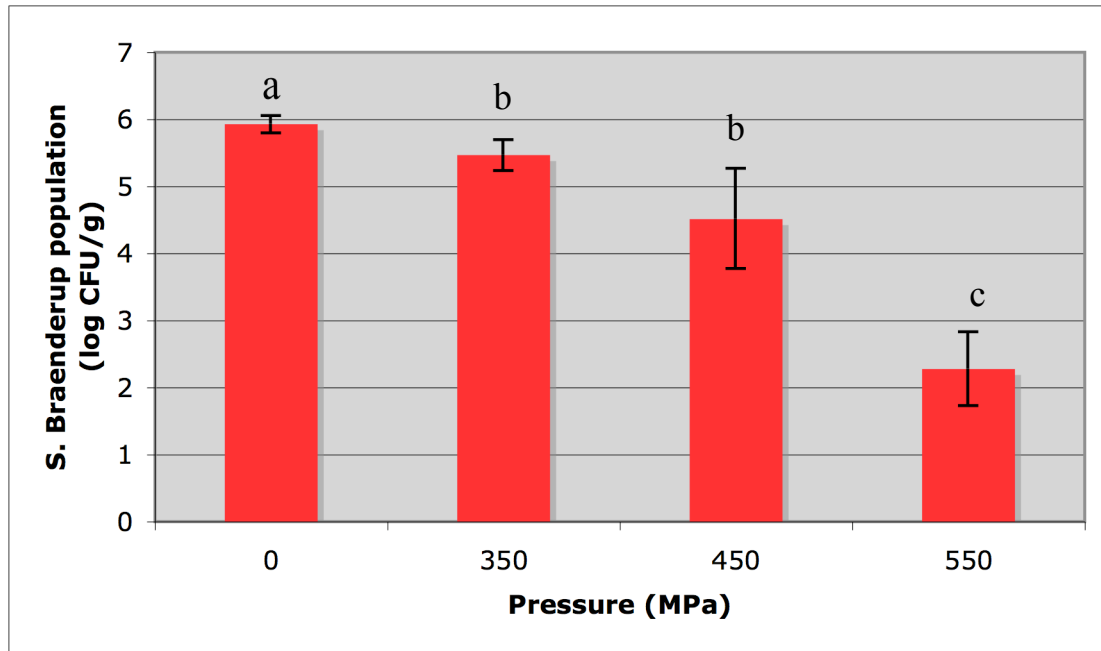


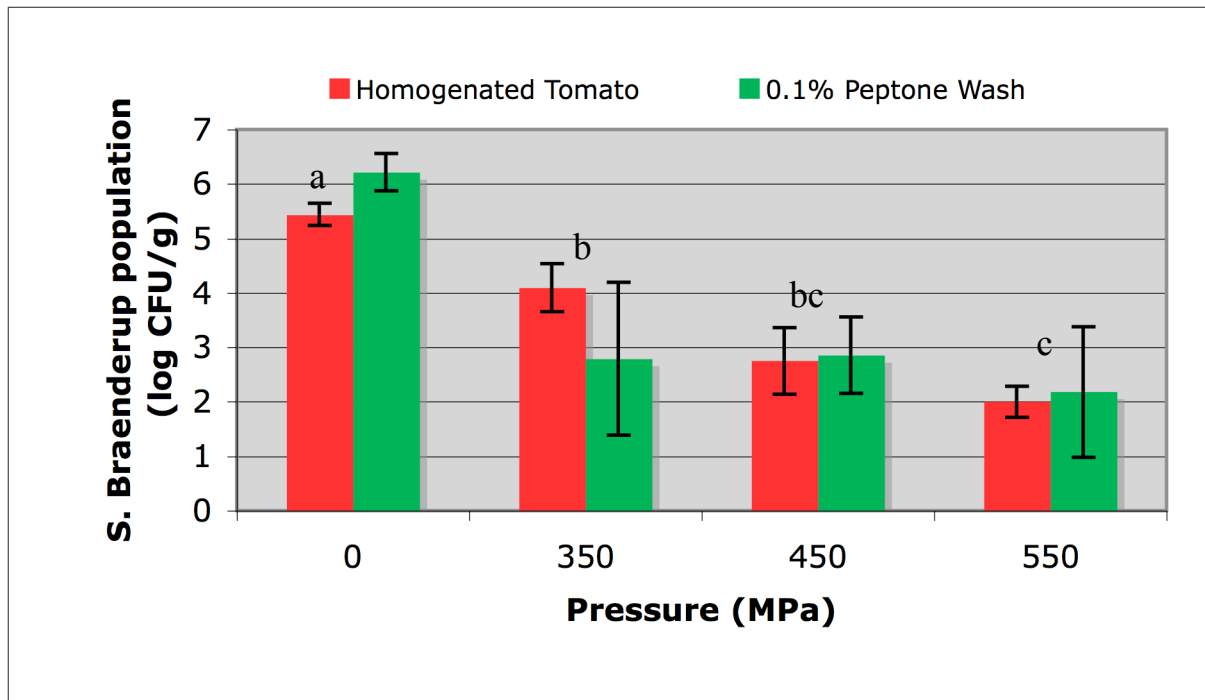
Figure 3.2 Population survival (log CFU/g) of *Salmonella enterica* serovar Braenderup in diced tomatoes pressurized at 350, 450, or 550 MPa for 120 seconds at 20°C.
n=9



*Columns with the same letter are not significantly different.

Figure 3.3 Population survival (log CFU/g) of *Salmonella enterica* Braenderup in whole red round tomatoes pressurized at 350, 450, or 550 MPa for 120 seconds at 20°C.
n=9

*Columns with the same letter are not significantly different.



Images:

Image 3.1. Comparison of physical characteristics of before and after HPP processed diced tomatoes at 550 MPa for 120s at 20°C.

Before Treatment

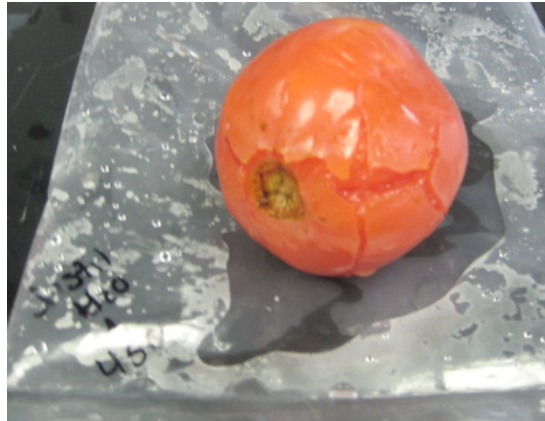


After Treatment



Image 3.2. Comparison of whole tomatoes packaged solutions of distilled H₂O, 1% NaCl, and 1% CaCl₂ after HPP processed at 450 MPa for 120 seconds at 20°C.

Distilled H₂O



1% NaCl



1% CaCl₂



Image 3.3 Comparison of physical characteristics of before and after HPP processed whole tomatoes inoculated and packaged in 1% CaCl_2 solution then run through 550 MPa for 120s at 20°C.

Before Treatment



After Treatment



Table 3.1 Average % weight gain of whole red round tomatoes after HPP in solutions of distilled water, 1% NaCl, and 1% CaCl₂.

* No significant difference in weight was found at any pressure.

* n=4

Average % Weight Gain			
Pressure (MPa)	Distilled H ₂ O	1% NaCl	1% CaCl ₂
350	2.9	3.1	2.8
450	4.8	2.9	2.6
550	4.7	3.5	3.3

Table 3.2 Average % weight gain of whole red round tomatoes after HPP.

* No significant difference in weight was found at any pressure.

* n=9

Pressure (MPa)	Average % Weight Gain
350	3.8
450	3.9
550	4.0

Chapter 4: Conclusion

Over the last two decades there has been a notable steady increase in outbreaks due associated with fresh produce. This increase could be due to multiple factors including a better developed tracking and surveillance system for foodborne outbreaks as well as and increased interest from consumers in eating fresh produce for health reasons. This work addressed the issue of controlling pre and post harvest contamination of tomatoes while still maintaining a fresh product through the use of high pressure. High pressure processing has been studied as an alternate to thermal processing for over a 100 years, but only recently has it been applied to fresh produce.

This study determined the effects of high pressure processing on *S. enterica* in broth, diced tomatoes, and whole tomatoes. Out of the four studied serovars (Newport, Javiana, Braenderup, and Anatum), *S. Braenderup* was found to be the most pressure resistant at 350, 450, and 550 MPa. In diced tomatoes, significant reductions were seen at 450 (1.44 log CFU/g) and 550 MPa (3.67 log CFU/g). In whole tomatoes significant reductions were seen at 350 (1.41 log CFU/g), 450 (2.25 log CFU/g), and 550 MPa (3.35 log CFU/g). All results were obtained with very little change in physical characteristics of both the diced and whole tomatoes. High pressure processing may be a successful strategy to reduce low levels of *S. enterica* contamination in whole and diced tomatoes while maintaining fresh characteristics.

Limitations and Pitfalls:

Many precautions were taken by the researchers to maintain a sterile environment and consistent data, however, some limitations could have affected the results. Ideally, the tomatoes would have been taken directly off of the same farm at the same point in

ripening to ensure similar microbial populations and texture. In the case of this study, tomatoes with the same expiration date were purchased from a local grocery store. This may have lead to discrepancies in ripening stages and natural microflora, which could have affected the results. Also it was difficult to keep the temperature of the high pressure processor at a consistent level throughout all replications. Although temperatures stayed around 20°C, there was an overall fluctuation of about 7°C, and this difference could have affected the results.

Future Research:

This study focused on the effect of high pressure processing on one particular microorganism in whole and diced tomatoes. In order to determine the real world application of this treatment, more research needs to be done on the way pressure affects other components of the tomato. It is important to understand how the natural enzymes will be affected because this could not only affect the taste but also the ripening process. There has been some research done on the effects of pressure on degrading enzymes in diced and cherry tomatoes. Shook et al found that HPP levels of 400 MPa and above had a significant affect ($P < 0.05$) of inactivating lipoxygenase (an enzyme that contributes to off flavors) and polygalacturonase (an enzyme that causes changes in tomato texture) in diced tomatoes. It would be important to see if the same inactivation occurs enzymes that maintain tomato quality. The study could also be expanded to examine the effects on contaminated unripe or green tomatoes, to see if intervening at this stage would produce a higher quality result. Also, this study used a very high level of starting inoculum (8 log CFU/ml). In order to more closely replicate field levels of contamination a much lower starting inoculum should be used to possibly produce a lower level of detection.

Reference:

Shook, C.M, T.H Shellhammer, and S.J. Schwartz. 2001. Polygalacturonase, Pectinesterase, and Lipoxygenase Activities in High-Pressure-Processed Diced Tomatoes. *J Agric Food Chem.* 49: 664-668.

Appendix A– Growth Curve and Strain Determination

Materials and Methods: Five different isolates from each of the four serovars were received from the CDC. These were: *S. Newport* (J1890, J1891, J1892, J1893, and J1894), *S. Javiana* (K2674, K2675, K2676, K2677, and K2678), *S. Anatum* (K2669, K2670, K2671, K2672, and K2673) and *S. Braenderup* (K2679, K2680, K2681, K2682, and K2683) were all prepared for growth curve analysis. Serial dilutions were used to dilute each of the samples to a 4-5 log CFU/ml concentration. Then, 100µl of diluted samples were combined with 300µL of TSB and transferred to a 100-well honeycomb plate (Growth Curves USA, Piscataway, N.J.). The samples were then run in a Bioscreen C growth curve machine (Growth Curves USA, Piscataway, N.J.) for 24 hours; turbidity of each sample was taken every 20 minutes at 37°C. The data was then exported to Microsoft Excel and growth curve graphs were created. One strain from each of the four serovars was selected based on the most consistent exponential growth and the most stable stationary phase.

Conclusion: Initially, five different strains of the four tomato outbreak related serovars were considered (*S. Newport* (J1890, J1891, J1892, J1893, and J1894), *S. Javiana* (K2674, K2675, K2676, K2677, and K2678), *S. Anatum* (K2669, K2670, K2671, K2672, and K2673) and *S. Braenderup* (K2679, K2680, K2681, K2682, and K2683). After 24-hour growth curve analysis was completed and data exported into Microsoft Excel graphs, one strain from each serovar was selected based on the most consistent exponential growth and the most stable stationary phase. The strains selected were *S. Newport* J1890, *S. Javiana* K2678, *S. Anatum* K2670, and *S. Braenderup* K2681 (Figures A.1, A.2, A.3, and A.4).

Figures:

Figure A.1 Growth of *Salmonella enterica* Newport samples at 37°C taken at 20 minute intervals over a 24 hour period.

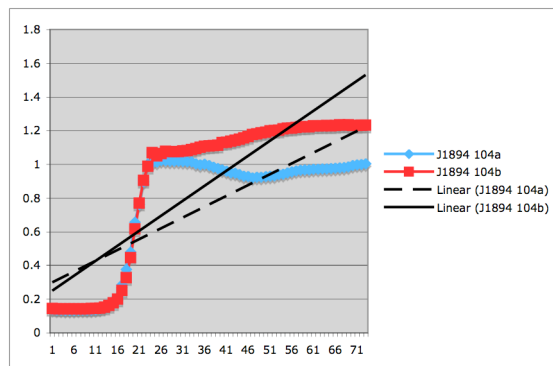
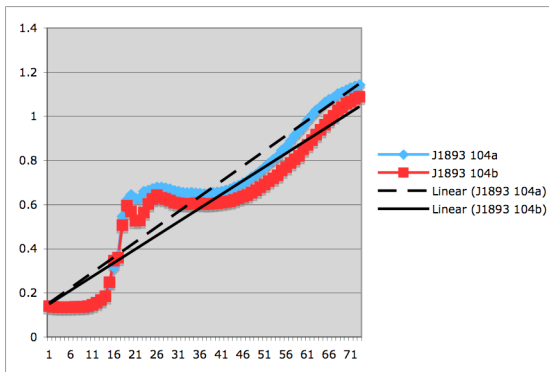
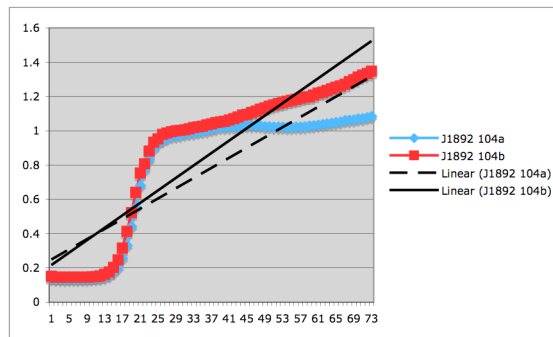
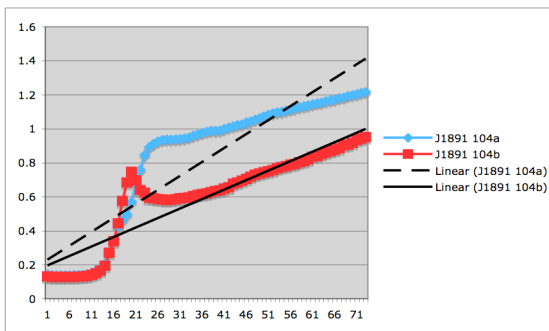
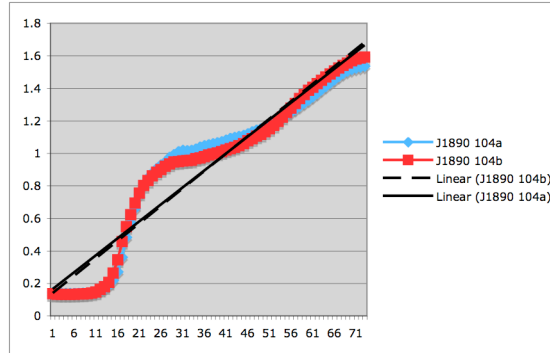


Figure A.2 Growth of *Salmonella enterica* Javiana samples at 37°C taken at 20 minute intervals over a 24 hour period.

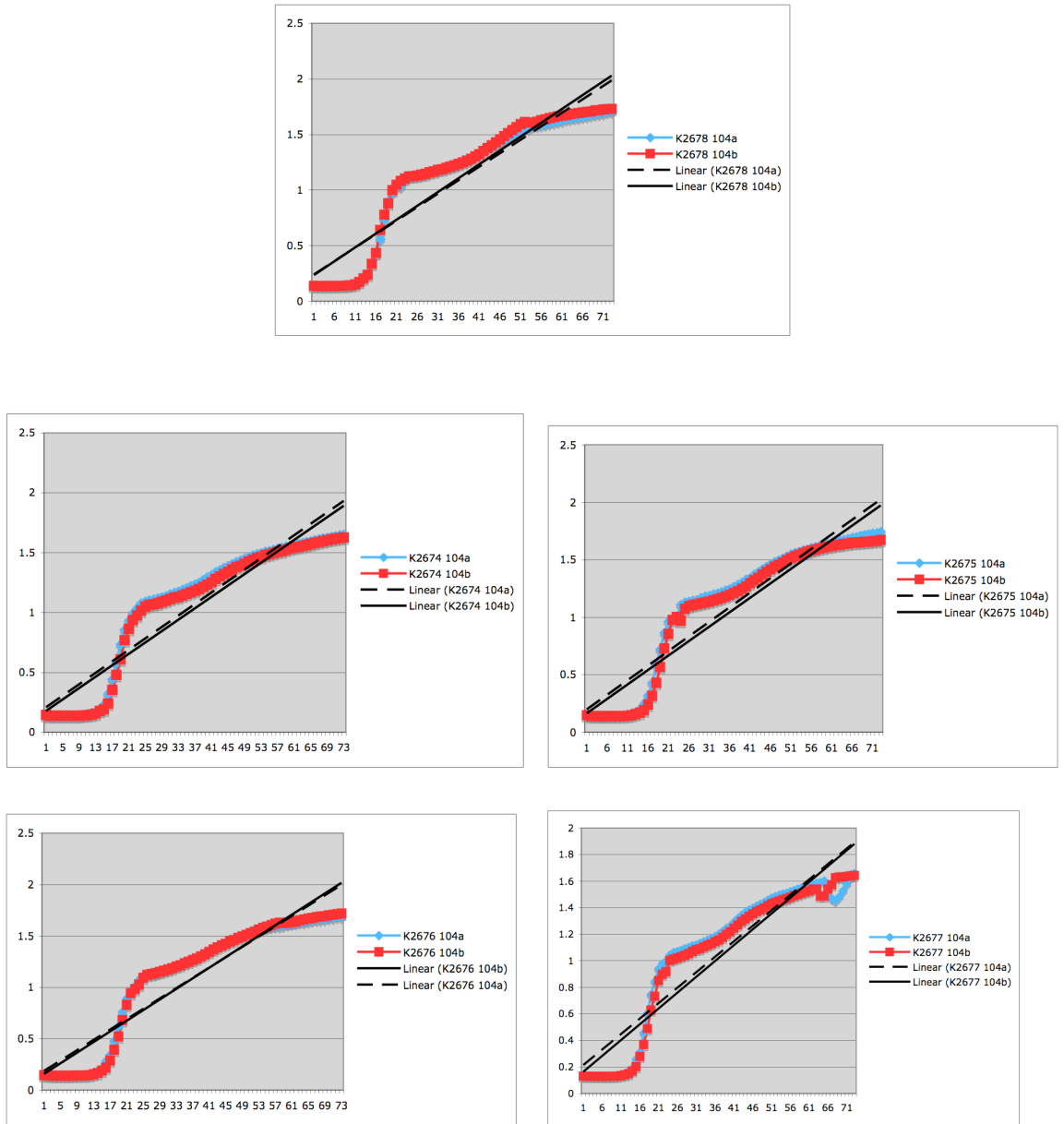


Figure A.3. Growth of *Salmonella enterica* Anatum samples at 37°C taken at 20 minute intervals over a 24 hour period.

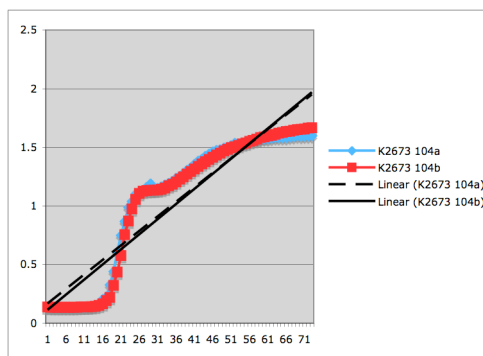
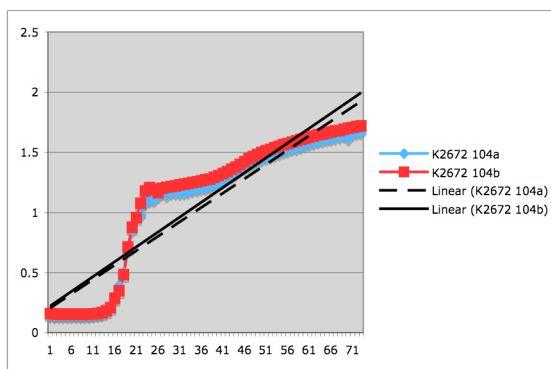
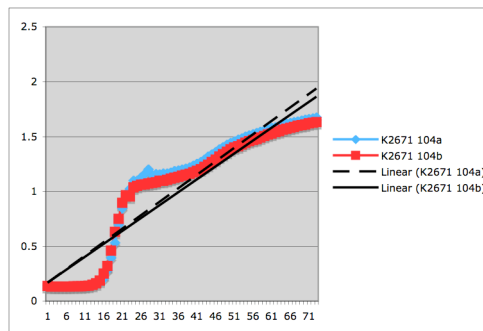
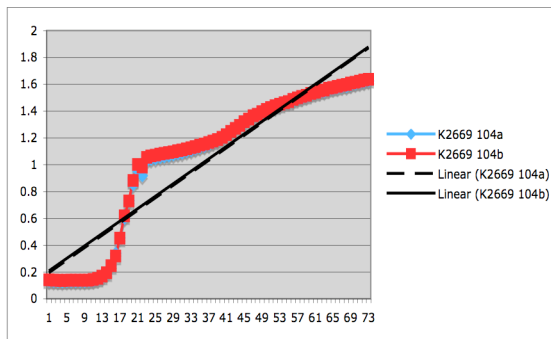
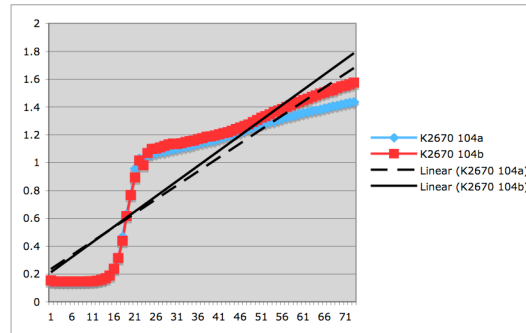
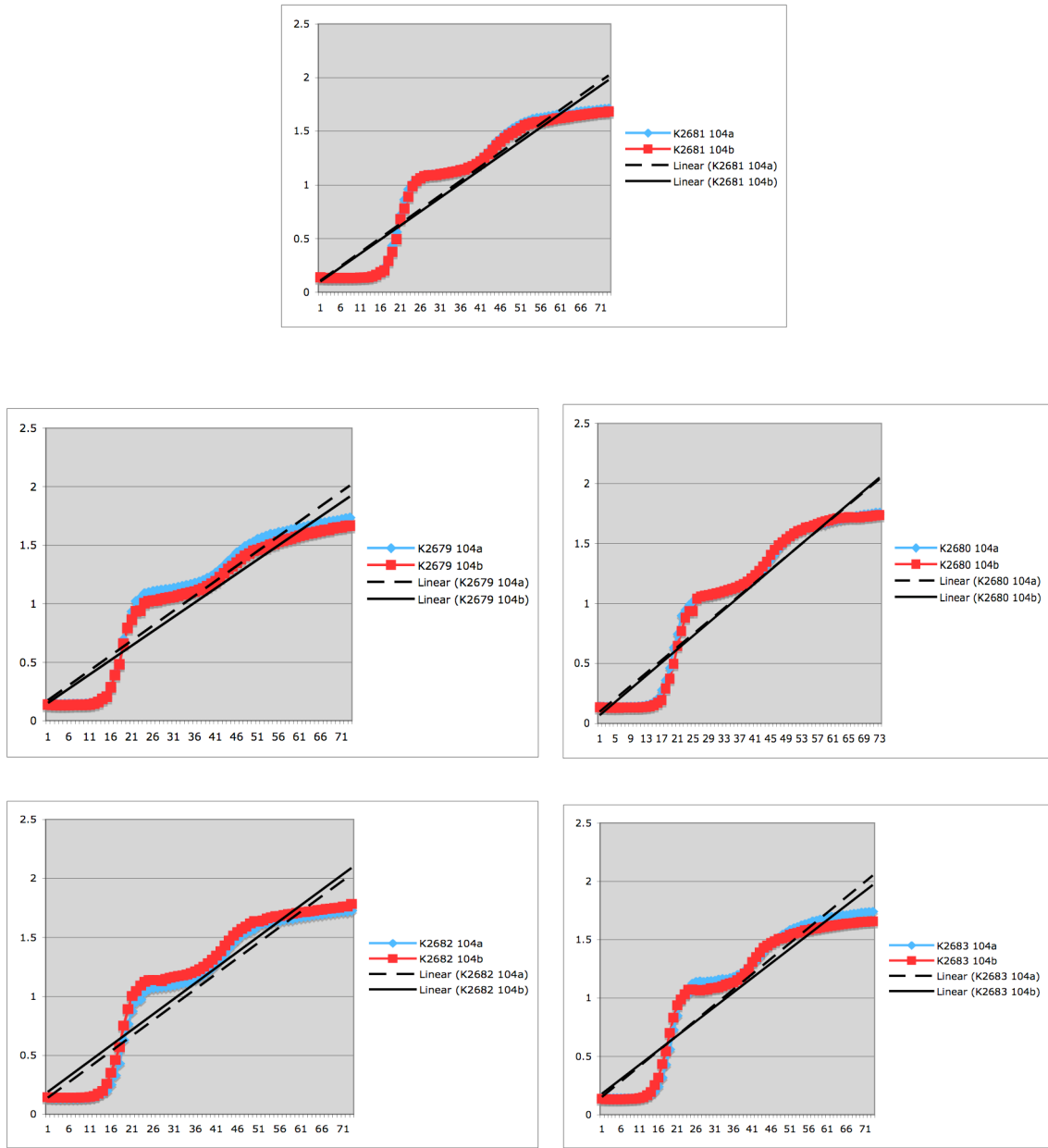


Figure A.4 Growth of *Salmonella enterica* Braenderup samples at 37°C taken at 20 minute intervals over a 24 hour period.



Appendix B – High pressure equipment specifications

Quintus Food Press QFP 35L-600
7XS-6000 Intensifier Pump

Operating temperature: 40-95°F (4-35°C) (excluding adiabatic temperature rise)
Temperature control accuracy: $\pm 4.5^\circ\text{F}$ ($\pm 2.5^\circ\text{C}$)

Process pressure range: 14,500 – 87,000 psi (100 – 600 MPa)

Cycle time: approximately 5 minutes at 87,000 psi (excluding hold time and loading/unloading)

Maximum hold time: 15 minutes

Process medium: water

Overall dimensions:

Maximum height (pressure vessel) 11.5 ft (3.5 m)
Height to hook (for loading and unloading baskets) – 13.0 ft (3.9 m)
Total press weight – 17,600 lbs (8,000 kg)
Pressure vessel volume – 9.25 gal (35 L)
Internal diameter – 7.5 in (190 mm)
Internal height – 48.0 in (1,220 mm)

Basket dimensions

Regular basket
Internal diameter – 6 $\frac{3}{4}$ in
Height – 46 in
Liner and basket
Internal diameter – 5 $\frac{3}{4}$ in
Height – 45 in