Influence of High Pressure Processing on Populations of *Salmonella enterica* in Fresh Green-Mature Tomato Fruits and Subsequent Ripening

By

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ABSTRACT

The objective of this work was to determine the effect of high pressure processing (HPP) on fresh tomato-associated outbreak isolates of *Salmonella enterica* in broth and on green mature tomato fruits. Nalidixic acid resistant (to 50 ppm) cultures of *Salmonella enterica* ser. Newport and *Salmonella enterica* ser. Braenderup were suspended in tryptic soy broth to a concentration of approximately 8 log CFU/ml and subjected to 350, 450, and 550 MPa for 120 s. Samples were serially diluted in peptone water, and surface plated onto tryptic soy agar supplemented with nalidixic acid (50 ppm; TSAN) and incubated at 35ºC for 48 h. Reductions of 5.64, 6.30, and 6.61 log CFU/ml in *S*. Newport, and reductions of 4.10, 5.22, and 6.35 log CFU/ml in *S*. Braenderup at 350, 450, and 550 MPa, respectively, were observed. Green tomato fruits inoculated with *S*. Newport or *S*. Braenderup to an initial concentration of approximately 6 log CFU/g were sealed in a bag containing 350 ml of 1% $CaCl_2$ and subjected to the same pressure treatments described above. The whole tomato fruits were pummeled in a stomacher and samples were surface plated onto TSAN supplemented with 1% pyruvic acid. Reductions of 1.55, 2.89, and 4.26 log CFU/g for *S*. Newport and 1.22, 2.26, and 3.77 log CFU/g for *S*. Braenderup at 350, 450, and 550 MPa, respectively, were observed. Bagged (350 ml 1% $CaCl_2$) samples of non-inoculated green tomato fruits were subjected to the same conditions described above. HPP treated tomatoes were then subjected to an ethylene gas (125 ppm; 0.7 cc/min) for 5 to 6 days. Pressured tomato fruits did not ripen. Even though HPP effectively reduced populations of *S. enterica*, it adversely affects the ripening characteristics of green mature tomato fruits.

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Chapter 1

Introduction and Justification

In the United States, consumption of fresh fruits and vegetables has increased substantially since the 1970's. Per capita annual consumption of fresh vegetables increased from 107.9 lbs. to 180.5 lbs. between 1970 and 2008 (USDA, 2008a). Following a similar trend, annual consumption of fresh fruits increased from 84.2 lbs. per capita in 1976 to 100.2 lbs. per capita in 2007 (USDA, 2008b). Increased consumption of fresh fruits and vegetables is believed to be due, in part, to greater consumer awareness of the reported benefits from fresh fruits and vegetables, including reduction in cardiovascular disease and obesity risks (Ibarra-Sanchez, 2004).

Increased consumption of fresh fruits and vegetables has coincided with a rise in the number of foodborne illness outbreaks associated with the consumption of fresh and minimally processed fruits and vegetables. Between 1996 and 2006, a total of 72 outbreaks of foodborne illness identified fresh produce as the implicated food (FDA, 2008). Factors that may have contributed to the observed increase in produce-related foodborne outbreaks include: year-round availability of domestic and imported produce, increased in-field cutting and packaging, and increased proximity of fruit and vegetable production areas to those of animal production (Lynch et al., 2008).

Among reported outbreaks of foodborne illness related to fresh produce, *Salmonella enterica* infections associated with consumption of fresh tomato fruit (*Lycopersicon esculentum* Mill) have been among the most common (CDC, 2007). It is estimated that *Salmonella enterica* associated with different foods is responsible for 1.5 million cases of infection, 15,000

hospitalizations, and 500 deaths annually in the United States (Mead et. al., 1999). During one two-year period (2005-2006) there were four multistate outbreaks of *Salmonella* human infections associated with consumption of raw tomatoes from different restaurants that resulted in 459 cases of illness in 21 states (CDC, 2007). Shortly after these outbreaks, the U.S. Food and Drug Administration (FDA) began a Tomato Safety Initiative whereby the FDA, state departments of agriculture and health (Virginia and Florida), university scientists, and produce industry members assessed current tomato production and handling practices on the eastern coast of the U.S. to identify potential routes of *Salmonella* contamination (FDA, 2007).

Although the exact mechanisms of tomato contamination have not been elucidated, many fresh tomato producers have implemented food safety practices that focus on reducing the risk of contamination from irrigation and spray water, manure, and farm or packinghouse workers (Beuchat, 1997; Tauxe, 1997). It is widely acknowledged that strategies to prevent *Salmonella* contamination on fresh tomatoes are of primary focus (Beuchat, 1998; CDC, 2007). However, no current approach has been shown to be completely effective at preventing contamination under commercial production conditions; therefore scientists are also considering post harvest tomato fruit treatment approaches to reduce or eliminate *Salmonella* already present on fresh tomato fruit (Beuchat, 1998). These approaches have included the use of sanitizers such as chlorine and physical treatments (Beuchat, 1998). One significant concern is the internalization of *Salmonella* into intact tomatoes (Zhuang et. al., 1995). Some studies have proposed that internalization could occur through the stem-scar of recently picked tomatoes when exposed to water temperatures that are more than 10°F cooler than the fruit, as it may occur during washing operations (Boyham, 2010; Cox, 2009; MFCL/NGMC/NARI, 2003; Zhuang et. al., 1995). Due

to pathogen entry into the interior of intact fruit, surface decontamination strategies, such as sanitizing agents may not be effective for ensuring the safety of fresh tomato fruit (Zhuang et. al., 1995).

Many processes employed to ensure the microbiological safety of foods, such as thermal processing, drying, and acidification, are not viable options for the treatment of fresh produce (Mañas, et. al., 2004). Applying these processes significantly changes the organoleptic properties of the fresh fruit or vegetable and results in products that are no longer accepted as fresh by consumers (Mañas et. al., 2004). However, high pressure processing (HPP), a technology that reduces microbial populations without the use of high temperatures, results in products with significantly improved microbiological safety and quality but without significant organoleptic changes (Basak et. al., 1998; Considine et. al., 2008; Norton et. al., 2007; Mañas et. al. 2004).

HPP is accomplished by pressurizing food products (up to 800 MPa) in a liquid medium; usually water (FDA, 2000). The treatment can be performed at temperatures as low as 0°C to greater than 50°C for a few s or several min, but often the products are processed at temperatures between 4°C and 30°C (FDA, 2000). Since the pressure is administered through a liquid medium in a closed chamber, the pressure is transmitted uniformly and instantaneously through the product (Douglas, 2002; Patterson, 2005; Rastogi et. al., 2007). Due to the use of low temperatures during pressure treatment, the nutrient content and many sensory properties, such as aroma and taste, are not negatively affected (Douglas, 2002; FDA, 2000; Patterson, 2005; Rastogi et. al., 2007).

Previous work has shown that HPP is effective for reducing populations of *Salmonella*

Braenderup on fresh, red, ripe tomatoes and diced, ripe tomato fruit. Reduction of populations of *Salmonella* Braenderup on whole red ripe tomato fruit in the study reached levels of 1.41, 2.25, and 3.35 log CFU/g when treated at 350, 450 and 550 MPa respectively. Reductions of 0.46, 1.44, and 3.67 log CFU/g at 350, 450, and 550 MPa on diced tomatoes were also reported (Maitland, 2009). Although this work demonstrates great promise for the use of HPP to control *Salmonella* in fresh, ripe tomato fruit, tomato fruit for the commercial fresh market are commonly harvested at the immature green or early red-ripe stages followed by ripening prior to distribution (Boukobsa, 2002).

Commercial ripening of mature-green tomato fruits consists of exposing the fruit to a flow of ethylene gas for 24 to 72 h at temperatures ranging from 20°C to 25°C at 85% to 90% relative humidity (MFCL/NGMC/NARI, 2003; Boyette et. al., 1995). The highest quality tomato fruit are those reaching the breaker stage within three days of ethylene exposure (Sargent, 2005). During the ripening process tomato fruits undergo several quantitative and qualitative changes in their chemical composition (Kader, 2002). Ripening of tomato fruit is characterized by: softening of the fruit, degradation of chlorophylls, lycopene formation, as well as the synthesis of acids and sugars (Kader, 2002). Once these changes have taken place, the tomato fruit is considered ripe. The effect of HPP on the subsequent ripening of mature green tomato fruit has not been previously reported.

Therefore, the objectives of this work were:

1. To determine the influence of HPP on populations of *Salmonella* Newport and *Salmonella* Braenderup in growth media (tryptic soy broth)
2. To determine the influence of HPP on populations of *Salmonella* Newport and *Salmonella* Braenderup in mature, green tomato fruit
3. To determine the influence of HPP on the ripening and quality of mature, green tomato fruits.

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Chapter 2

Literature Review

Foodborne Illnesses Associated with Fresh Produce

Fresh fruits and vegetables are considered a great source of different nutritional elements, such as, fiber, beta-carotenes, and vitamins and are known to maintain a healthy diet in general (Zink, 1997). As a result, many countries have adopted different incentives to encourage consumers to introduce higher amounts of fresh fruits and vegetables to their diet (FAO/WHO, 2008; Sivapalasingam et. al., 2004; Zink, 1997). Therefore, an increase in the consumption of fresh produce has been observed in the past few years (Zink, 1997).

The increased demand for fresh or minimally processed produce commodities in the United States and other countries have supported an increased importation of produce from different regions (Beuchat, 1996). At the same time, an increased occurrence of foodborne outbreaks associated with the consumption of fresh or minimally processed produce has also been observed (Beuchat, 1996; Sivapalasingam et. al., 2004). Several foodborne outbreaks have been linked to the consumption of contaminated vegetables and sometimes even fruits (Beuchat, 1996).

The CDC has estimated that foodborne diseases are responsible for approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths annually in the United States (CDC, 2005a). These outbreaks include foodborne outbreaks associated with the consumption of fresh produce commodities (CDC, 2005a; Mead et. al., 1999). An analysis of the records in the database of the Center for Science in the Public Interest (CSPI) showed that between 1990 and 2004, an estimated of 713 outbreaks resulted in 34,049 individual cases of illness that were

associated with the consumption of fresh produce (DeWaal et. al., 2007). Produce outbreaks accounted for 13% of total foodborne outbreaks and 21% of illnesses (DeWaal et. al., 2007). Between all the produce outbreaks reported in this period of time Norovirus accounted for a 40% of outbreaks, while *Salmonella enterica* was found to be responsible for 18%, and *Escherichia coli* was responsible for 8% of the outbreaks (DeWaal et. al., 2007). The most common pathogens found in outbreaks associated with vegetables were Norovirus (39%), *S. enterica* (21%), and *Clostridium* (12%) (DeWaal et. al., 2007). Similarly, between 1996 and 2004, the Food and Drug Administration reported at least 14 outbreaks of foodborne illness with lettuce and fresh tomato fruits as confirmed or suspected vehicles. The causative agents included *S. enterica* in fresh tomato fruits and *E. coli* O157:H7, *Cyclospora,* and Hepatitis A virus in lettuce (FDA, 2004). However, not all the cases of foodborne outbreaks are reported, and these numbers are only estimates of the real numbers of foodborne outbreaks that occur each year (CDC, 2005b).

From all the foodborne infections that take place every year in the United States, many infections especially milder cases are undiagnosed and not detected through routine surveillance (CDC, 2005b; Mead et. al., 1999; Tauxe, 1997). The short shelf life of produce and the complex distribution system throughout the country has made investigations of foodborne illnesses outbreaks more difficult (Harris et. al., 2006). However, improved investigations and detection methods all together with a surveillance system have contributed to a better documentation of produce related foodborne illnesses in the past few years (CDC, 2005b; Harris et. al., 2006).

The real mechanisms of contamination of fresh produce with pathogenic microorganisms remain still unknown (CDC, 2007a; Tauxe et. al., 1997). A better understanding of the complex

interactions of microorganisms with produce and the mechanisms of contamination can help develop measures to prevent and reduce future contamination from the farm to table (CDC, 2006a; CDC, 2007a; Sivapalasingam et. al., 2004). Handling, processing, and distribution practices of fresh produce are receiving great attention for the identification and control of microbiological hazards (Beuchat, 1996). In addition, many producers are implementing the hazard analysis critical control point (HACCP) program to reduce the risk of contamination of fresh produce (Beuchat, 1996).

Contamination of fresh produce can occur anywhere during preharvest and postharvest practices (Beuchat et. al., 1997). During preharvest, the use of treated manure, uncontaminated irrigation and spray water, good field-workers hygiene practices, and the prevention of contact with wild animals, human and bird's feces are important practices for the prevention of contamination of produce (Beuchat et. al., 1997; Harris et. al., 2006; McGlynn et. al., 2009). During postharvest sanitation of equipment and contact surfaces as well as good hygiene maintained by workers during handling, packaging and distribution of produce are also important in the prevention of contamination (Beuchat et. al., 1997; McGlynn et. al., 2009; Harris et. al., 2006).

Manure is widely used in the production of fresh produce, the correct treatment of manure before application to the fields is an important step to prevent contamination (Beuchat et. al., 1997). The application of untreated manure can increase the risk of fecal contamination of fresh produce (Beuchat, 1996). Pathogenic bacteria can also access production fields through contact with feces from wild and farm animals or contact with birds (Beuchat et. al., 1997; Harris et. al., 2006). While contamination of the production areas and growing operations through

domestic animals can usually be controlled, control of contamination of fresh produce by contact with birds or wild animals is usually limited (Beuchat et. al., 1997; Harris et. al., 2006). Water used during irrigation, spraying, and cleaning should be microbiologically safe to prevent contamination of produce through preharvest and postharvest practices (Beuchat et. al., 1997; Fonseca et. al., 2007; Tauxe, 1997). The type of irrigation system will also determine the risk of contamination of fresh produce (Fonseca et. al., 2007).

Field and packing house workers hygiene is also very important in the prevention of contamination of fresh produce (Brackett, 1999; Fonseca et. al., 2007). Workers should be well trained on the importance of hygiene practices since they handle produce from harvesting, during packaging, preparation, and retail (Beuchat et. al., 1997; Harris et. al., 2006). Sanitation of surfaces, harvesting equipment as well as packaging and transportation equipment are essential in the prevention of contamination of fresh produce (Beuchat et. al., 1997; Brackett, 1999).

Commonly, water used for washing, spray, and flume of fresh produce contains chlorine (Beuchat et. al., 1997). Chlorine is used as a sanitizer and its microbial activity depends on the amount of free available chlorine (Beuchat et. al., 1997). However the use of chlorine by the industry to wash fresh produce does not ensure the elimination of pathogenic bacteria and is only targeted to prevent the spread of contamination (Beuchat et al., 1997). On the other hand once pathogens had been internalized into fresh produce sanitizing methods are not longer effective in preventing contamination (Lynch et. al., 2009). The minimum processing applied to fresh and fresh-cut produce, does not include any effective microbial elimination step and results in produce that might carry potentially hazardous microorganisms (Harris et. al., 2006).

Foodborne Illnesses Associated with *Salmonella enterica* and Tomato Fruits

S. enterica has been considered the second most common bacteria responsible for foodborne diseases in the United States (CDC, 2008). An estimated of 1.4 million foodborne illnesses annually result from *S. enterica* contamination, yet, only 40,000 laboratory-confirmed cases of *S. enterica* are reported to the CDC each year (CDC, 2008). According to FoodNet there were 15 reported laboratory-confirmed infections per 100,000 people in 2007 (CDC, 2008). Also the surveillance data from FoodNet and related surveys from 1996-1999 estimated that 1.4 million people are infected with *S. enterica*, that results in 15,000 hospitalizations and near to 400 deaths annually in the United States (Voetsch et. al., 2004). However, the real number of *S. enterica* foodborne infections can only be estimated due to the occurrence of mild cases that are not diagnosed or even reported (CDC, 2010).

The number of foodborne outbreaks of *S. enterica* associated with the consumption of fresh tomato fruits has increased in recent years as reported by the CDC (CDC, 2006b). Between 1990 and 2004, an estimated of 1,616 reported illnesses of *S. enterica* infections resulted from nine outbreaks (CDC, 2005b). However, this number increases to approximately 60,000 illnesses if the proportion of unreported cases are taken into account (Voetsch et. al., 2004). In 2006 alone there were 121 *S. enterica* outbreaks that resulted in more than 3,300 illnesses as reported to the CDC Foodborne Outbreak Reporting System (CDC, 2006b). During this period the most common serotypes involved include *S.* Enteriditis, *S.* Typhimurium, *S.* Newport, and *S.* Heidelberg (CDC, 2006b). Although, a higher number of *S. enterica*-tomato associated outbreaks have been reported, many cases that are not reported make difficult the estimation of the real

number of outbreaks as well as the estimation of possible sources of contamination of tomatoes with *S. enterica* (CDC, 2006b).

During the summer of 2004 three multistate outbreaks of *S. enterica* associated with the consumption of Roma tomato fruits resulted in 561 illnesses in 18 states and one province of Canada (CDC, 2005b). Different *S. enterica* serotypes were found in one multistate U.S. outbreak and cases were associated with the consumption of Roma tomato fruits from different locations of a chain delicatessen (CDC, 2005b). *S.* Braenderup was implicated in the second multistate outbreak and *S.* Javiana in the third outbreak in Canada (CDC, 2005b). Most of the tomato fruits were traced back to a single packinghouse in Florida, but other growers and packers could have also supplied the Roma tomato fruits (CDC, 2005b).

Four multistate outbreaks of *S. enterica* infections associated with the consumption of raw tomato fruits at restaurants occurred between 2005-2006. The outbreaks resulted in 459 cases in 21 states (CDC, 2007b). The investigation showed that tomato fruits were supplied from the fields in Florida, Ohio, and Virginia (CDC, 2007b). A recent multistate outbreak in 2008 resulted in a total of 1,442 cases of infection, 286 hospitalizations, and at least two deaths in 43 states, the District of Columbia, and Canada (CDC, 2008). *S.* Saintpaul was identified as the implicated *S. enterica* serovar (CDC, 2008). Jalapeño peppers, Serrano peppers and tomato fruits were believed to have served as a vehicle during this outbreak (CDC, 2008). The mechanisms of contamination of these produce items have not been determined, but contamination may have taken place in the farm or during postharvest processing or distribution (CDC, 2008).

Raw tomato fruits have been increasingly associated with foodborne outbreaks of *S. enterica* infections (CDC, 2005a). Several investigations of these outbreaks proposed that

contamination of tomato fruits could take place in the fields while tomatoes are growing or after harvest during processing or transportation (CDC, 2007b). Commercial tomato fruits are usually grown in open fields were they could get contaminated by many known *S. enterica* reservoirs (CDC, 2007b) such as feces from domestic or wild animals (e.g., reptiles, amphibians, or birds), and water from contaminated sources, such as ponds or drainage ditches (CDC, 2005a; CDC, 2007b).

The real mechanisms of contamination of tomatoes before and after harvesting are still not well understood (CDC, 2007a). Further research and investigations are needed to determine the rotes of contamination, the ways that microorganism get internalized in tomatoes, the stages of growth were plants are more susceptible to contamination, and strategies to reduce or eliminate contamination (CDC, 2007a). Results from a study have suggested that attachment of *S. enterica* to the stems and flowers of tomatoes plants could contribute and serve as possible rotes for contamination of tomato fruits during development in the plant (Xuan et. al., 2001). Mechanical injury of the stems and fruits of tomato plants could occur in the field and during postharvest handling making tomato plants and fruits more susceptible to internalization of *S. enterica* (Xuan et. al., 2001). Therefore contact of tomato fruit and plant with contaminated soil, manure, irrigation water and surfaces should be avoided at all points of growth and production of tomato fruits (Xuan et. al., 2001).

Salmonella

Salmonella enterica is a gram-negative, non-spore forming, rod-shaped bacilli bacterium. *S. enterica* belongs to the *Enterobacteriaceae* family and it is a facultative anaerobic, motile

bacterium with flagella (FDA, 2009; Bailey et. al., 2009; Scheneider et. al., 2009; USDA, 2006). There are more than 2,500 known serotypes of *S. enterica,* most of them are capable of causing disease in humans, and are easily adaptable to a variety of environmental conditions (Bailey et. al., 2009; CDC, 2010; USDA, 2006; WHO/FAO, 2002). The bacteria can grow at temperatures between 8ºC and 45ºC (46ºF and 113ºF) depending on the *S. enterica* strain and the food matrix, but the optimal temperature for growth is between 35ºC and 40ºC (95ºF and 104ºF) (Bailey et. al., 2009; WHO/FAO, 2002). The optimal pH value for the growth of *S. enterica* is between 6.5 and 7.5, yet the bacteria can still grow at a pH value between 4 and 8 (Bailey et. al., 2009; WHO/FAO, 2002). Meat and poultry products have a pH between 5.1 to 6.4, fish and shellfish a pH between 5.5 to 7.0, most fruits a pH between 1.8 and 6.7, and vegetables a pH between 3.8 to 7.3 (Bailey et. al., 2009; WHO/FAO, 2002). *S. enterica* requires a water activity greater than 0.93 to grow (Bailey et. al., 2009).

S. enterica is widespread in the environment and a natural habitat for the bacteria is the intestinal track of animals including mammals, birds, reptiles, amphibians and even humans (CFIA, 2009; Scheneider et. al., 2009). The pathogen has also been found in water, soil, insects, and animal feces (Scheneider et. al., 2009). Foods that had been implicated in foodborne illnesses outbreaks of *S. enterica* include raw meat, raw poultry, raw eggs, raw seafood, milk and even fruits and vegetables (Bailey et. al., 2009; FDA, 2009; Scheneider et. al., 2009; USDA, 2006). Usually these foods become contaminated by direct or indirect contact with fecal matter (Bailey et. al., 2009). Food may also become contaminated when in contact with contaminated surfaces and also by contact with the contaminated hands of infected food handlers (CDC, 2010; USDA, 2006). In some cases people infected with *S. enterica* do not show symptoms, and might

become carriers spreading the infection to others (WHO/FAO, 2002). *S. enterica* can be spread from person-to-person, animal-to-person, and by consumption of contaminated food (CFIA, 2009, WHO/FAO, 2002).

S. enterica is typically responsible for self-limiting gastroenteritis in infected people (WHO/FAO, 2002). Symptoms usually develop after 12 to 72 h of consumption of the contaminated food, and include fever, diarrhea, abdominal cramps, headaches, nausea, and vomiting (CDC, 2010; CFIA, 2009; FDA, 2009; Scheneider et. al., 2009; USDA, 2006). The illness usually last from four to seven days and in most cases infected people recover without any treatment (CDC, 2010; FDA, 2009; Scheneider et. al., 2009; USDA, 2006; WHO/FAO, 2002). However some cases may lead to complications and the development of long-term conditions associated with the illness (Scheneider et. al., 2009). The elderly, infants, pregnant women, young children, and those with impaired immune systems are at a higher risk of developing severe illness and complications (CDC, 2010; Scheneider et. al., 2009; USDA, 2006). Moreover, depending on the host, food vehicle, and strain the infective dose for *S. enterica* may vary from 20 to 10^6 cells (Acheson et. al., 2004; FDA, 2009; Scheneider et. al., 2009).

Salmonella enterica in Fresh Produce

Several changes in agronomic practices, processing, packaging, distribution and marketing of the produce industry have enabled a greater distribution of high quality fresh produce in most countries throughout the year (Beuchat, 2002). On the other hand, many of these changes have also promoted an increasing risk of human illnesses associated with pathogenic microorganisms (Beuchat, 2002). The increasing number of documented produce associated outbreaks of human

illnesses could also be due to changes in food consumption patterns, demographics, a better surveillance system, distributions of produce items, and the production of minimally processed fruits and vegetables (Beuchat, 2002; Doyle et. al., 2008). All this practices could have contributed to changes in the ecological behavior of pathogens increasing the risk of contamination of produce commodities (Beuchat, 2002).

Although infections caused by enteric pathogens had been linked to the consumption of products of animal origin, in recent years fruits and vegetables that are consumed raw are increasingly being recognized as new vehicles for the transmission of infections associated with pathogenic bacteria (Berger et. al., 2010; Sivapalasingam et. al., 2004; Tauxe, 1997). However, the paths of contamination of fresh produce in the food supply chain and the mechanisms by which human pathogens attach and survive on or in fresh produce commodities remain still unknown (Berger et. al., 2010; Doyle et. al., 2008). As a result, the fresh produce industry has adopted several risk management practices designed to prevent contamination of fresh produce in the field and after harvest (Berger et. al., 2010). According with the studies performed by Sivapalasingam and others (2004), *S. enterica* was considered one of the most common pathogens involved in several outbreaks of foodborne illnesses associated with the consumption of fresh produce (Sivapalasingam et. al., 2004). Some of the produce commodities most commonly linked to *S. enterica* illnesses are tomatoes, seed sprouts, cantaloupe, watermelon, apple juice, and orange juice (Burnett et. al., 2001).

Pathogen contamination of fruits and vegetables may occur while growing in the fields, during harvest, postharvest handling or during distribution (Beuchat, 2002, Tauxe, et. al., 1997). Several studies have been performed to investigate potential sources of contamination of fresh

produce during preharvest and postharvest practices (Berger et. al., 2010). Attachment and establishment of *S. enterica* and other pathogens on growing crops could take place during preharvest, yet during postharvest proliferation or further contamination with pathogenic bacteria populations could increase the risk (Berger et. al., 2010). During preharvest some potential sources of *S. enterica* to be consider include inadequately composed manure, feces, irrigation water, water used for pesticides, insects, wild and domestic animals, and produce handlers (Beuchat, 2002; Tauxe, 1997). Possible sources of *S. enterica* and other microorganisms during postharvest include human handling, equipment, containers, the presence of animals, insects, rinse water, ice, transport equipment, and processing equipment (Beuchat, 2002; Tauxe, 1997). On the other hand, attachment and survival of *S. enterica* on or in raw and minimally processed fresh produce is necessary for its growth and multiplication to levels that could cause illness (Kroupitski et. al., 2009).

The surfaces of fruits, stems, plants, roots, florets, and leaves are characterized by specific microenvironments (Beuchat, 2002). The colonization of the plant and plant tissues with these unique types of microflora will depend on the environment in which plants grow as well as on the type of plant, protective cuticle, tissue pH and presence of antimicrobial substances (Beuchat, 2002). As a result each characteristic microenvironment will influence attachment and survival of other types of bacteria, yeast, fungus, parasites and viruses (Beuchat, 2002). Different postharvest operations of fresh produce result in mechanical injury produced by cutting, shredding, dicing, or peeling of fresh produce (Doyle et. al., 2008). These operations create surfaces where enteric pathogens such as *S. enterica* could attach and survive under suitable conditions (Doyle et. al., 2008; Iturriaga et. al., 2007). The cut surfaces may provide different

amounts of nutrients necessary for the attachment and survival of pathogenic bacteria (Doyle et. al., 2008).

S. enterica has the ability to survive outside its host and this property enables this pathogen to sporadically contaminate and survive on fresh produce (Kroupitski et. al., 2009). The formation of biofilms is a characteristic of *S. enterica* and many other pathogens that could increase the probability of the bacteria to survive on the surfaces of fresh produce and outside their host (Kroupitski et. al., 2009). During a study performed by Kroupitski and others (2009), observations using confocal microscopy showed that *S.* Typhimurium formed aggregates/biofilms on the intact surface and on the cut surface of lettuce leaves after incubation for three days at 30ºC (Kroupitski et. al., 2009). However, bacterial aggregates on the intact surface of the lettuce leaf were more scattered compared to those formed on the cut surfaces (Kroupitski et. al., 2009). *S.* Typhimurium showed higher levels of attachment on the cut surfaces of lettuce leaves compared to intact surfaces (Kroupitski et. al., 2009). In the same study attachment of seven different serovars of *S. enterica* to lettuce leaves were evaluated (Kroupitski et. al., 2009). Results showed that after inoculation of the lettuce leaves at 25ºC for 2h, five serovars of *S. enterica* (*S.* Enteriditis, *S.* Virchow, *S.* Thompson, *S.* Typhimurium, and *S.* Newport) resulted in high levels of attachment, while the other three serovars (*S.* Hadar, *S.* Poona, and *S.* Amager) showed relatively low levels of attachment (Kroupitski et. al., 2009).

Attachment of pathogenic bacteria to the surfaces of fresh produce is an important step for its colonization and survival (Brandl, 2006). Several studies have proposed that contamination of fresh produce with pathogenic microorganisms might depend on the attachment of foodborne pathogens to plant tissue (Barak et. al., 2002; Takeuchi et. al., 2000). During storage and

distribution of produce several factors such as storage temperature, relative humidity, nutrient availability, and competitive bacteria might affect the survival of pathogenic bacteria on produce (Doyle et. al., 2008). Additionally, damage to produce items or produce affected by soft rot increases the likelihood of survival and multiplication of attached pathogens (Doyle et. al., 2008; Wells et. al., 1997). Wells and others, 1997 reported a higher percentage of fresh produce collected from the market and that were affected by soft rot were also positive for suspected strains of *S. enterica* compared to healthy samples (Wells et. al., 1997). Furthermore, samples of carrot, potato and pepper that were inoculated with a soft rot bacteria (*Erwinia carotovora*) and *S.* Typhimurium and incubated for 72 h at room temperature, contained ten times the concentration of *S.* Typhimurium compared to those samples that were inoculated only with *S. enterica* (Wells et. al., 1997).

Populations of *S.* Montevideo survived on the surface of tomato fruits after ten days of storage at 30ºC (Iturriaga et. al., 2007). Furthermore *S.* Montevideo population increased as the relative humidity during storage was also increased (Iturriaga et. al., 2007). Other studies showed that *S. enterica* was able to survive for a period of 10 to 12 days on shredded lettuce (Chang et. al., 2007). Additionally, *S.* Typhimurium populations were able to attach to the intact surface as well as on to cut edges of iceberg lettuce (Takeuchi et. al., 2000). The results of this study also suggested that some cells of *S. enterica* were able to penetrate the internal tissue of the lettuce through the cut edge surfaces (Takeuchi et. al., 2000). Although, different practices during preharvest, harvest, and postharvest activities could contribute to the contamination of fresh produce with *S. enterica*; attachment, multiplication, and survival of this pathogen on and in fresh produce commodities may be dependant on the type of fresh produce, temperature of

storage of the produce, humidity, and several other factors.

Internalization of *S. enterica* into the core of tomato fruits during washing or sanitation of tomato fruits in water baths with a lower temperature than the fruit was reported in another study (Zhuang et. al., 1995). Temperature differentials between the water used in tomato fruits dip tanks and the temperature of the tomato fruit could potentially lead to an internalization of *S. enterica* into the tomato fruits (Zhuang et. al., 1995). Water that is 10ºC to 15ºC colder than the tomato fruit can create a possibility of internalization of *S. enterica* into the tomato fruit when immersed into this water (Zhuang et. al., 1995). Temperatures between 20ºC and 30ºC could potentially contribute to multiplication of *S. enterica* in the surface and core of tomato fruits during storage, transportation and ripening period (Zhuang et. al., 1995). Additional results showed that the use of chlorine in dip tanks could potentially reduce *S. enterica* contamination from the surface of tomato fruits, but it does not have an effect on internalized bacterial cells (Zhuang et. al., 1995). The concentration of chlorine in dip tanks should be maintained at a free chlorine concentration greater than 100 ppm and preferably near 200 ppm, and at a temperature closest to tomato temperature to prevent possible internalization of *S. enterica* (Zhuang et. al., 1995).

The temperature and humidity at which tomato fruits are stored are two important parameters to control during postharvest operations, storage and transportation of tomato fruits. The results of a study showed that *S.* Montevideo populations on the surface of tomato fruits previously inoculated increased remarkably after storage at 22ºC and 30ºC and humidity levels of 60% to 97% for ten days (Iturriaga et. al., 2007). The results showed the importance of maintaining tomato fruits at temperatures and humidity levels that do not support growth of

pathogenic bacteria during storage (Iturriaga et. al., 2007). Bacterial biofilm development was also observed in the surface of tomato fruits during storage at 22ºC and 30ºC and 97% relative humidity (Iturriaga et. al., 2007). Storage of tomato fruits at improper temperatures and humidity could potentially affect the survival, colonization, and biofilm formation of pathogenic bacteria on the surface of tomato fruits (Iturriaga et. al., 2007).

Survival and growth of *S. enterica* populations in the surface of tomato fruits, growth cracks and stem scars after a few days of storage were observed in a past study (Wei et. al., 1995). *S. enterica* populations were able to survive for up to two days on the intact skin of tomato fruits; additionally, bacterial populations were able to survive for up to seven days in the stem scar of tomato fruits (Wei et. al., 1995). Populations of the bacteria were able to increase rapidly on puncture wounds and on tomato fruit slices; the pH of tomato fruits did not show an effect on the inhibition of bacterial populations (Wei et. al., 1995). The study also reported that the use of 100 ppm of aqueous chlorine for up to two min did not eliminate the bacteria in these locations (Wei et. al., 1995). Contamination of tomato fruits with *S. enterica* can occur in any step of the production and processing of tomato fruits, but the place (surface of intact skin, stem scar, cracks or wounds) where bacterial cells attach in the tomato fruit could have a big impact in the survival and growth of populations of the bacteria postharvest, during storage or transportation (Wei et. al., 1995).

Fresh Tomato Fruit Production

Tomato fruit (*Lycopersicon esculentum* Mill) belongs to the botanical family *Solanaceae* and is one of the most widely grown vegetables in the United States (Boyham et. al., 2010; Le

Strange et. al., 2000; Orzolek et. al., 2006). Peppers, eggplant, Irish potatoes and tobacco are all members of the *Solanaceae* family (Boyham et. al., 2010; Orzolek et. al., 2006). Tomato fruits are originally from South America (Boyham et. al., 2010; Orzolek et. al., 2006). For many years tomato fruits were used only as an ornament; people believed that tomato fruits were poisonous and were not consumed as a food until the 18th century (Boyham et. al., 2010; Orzolek et. al., 2006). In recent years United States tomato fruit production exceeds 14 million tons a year (Boyham et. al., 2010). In the United States, California and Florida are leaders in the production of fresh market tomato fruits (Boyham et. al., 2010).

According to growth habits, tomato plants can be classified in two groups: determinate and indeterminate (Orzolek et. al., 2006). The first group determinate plants are those that grow to a specific height and have a defined period to produce flowers and fruits (Boyham et. al., 2010; Orzolek, 2006). Tomato fruits produce by this type of plants are firm and can better withstand handling and shipment on packing house operations (Rutledge et. al., 1999). On the other hand, indeterminate plants do not have a specified height and produce flowers and fruits throughout the entire season (Boyham et. al., 2010; Rutledge et. al., 1999). The tomato fruits of indeterminate plants are usually softer and usually better adapted for local markets or processing (Rutledge et. al., 1999). The most widely grown commercial cultivars belong to the determinate type (Rutledge et. al., 1999).

Tomato fruit is considered a warm season vegetable crop that is sensitive to cold temperatures at all stages of growth (Boyham et. al., 2010; Le Strange et. al., 2000). During seed germination the optimum temperature of the soil is 20ºC (68ºF) or above and during plant growth, fruit set, and development the optimum temperature is between 21ºC and 27ºC (Boyham

et. al., 2010; Le Strange et. al., 2000). Tomato fruit set and quality can be adversely affected if day temperatures fall below 20ºC and temperatures below 10ºC at night can produce chilling injury in plants and fruits (Le Strange et. al., 2000).

Tomato plants can grow in different types of soil, but optimal grow is obtained in deep, medium textured sandy loam, fertile, well-drained soils, and with good levels of organic matter (Boyham et. al., 2010; Le Strange et. al., 2000; Rutledge et. al., 1999). The soil should provide physical support, nutrients and water to tomato plants (Boyham et. al., 2010). Tomato fruit growers commonly use greenhouse grown tomato plants instead of seeds in the fields (Boyham et. al., 2010). Tomato transplants are hardened off one week before transplanting them to the fields (Boyham et. al., 2010; Rutledge et. al., 1999). During this process the temperature is reduced, the amount of water is also reduced, and plants are subjected to increased ventilation and to direct sunlight (Boyham et. al., 2010; Rutledge et. al., 1999). This technique helps plants in the transition to less favorable conditions in the field (Boyham et. al., 2010; Rutledge et. al., 1999).

Once tomato plants are growing in the fields staking and pruning of the plants are necessary for the production of high quality tomato fruits (Boyham et. al., 2010; Rutledge et. al., 1999). Pruning usually helps to increase the size of the fruit and increase the early yield (Boyham et. al., 2010; Rutledge et. al., 1999). The use of plastic mulch improves growth of the plant by increasing the temperature of the soil, reduces the development of weeds, and improves the application of fertilizer (Boyham et. al., 2010; Rutledge et. al., 1999). Irrigation is a very important step during production of tomato fruits; a well-irrigated field improves the uptake of nutrients, activation of herbicides, and also improves the size and shape of the tomato fruit

(Rutledge et. al., 1999). Two types of irrigation systems are the most commonly used by tomato fruit producers (Rutledge et. al., 1999). Over the top sprinkler irrigation systems are most commonly used when plastic mulch is not applied to the field (Rutledge et. al., 1999). On the other hand, drip irrigation systems can be use with or without the use of plastic mulch (Boyham, 2010). This systems releases water at the base of the plant near the root zone and reduces the possibility of diseases because it reduces the contact of water and soil with plant leaves and fruits (Le Strange et. al., 2000; Rutledge et. al., 1999).

The tomato fruit can be harvested at different stages of maturity depending on the intended market destination (Orzolek et. al., 2006). Tomato fruit that is harvested at the green mature stage of ripening can better resist the stress of handling and transportation, and it reduces the risk of becoming over ripe before reaching the market (MFCL/NGMC/NARI, 2003; Orzolek et. al., 2006). On the other hand, tomato fruit that is left in the vine until breaker stage is intended for local markets were long distant transportation is not necessary (MFCL/NGMC/NARI, 2003). Tomato fruits in this stage of ripening can be marketed as vine-ripe tomato fruits (MFCL/NGMC/NARI, 2003). Tomato fruit in the green mature stage can be characterized by the white stripes in form of a star that appear at the blossom end of the fruit (Rutledge et. al., 1999). At this stage of ripening the seeds are completely developed and the cavity of the tomato fruit is filled with a jelly-like material in each of the locules (Boyham, 2010; Le Strange et. al., 2000; MFCL/MGNC/NARI, 2003).

Methods for harvesting of tomato fruits vary according to the preference of growers and also according to the level of maturity of the fruit (Boyham, 2010). Green mature tomato fruits are usually hand picked and placed in large bulk bins that are then transported to the

packinghouse. In the packinghouse tomato fruits are graded and separated into the different sizes, cleaned, ripened, and packed for shipping (Boyham, 2010; Le Strange et. al., 2000; Rutledge et. al., 1999). In the field and during harvesting, workers perform a preliminary grading to remove fruit showing signs of decay from the field and to separate them from the rest of the fruit (Boyham, 2010). This practice reduces the risk of contamination of healthy tomato fruits (Boyham, 2010). On the other hand, some tomato fruit producers prefer to field packed tomato fruits in the breaker stage to reduce further damage during harvesting and handling (Rutledge et. al., 1999; Boyham, 2010). Handling of the tomato fruit during harvesting and postharvest practices could produce bruising and injury of the tomato fruit tissue and could affect ripening of the fruit increasing the risk of contamination with decay-causing microorganisms (MFCL/NGMC/NARI, 2003).

Postharvest practices during production of fresh tomato fruits include cleaning, grading, packing, and ripening. The first postharvest operation is cleaning. There are different methods to clean freshly harvested tomato fruits, but one of the most common is the use of a dump tank (Boyette et. al., 1995). Cleaning of the tomato fruits removes dirt, and other foreign materials from the surface of the tomato fruit (Boyette et. al., 1995; MFCL/NGMC/NARI, 2003). Several studies have proposed that infiltration of potential pathogens such as *Salmonella enterica* could occur through the stem scar or harvest cuts of fresh tomato fruits when immersed in dump tanks (Boyham, 2010). Other studies have proposed that internalization of microorganisms could be due to the difference in temperature between the water and the tomato fruits (Boyham, 2010; Cox, 2009; MFCL/NGMC/NARI, 2003; Zhuang et. al., 1995). Water used in the tanks should be heated to a temperature a few degrees Celsius higher than the temperature of the tomato fruits to

be cleaned to reduce the risk of internalization of decay and pathogenic microorganisms inside the tomato fruit (Boyham, 2010; Cox, 2009; MFCL/NGMC/NARI, 2003; Zhuang et. al., 1995). If the temperature of the water is lower than the temperature of the tomato fruits, the air spaces of the fruit tissue may contract causing a vacuum, which draws water and microorganism inside the tomato fruit tissue through the stem scar (Boyham, 2010; Cox, 2009; MFCL/NGMC/NARI, 2003). Therefore, the temperature of the water tank should be maintained above the highest tomato fruit pulp temperature (Boyham, 2010; MFCL/NGMC/NARI, 2003). Infiltration could also occur if tomato fruits are submerged to deeply in the water or for a long period of time (Boyham, 2010, Cox, 2009; MFCL/NGMC/NARI, 2003). The water in dump tanks should also be treated with a chemical sanitizer solution to reduce the potential of harboring pathogens that could contaminate tomato fruits (Cox, 2009). Chlorine is used in the water to prevent the concentration and spread of decay and pathogenic microorganism to tomato fruits (Boyham, 2010).

Sorting and grading of the tomato fruits takes places after cleaning and before packing (MFCL/NGMC/NARI, 2003). Tomato fruits are first separated according to their size, and then fruit with cracks, bruises, open cuts, and with signs of decay are separated (MFCL, 2003). Soft and overripe fruits are also separated as they bruise easily and do not withstand handling and transportation (MFCL/NGMC/NARI, 2003). Green mature fruits are usually packed in fiberboard containers containing a net weight of 25 pounds (MFCL/NGMC/NARI, 2003). Tomato fruits in the breaker stage are usually packed in two-layer 20 pounds cartons (MFCL/NGMC/NARI, 2003).

Green mature tomato fruits are then transported to ripening rooms to be ripened before

reaching the market (Anonymous, 2003; Boyette et. al., 1995). Ethylene is a gas that is naturally produced by tomato fruits and other produce items in the ripening stage (Anonymous, 2003; Boyette et. al., 1995). Ethylene is also used commercially to initiate the ripening of green mature fruit (Boyette et. al., 1995). Tomato fruits are exposed to an ethylene concentration of 100 to 150 ppm for 24 to 72 h, at a temperature of 20ºC to 25ºC (68ºF to 77ºF), and a relative humidity of 85% to 95% (Boyette et. al., 1995). A fairly airtight room is necessary for the application of ethylene, which can be applied by a shot method, a generator, or a flow-through system (Anonymous, 2003; Boyette et. al., 1995).

After harvest cooling of the harvested tomato fruits is a very important step to maintain their quality and to prevent them from becoming overripe before reaching the consumer (Boyette et. al., 1995; Le Strange et. al., 2000). Temperatures of storage after cooling depend on the stage of maturity of the fruit (Boyette et. al., 1995). Green mature tomato fruits can be stored at 13ºC to 14ºC (55ºF to 58ºF) for two weeks before ripening without significant changes in the ripening rate, color development, or sensory quality (Boyette et. al., 1995; Le Strange et. al., 2000). Temperatures between 14ºC and 15ºC (58ºF and 60ºF) reduce the ripening speed of mature green tomato fruits and prevent possible decay (Boyette et. al., 1995). Different studies showed that tomato fruits stored at 90% humidity or higher might increase the incidence of decay (Boyette et. al., 1995). After harvesting and after cooling tomato fruit at the breaker and semi-ripe stages of maturity can be stored in cool rooms at 10ºC (50ºF) for about ten days at 95% relative humidity (Boyette et. al., 1995; Boyham, 2010; Le Strange et. al., 2000). If tomato fruits are held at this temperature for longer periods its retail shelf life is reduced remarkably (Boyette et. al., 1995). Completely ripe tomato fruits are usually stored at 4.4ºC to 10ºC (40ºF to 50ºF) for a couple of

days, longer storage can result in a loss of color, shelf life, and firmness (Anonymous, 2003; Boyette et. al., 1995). Since tomato fruit is a tropical fruit, it can be greatly affected by exposure to low temperatures during storage (Anonymous, 2003; Boyham, 2010; MFCL/NCMG/NARI, 2003). The effects of exposure to low temperatures can have a cumulative effect on the tomato fruit that develops signs of chill injury (Anonymous, 2003; MFCL/NCMG/NARI, 2003). Chill injury can be developed in ripe tomato fruits exposed to temperatures below 10ºC (50ºF) and in green mature fruit exposed to temperatures below 12.5ºC (55ºF) (Boyham, 2003; MFCL/NGMC/NARI, 2003). Signs of chill injury in tomato fruits include irregular color development, softening, surface pitting, water-soaked lesions, browning of seeds, off-flavor development, and increased postharvest decay (Boyham, 2010; MFCL/NGMC/NARI, 2003).

Current Processes Applied to Fresh Produce

According to the definition established in the Code of Federal Regulations, the term "fresh" is used for a food product in its raw state (CFR, 2008). An unprocessed food product that has not been frozen, and that has not been subjected to any form of thermal processing or preservation treatment is considered fresh (CFR, 2008). Furthermore, the use of approved waxes, postharvest pesticides, the application of chlorine wash or mild acid wash on produce, the application of refrigeration temperatures, and the use of ionizing radiation at a maximum dose of 1 Kilo Gray do not exclude the food product from been considered fresh (CFR, 2008).

Following this definition, fresh produce items are considered fresh because they are in its raw state and they do not undergo any treatment that changes its freshness such as thermal processing, addition of preservatives, or any other type of processing (CFIA, 2009; De Roever,

1998; Velez et. al., 2005). Fresh produce commodities can become contaminated with pathogens at any step from production, packinghouse operations or even distribution and due to the lack of a lethal treatment that ensures the complete elimination of pathogens, microorganisms might be able to survive and be present when produce is consumed (De Roever, 1998; FDA, 2001). As a result, prevention of contamination at the farm level and at the packinghouse is more important in the production of fresh produce rather than the application of corrective actions once contamination occurs (FDA, 2001; Harris et. al., 2002; Velez et. al., 2005).

Many postharvest operations of fresh produce include the separation of foreign objects, sorting to remove substandard items, sorting and grading according to size categories, washing, cleaning, application of wax and packing into a shipping container (Burden, 1997; Shewfelt et. al., 2009). Most of these treatments are intended to control postharvest diseases (Burden, 1997). Washing of fresh produce is usually performed to improve the appearance of the commodity, lower the produce temperature, and to reduce microbial load on the surface of produce that will improve the quality, shelf life, and safety of the produce item (Beuchat, 1998; Harris et. al., 2002; Herdt et. al., 2009; Sapers, 2009; Shewfelt et. al., 2009). However, water used during washing of produce may become contaminated with pathogenic bacteria when contaminated produce coming from the fields is washed in that water (FDA/HHS/CFSAN, 1998; Harris et. al., 2002; Sapers, 2009; Velez et. al., 2005). If pathogenic microorganisms are not removed or controlled they could consecutively spread and cross contaminate new batches of produce during subsequent washing operations (FDA/HHS/CFSAN, 1998; Harris et. al., 2002; Herdt et. al., 2009; Sapers, 2009).

The use of a chemical sanitizer in the water during washing, cleaning, and cooling fresh

produce is a common practice in the production of fresh produce to enhance control of microorganisms found in the surface of incoming fresh produce and to reduce microbial accumulation in the water (Beuchat, 1998; FDA, 2001; FDA/HHS/CFSAN, 1998; Herdt et. al., 2009; Sapers, 2009). As these treatments are applied at concentrations that will not cause changes in the sensory qualities of fresh produce; they should be considered as methods of disinfection that reduce populations of microorganisms but do not ensure their complete elimination (Beuchat, 1998; Chaidez et. al., 2007; FDA, 2001; FDA/HHS/CFSAN, 1998). The most common sanitizers and mitigation treatments used for fresh produce and equipment are chlorine, chlorine dioxide, organic acids, quaternary ammonium compounds, bromide, iodine, ozone, ionizing irradiation, and modified atmosphere packaging (CFIA, 2009; Harris et. al., 2002; Sela et. al., 2009; Seymour et. al., 2001). From these common sanitizers some are appropriate for use in direct contact with wash waters, while others can only be used for equipment and containers used in the packinghouse, during storage and distribution of fresh produce (Beuchat, 1998).

Chlorine is the most widely used sanitizer in packinghouse operations (Chaidez et. al., 2007; Gonzalez et. al., 2004; Harris et. al., 2002). Chlorine is used for the disinfection of equipment and contact surfaces in the fresh produce industry (Beuchat, 1998); however the primary application of chlorine is to reduce the risk of cross contamination with microorganisms during washing operations (Chaidez et. al., 2007; Doyle et. al., 2008; Gonzalez et. al., 2004; Harris et. al., 2002). The concentration of chlorine in the water used in packinghouse operations of fresh produce should be maintained at 100 to 200 ppm total chlorine with a pH of 6.0 to 7.5, and with a contact time of one to two min (CFIA, 2009; Beuchat, 1998; Chang et. al., 2007;

FDA/HHS/CFSAN, 1998). The highest bactericidal activity against a wide range of microorganisms is provided by the availability of free chloride in the form hypochlorous acid (HOCl) (Beuchat, 1998; Doyle et. al., 2008; FDA, 2001). The concentration of hypochlorous acid in aqueous solutions increases as the pH decreases (Beuchat, 1998; FDA, 2001). However, a pH of 6.0 to 7.5 is maintained in sanitizer solutions to reduce the risk of corrosion of equipment while retaining an acceptable chlorine efficacy (Beuchat, 1998; FDA, 2001). Temperature, organic matter, light, air, and metals are known to reduce the concentration and microbial activity of hypochlorous acid (Beuchat, 1998; Doyle et. al., 2008). A greater solubility of chlorine is obtained at a water temperature of 4ºC (Beuchat, 1998; FDA, 2001); however the temperature of the water should be at least 10°C higher than the temperature of incoming produce to minimize infiltration of water and bacteria into the produce item (Bartz et. al., 1981; Zhuang et. al., 1995).

Another sanitizer compound used as a disinfectant for fresh produce is chlorine dioxide (ClO_2) (Beuchat, 1998). This chemical is less affected by changes in pH and organic matter, but it is unstable and requires on-site generation (Beuchat, 1998; FDA, 2001). In the United States a maximum concentration of 200 ppm is permitted for the disinfection of processing equipment and surfaces, and is also permitted for washing whole produce at a concentration of three to five ppm (Beuchat, 1998).

Quaternary ammonium compounds (Quats) are commonly used for the sanitation of floors, walls, equipment and food-contact surfaces (Beuchat, 1998). Due to the surfactant activity, Quats have a good penetrating action and form a residual antimicrobial film on treated surfaces (Beuchat, 1998; FDA, 2001). This sanitizer is stable in the presence of organic matter, they are

odorless and colorless when diluted, but require a pH between 6.0 and 10.0 to be effective (Beuchat, 1998; FDA, 2001). This disinfectant is not approved for the use on fresh produce (Beuchat, 1998; Chaidez et. al., 2007).

Bromine has had very limited use alone or in combination with chlorine in the treatment of water, but there is very little known about its usefulness as a disinfectant for fresh produce (Beuchat, 1998; FDA, 2001). On the other hand iodine compounds are widely used for the sanitation of equipment and contact surfaces in food processing environments (Beuchat, 1998). These sanitizers may stain equipment surfaces and react with starch forming a blue-purple coloration (Beuchat, 1998; FDA, 2001). Therefore, the direct use of this sanitizer with fresh produce has limited potential (Beuchat, 1998; FDA, 2001).

Organic acids can be naturally found in many fresh produce items especially fruits (Herdt et. al., 2009) and some of the most common are acetic, citric, succinic, malic, tartaric, benzoic and sorbic acids (Beuchat, 1998; FDA, 2001). Many of these organic acids are generally recognized as safe and are used as antimicrobials in food preservations (Herdt et. al., 2009). However due to the low pH, organic acids can sometimes negatively affect organoleptic properties of some produce items (Herdt et. al., 2009). Organic acids are stable in the presence of organic matter and do not transfer off-odors (Herdt et. al., 2009).

Ozone is widely used for the disinfection of wash water and flume-water used during postharvest operations of fresh produce (Beuchat, 1998). As a sanitizer ozone is very effective at concentrations of 0.5 to 2 ppm in water free of organic matter and soil particles (Suslow, 1998). Ozone is a highly reactive oxidizing gas and its effectiveness is only slightly affected at a water pH from 6.0 to 8.5 (Sela et. al., 2009; Suslow, 1998). Once ozone contacts water it becomes very

unstable and decomposes to oxygen in a very short time (Sela et. al., 2009; Suslow, 1998). Due to ozone instability, some packinghouses using ozone as a disinfectant for wash water add a small quantity of chlorine to provide a residual disinfecting effect on the water (Suslow, 1998). On the other hand, the instability of ozone is also considered beneficial since it decomposes to oxygen and does not create off-odors or changes in the quality of water (Herdt et. al., 2009; Sapers, 2009; Suslow, 1998). Penetration of ozone to natural openings or wounds of fresh produce is limited (Herdt et. al., 2009; Suslow, 1998). However, contact times for microbial action are usually four to five times shorter compared to chlorine (Suslow, 1998).

Currently postharvest irradiation of fresh produce at doses up to 1 kGy is permitted to destroy insects and to extend the shelf life (FDA, 2001; Sela et. al., 2009); however, the FDA does not approve the use of irradiation to reduce populations of pathogenic microorganisms on produce (Groth, 2007; Sela et. al., 2009). Treatment with irradiation preserves the quality and shelf life of food products by eliminating spoilage microorganisms and retarding ripening (Groth, 2007; Sela et. al., 2009). The irradiation doses necessary to eliminate pathogenic microorganisms to safe levels on fresh produce results in unacceptable changes on the sensory quality of produce (Beuchat, 1998; Groth, 2007). Irradiation doses that prevent the changes on quality on fresh produce are low and might not be sufficient to ensure the reduction of pathogens to safe levels (Groth, 2007).

Modified atmosphere packaging (MAP) is also commonly used to reduce quality changes during storage and to increase the shelf life of fresh and fresh-cut produce (De Roever, 1998). Gas-permeable films can allow fresh produce packaged in this type of packages to modify its own atmosphere (Harris et. al., 2002). During modification of the packaging atmosphere three

processes take place: respiration of the produce item, gas diffusion through the produce, and gas transmission through the film; as a result the concentration of oxygen is reduced and the concentration of carbon dioxide is increased (Harris et. al., 2002). The reduction on the concentration of oxygen inside the package decreases the rate of respiration of the fresh produce item increasing its shelf life (FDA, 2001). The reduction in oxygen concentration suppresses the growth of some aerobic spoilage bacteria, but it has little effect in the growth of most human pathogenic bacteria (De Roever, 1998; Harris et. al., 2002).

Limitations of Treatments Applied to Fresh Produce

Several studies have reported that the use of chlorine and other sanitizers permitted by the FDA and the EPA cannot reduce microbial populations attached to the surface of produce more than one to three logs (Beuchat, 1998; Sapers, 2009). The use of sanitizers can aid in the reduction of microbial load on the surface of produce, but is not sufficient to ensure its safety (Sapers, 2009). Moreover, the effectiveness of antimicrobial treatments can be influenced by different treatment factors such as: chemical and physical state, water temperature, concentration of the sanitizer, pH, buildup of organic material, contact time, and the resistance of pathogens (Beuchat, 1998; Herdt et. al., 2009; Sela et. al., 2009; Velez et. al., 2005). However, strong attachment of bacterial cells to the surface of produce, the formation of protective barriers such as biofilms that could potentially protect bacterial populations from contact with sanitizers, infiltration into the core tissues of produce, and the inability of sanitizers to access puncture wounds, pores, cracks, crevices, and other natural irregularities on the surface of produce can as well influence and limit the effectiveness of sanitizers in reducing populations of

microorganisms from the surface of produce (Beuchat, 1998; Burnett et. al., 2001; Doyle et. al., 2008).

Several studies have evaluated the survival, attachment, and growth characteristics of pathogenic bacteria inoculated on the surface, stem scar, cut surfaces, and the internal tissues of produce items. These studies have also observed the survival of pathogens during storage of inoculated produce items and the reductions obtained in the populations of different pathogens when in contact with chlorine solutions at different concentrations. During a study conducted by Weissinger and others (2000), samples of shredded lettuce and diced tomato fruits were inoculated with *S*. Baildon (Weissinger et. al., 2000). Results showed that bacterial cells attached to the lettuce and tomato fruit samples were not reduced to undetectable levels after storage for 12 days at 4ºC (Weissinger et. al., 2000). Moreover, populations of this pathogen increased by 4.53 log CFU/g and 6.0 log CFU/g when inoculated on diced tomato fruits and stored at 21ºC and 30ºC for 24 h (Weissinger et. al., 2000). In addition, populations of *S*. Baildon on shredded lettuce and diced tomato fruits were reduced by less than 1 log CFU/g when treated with 120 ppm or 200 ppm free chlorine solution for 40 s (Weissinger et. al., 2000). Results from another study showed that populations of *S*. Montevideo inoculated onto the skin of tomato fruit were able to survive for 48 h, but were not detected after 5 days (Wei et. al., 1995). On the other hand, populations of *S*. Montevideo inoculated on the stem scar of tomato fruit survived for seven days and were only reduced by one to two log units (Wei et. al., 1995). Wei and others (1995) reported that bacterial populations increased rapidly on puncture wounds, but decreased on the unbroken skin and stem scar of tomato fruits (Wei et. al., 1995). However, treatment with 100 ppm of chlorine for 2 min failed to eliminate bacteria in this inoculation sites (Wei et. al., 1995).

The results of a study conducted by Zhuang and others (1995) showed the ability of *S*. Montevideo to survive on the surface of inoculated tomato fruits after extended storage for 18 days at 10ºC (Zhuang et. al., 1995). Additionally, a significant increase in population of *S*. Montevideo on the surface of tomato fruits was reported when tomato fruit were stored for seven days and one day at 20ºC and 30ºC (Zhuang et. al., 1995). Significant reductions on the populations of the pathogen on the surface of tomato fruit and in the core tissues were reported after dipping tomato fruits for two min in a 60 or 110 ppm chlorine solution, but concentrations of 320 ppm chlorine did not achieve complete elimination (Zhuang et. al., 1995). Then again, the study observed that populations of *S*. Montevideo remained unchanged when inoculated on chopped tomato fruits and stored at 5ºC for 9 days, but increased after storage for 96 h or 22 h at 20ºC or 30ºC, respectively (Zhuang et. al., 1995). Kroupitski and others (2009) evaluated the attachment patterns of *S*. Typhimurium to intact and cut surfaces of Romaine lettuce leaves (Kroupitski et. al., 2009). A higher attachment of cells was observed after 2 h of contact with the lettuce leaves at 25ºC, compared to contact for 2 min at 25ºC (Kroupitski et. al., 2009). Attachment of cells was also higher on the cut surfaces of the leaves compared to attachment observed on the intact surfaces of lettuce leaves for the two incubation times (Kroupitski et. al., 2009). Results showed that treatment of the inoculated leaves with 200 ppm chlorine solution for two min after the two h of incubation resulted in higher reductions on intact surfaces compared to the reductions obtained on the cut surfaces of leaves (Kroupitski et. al., 2009). Observations using confocal microscopy showed a higher bacterial attachment to the cut edge surfaces of leaves compared to those attached to the intact surfaces as reported in this study (Kroupitski et. al., 2009). Additionally, higher attachment was reported after incubation for 2 h at 25ºC

compared to the number of cells attached after incubation for 18 h at 4ºC (Kroupitski et. al., 2009). The study also reported the formation of biofilms in both surfaces of lettuce leaves (Kroupitski et. al., 2009). From the reported results in these studies attachment, growth, and survival of pathogenic bacteria might appear to be higher in cut surfaces, injured tissue, and stem scars than it was on the intact surface and skin of produce. Complete bacterial reductions after contact with chlorine solutions of different concentrations were not achieved in any of these studies; additionally, reductions of bacterial cells were limited by attachment of the cells to cut or wounded tissues compared to those attached to the intact surface of produce. According to Beuchat (1998), the presence of cracks, creases, crevices, and natural openings in the skin of produce that shelter microbial cells and limits contact with chlorine, might contribute to chlorine lack of effectiveness (Beuchat, 1998; Burnet et. al., 2001).

The potential for internalization of pathogenic bacteria inside the internal tissue of produce is also of concern (Aruscavage et. al., 2006; FDA, 2009). Internalized pathogenic bacteria might be less likely to be reduced during postharvest operations especially during washing and consequently limiting the effectiveness of treatment with sanitizers (Aruscavage et. al., 2006). The infiltration of pathogenic bacteria inside the core tissues of produce is believed to occur when produce is submerged or when it comes in contact with cells suspended in water (Burnett et. al., 2001) especially when a negative temperature differential exist between the water and the produce item (Beuchat, 1998). A study performed by Zhuang and others (1995) evaluated the ability of *S*. Montevideo to infiltrate into the internal tissue of tomato fruit when subjected to bacterial suspensions at different temperatures (Zhuang et. al., 1995). As reported in this study, a larger number of bacterial cells were infiltrated when the temperature of the suspension was

15ºC colder than the tomato fruit, compared with the number of cells infiltrated when tomato fruits were exposed to suspensions having the same temperature as the tomato fruit and suspensions 12ºC higher than the temperature of the tomato fruit (Zhuang et. al., 1995). Moreover, populations of the pathogen remained constant after storage of the infiltrated tomato fruits at 10ºC for eight days, but storage at 20ºC resulted in significant increases in populations of the pathogen (Zhuang et. al., 1995). On the other hand, effectiveness of chlorine in reducing populations of *S*. Montevideo in the core tissue of tomato fruit was less than its effectiveness in reducing populations of this pathogen on the surface (Zhuang et. al., 1995). A treatment with 60 ppm of chlorine solution showed no significant reductions, but treatment with 110 ppm and 320 ppm reduced populations of the pathogen at significant levels (Zhuang et. al., 1995).

Bartz and others (1981) reported the infiltration of water and bacterial cells inside the tissues of tomato fruit occurred when they were immersed in bacterial suspensions at a lower temperature than the temperature of the tomato fruit (Bartz et. al., 1981). An increase in weight after immersion of tomato fruit on a suspension with negative temperature differential (temperature of the suspension colder than the temperature of the tomato fruit) was reported in this study (Bartz et. al., 1981). Whereas tomato fruit immersed in suspensions with no temperature differential or with a positive temperature differential did not show a significant increment in weight and rarely became diseased (Bartz et. al., 1981). Infiltration of water and bacteria was reduced when the temperature of the water was significantly warmer than the temperature of the tomato fruit (Bartz et. al., 1981). Green and pink tomato fruits suffer greater infiltration compared to ripe fruit (Bartz et. al., 1981).

Burnett and other (2000) evaluated attachment and internalization of *Escherichia coli*

O157:H7 in the surface, puncture wounds and internal tissues of apples (Burnett et. al., 2000). *E. coli* O157:H7 attached to the surface and to damage tissue surrounding puncture wounds (Burnett et. al., 2000). Infiltration through the floral tube and attachment to seeds, cartilaginous pericarp, and internal trichomes was observed for apples inoculated at the three inoculation temperature differentials (negative, positive, and zero temperature differential between the apple and the inoculum) (Burnett et. al., 2000). Additionally, a higher number of cells attached and infiltrated intact skin and lenticels, bruised areas, and the floral tube when apples were exposed to a inoculum at a negative temperature differential compared to those inoculated under no temperature differential (Burnett et. al., 2000). This study suggested that *E. coli* O157:H7 could attach to internal core tissues and within tissues of apples reducing contact with sanitizers and consequently reducing chemical sanitizer efficacy (Burnett et. al., 2000). Results form these studies confirm that infiltration of pathogenic bacteria inside the internal tissues of fresh produce might potentially reduce the effectiveness of sanitation treatments in reducing bacterial populations by providing protection sites for bacterial cells and increasing the opportunity for bacteria to grow and survive.

The growth of microorganism in protected areas on produce surfaces can result in the formation of biofilms (Carmichael et. al., 1999). The formation of biofilms improves the ability of bacteria to colonize and survive the harsh environment of the surface of produce (Annous et. al., 2005). As a result, a well-developed biofilm can provide protection for the pathogen reducing the effectiveness and penetration of sanitizers (Carmichael et. al., 1999; Annous et. al, 2005). Iturriaga and others (2007) evaluated the influence of temperature and humidity during storage on the survival and growth of *S.* Montevideo on the surface of tomato fruit (Iturriaga et. al.,

2007). After storage for 10 days at 30ºC populations of *S*. Montevideo increased by 0.7, 1.0, 1.2, and 2.2 log CFU per tomato when exposed to 60, 75, 85, and 97% relative humidity respectively (Iturriaga et. al., 2007). Formation of a well-defined biofilm on the tomato fruit cuticle was reported after storage for 10 days (Iturriaga et. al., 2007).

Another study evaluated the formation of biofilms on the surface of melons that were previously spot inoculated with two strains of *S. enterica* (Annous et. al., 2005). Bacterial cells were able to form a biofilm on the surface of melons after inoculation and storage for two h at 20ºC; additionally, bacterial cells were found enclosed in an extracellular polymeric material after 24 h at 10ºC and 20ºC (Annous et. al., 2005). This study showed that pathogenic bacteria can develop biofilms on the surface of produce and that biofilm formation may be responsible for the reduced efficacy of sanitizers (Annous et. al., 2005).

Cluster formations of biofilm and individual bacterial cells were found in the surface of lettuce leaves previous to treatment with a sanitizer (Carmichael et. al., 1999). Results form this study showed that the use of a sanitizer reduced bacteria populations from the surface of the lettuce, but formation of new biofilms was observed after four days of storage post sanitation treatment (Carmichael et. al., 1999). Biofilms were localized on the intercellular junctions of the leaf, and continued to grow with storage time becoming multilayered (Carmichael et. al., 1999). Bacterial cells have been found to attach and survive on the surface of produce especially in protected sites (Brandl, 2006; Solomon et. al., 2009). Attachment of bacterial cells on these protected sites might result in the formation of aggregates or strong biofilms that will provide additional protection for bacterial cells (Brandl, 2006; Burnett et. al., 2000). The inability of sanitizers to access this protected sites and to contact bacterial cells in biofilms might possibly

limit the effectiveness of sanitizers (Burnett et. al., 2000).

Use of High Pressure Processing (HPP) for Fresh Products

High pressure processing (HPP) is a potential non-thermal food processing technology alternative to heat pasteurization (Basak et. al., 1998; Considine et. al., 2008). This technology has the capability to reduce populations of pathogenic and spoilage microorganisms, improving food safety and increasing the shelf life of food while preserving fresh attributes, nutrient substances and minimal treatment of foods (Considine et. al., 2008; Douglas, 2002; Norton et. al., 2007). Foods treated with this technology show improved quality, safety and greater retention of nutritional and organoleptic attributes of foods (Considine et. al., 2008; Douglas, 2002; Norton et. al., 2007). Furthermore, this processing technology offers great potential for the retention of quality and nutritional attributes of fruits and vegetables that are negatively affected by conventional heat treatments (Basak et. al., 1998).

Pressures between 100 and 800 MPa may be applied to liquid or solid foods with or without packaging during HPP (FDA, 2000). Temperatures applied during treatment vary between 0ºC and 100ºC and the treatment time can range from milliseconds to 20 min (FDA, 2000). Depending on the time and temperature selected for HPP, different chemical changes in the food may take place (FDA, 2000). At room temperature foods treated with HPP will not experience significant chemical alterations due to the pressure treatment itself (FDA, 2000). However, the application of higher pressures and longer pressurization times increases the potential for changes in the structure of fragile fresh foods such as, strawberries or lettuce (FDA, 2000). Cell deformation and membrane damage of the food can result in tissue softening and a

food with the characteristics of a processed product (FDA, 2000; Norton et. al., 2007; Rastogi et. al., 2007). On the other hand, during pressurization of foods covalent bonds remain unaffected and as a result many of the components responsible for the organoleptic and nutritional quality of foods are preserved (Douglas, 2002; Patterson, 2005; Rastogi et. al., 2007).

The pressure is applied rapidly and uniformly throughout the pressure medium and the food regardless of size, shape and composition obtaining a very homogeneous food (Douglas, 2002; FDA, 2000; Patterson, 2005; Yuste et. al., 2001). During pressurization a rise in temperature due to adiabatic heating from the work of compression occurs (FDA, 2000; Patterson, 2005). Temperature increases approximately 3ºC per 100 MPa depending on the composition of the food; however, the temperature returns to its original level during decompression if no heat has been gained or lost from the walls of the pressure vessel during holding time (FDA, 2000; Rastogi et. al., 2007).

The first report on the use HPP for food preservation was in the study performed by Bert Hite in 1899 (Hite, 1899). Hite reported that treatment of milk at 600 MPa for one h at room temperature extended the shelf life of milk for approximately 4 days and that the milk was still sweet after this period of time (Hite, 1899). In a latter study Hite and others (1914) reported that most fruits treated with pressures between 400 to 829 MPa remained commercially sterile for at least five years (Hite et. al., 1914). However, this study also found that pressure treatment was not successful for the treatment of vegetables due to the presence of spore forming bacteria (Hite et. al., 1914). The first commercial product treated with HPP was fruit jam and sauces that were produced in Japan in the early 1990's (Patterson, 2005; Rastogi et. al., 2007). Currently some HPP products are already available in the market, this include fruit jellies, jams, fruit juices,

salad dressing, raw squid, ham, guacamole, meal kits, peppers, onions, avocado, and raw oysters (Douglas, 2002; FDA, 2000; Patterson, 2005).

In the past few years several studies have been performed on food products to determine the effectiveness of HPP in reducing populations of different spoilage and pathogenic microorganism, as well as the effects of HPP on the sensory and nutritional quality of foods, and the inactivation of enzymes. Recent studies have focused specially on the application of HPP to different fresh products and between them fresh vegetables and fruits. The results of a study performed by Goodridge and others (2006) showed that two strains of *S*. Enteriditis inoculated into raw almonds were reduced by approximately one log after six cycles of pressure treatment at 60,000 psi (414 MPa) at 50ºC for 20 s (Goodridge et. al., 2006). The low water activity of almonds was believed to have a baroprotective effect since reduction of the two *S*. Enteriditis strains increased to 3.37 logs when the almonds were suspended on water during pressure treatment (Goodridge et. al., 2006). Another study showed that *S*. Senftenberg was more sensitive to pressure levels between 2,380 atm (241.15 MPa) and 3,400 atm (344.50 MPa) compared to *S*. Typhimurium (Metrick et. al., 1989). Reductions of populations of both strains of Salmonella were reported to be higher when inoculated onto phosphate buffer compared to reductions obtained in chicken medium (Metrick et. al., 1989). Recovery of injured cells after pressure treatment at 37ºC was possible in chicken medium but not in buffer (Metrick et. al., 1989).

Whitney and others (2008) observed the reductions of populations of six strains of *E. coli* O157:H7 and five serovars of *S. enterica* after pressure treatment between 300 and 550 MPa at 6ºC for two min on different mediums (Whitney et. al., 2008). Results from this study showed

that reductions on the population of *E. coli* O157:H7 after treatment at 550 MPa were between 0.28 and 4.39 log CFU/ml, while reductions on the populations of *S. enterica* were greater than 5 logs (Whitney et. al., 2008). The observations of the study showed greater bacterial reductions in orange and apple juices compared to reductions obtained in TSB and distillated water (Whitney et. al., 2008). Storage of samples for 24 h at 4ºC after pressurization at 550 MPa showed an increase in the reduction levels on the populations of *E. coli* O157:H7 while reductions on the populations of *S. enterica* were greater than 5 logs (Whitney et. al., 2008). Another study compared the reductions on populations of different strains of *Staphylococcus aureus, Listeria monocytogenes, E. coli* O157:H7, *S.* Typhimurium, and *S.* Enteriditis after different variation on pressure level, temperature, time and pH (Alpas et. al., 2000). This study concluded that the populations of all pathogens but *S. aureus* were reduced by more than 8 log cycles at 345 MPa, 50ºC for five min, and that the addition of citric and lactic acid increased reductions by 1.2-3.9 log cycles at a pH level of 4.5 and a pressure of 345 MPa (Alpas et. al., 2000).

During a study conducted by Maitland and others (2009), results showed significant reductions on the population of *S.* Braenderup in tryptic soy broth after pressure treatment at 350, 450, and 500 MPa at 20ºC for 120 s (Maitland et. al., 2009). However, reductions on the population of this pathogen in diced and whole tomato fruits were smaller (Maitland et. al., 2009). Reported reductions on the population of *S.* Braenderup reach levels of 3.67 log cycles in diced tomato fruits, and 3.35 log cycles in whole tomato fruits both after treatment at 550 MPa (Maitland et. al., 2009). The visual appearance of tomato fruit samples after pressure treatment was reported as being similar to the appearance of control tomato fruits with no treatment (Maitland et. al., 2009). Furthermore, results from a study conducted by Arroyo and others

(1997), showed that a pressure level of 350 MPa reduced populations of gram-negative bacteria and fungi, and a pressure of 400 MPa could not completely reduce populations of gram-positive bacteria (Arroyo et. al., 1997). Additionally, populations of aerobic mesophiles, fungi, and yeast in lettuce and tomato fruits treated at 300 MPa and above were reduced by one log unit (Arroyo et. al., 1997). However, the skin of tomato fruits peeled away and browning of lettuce occurred after treatment (Arroyo et. al., 1997). Additionally, following HPP treatment mango slices experienced declined fresh flavor and increased off-flavors during storage at 3ºC, but color, texture and other organoleptic attributes were just slightly affected (Boynton et. al., 2002). After nine weeks of storage, microbial levels on the control mango slices were two and three log cycles greater than samples treated at 300 MPa and 600 MPa (Boynton et. al., 2002). Carambola slices that were treated at 800 MPa showed reduce browning after four weeks of storage at 3ºC and air exposure compared to control samples (Boynton et. al., 2002).

Texture loss and increase deformability of carrots was reported in a study were different pressure levels were applied to carrots (Trejo-Araya et. al., 2007). Hardness losses were higher in carrots treated at 300 MPa, but no further increase in texture losses occurred at higher-pressure levels (Trejo et. al., 2007). However texture recovery was observed during pressure holding time at pressures above 300 MPa (Trejo-Araya et. al., 2007). Additionally, as reported in another study, different fruits (apple, pear, orange, and pineapple) and vegetables (carrot, celery, green pepper, and red pepper) treated with HPP experienced an initial loss of texture due to the instantaneous initial application of pressure followed by an increase in texture during pressure holding time (Basak et. al., 1998). The firming effect during pressure holding time was reported for all fruits and vegetables but apples, carrots, and green peppers (Basak et. al., 1998). The

results of the study found that the samples of vegetables and fruits resembled the appearance of mildly heat-treated samples (Basak et. al., 1998).

Bayindirli and others (2006) evaluated the efficacy of HPP (350-450 MPa) in combination with mild heat (40ºC- 50ºC) treatments on the reduction of three pressure resistant pathogens (*S. aureus* 485, *E. coli* O157:H7 933, and *S.* Enteriditis FDA) and enzymes (polyphenol oxidase and pectinesterase) in fruit juices (apple, orange, apricot, and sour cherry) (Bayindirli et. al., 2006). Results showed that a treatment of 350 MPa at 40ºC for 5 min reduced all the pathogenic populations by more than 8 log cycles in the different fruit juices studied (Bayindirli et. al., 2006). The activity of polyphenol oxidase in apple juice and pectinesterase in orange juice were reduced to 9% and 7% after application of 450 MPa 50ºC for 60 and 30 min respectively (Bayindirli et. al., 2006). This study showed that enzymes require higher-pressure levels, and times to be inactivated compared to pathogenic bacteria (Bayindirli et. al., 2006). Similarly, the enzyme lipoxygenase (LOX) in tomato juice was completely inactivated after HPP treatment at pressures higher than 550 MPa at 20ºC for 12 min (Rodrigo et. al., 2007). The activity of another enzyme found in tomato juice known as hydroperoxide lyase (HPL) was reduced by 20% after pressure treatment at 300 MPa (Rodrigo et. al., 2007). However, a residual fraction of 20% remains active even after 650 MPa at 20ºC for 12 min (Rodrigo et. al., 2007). In another study conducted by Terefe and others (2008) results showed that the activity of peroxidase in fresh strawberries was inactivated to a maximum of 58% after treatment at 600 MPa at 60ºC for 10 min, but the activity of polyphenol oxidase was not affected under this conditions of treatment (Terefe et. al., 2008). Additionally, treatment of the strawberries at this pressure and temperature levels did not have a negative effect on polyphenol and total anthocyanins content (Terefe et. al.,

2008). Result of the study also showed that strawberries HPP treated at a temperature between 20ºC and 40ºC looked similar to fresh strawberries with no treatment (Terefe et. al., 2008). A HPP treatment of strawberries at room temperature and refrigerated storage resulted in a high quality product over at least three months (Terefe et. al., 2008).

During the application of HPP to tomato and carrot purée, Patras and other (2008) found that the antioxidant activity of HPP treated tomato and carrot purée was higher than that of untreated or thermally treated samples (Patras et. al., 2008). In tomato purée, 90% of ascorbic acid was retained after treatment at 600 MPa at 20ºC for 15 min, and phenolic content was not affected (Patras et. al., 2008). The color of both purées was better retained when HPP treated at this level of pressure compared to thermal treatment (Patras et. al, 2008). Butz and others (2002) found that HPP treatment of carrots, tomatoes and broccoli at a pressure level of 600 MPa at 70ºC did not affect chlorophyll a and chlorophyll b in broccoli, and that even after 60 min of treatment the total concentration of lycopene and β-carotene was maintained with no isomers formation detected (Butz et. al., 2002). The study also reported that antioxidant activity of carrot and tomatoes was slightly affected by the pressure treatment (Butz et. al., 2002).

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Chapter 3: Influence of High Pressure Processing on Populations of *Salmonella enterica* in Fresh Green-Mature Tomato Fruits

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Abstract:

The objective of this study was to determine the effect of high pressure processing (HPP) on fresh tomato-associated outbreak isolates of *Salmonella enterica* in broth and on green mature tomato fruits. Nalidixic acid resistant (to 50 ppm) cultures of *Salmonella enterica* ser. Newport and *Salmonella enterica* ser. Braenderup were suspended in tryptic soy broth to a concentration of approximately 8 log CFU/ml. Portions containing five ml of this broth were packaged in sterile stomacher bags and subjected to one of three different pressures (350, 450, or 550 MPa) for 120s. After pressure treatment, samples were serially diluted in peptone water, and surface plated onto tryptic soy agar supplemented with nalidixic acid (50 ppm; TSAN) and incubated at 35ºC for 48 h. Reductions of 5.64, 6.30, and 6.61 log CFU/ml in *S*. Newport, and reductions of 4.10, 5.22, and 6.35 log CFU/ml in *S*. Braenderup at 350, 450, and 550 MPa respectively were observed. Green tomato fruits inoculated with *S*. Newport or *S*. Braenderup to an initial concentration of approximately 6 log CFU/g were sealed in a sterile bag containing 350 ml of 1% $CaCl_2$ and subjected to the same pressure treatments as described above. The whole tomato fruits were then homogenized in a stomacher and samples were surface plated onto TSAN supplemented with 1% pyruvic acid. Significant reductions of 1.55, 2.89, and 4.26 log CFU/g for *S*. Newport and 1.22, 2.26, and 3.77 log CFU/g for *S*. Braenderup at 350, 450, and 550 MPa, respectively, were observed. HPP could be considered a potentially effective method for the reduction of populations of *S. enterica* in green mature tomato fruit.

Introduction:

The number of foodborne outbreaks of *Salmonella enterica* associated with the consumption of fresh tomato fruits has increased in recent years as reported by the CDC (CDC, 2006). Between 1998 and 2006, the number of outbreaks associated with tomatoes reported to the FDA accounted for a 17% of the total produce-related outbreaks (FDA, 2010). Additionally, between 1996 and 1999, it was estimated that 1.4 million persons were infected with nontyphoidal *Salmonella,* that resulted in 15,000 hospitalizations and 400 deaths annually in the United States as estimated using FoodNet surveillance data and other related surveillance (Voetsch et. al., 2004). In 2006 alone there were 121 *S. enterica* outbreaks that resulted in more than 3,300 illnesses as reported to the CDC Foodborne Outbreak Reporting System (CDC, 2006). In 2004 three multistate outbreaks of *S. enterica* associated with the consumption of roma tomato fruits from a packinghouse in Florida resulted in 561 illnesses (CDC, 2005). Additionally, between 2005 and 2006 four multistate outbreaks of *S. enterica* infections also associated with the consumption of raw tomato fruits from Florida, Ohio, and Virginia resulted in 459 cases (CDC, 2007). Finally in 2008 another multistate outbreak of *S. enterica* associated with the consumption of jalapeño peppers, Serrano peppers and tomato fruits resulted in 1.442 cases of infection, 286 hospitalizations, and at least two deaths (CDC, 2008).

These multistate outbreaks emphasize the need to prevent contamination of tomato fruits during production in the field, during harvesting, and during packing operations and distribution (Beuchat, 1997; CDC, 2005; FDA, 2009). However, because most produce is grown in a natural environment unexpected pathogenic contamination may occur (FDA, 2004). Since produce is often consumed raw without any type of intervention that would reduce, control or eliminate

pathogens before consumption, produce may become a potential source of foodborne illnesses (FDA, 2004).

The potential for infiltration or internalization of *S. enterica* inside the internal tissue of tomato fruits is also a concern (Aruscavage et. al., 2006; FDA, 2009). Infiltration of *S. enterica* is believed to occur when tomato fruits are submerged in contaminated water with a negative temperature differential between the water and the tomato fruit (temperature of the water being lower than the temperature of the tomato fruit) (Bartz et. al., 1981; Burnett et. al., 2001; FDA, 2010; Zhuang et. al., 1995). The temperature of the water should be at least 10°F warmer than the temperature of the tomato fruit to prevent infiltration (Bartz et. al., 1981; FDA, 2010). Internalized pathogenic bacteria might be less likely to be removed during postharvest operations especially during washing and sanitizing operations (Aruscavage et. al., 2006; FDA, 2010; Zhuang et. al., 1995).

Postharvest operations for the surface cleaning of tomato fruits involve the use of chemical and physical treatments (FDA, 2009). Producers commonly use soft brushes during cleaning for the removal of soil and debris in conjunction with water washes and sanitizing rinses (Boyette et. al., 1995; FDA, 2009; MFCL/NGMC/NARI, 2003). Washing efficiency depends on the type of produce, type of washing system, type of soil, contact time, sanitizer used, and water temperature (FDA, 2009). However, these treatments are not targeted for the elimination of pathogenic bacteria in the surface or inside the tissue of the produce item, but to provide a barrier against cross contamination of produce during cleaning (Beuchat, 1997; FDA, 2009).

Physical preservation methods (heating, freezing, dehydration, and packaging) as well as, chemical preservation methods (pH and preservatives) are still being widely used in the food industry for the processing of food products (Mañas et. al., 2005). However, such a treatments cannot be applied to fresh produce due to the unwanted changes in sensory, nutritional, and functional properties of food that result after treatment (Lund, 2002; Mañas et. al., 2005). On the other hand non-thermal technologies such as high pressure processing allow the inactivation of microorganisms at low temperatures thus better preserving the sensory, nutritional and functional properties of foods (Lund, 2002; Mañas et. al., 2005).

High pressure processing (HPP) is a non-thermal food processing technology (Basak et. al., 1998; Considine et. al., 2008). Foods treated with this technology show improved quality, safety and greater retention of nutritional and organoleptic attributes of foods (Considine et. al., 2008; Douglas, 2002; Norton et. al., 2007). Pressures of 100 to 800 MPa may be applied to liquid or solid foods with or without packaging during HPP (FDA, 2000). The pressure is applied rapidly and uniformly throughout the pressure medium and the food, regardless of size, shape and composition obtaining a very homogeneous food (Douglas, 2002; FDA, 2000; Patterson, 2005; Yuste et. al., 2001). The first report on the use HPP for food preservation was in the study performed by Bert Hite in 1899 (Hite, 1899), who tried to extend the shelf life of milk (Hite, 1899). The application of HPP on ripe diced and ripe whole tomato fruits reduced the population of *Salmonella enterica* ser. Braenderup (Maitland et. al., 2009). Additionally, results from this previous study showed that no significant visual changes in the appearance of diced and whole tomato fruit samples were observed after HPP treatment (Maitland et. al., 2009).

Tomato fruit can be harvested at different stages of maturity depending on the intended

market destination (Orzolek et. al., 2006). Commercially produced fresh market tomato fruits are harvested at the green mature stage (Boukobza et. al., 2002; Wang et. al., 2007). Tomato fruit that is harvested at the green mature stage of ripening can better resist the stress of handling and transportation, and it reduces the risk of becoming over ripe before reaching the market (MFCL/NGMC/NARI, 2003; Orzolek et. al., 2006; Wang et. al., 2007). Commercial tomato producers use ethylene to promote the ripening of tomatoes, and since tomato fruits are harvested at the green mature stage producers have a better control of the ripening and shelf life of the tomato fruits (Wang, 2007; Boukobza, 2002). No previous work has been done on the efficacy of HPP to reduce *S. enterica* population on green mature tomato fruits and the effects of high pressure processing on the artificial ripening of tomatoes with ethylene has also not been reported.

The objectives of this work are:

1. To determine the effects of HPP at three levels (350, 450, and 550 MPa) for 120 s at 21ºC on populations of *S.* Newport and *S.* Braenderup in broth and in green mature tomato fruit.
2. To determine the effects of three levels of HPP (350, 450, and 550 MPa) at 21ºC for 120 s on the development of ripening of green mature tomato fruits after exposure to ethylene gas.

Materials and Methods

Identification of Cultures and Culture Maintenance

In this study, two serovars of *Salmonella enterica* originally isolated from tomato fruit outbreaks were used. These are *Salmonella enterica* ser. Newport and *Salmonella enterica* ser. Braenderup. The selected isolates were obtained from the Center for Diseases Control and Prevention (CDC; Atlanta, GA) frozen culture collection and were maintained frozen at -80ºC until use. Serial transfers into tryptic soy broth (TSB) (9ml) were used to activate cultures at 24 h intervals for three consecutive days at 35ºC prior to testing.

Inoculum Preparation and Conservation

The two serovars were made nalidixic acid resistant. To achieve this resistance the selected isolates were first inoculated onto tryptic soy agar plates supplemented with 5 μg/ml of nalidixic acid and incubated for 24 h at 35ºC until colonies were resistant to this initial concentration of nalidixic acid. An isolated colony was chosen, and then consecutively transferred onto TSA plates with increasing concentrations of nalidixic acid (10 μg/ml, 25 μg/ml, and 50μg/ml) every 24 h until colonies were resistant at a level of 50 μg/ml. The nalidixic acid strains were then prepared, in a 20 % glycerol solution, and stored at -74ºC in a freezer (Forma Scientific 5479, Marjetta, OH) (Appendix C) housed in the Food Science and Technology department at Virginia Tech until needed.

Pressure Resistance Study

A broth culture study was performed to determine and compare the pressure resistance of both serovars of *S. enterica* to HPP. Cultures of the two nalidixic acid resistant serovars were obtain from the -74ºC frozen collection and activated by consecutive 24 h transfer onto TSB supplemented with 50 µg/ml of nalidixic acid (TSBN) (9 ml) tubes over three days with incubation at 35ºC.

Flasks containing 99 ml of sterile TSB were inoculated with 1 ml of activated culture to produce approximately an 8 log CFU/ml starting population, and 5ml portions were then packed into sterile stomacher bags (Fisher Brand Secure T, Pittsburg, PA). An inoculated sample was plated on TSAN plates to confirm the initial population of *S. enterica* in broth samples. The bags were vacuum-sealed with a 1.25-hp vacuum (Koch UltraVac 250, Kansas City, MO). Each sample was inserted in a second and finally in a third bag containing 10 ml of disinfectant solution (120 ppm QUAT) to ensure no contamination of the pressure chamber with potentially leaked viable cells.

The bagged broth cultures samples were then subjected to three different pressures. Three samples were subjected at 350 MPa, the next three samples were subjected to 450 MPa and the last three samples were subjected at 550 MPa for 120 s using a Quintus Food Press QFP 35L-600 (Avure Technologies, Kent, WA) (Appendix A) at approximately 21ºC. Once the pressure process was complete each of the samples was plated onto tryptic soy agar supplemented with 50 μg/ml of nalidixic acid (TSAN) and incubated at 35ºC for 48 h. Three bags from each serovar were run during each pressure treatment. The broth study was repeated twice to verify the results (n=6).

Tomato Fruits

Freshly harvested whole green tomato fruits at the mature green stage, with approximately one inch of the stem attached were collected from the fields in Virginia and Florida. Virginia tomato fruits were collected in October 2009 and Florida tomato fruits were collected in March 2010. Tomato fruits were packed individually wrapped in paper and were shipped overnight in carton boxes containing approximately 50 fruits each. Upon arrival, tomato fruits were unpacked and maintained in a refrigerator at 14ºC and 70% relative humidity for no more than 5 days prior to use. This temperature was chosen given that green mature tomato fruits stored at 13ºC to 14ºC can be stored for two weeks before ripening without significant changes in the ripening rate, color development, or sensory quality. This temperature also reduces the ripening speed of green mature tomato fruits for the duration of the storage.

Inoculation of the Tomato Fruits and HPP Treatment

Cultures of the two serovars were obtained from the frozen cultures. Three serial 24-h transfers into TSBN media with incubation at 35ºC were performed to activate the cultures before use, as described previously. After the third inoculation in TSBN was completed, cells were centrifuged at 10,000 x g for 5 min using a Fisher Scientific AccuSpin 400 (FisherBrand, Pittsburg, PA). The cells were then washed twice with 0.1% of sterile peptone water and re-suspended in sterile de-ionized water (10 ml) to obtain an inoculum of approximately 8 log CFU/ml. Green tomato fruits (without the stem) at room temperature (24ºC) were weighed and then spot inoculated at the stem scar with 0.1 ml of inoculum to obtained a initial population of approximately 6 log CFU/g, using a vacuum chamber at approximately 0.6 MPa for 2 min. After

the first vacuum treatment was applied the pressure was allowed to equilibrate with the atmospheric pressure, and then the vacuum procedure was performed again repeating these procedure three times to draw the inoculum inside the tomato fruits. Tomato fruits were allowed to air dry in a laminar flow-through hood (NAAIRE Biological Safety Cabinets NU-425-400, Plymouth, MN) for 30 min post-inoculation. An inoculated tomato fruit was homogenized and plated onto TSAN plates to obtain the initial population inside the tomato fruits. After the inoculation, the tomato fruits were packed into sterile stomacher bags containing approximately 350 ml of a 1% solution of $CaCl_2$. The bags were then vacuum-sealed at 95% vacuum, and bagged for a second time to prevent possible leakage during the pressure treatments. The bagged tomatoes were subjected to the three different levels of pressure (350, 450, and 550 MPa) for 120 s at 21°C (+/-4°C) (initial temperature). Three tomato fruits for each pressure were treated, and the study was repeated three times for tomato fruits from each location (Virginia and Florida) (n=18).

Microbiological Enumeration of *S.* Newport and *S.* Braenderup in Tomato Fruits After HPP

After the pressure treatment, tomato fruits were weighed. Each of the vacuum-sealed bags containing the HPP treated whole tomato fruit was cut open across the top using sterilized scissors and processing solution was poured out. The whole tomato fruit remaining in the sterile bag was transferred to a new sterile stomacher bag and 10 ml of 0.1% sterile peptone water was added and then the sample was homogenized for one min. The homogenate was serially diluted

in 0.1% peptone, surface plated onto TSAN supplemented with 1% pyruvic acid (TSANP) and incubated at 35ºC for 48h to enumerate *S. enterica*.

Statistical Analysis

Results were statistically analyzed in accordance with a split plot design. The experiment was repeated six times for both serovars of *S. enterica (S.* Newport and *S.* Braenderup). The first three repetitions were performed using tomato fruits from Virginia and the following three repetitions using tomato fruits from Florida. Three culture preparations for each of the two serovars and for each experiment were prepared (n=18). A general linear model procedure was used to determine the least squares means of Log CFU reductions, together with Duncan's multiple range tests. The Statistical Analysis System (SAS Institute Version 9.1, 2002, Cary, NC) was used. The P-value used for the statistical analysis was 0.05.

Results and Discussion

The influence of three different levels of pressure (350, 450, and 550 MPa) on the reduction of *S. enterica* populations in whole mature green tomato fruits treated for 120 s at a tomato ripening temperature (approximately 21ºC) was evaluated. Un-inoculated tomato fruits subjected to treatment served as control. *S. enterica* contamination was not detected in any un-inoculated tomato fruits samples under the protocol of the study.

Bacterial Resistance to Pressure in Broth Study

Significant reductions of *S*. Newport and *S*. Braenderup were observed in TSB during pressure treatments at 350, 450, and 550 MPa for 120 s at an initial temperature of 21ºC (+/- 4ºC) ($P < 0.05$). Reduction levels were obtained from an initial inoculum of 8.04 log CFU/ml for *S*. Newport, and an initial inoculum of 8.17 log CFU/ml for *S*. Braenderup in TSB. After treatment in TSB for 120 s at 350, 450, and 550 MPa, reductions in the populations of *S*. Newport were 5.64, 6.30, and 6.61 log CFU/ml, respectively ($P < 0.05$) (Figure 1). Reductions of *S*. Braenderup populations in TSB after treatment were: 4.10, 5.22, and 6.35 log CFU/ml at 350, 450, and 550 MPa, respectively ($P < 0.05$) (Figure 1). *S*. Newport was significantly more sensitive to HPP than *S*. Braenderup ($P < 0.05$). Similarly, a study performed by Maitland and others (2009), reported differences in resistance to HPP of four serovars of *S*. *enterica* (*S*. Newport, *S*. Javiana, *S*. Anatum, and *S*. Braenderup) in TSB (Maitland et. al., 2009). During the study *S*. Braenderup was found to be the most pressure resistant serovar with reductions of 4.53, 5.74, and 7.09 log CFU/ml at 350, 450, and 550 MPa while *S*. Newport reductions levels were reported as 6.05, 7.83, and 7.86 CFU/ml at 350, 450, and 550 MPa (Maitland et. al., 2009).

Another study showed that *S*. Senftenberg was more sensitive to pressure levels between 2,380 atm (241.15 MPa) and 3,400 atm (344.50 MPa) compared to *S*. Typhimurium (Metrick et. al., 1989). Additionally, reductions of populations of both strains of *Salmonella* were reported to be higher when inoculated onto phosphate buffer compared to reductions obtained in chicken medium (Metrick et. al., 1989). Whitney and others (2008) observed the reductions of populations of five serovars of *S*. *enterica* (*S*. Agona, *S*. Baildon, *S*. Gaminara, *S*. Michigan and *S*. Typhimurium) after pressure treatment between 300 and 550 MPa at 6ºC for two min on

different mediums (Whitney et. al., 2008). Results from this study showed that at 550 MPa most *S. enterica* populations were reduced by more than 5 logs (Whitney et. al., 2008). Additionally, *S.* Agona was reported as the most resistant serovar tested, having a decrease of 3.79 log CFU/ml at 550 MPa in TSB (Whitney et. al., 2008). Bacterial populations in orange juice showed larger decreases than populations in TSB and distilled water (Whitney et. al., 2008).

In another study, Chen and others (2006) subjected milk inoculated with *S.* Enteritidis to pressures ranging from 350 MPa to 700 MPa at 21.5°C for 10 min (Chen et. al., 2006). The results showed no significant reduction in *S.* Enteritidis populations at 350 MPa, but *S.* Enteriditis population was reduced by approximately 2 log CFU/ ml at 450 MPa and 6 log CFU/ml at 550 MPa (Chen et. al., 2006). The results of another study showed reductions on the population of *S.* Enteriditis below detectable levels when populations were treated at 400 MPa for 15 min in TSB (Fioretto et. al., 2005).

HPP Effect on Populations of *S.* Newport and *S.* Braenderup in Whole Green Mature Tomato Fruits

Significant reductions of *S.* Newport and *S.* Braenderup were observed in whole green mature tomato fruits during pressure treatments at 350, 450, and 550 MPa for 120 s at a initial temperature of 21ºC (+/- 4ºC) ($P < 0.05$). Reduction levels were obtained from an initial inoculum inside the tomato fruits of 6.19 log CFU/g for *S.* Newport, and an initial inoculum in the tomato fruits of 6.46 log CFU/g for *S.* Braenderup. *S. enterica* was not detected on any un-inoculated tomato fruit samples under the protocol of the study. The location (Florida or

Virginia) from where the tomato fruits were obtained did not effect reductions in *S. enterica* populations under the protocol of this study.

The pressure treatment tomato fruits were subjected during the study resulted in significant reduction levels of 1.55, 2.89, and 4.26 log CFU/g at 350, 450, and 550 MPa respectively for *S*. Newport (Figure 2). *S*. Branderup reduction levels were significantly smaller than those achieved for *S*. Newport ($P < 0.05$). Reduction levels for *S*. Braenderup were 1.22, 2.26, and 3.77 log CFU/g for 350, 450, and 550 MPa respectively (Figure 2). *S*. Newport was significantly more sensitive to pressure compared to *S*. Braenderup when inoculated on whole mature green tomato fruits ($P < 0.05$). The reduction levels obtained for *S*. Braenderup in the present study were similar to those obtained in another study that evaluated the effects of three levels of HPP (350, 450, and 550 MPa) on reductions of *S*. Braenderup on whole and diced ripe tomato fruits treated for 120 s at 20ºC (Maitland et. al., 2009). Reductions of S. Braenderup population were 1.41, 2.25, and 3.35 log CFU/g for 350, 450, and 550 MPa respectively (Maitland et. al., 2009). After comparing the result of the present study with the result of this past study, the maturity stage of tomato fruits seem to have no effect on the reduction of populations of *S*. Braenderup.

Even though, no other studies have been performed on the evaluation of HPP in the reduction of populations of these two *S. enterica* serovars on green mature tomato fruits, some studies have been performed on the HPP reduction of other pathogenic bacteria in tomato fruits and products. Arroyo and others (1997) showed that a pressure level of 350 MPa reduced populations of gram-negative bacteria and fungi, and a pressure of 400 MPa could not completely reduce populations of gram-positive bacteria (Arroyo et. al., 1997). Additionally,

populations of aerobic mesophiles, fungi, and yeast in lettuce and tomato fruits treated at 300 MPa and above were reduced by one log unit (Arroyo et. al., 1997). However, the skin of tomato fruits peeled away and browning of lettuce occurred after treatment at 400 MPa (Arroyo et. al., 1997). A similar study conducted by Arroyo and others (1999) reported that pressurization at 400 MPa resulted in almost complete elimination (> 10 CFU/g) of viable aerobic mesophiles as well as yeasts and fungi while preserving texture and flavor in whole tomato fruits processed in water (Arroyo et. al., 1999). Additionally, Dede and others (2007) showed that the application of 250 MPa at 35ºC for 15 min to tomato and carrot juices reduce total aerobic counts below the detection limit (<1 CFU/ml) (Dede et. al., 2007).

In the present study, reductions of *S. enterica* population inoculated on TSB were higher than the reductions obtained on *S. enterica* population inoculated on green mature tomato fruits. Additionally, the original location from where the tomato fruits were obtained for the present study did not show a significant effect on the reduction of populations of the two *S. enterica* serovars used. Therefore, the origin of the tomato fruits might not be a potential factor that affects the reduction of *S. enterica* populations by HPP. The results of this study suggest that HPP seems to be a potentially effective method to reduce populations of *S. enterica* on green mature tomato fruits.

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Figure 1. Populations (log CFU/ml) of *Salmonella enterica* ser. Newport and *Salmonella enterica* ser. Braenderup in tryptic soy broth following HPP (350, 450, and 550 MPa) for 120s at 21ºC (initial temperature).

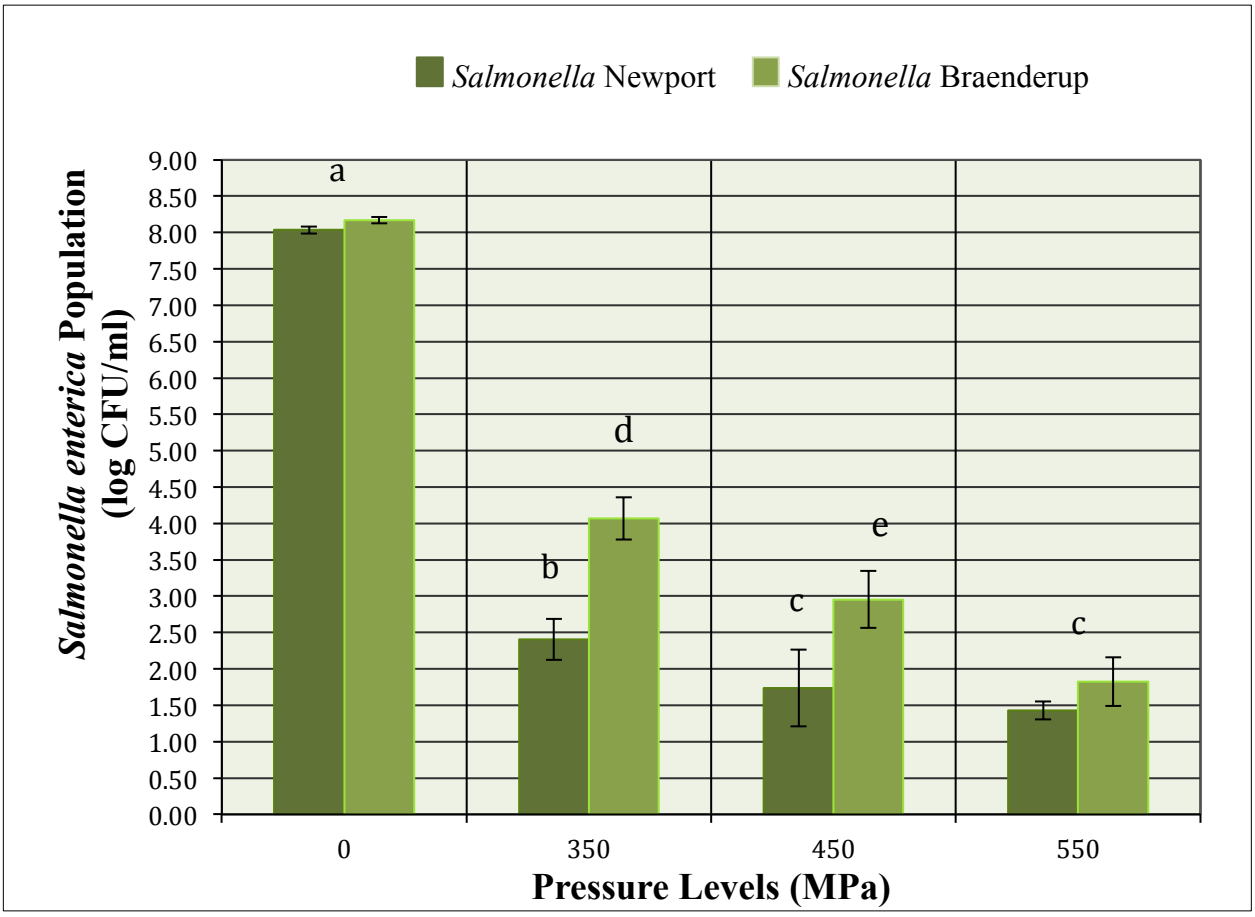
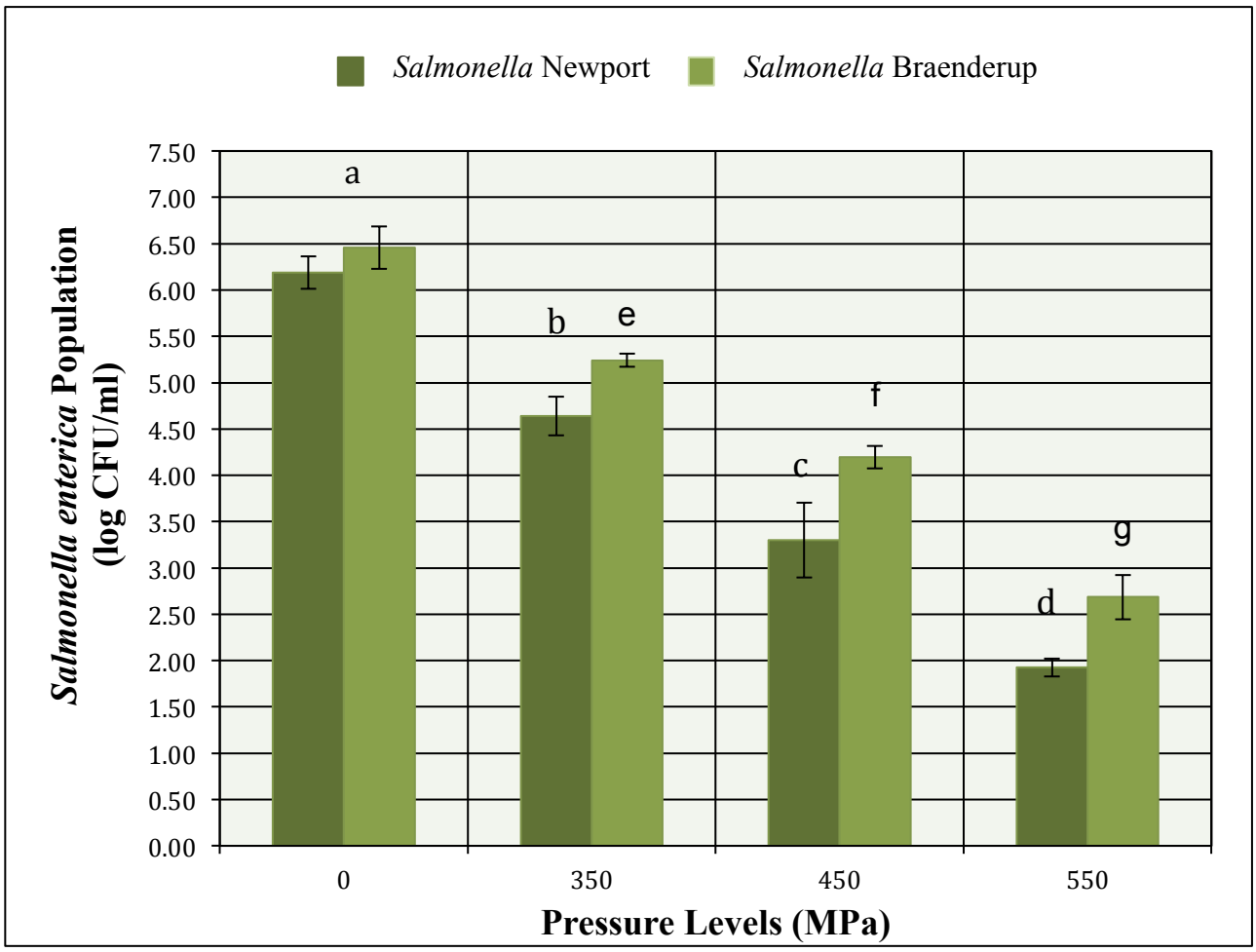


Figure 2. Populations (log CFU/g) of *Salmonella enterica* ser. Newport and *Salmonella enterica* ser. Braenderup in whole, green mature tomato fruits from two locations (Virginia and Florida) following HPP (350, 450, and 550 MPa) for 120s at 21ºC (initial temperature). n=18
* Same letter on different columns indicates not significant difference.



Chapter 4: Influence of High Pressure Processing on Ripening and Quality of Green Mature Tomato Fruits

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Abstract:

The objective of this study was to evaluate the effect of high pressure processing (HPP) on the ripening and quality characteristics of green mature tomato fruits. Green mature tomato fruits were packaged in sterile stomacher bags containing 350 ml of a 1% $CaCl_2$ solution and subjected to one of three different pressures (350, 450, and 550 MPa) for 120 s at 21ºC. After, pressure treatment samples were placed in an incubator and subjected to an ethylene gas (125 ppm; 0.7 cc/minute) at 22ºC and 75% to 85% relative humidity for 5 to 6 days. The weight of pressurized tomato fruits increased after pressure, but a higher reduction on the weight of these tomato fruits resulted after the ripening period compared to tomato fruits that were not HPP treated. Pressured tomatoes did not ripen while control tomato fruit samples (tomato fruits that were not HPP treated) were completely ripened after the ripening period. Even though HPP effectively reduced populations of *Salmonella enterica*, it adversely affects the development of ripening and quality of green mature tomatoes.

Introduction:

Depending on the planned market destination tomato fruits are harvested at different stages of maturity (Orzolek et. al., 2006;). Commercially produced market tomato fruits are harvested at the green mature stage (Boukobza et. al., 2002; Orzolek et. al., 2006; Wang et. al., 2007). Tomato fruits at this stage of maturity can resist better the stress of handling during shipping and distribution through the market preventing over ripening and damaged to fruit (Boyette et. al., 1995; Orzolek et. al, 2006; Wang et. al., 2007). On the other hand, tomato fruit that is left in the vine until breaker stage is intended for local markets were long distant transportation is not necessary (Orzolek et. al., 2006).

Commercial tomato fruit producers use ethylene to promote the ripening of tomato fruits (Boyette et. al., 1995). Since tomato fruits are harvested at the mature green stage the use of external ethylene gas allows producers to have a better control of the ripening and shelf life of the tomatoes (Wang, 2007; Boyette et. al., 1995; Boukobza, 2002). Ethylene gas is a tasteless and odorless gas naturally produced by tomato fruits at the ripening stage (Boyette et. al., 1995). Commercially produced tomato fruit at the green mature stage are exposed to an ethylene concentration of 100 to 150 ppm for 24 to 72 h, at a temperature of 20ºC to 25ºC (68ºF to 77ºF), and 85-95% relative humidity (Boyette et. al., 1995). A fairly airtight room is necessary for the application of ethylene, which can be applied by a shot method, a generator, or a flow-through system (Boyette et. al., 1995).

A previous study evaluated the efficacy of high pressure processing (HPP) at eliminating *Salmonella enterica* in ripe whole and diced tomato fruits (Maitland et. al., 2009). Results form this study showed significant reduction in the population of *Salmonella enterica* ser. Braenderup

in whole and diced tomato fruits after HPP treatment at three levels (350, 450, and 550 MPa) (Maitland et. al., 2009). Additionally, results showed that no significant visual changes in the appearance of diced and whole tomato fruits were observed after HPP treatment, and no significant changes in weight occurred in whole tomato fruits after the treatment (Maitland et. al., 2009). However, the application of HPP on green mature tomato fruits after harvesting has not yet been reported. Additionally, no other studies have been done examining the effects of HPP on the quality and development of ripening of green mature tomato fruits after treatment.

Consumer's preference of fresh tomato fruits depends primarily on the external characteristics such as color and firmness of the fruit. A second important parameter for consumers to choose a specific tomato fruit is the flavor and eating quality (Kader, 1996). All these quality parameters are also used in the determination of the maturity level of tomato fruits (Brandt et. al., 2006). Color in tomato fruits is largely determined by their lycopene content (Brandt et. al., 2006; Radzevicious et. al., 2008) and flavor of tomato fruits is determined by the sugar (estimated by soluble solids content) and the acid (estimated by titratable acidity) composition of the fruit (Flores et. al., 2009; Jimenez et. al., 1996).

Therefore, the objectives of this work are:

1. To determine the effects of HPP at three levels (350, 450, and 500 MPa) at 21ºC for 120 s on the development of ripening of green mature tomato fruits subjected to 125 ppm of ethylene gas for 5 to 6 days.
2. To determine the effects of HPP at three levels (350, 450, and 500 MPa) at 21ºC for 120 s on the quality of tomato fruits after treatment and after the ripening period.

Materials and Methods

Tomato Fruits

Freshly harvested whole green tomato fruits at the mature green stage, with approximately one inch of the stem attached were collected from the fields in Virginia and Florida. Virginia tomato fruits were collected in October 2009 and Florida tomato fruits were collected in March 2010. Tomato fruits were packed individually wrapped in paper and were shipped overnight in carton boxes containing approximately 50 fruits each. Upon arrival, tomato fruits were unpacked and maintained in a refrigerator at 14ºC and 70% relative humidity for no more than 5 days prior to use. Green mature tomato fruits stored at 13ºC to 14ºC can be stored for two weeks before ripening without significant changes in the ripening rate, color development, or sensory quality. This temperature reduces the ripening speed of mature green tomato fruits during storage before ripening.

High Pressure Processing (HPP) Treatment of Tomato Fruits

Mature green tomato fruits at room temperature (24ºC) were weighed without the stem and then packed into sterile stomacher bags (Fisher Brand Secure T, Pittsburg, PA) containing approximately 350 ml of a 1% solution of CaCl2. The bags were then vacuum-sealed with a 1.25-hp vacuum (Koch UltraVac 250, Kansas City, MO) at 95% vacuum, and bagged for a second time to prevent possible leakage during the pressure treatments. The bagged tomatoes were subjected to the three different levels of pressure (350, 450, and 550 MPa) for 120 s at 21°C (+/-4°C) (initial temperature) in the HPP equipment. Four tomato fruits for each pressure

were treated, and the study was repeated three times for tomato fruits from Virginia and three times for tomato fruits from Florida (n = 24).

Ripening Process After HPP of Mature Green Tomato Fruits

After tomato fruits were subjected to HPP, they were weighed. Tomato fruits were then placed inside an insulated incubator (Microprocessor Controlled Low Temperature Illuminated Incubator 818, Austin, TX) (Appendix B) for ripening (up to 6 days). The temperature inside the chamber was monitored three times a day and maintained at 22°C (71.6°F), the relative humidity was also monitored and maintained between 75% and 85%. Ethylene was applied in the incubator by a flow-through system. The optimum concentration of ethylene for the treatment of tomato fruits was set at 125 ppm of ethylene. Good air circulation was maintained by the ventilation system inside the incubator to ensure temperature uniformity within the ripening chamber and to prevent the accumulation of CO_2.

Analytical, Physical and Quality Analysis of Tomato Fruit After Ripening

Tomato fruit that had received HPP treatment and tomato fruits that did not receive HPP treatment were analyzed and visually compared. Two physical analyses were performed on green mature tomato fruit samples. Color and texture-firmness were measured and then four components of tomato fruit internal quality were analyzed: pH, soluble solids, titratable acidity and lycopene content.

Color and Texture-Firmness Analysis

Color was measured on the puree of the tomato fruit using a hand-held colorimeter (Minolta chromameter CR-200, Minolta Corporation, Japan). The colorimeter was configured for Hunter Lab's *L*, *a*, and *b* scale with daylight (D65) and a 10° observer. It was fitted with a 2.5 cm diameter aperture. The instrument was calibrated using the white tile, against a standard white color plate (*Y*=93.16, *x*=0.3189, *y*=0.3360). Color was expressed in Hunter Lab units *L*, *a,* and *b* (*L* = lightness, *a* ranging from green to red, *b* ranging from blue to yellow). Samples of tomato puree were poured into glass tubes (2.2 cm diameter) taking care to exclude air bubbles and then the tubes were placed under the aperture of the colorimeter. Eight replicate measurements were performed and results were averaged. In addition, hue angle and chroma were calculated by the following equations.

Hue angle= $\tan^{-1}(b^*/a^*)$

Chroma=$\sqrt{(a^2+b^2)}$

The three-color variables were used to compute the tomato color index (TCI);

TCI = $2000a/L(a^2 + b^2)^{1/2}$. This is a single-number criteria used to measure tomato fruit color.

Texture- Firmness of Tomato Fruits

A destructive deformation test was used by recording force and deformation values to determine the levels of firmness of tomato fruits. Firmness was measured at the furthest two points apart on the equator of each fruit (i.e. equidistant between the top and bottom of each fruit) approximately 120° apart and perpendicular to the stem-bottom axis, with a Texturometer (TA. XT. Plus, Texture Technologies Corp., Scarsdale, NY). The analyzer was calibrated with a

2 kg weight prior to the first test. In the firmness measurements a 2 mm diameter cylindrical stainless steel probe with a flat end was used for the penetration test and a 75 mm diameter acrylic plate for the compression tests. Equipment settings were set as follow: test speed 0.10 mm/s; distance 10 mm into the tomato fruit. Results were expressed in terms of the force (in Newton's (N)) required to break the radial pericarp (i.e. skin/surface) of each tomato. The deformation (mm) values during penetrations were recorded. Deformation was defined as the distance (mm) traveled by the probe from first contact with the tomato skin to the bioyield point. Firmness (N mm^{-1}) is defined as the average slope of the force/deformation curve.

pH Measurement

The pH of the juice samples was determined after compression and puncture tests, using the pH meter (Accumet Fisher Scientific 15, Arvada, CO). Tomato samples were blended into a puree using a food processor and the juice was filtered and separated before measurements. The pH values were recorded for each sample of tomato. The pH was measured directly in the filtered juice.

Soluble Solids Measurement

A portion of 5 ml of the filtered tomato juice was used for the determination of soluble solids. The soluble solids content was determined using an Abbe refractometer ($B^{+}S$ 60/70 Model No. A-90067, England). The refractometer had a range of 0-32 °Brix, and a resolution of 0.2 °Brix. One or two drops of clear juice were placed on the prism and the °Brix were recorded. All the readings were performed at room temperature (20ºC to 24 °C).

Titratable Acidity Measurement

A portion of the filtered and decanted tomato juice was used for the analysis. The titratable acidity, expressed as percentage citric acid, was obtained by titrating 10 ml of tomato juice with 0.1N NaOH to pH 8.1. The following formula was used for calculation: $Z = (V \times N \times \text{Meq} \times 100) \div Y$, where Z = titratable acidity (as % citric acid), V = volume of NaOH used, N = normality of NaOH, Meq = weight of a milliequivalent of citric acid (0.064 g), and Y = volume of tomato extract used (10 ml).

Lycopene Content Measurement

Lycopene content was determined according to the reduced volumes of organic solvents method. About 0.6 g of unfiltered whole tomato puree was weighed precisely (to the nearest 0.01 g), and then it was added to a 40 ml amber vial containing 5 ml of acetone with 0.05% butylated hydroxytoluene (BHT), 5 ml of ethanol, and 10 ml of hexane. The mixture was placed on an orbital shaker (Lab-Line Instruments, Melrose park, IL) at 180 rpm for 15 min. Three milliliters of water were then added, prior to an additional 5 min on the shaker. Afterwards, the vial was left in an upright position at room temperature (20ºC to 24 °C) for 5 min to allow for phase separation. The upper phase (hexane) was sampled to obtain an absorbance reading at 503 nm using a UV-VIS spectrophotometer (Shimadzu Suzhou Instruments 2550, Kyoto, Japan). The absorbance of the hexane (upper) layer was measured in a 1 cm path length quartz cuvette at 503 nm versus a blank of hexane solvent. The following relationship was used for estimation of lycopene content:

Lycopene (mg/kg) = $(A_{503} \times 31.2) \div$ (quantity of tissue used (g)).

Statistical Analysis

Results were statistically analyzed according to a split plot design. The experiment was repeated three times for tomato fruit from each of the two different locations (Virginia and Florida). Four tomato fruits were used for each pressure level, and four-control tomato fruits were also use for the ripening (n=24). A general linear model procedure was used to determine the least squares means of percentage weight gain and percentage weight loss, together with Duncan's multiple range tests. The Statistical Analysis System (SAS Institute, Version 9.1, 2002, Cary, NC) was used. The P-value used for the statistical analysis was 0.05.

Results and Discussion

Tomato Weight After HPP and After Exposure to Ethylene and Ripening

The percentage weight gained of mature green tomato fruits after HPP treatment, previous to ripening, was not significant for any of the tomato samples ($p > 0.05$). There were no significant differences in the percentage weight gained for tomato fruits from the two locations. The mean percentage weight gained was 3.75% for Florida and 2.97% for Virginia. Although the average percentage weight gain of tomato fruits subjected at 550 MPa was slightly larger, no significant difference was observed in the values of percentage weight gained for tomato fruits subjected to 350, 450, and 500 MPa ($P > 0.05$). The average percentage weight gained for tomato fruit samples were as follows: 3.04%, 3.19%, and 3.85% for samples treated at 350, 450, and 500 MPa respectively (Figure 3). Similar results were seen in a study of the application of three levels of HPP (350, 450, and 550 MPa) to ripe whole tomato fruits (Maitland et. al., 2009). The study concluded that the percentage weight gained by tomatoes after HPP was not significant in

any of the three pressure levels (Maitland et. al., 2009). The percentages of weight gained found in this study were 3.8%, 3.9%, and 4.0% for 350, 450, and 550 MPa respectively (Maitland et. al., 2009).

After ripening, no significant difference was seen between the percentages of lost weight of HPP-treated tomato fruits from the two different locations (Virginia and Florida) ($P > 0.05$). The percentage of lost weight of tomato fruits treated with HPP at 350, 450, and 550 MPa was not significantly different from each other ($P > 0.05$). On the other hand, the percentage of lost weight after ripening was significantly different between control tomato fruits that were not subjected to HPP and tomato fruits that were subjected to HPP ($P < 0.05$). Control tomato fruits had an average percentage of lost weight of 7.64%, and for HPP tomato fruits the average percentage of lost weight after ethylene exposure was 10.57%, 10.71%, and 10.13% for 350, 450, and 550 MPa respectively (Figure 4). Although control tomato fruits experienced a certain percent of lost weight after ripening, it was clearly identified that HPP treated tomato fruits suffered a greater loss in weight after the ripening period.

No other studies were found that evaluated the effects of HPP on the weight and quality parameters of green mature tomatoes after pressure treatment. However, some studies have been performed to evaluate the effects of HPP on the quality and inactivation of enzymes of fresh produce items and other food products after HPP treatment in different conditions. In a study performed by Maitland and others (2009), results showed that no visual changes in the appearance of whole ripe tomato fruits were observed after HPP treatment even at the highest pressure level (550 MPa) for 120 sec at 20°C (Maitland et. al., 2009). Results from another study suggested that HPP treated whole ripe cherry tomato fruits experienced an increasing textural

damage with increasing pressures up to 400 MPa (Tangwongchai et. al., 2000). However, treatment of the cherry tomato fruits with pressures above 400 (500 to 600 MPa) showed less apparent damage to the texture, and resulted in samples similar in appearance to untreated cherry tomato fruits (Tangwongchai et. al., 2000). HPP at the highest-pressure level (600 MPa) had no effect on pectinmethylesterase activity, while polygalacturinase was almost completely inactivated after HPP at 500 MPa (Tangwongchai et. al., 2000). Similar results were obtained in a study performed by Marigheto and others (2009) were textural damage on unripe tomato fruit samples occurred after HPP treatment at 200 and 400 MPa compared to untreated samples and tomato fruits treated at 600 MPa (Marigheto et. al., 2009). The texture of tomato fruits treated at 600 MPa was similar to the texture of untreated samples (Marigheto et. al., 2009). Ripe tomato fruits treated at 400 MPa experienced almost complete loss in texture, while a smaller reduction in the texture of tomato fruits occurred after treatment at 600 and 200 MPa (Marigheto et. al., 2009). The activity of pectinmethylesterase was not affected in tomato fruit samples even after the highest-pressure level (600 MPa), but polygalacturonase in ripe tomato fruits was completely inactivated after pressure treatment at 600 MPa (Marigheto et. al., 2009).

Results from another study showed that texture of lettuce and spinach remained firm after HPP treatment at 300 MPa, but browning of the leaves started to develop after treatment (Arroyo et. al., 1999). The texture of tomato fruit, cauliflower, asparagus, and onions also remained firm after HPP treatment at 350 MPa (Arroyo et. al., 1999). However, treatment at 400 MPa resulted in peeling of the skin of tomato fruits even though the texture remained firm (Arroyo et. al., 1999). The color of asparagus, tomato fruit, and onion was not changed after HPP treatment (Arroyo et. al., 1999). Moreover, the activity of peroxidase remained unchanged after treatment

at 400 MPa (Arroyo et. al., 1999). Additionally, as reported in another study, different fruits (apple, pear, orange, and pineapple) and vegetables (carrot, celery, green pepper, and red pepper) treated with HPP experienced an initial loss of texture due to the instantaneous initial application of pressure followed by an increase in texture during pressure holding time (Basak et. al., 1998). The firming effect during pressure holding time was reported for all fruits and vegetables but apples, carrots, and green peppers (Basak et. al., 1998). The results of the study showed that the samples of vegetables and fruits resembled the appearance of mildly heat-treated samples (Basak et. al., 1998). Shook and others (2001) found that complete inactivation of lipoxygenase and polygalacturonase in diced tomato fruits occurred after treatment at 800 MPa for 5 min, but pectinesterase was very resistant to pressure (Shook et. al., 2001). The percentage of soluble solids, titratable acidity, and color were not affected by any of the pressure treatments (Shook et. al., 2001).

In another study antioxidant activity, ascorbic acid content, and carotenoids content were better preserved in tomato and carrot puree after HPP (400-600 MPa) treatment compared to those thermally treated samples (Patras et. al., 2009). The color intensity of the puree was also better preserved by HPP than thermal treatment (Patras et. al., 2009). As reported in a study performed by Butz and others (2002), the concentration of lycopene and β-carotene on tomato and carrot homogenate remained unchanged after HPP treatment (Butz et. al., 2002). Additionally, the antioxidant activity of both homogenates remained unaffected as reported in this study (Butz et. al., 2002). The results of a stuffy performed by Qiu and others (2006), showed that the content of lycopene in tomato puree after HPP at 600 MPa was reduced and the concentration of lycopene isomers increased with storage time (Qiu et. al., 2006). However,

treatment at 500 MPa resulted in better stability of lycopene stored at 4ºC (Qiu et. al., 2006).

Ripening of Tomato Fruits and Ethylene Exposure

Control Tomato Fruits

Mature green tomato fruits that were not treated with HPP were identified as control tomato fruits. Control tomato fruits were exposed to 125 ppm of ethylene gas, 75% (+/- 5%) relative humidity, and at 22ºC in the ripening incubator. Characteristics of tomato samples changed during the different days of exposure to ethylene gas. These characteristics are described below:

Second day of exposure to Ethylene gas: tomato control samples started the breaker stage of ripening by turning the skin color to a light green, and some slightly pink spots were visible in the blossom end (Image 1). Third day of exposure: A larger area of the surface of the tomato fruits showed a light red-orange coloration. The rest of the surface remained slightly green (Image 2). Fourth day of exposure: The whole surface of the tomato fruit showed a red coloration with very few orange spots and almost no light green spots (Image 3). Fifth and sixth day of exposure: The whole surface of the tomato fruits turned to a dark red and a characteristic tomato aroma was easily perceivable (Image 4).

Commercial tomato producers use ethylene to start ripening of mature green tomato fruits and to produce a faster and more uniform ripening (MFCL/NGMC/NARI, 2003; Boyette et. al., 1995). According to previous studies, tomato fruits should be exposed to an ethylene concentration of 100 to 150 ppm at a temperature of 20ºC to 22ºC (68ºF to 72ºF) with 80% to 90% relative humidity (Sargent et. al., 2005; MFCL/NGMC/NARI, 2003; Boyette et. al., 1995).

Under these conditions tomatoes usually reach breaker stage after 24 to 72 h of ethylene exposure (Sargent et. al., 2005; MFCL/NGMC/NARI, 2003; Boyette et. al., 1995). As reported in these studies, control tomato fruits from the present work were able to reach breaker stage after 48 h of exposure to ethylene in similar conditions, and the ripening of these samples was uniform.

HPP Tomato Fruits

After HPP treatment at 350, 450, and 550 MPa tomato fruits were less firm when touched, and coloration changed from a shiny green to an opaque light brown-green (Image 5). A similar study suggested that textural damage on unripe tomato fruit samples occurred after HPP treatment at 200 and 400 MPa compared to untreated samples and tomato fruits treated at 600 MPa (Marigheto et. al., 2009). The texture of tomato fruits treated at 600 MPa was similar to the texture of untreated samples (Marigheto et. al., 2009). Moreover, ripe tomato fruits treated at 400 MPa experienced almost complete loss in texture, while the texture of tomato fruits treated at 600 MPa was similar to the texture of samples treated at 200 MPa (Marigheto et. al., 2009). Additionally, results obtained in another study performed by Tangwongchai and others (2000) showed that HPP treated whole ripe cherry tomato fruits experienced an increasing textural damage with increasing pressures up to 400 MPa (Tangwongchai et. al., 2000). However, treatment of the cherry tomato fruits with pressures above 400 (500 to 600 MPa) showed less apparent damage to the texture, and resulted in samples similar in appearance to untreated cherry tomato fruits (Tangwongchai et. al., 2000). The tomato fruit samples in these two past studies were treated at pressure levels similar to those used in the present study, and most of these

tomato fruits (unripe and ripe) experienced similar texture damage, compared to the tomato fruit samples used in the present work.

Tomato fruits treated with HPP did not ripen and some fluid was dripping through the stem scar during the first few days. After 5 days of exposure to ethylene, fungi started growing in the surface and stem scar of the tomato fruit (Image 6). The observations were the same for tomato fruit treated at 350, 450, and 550 MPa of pressure. Although no other studies have been done evaluating the effects of HPP on the ripening of green mature tomato fruits, previous work has been done determining the effects of HPP on the activity of softening enzymes in tomato fruit. The results of a study performed by Tangwongchai and others (2000), showed that no significant inactivation of pectinmethylesterase in cherry tomato fruits occurred after HPP treatment at 600 MPa for 20 min (Tangwongchai et. al., 2000). On the other hand, polygalacturonase was almost completely inactivated after HPP treatment at 500 MPa for 20 min (Tangwongchai et. al., 2000). The study concluded that HPP could change the permeability of tomato fruit cell walls allowing the release of water and permitting different modifications in enzyme activity (Tangwongchai et. al., 2000). Due to changes inside the cell substrates, ions and enzymes might interact with each other producing changes during and after application of HPP (Tangwongchai, 2000). Similarly, results from a study performed by Marigueto and others (2009) showed that the activity of pectinmethylesterase in ripe tomato fruit was not affected after HPP treatment at 600 MPa (Marigueto et. al., 2009). An apparent increased activity of polygalacturonase in ripe tomato fruits was observed after HPP treatment at 200 MPa (Marigueto et. al., 2009). However complete inactivation of polygalacturonase in ripe tomato fruits was

observed after HPP treatment at 600 MPa (Marigheto et. al., 2009). As seen in these past studies it seems possible that the activity of different enzymes could be affect by HPP.

Some enzymes responsible for the development of color, changes in texture, degradation of chlorophyll, formation of lycopene, and other characteristic transformations that take place during ripening of tomato fruits may have been affected when HPP was applied. The immediate change of color and texture as well as the lack of color and ripening development during ethylene gas exposure could have been a consequence of changes in the activity of different enzymes in the tomatoes after HPP. Additionally, the results from a past study conducted by Rodrigo and others (2007) showed no significant change on color of tomato puree after HPP treatment at 300 to 700 MPa, for 60 min at 65ºC (Rodrigo et. al., 2007). According to Oey and others (2008), some undesired chemical reaction and inactivation of enzymes could take place during storage of HPP fruits and vegetables (Oey et. al., 2008). Chemical reactions such as oxidation can change the color of the food and might take place due to an incomplete inactivation of enzymes or other microorganisms (Oey et. al., 2008).

Tomato fruits from the Eastern Shore of Virginia were obtained at the end of October near the end of the tomato production season in this region. Some changes on the surface of the tomato fruits occurred during ripening of these samples. Although the red color of the samples was completely developed at the end of ripening, some parts of the skin of the tomato fruit showed scarring and decay of the tissue especially near the stem scar starting on the third day of exposure to ethylene in the ripening incubator. Samples seemed to show possible signs of chill injury in their surface such as slight softening of the tissue and dry skin areas near the stem scar (Image 7).

Different studies concluded that tomato fruit that had suffered from chill injury, would present poor flavor and color development as well as water-soaked spots, pitting, tissue collapsed and decay (Sargent et. al., 2005). Green and breaker tomato fruits are more susceptible to chill injury than ripe tomatoes (Sargent et. al., 2005). Even though tomato samples collected from Virginia seem to have suffered from some tissue decay on the surface, no other changes in color development or water-soaked spots, and pitting were observed. Therefore it was not possible to conclude that the tomato fruits obtain from Virginia had suffered from chill injury.

Relative humidity, temperature and ethylene concentration were measured two times a day for the duration of the ripening process. Relative humidity levels were maintained at 75% (+/- 5%), the temperature at 22ºC and ethylene levels at 125 ppm. The chamber was completely ventilated twice a day for half an hour.

Quality Analysis of Green Mature Tomato Fruits

High-pressure processed tomato fruits were not analyzed for quality because they never ripened after the pressure treatment. On the other hand, control green mature tomato fruits were analyzed to determine their quality before ripening.

Color, Firmness, pH, Soluble Solids, Citric Acid and Lycopene Content of Green Mature Tomato Fruit

Four green mature tomato fruits were used for the determination of color, firmness, pH, soluble solids, percentage of citric acid, and lycopene content and measurements were repeated three times.

Table 1. Quality parameters of green mature tomato fruits that did not undergo HPP treatment and quality parameters of commercial ripe and green mature tomato fruits. n=12.

Quality Values of Green Mature Tomato Fruits and Commercial Quality Values for Ripe and Green Mature Tomato Fruits

	Color			Firmness (N/mm)	
	Color Index	**Hue angle**	**Croma**	**Penetration**	**Compression**
Green Tomato Fruit	-17.47+/-2.17*	75.37+/-3.13*	26.11+/-2.18*	3.73+/-0.81*	19.63+/-3.50*
Commercial Ripe	25.53-36.52 [1]	39 [3]		1.22-1.46 [4]	
Commercial Green	-15.7 [2]	80-100 [3]			
	pH	**Soluble Solids ºBrix**	**Citric Acid (%)**	**Lycopene (mg/Kg)**	
Green Tomato Fruit	*4.43+/-0.12	*3.95+/-0.30	*0.43+/-0.045	*1.25+/-0.31	
Commercial Ripe	4.28-4.44 [1]	4.0-5.0 [1]	0.66 [2]	12.4 [3]	
Commercial Green	4.01-4.08 [1]	3.5 [2]	0.42 [2]	1.5 [3]	

* Value represent: measured value +/- Standard deviation.

Sources:
[1]Gomez et. al., 2001.
[2]Clement et. al., 2008.
[3]Radzevicius et. al., 2008
[4]Batu, 2004

The results of this study suggest that HPP may have a negative effect on the quality, the activity of different enzymes, and the ripening of green mature tomato fruits after HPP treatment. Evaluation of the enzymes in green mature tomato fruits that could possibly be affected by HPP could aid in the determination of pressure levels or packaging solutions that might prevent such a changes in future studies. Additionally, an evaluation of changes in cell permeability and enzyme activity after HPP and during storage of the green mature tomato fruits may aid in a better

understanding of the changes occurred after the application of HPP to green mature tomato fruits.

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Figure 3. Percentage weight gained for green mature tomato fruits after high-pressure processing at 350, 450, and 550 MPa for 120s at 21ºC (initial temperature). n=24
* Columns with the same letter are not significantly different.

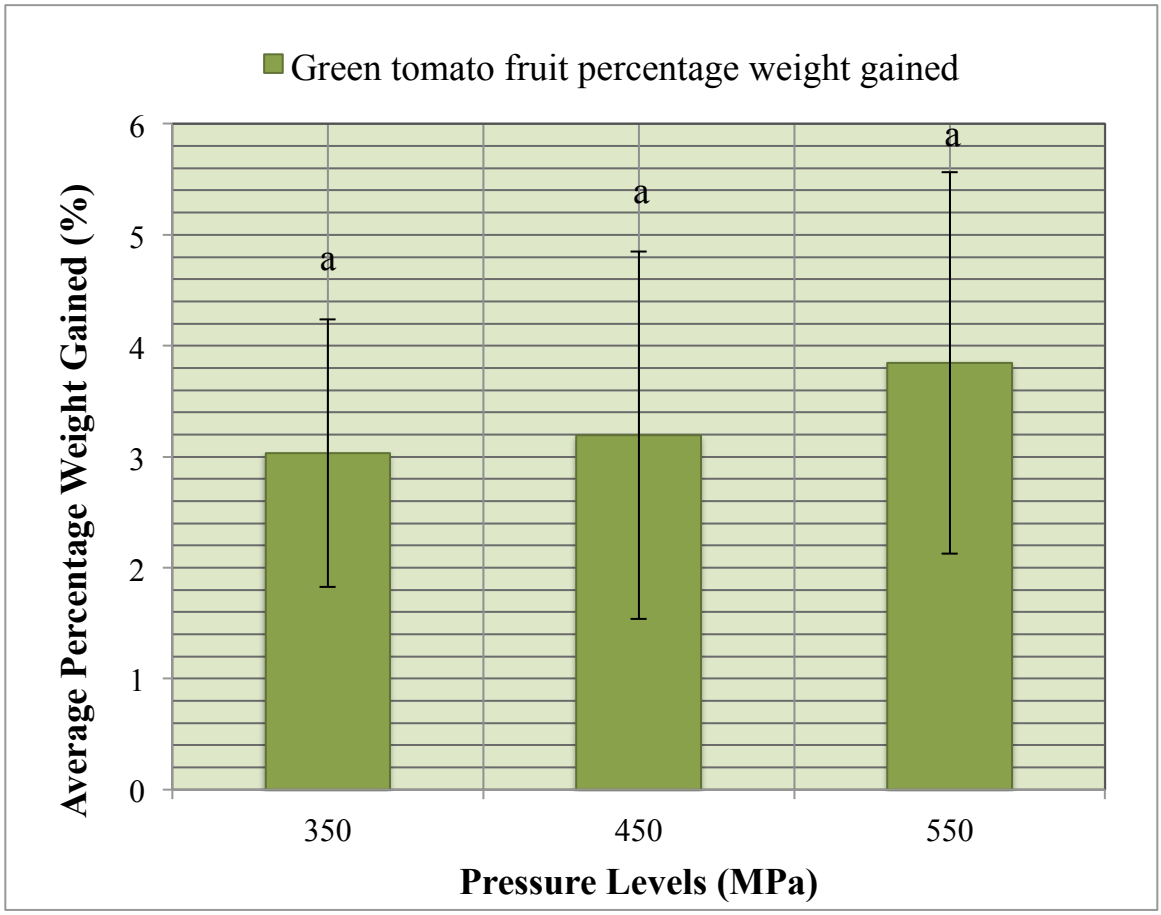

Figure 4. Percentage weight loss of HPP treated and control green mature tomato fruits after 5 days of exposure to ethylene gas (125 ppm, 70% humidity, and 22ºC).
* Columns with the same letter are not significantly different.

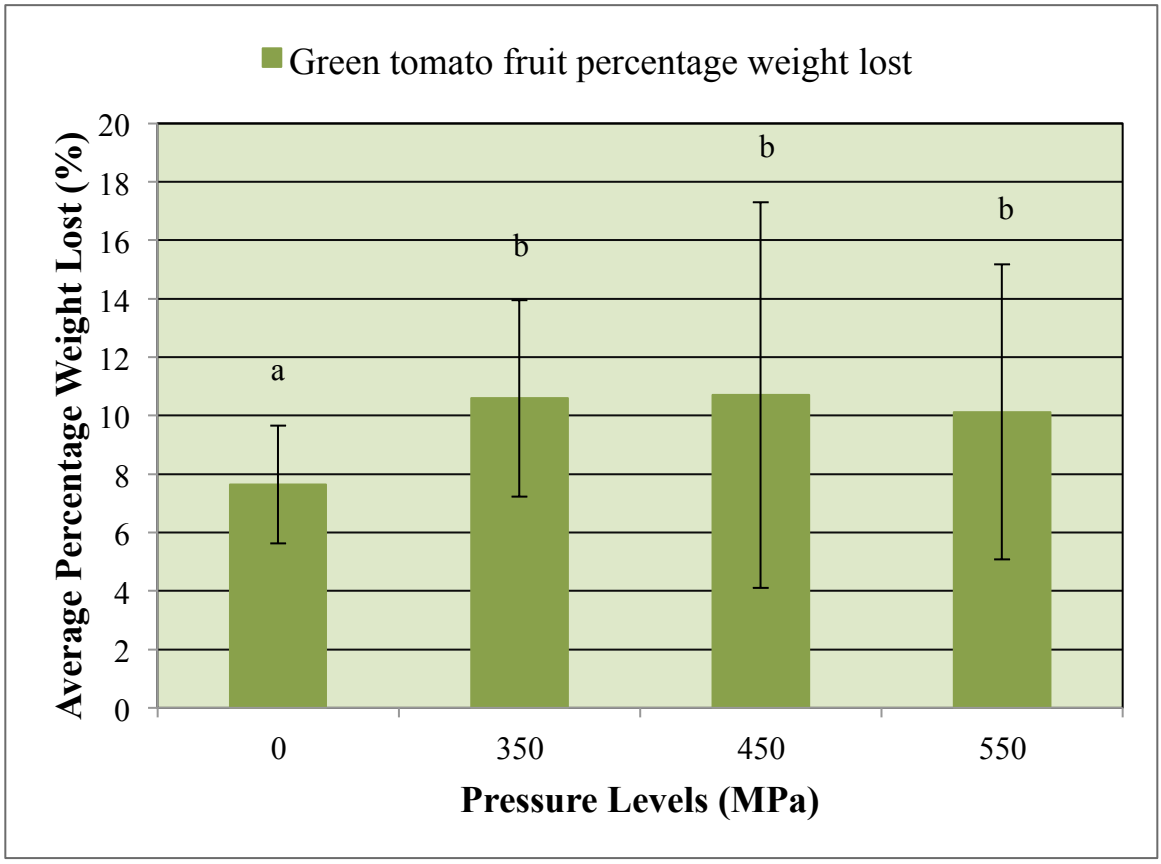

Image 1. Control tomato fruits (tomato fruits that did not undergo HPP treatment) on the second day of exposure to ethylene 125 ppm, 70% humidity, and 22ºC.

Image 2. Control tomato fruits (tomato fruits that did not undergo HPP treatment) on the third day of exposure to ethylene gas 125 ppm, 70% humidity, and 22ºC.

Image 3. Control tomato fruits (tomato fruits that did not undergo HPP treatment) on the fourth day of exposure to ethylene gas 125 ppm, 70% humidity, and 22ºC.

Image 4. Control tomato fruits (tomato fruits that did not undergo HPP treatment) on the fifth and sixth day of exposure to ethylene gas 125 ppm, 70% humidity, and 22ºC.

Image 5. Tomato fruits after HPP at 350, 450, and 550 MPa for 120 s at 21ºC (initial temperature), and before ethylene exposure.

Image 6. Tomato fruits after HPP treatment on the third and fourth days of exposure to ethylene gas 125 ppm, 70% humidity, and 22ºC.

Image 7. Control tomato fruits (tomato fruits that did not undergo HPP treatment) harvested in Virginia showing possible sings of chill injury, after a few days of exposure to ethylene.

Chapter 5:

Conclusions

Current food processing technologies need to comply with two specifications: the technology should be capable of reducing populations of pathogenic microorganisms to safe levels, and it should preserve nutritional, and sensory characteristics in fresh and processed foods. The present study was focused on the concern of controlling contamination before and after harvest of fresh green mature tomato fruit while retaining the fresh characteristics of the tomato fruits through the application of high pressure. High pressure processing (HPP) is currently used in the processing of several food products in the market, and has proven to be successful in reducing populations of pathogenic microorganisms while retaining their freshness and quality.

During the development of this study, the influence of HPP at 350, 450, and 550 MPa on populations of *Salmonella enterica* in broth and in green mature tomato fruits and its effects on ripening and quality of tomato fruits was evaluated. Results of the broth study showed that *Salmonella enterica* ser. Braenderup had a higher-pressure resistance than *Salmonella enterica* ser. Newport. After pressure treatment *S*. Newport populations were reduced by 5.64, 6.30, and 6.61 log CFU/ml while *S*. Braenderup populations were reduced by 4.10, 5.22, and 6.35 log CFU/ml at 350, 450, and 550 MPa respectively for both serovars.

On the other hand, reductions of populations of both serovars of *S. enterica* in whole green mature tomato fruits were smaller compared to reductions in broth cultures. *S*. Newport reductions reached levels of 1.55, 2.89, and 4.26 log CFU/g. Reductions of *S*. Braenderup were 1.22, 2.26, and 3.77 log CFU/g. As seen before in the results obtained from the broth cultures, *S*.

Braenderup is more resistant to pressure than *S*. Newport. HPP treatment could be considered a potentially effective method for the reduction of populations of *S*. Newport and *S*. Braenderup on green mature tomato fruit postharvest.

During the determination of the effects of HPP at three levels (350, 450, and 550 MPa) for 120 s at 21° C on the ripening of green mature tomato fruits and its quality attributes, results showed that the weight of tomato fruits after HPP treatment was increased by approximately 3%. The weight loss of tomato fruits after the ripening period was approximately 10% for HPP treated tomato fruits and approximately 7% for control tomato fruits that were not HPP treated. This difference in percentage weight loss between HPP tomato fruits and control tomato fruits may be attributed to the weight gained by the former tomato fruits during HPP. Tomato fruits might have experienced infiltration of the packaging solution during the HPP treatment.

HPP treated tomato fruits were not able to ripen after exposure to 125 ppm of ethylene gas for five days while control untreated tomato fruits were completely ripened after the exposure period to ethylene gas in the same conditions as HPP green mature tomato fruits. After the analysis of the experiment, it could be concluded that HPP might have a negative effect on ripening of green mature tomato fruits. Although, HPP green mature tomato fruits did not showed remarkable visual changes in some quality parameters right after pressure treatment; the texture, color and general appearance of HPP tomatoes did remarkably change after a few days in the ripening incubator. Even though, HPP might be an effective method of reducing populations of *S*. enterica in green mature tomato fruits; the effects of the treatment on the ripening of the tomato fruits might result in changes or inactivation of components or enzymes responsible for the development of ripening and retention of quality of the tomato fruit.

Future Research

Future research should focus on the determination of the mechanism of ripening, and tomato fruit components or enzymes responsible for the ripening of green mature tomato fruits. Research is needed to understand which components or enzymes of green mature tomato fruits may be affected during and after HPP and how this changes in the activity of the enzymes might affect the development of ripening and the preservation of the quality attributes of green mature tomato fruits. Possible modifications in the treatment of green mature tomato fruits with HPP should also be evaluated to determine possible ways to prevent damage of tomato fruit components responsible for the development of ripening.

Different studies have reported different levels of inactivation of different softening enzymes in tomato fruits after HPP treatment. A study performed by Tangwongchai and others (2000), reported that no significant inactivation of pectinmethylesterase in ripe cherry tomato fruits occurred after HPP treatment at 600 MPa for 20 min (Tangwongchai et. al., 2000). On the other hand, polygalacturonase was almost completely inactivated after HPP treatment at 500 MPa for 20 min (Tangwongchai et. al., 2000). Similarly, a study performed by Marigueto and others (2009) showed that the activity of pectinmethylesterase in ripe tomato fruit was not affected after HPP treatment at 600 MPa (Marigueo et. al., 2009). An apparent increased activity of polygalacturonase in ripe tomato fruits was observed after HPP treatment at 200 MPa (Marigueto et. al., 2009). However complete inactivation of polygalacturonase in ripe tomato fruits was observed after HPP treatment at 600 MPa (Marigheto et. al., 2009). Shook and others (2001) reported that lipoxygenase (responsible for the production of off-flavors) and polygalacturonase (responsible for changes in texture) in diced tomato fruits were completely

inactivated at 800 MPa. However, pectinesterase (responsible for changes in texture) was very resistant to pressure (Shook et. al., 2001). Since the activity of these enzymes was affected by HPP an evaluation of the effects of HPP on the activity of other enzymes in tomato fruits, after and during HPP, might aid in a better understanding of the effects of HPP on the ripening of tomato fruits and on changes of the sensory quality characteristics of tomato fruits. Finally, different packaging solutions should also be evaluated to produce the least changes in weight of tomato fruit samples.

References

Marigheto N.A., G.K. Moates, M.E. Furfaro, K.W. Waldron, and B.P. Hills. 2009. Characterization of ripening and pressure-induced changes in tomato pericarp using NMR relaxometry. *Journal of Applied Magnetic Resonance*. 36(1): 35-47.

Shook C.M., Shellhammer T.H., and S.J. Schwartz. 2001. Polygalacturonase, pectinesterase, and lipoxygenase activities in high pressure processed diced tomatoes. *Journal of Agriculture and Food Chemistry*. 49(2): 664- 668.

Tangwongchai R., D.A. Ledward, and J.M. Ames. 2000. Effects of high-pressure treatment on the texture of cherry tomato. *Journal of Agriculture and Food Chemistry*. 48(5): 1434-1441.

Appendix A. High Pressure Equipment

Name: Quintus Food Press

Model: QFP 35L-600

Temperature of operation: 4 to 35ºC

Control temperature accuracy: 2.5ºC

Pressure Range: 100-600 MPa

Max. hold time: 15 min.

Processing Medium: Water

Dimensions:

Weight: 17,600 Lbs.

Volume inside vessel: 9.25 Gal.

Product holding Basket

Height: 46 in.

Diameter: 6 ¾ in.

Appendix B. Ripening Incubator

Name: Microprocessor Controlled Low Temperature Illuminated Incubator

Model: 818 (3758)

Volume: 17.8 (cu. Ft).

Temperature:

Without illumination: - 10ºC to + 50ºC

With illumination: + 10ºC to + 50ºC

Temperature Uniformity:

Without illumination: +/- 2.0ºC at -10ºC, +/- 1.8ºC at 31.0ºC

With illumination: +/- 0.6ºC at 20ºC, +/- 1.5ºC at 20ºC, +/- 0.7ºC at 50ºC

Temperature Sensitivity: +/- 0.2ºC

Recovery time after 30 s door opening: 10 min at 20ºC

Shelves:

Interior Shelf Area: 15.8 sq. ft (1.47 sq. m)

Supplied: 6

Electrical:

115 VAC 60 Hz, 1 phase, 860 watts, 7.5 FLA

230 VAC 50 Hz, 1phase, 860 watts, 3.75 FLA

BTU output: 2935

Appendix C. Commercial Refrigerator and/or Freezer

Name: Commercial Refrigerator and/or Freezer VWR brand Forma Scientific, Inc.

Model: 5479

Design Press: (high): 2863 KPa (400 psig)

(Low): 690 KPa (85 psig)

Low stage: R-290: 25 g (0.9 oz.)

R-23: 232 g (8.2oz.)

Design Press: (high): 2518 KPa (350 psig)

(Low): 1690 KPa (230 psig)

Charge R-290 from vac – 0 psig

Charge R-23 from 0-70 psig

Temperature: -72ºC.