**Inactivation of *Salmonella enterica* and *Listeria monocytogenes* in cantaloupe puree by
high hydrostatic pressure with/without added ascorbic acid[†]**

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Running Title: Development of nonthermal method for cantaloupe puree

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Abstract

The objective of this research was to evaluate and develop a method for inactivation of *Salmonella enterica* and *Listeria monocytogenes* in cantaloupe puree (CP) by high hydrostatic pressure (HHP). Cantaloupe being the most netted varieties of melons presents a greater risk of pathogen transmission. Freshly prepared CP with or without 0.1% ascorbic acid (AA) was inoculated with a bacterial cocktail composed of a three serotype mixture of *S. enterica* (*S. Poona*, *S. Newport* H1275 and *S. Stanley* H0558) and a mixture of three strains of *L. monocytogenes* (Scott A, 43256 and 51742) to a population of ca. 10^8 CFU/g. Double sealed and double bagged inoculated CP (ca. 5 g) were pressure treated at 300, 400 and 500 MPa at 8 °C and 15 °C for 5 min. Data indicated increased inactivation of both *Salmonella* and *Listeria* spp. with higher pressure. Log reduction for CP at 300 MPa, 8 °C for 5 min was 2.4 ± 0.2 and 1.6 ± 0.5 log CFU/g for *Salmonella* and *Listeria*, respectively. Survivability of the pathogens was significantly compromised at 400 MPa and 8 °C, inactivating 4.5 ± 0.3 log CFU/g of *Salmonella* and 3.0 ± 0.4 log CFU/g of *Listeria* spp. Complete inactivation of the pathogens in the puree (log reduction > 6.7 log CFU/g), with or without AA, was achieved when the pressure was further increased to 500 MPa, except that for *Listeria* containing no AA at 8 °C. *Listeria* presented higher resistance to pressure treatment compared to *Salmonella* spp. Initial temperatures (8 and 15°C) had no significant influence on *Salmonella* log reductions. Log reduction of pathogens increased but not significantly with increase of temperature. AA did not show any significant antimicrobial activity. Viable counts were about 0.2-0.4 log CFU/g less in presence of 0.1% AA. These data validate that HHP can be used as an effective method for decontamination of cantaloupe puree.

Keywords: Cantaloupe puree, high hydrostatic pressure processing, *Salmonella enterica*; *Listeria monocytogenes*; antimicrobial.

1. Introduction

Cantaloupe is one of the most popular fruits in the human diet worldwide (Ukuku, et al., 2005). It is a good source of vitamins A, C, beta-carotene, potassium and has high water content with a low caloric value (Gil et al., 2006; Ismail et al., 2010; Laur and Tian, 2011). Production, consumption and trade in this fruit is increasing due to its high nutritive value as well as its desirable taste, texture and versatility. Soft texture and low acidity makes cantaloupe appealing to the young, elderly and infirm who have been shown epidemiologically to be at increased risk when their food is prepared in an institutional setting (FAO, 2011). The inherent characteristics of this fruit render them susceptible to contamination and potential vehicles for foodborne illness transmission. Since melons are grown at ground level, their outer skins can be contaminated with pathogenic and spoilage bacteria during production and processing (Beuchat, 2002; Bowen et al., 2006; Richards and Beuchat, 2005a, 2005b). Cantaloupe being the most netted varieties of melons presents a greater risk of pathogen transmission during food preparation and the flesh and pulp is likely to be contaminated with bacteria during the cutting process. Bacterial pathogens are able to survive and grow in the low acid environment of melon flesh or pulp rapidly to high levels before spoilage is apparent (Castillo et al., 2009)

Since 1990, more than twenty-five outbreaks have been linked to cantaloupes (Barak et al., 2003,; CDC, 1991; FDA, 2001; Tamplin,1997) with a total of 1434 people ill, 42 hospitalized, and two deaths (Bowen et al., 2006). Most of these outbreaks are caused by *Listeria monocytogenes* and *Salmonella* (Bowen et al., 2006; Johnston et al., 2005; Ukuku and Fett, 2006). The predominant *Salmonella* serotype responsible for national and international outbreaks associated with consumption of cantaloupe has been *Salmonella* Poona (Richards and Beuchat, 2005a, 2005b).

Cantaloupe has a brief harvest season and large amounts of cantaloupes rot away in farmlands every year due to the lack of processing (Rastogi et al., 2007). As with most other produce, cantaloupe receives minimal processing before consumption. The outbreaks related to cantaloupe highlight the need for effective decontamination method for cantaloupe products. There is an increased market interest for preserved cantaloupe and we were recently contacted by the industry for a suitable method of preservation for this fruit as a puree to minimize the waste. Due to this market inquiry, experiments were conducted to prepare and process cantaloupe as puree. Cantaloupe flesh is heat sensitive (Rastogi, 2013) and hence thermal processing may not be an appropriate method as the heat of thermal processing adversely affects the color, nutrients and characteristics flavor of fruits (Cao et al., 2011). Since non-thermal processing does not employ heat, it remains the preferred choice of processing for fruits and vegetables. High hydrostatic pressure processing (HHP) is one of the most attractive non-thermal processing options which employs elevated pressures of approximately 300-700 MPa for periods of approximately 30 s to a few minutes to destroy pathogenic bacteria, vibrio, natural microflora including yeasts, and molds that cause spoilage of food (Jofre et al., 2010; Manas and Pagan, 2005; Munoz et al., 2007). Inactivation of microorganisms by HHP is due to a number of reasons including changes in cell wall, cell membrane lipids, and cell morphology; dissociation of ribosome, loss of genetic functions and altered biochemical reactions (Ritz et al., 2000; Wouters et al., 1998). HHP also causes limited reduction in enzymatic activity (Wang et al., 2013). HHP processing of plant products is gaining popularity in the food industry because of its ability to inactivate microorganisms and some enzymes near room temperature with little impact on flavor or nutritional attributes of the food (Bala et al., 2008; Baron et al., 2006; Bayındırlı et al., 2006; Hiremath and Ramaswamy, 2012; Oey et al., 2008; Verbeyst et al., 2010). There are a few

reports where HHP has been employed successfully to preserve purees of tomato (Iizuka et al., 2013; Knockaert, et al., 2012; Viljanen et al., 2011), carrot (Patras, 2009 a), apple (Keenan, et al., 2011; Landl, et al., 2010), plum (Garcia-Parra, et al., 2014), pineapple (Chakraborty et al., 2015), blackberry (Patras et al., 2009 b) and strawberry (Bodelon et al., 2013; Huang et al., 2013; Marszałek, et al., 2015). However, no information is available regarding the impact of HHP on target pathogens in cantaloupe puree. Many food processing methods can induce color changes due to oxidation, and ascorbic acid being an antioxidant (Padayatty et al, 2003) can minimize the oxidative changes during processing (Garzarella et al., 1990; Lattanzio et al., 1989). Hence the objectives of the present research were to investigate the efficacy of HHP to inactivate *Salmonella enterica* and *Listeria monocytogenes* in cantaloupe puree in the presence or the absence of ascorbic acid, and to investigate the survival of these pathogens in processed puree during storage at refrigeration temperature. To the best our knowledge, this is the first report on HHP decontamination of target pathogens in cantaloupe puree.

2. Material and Methods

2.1. Cantaloupes

Cantaloupes (*Cucumis melo L. var. cantalupensis Naud*) were purchased at a local grocery store the day before each experiment and stored at 4 °C until use. Cantaloupes used in experiments were ripe with no visible microbial growth or visible physical defects. Cantaloupes were washed in potable tap water for about 2 min and air dried at 22 °C for approximately an hour in a biosafety cabinet (Nuare™, Plymouth, MN, USA) before cutting to prepare puree.

2.2. Strain, growth conditions, and inoculum preparation

A bacterial cocktail composed of a three serotype mixture of *S. enterica* (*S. Poona* RM 2350, *S. Newport* H1275, and *S. Stanley* H0558) and a mixture of three strains of *L. monocytogenes* (Scott A, 43256 and 51742) was used in this work. Selection of these strains was based on their association, mainly with cantaloupe and other produce related outbreaks. Both *S. Newport* H1275 and *S. Stanley* H0558 were associated with alfalfa sprout-related outbreaks and were obtained from Dr. Patricia Griffin, Center for Disease Control and Prevention, Atlanta, GA. *Salmonella* Poona RM2350 (associated with a cantaloupe-related outbreak) was obtained from Ms. Sharon Abbott and Dr. Michael Jordan, California Dept. of Health Services. *L. monocytogenes* strains Scott A (a clinical isolate) and 43256, 51742 (food isolates) were obtained from the USDA-ARS-ERRC culture collection. The bacterial strains were grown by two successive loop transfers of individual strains incubated at 37 °C for 24 h in 5 ml Tryptic Soy Broth (TSB) with 0.6% yeast extract (TSBYE, Difco, Sparks, MD, USA). A final transfer of 0.2 ml was made into 50 ml TSBYE with incubation at 37°C for 18 h. The bacterial cells were harvested by centrifugation (4,000 x g, 10 min) at 4°C. Resulting cell pellets were washed twice in 0.1% (w/v) peptone water (PW) and finally suspended in PW. Equal volumes of individual cultures were then mixed to obtain a 3-strain cocktail of *Salmonella* and *Listeria* of cell population of about 10⁸ CFU/ml in the inoculums.

2.3. Preparation of cantaloupe purees (CP)

Medium size ripe cantaloupe (approx. 1380 g) were washed thoroughly in potable tap water and cut in a half using a sterile knife. The outer rind and the seeds were removed and the flesh was diced into small cubes. The cubes were then blended in 100 g batches in a large blender for

about 1 min on high setting. Blended batches were mixed together and reblended for 30 s to a consistent homogeneous puree.

2.4. Inoculation of cantaloupe puree

Freshly prepared cantaloupe puree as above was divided into two parts. To one part, food grade ascorbic acid (AA, Sigma-Aldrich, St. Louis, MO, USA) was added to achieve a concentration of 0.1% AA (w/w) in the puree (CP-AA); while no AA acid (NAA) was added to the other part of puree (CP-NAA). Cantaloupe puree (50 g) containing AA and NAA was inoculated with 5 ml working inocula of *S. enterica* or *L. monocytogenes* separately to a inoculation level of about 10^7 CFU/g and pummeled in a stomacher (Seward 400, Seward, London, U.K.) for 30 s to homogenize. Following inoculation, CP was subjected to HHP treatment as described below.

2.5. High Hydrostatic Pressure treatment of cantaloupe puree

Cantaloupe puree containing AA and NAA, inoculated with *S. enterica* or *L. monocytogenes* as described above was placed into individual sterile stomacher bags, double-sealed and double-bagged (ca. 5 g/bag). Samples were then pressure-treated at levels ranging from 300 to 500 MPa (in 100 MPa increments) for 5 min at 8 °C and 15 °C using a laboratory-scale, 0.5 L volume pressure unit (Mini Food lab FPG5620, Stansted Fluid Power Ltd., Essex, UK), where heat transfer fluid (a mixture of ethanol and castor oil) continuously circulated through a refrigerated liquid chiller (Proline RP 855, Lauda, Germany) to maintain temperature. The initial temperature in the processing chamber with samples was 8 °C and 15 °C and reached to a maximum of 35 °C at the highest working pressure (500 MPa). The pressure come-up rate was approximately 100

MPa/15 s and the pressure release was rather fast (~100 MPa/10 s.; Fig. 2 A., 2 B.). Pressurization time reported in this study did not include the pressure-come-up or release times. The chamber temperature was recorded by a built-in sensor (a T-type thermo couple) which was calibrated against a standard mercury thermometer with accuracy ± 0.5 °C. The temperature sensor was immersed in the working chamber close to puree samples, which was filled with the recirculation fluid. A thermo couple was inserted close to the bags containing puree samples inside the chamber to record puree sample temperature during experiment. All HHP treatments were conducted in duplicate in random mode and three samples were analyzed in each experimental replication. After pressure treatments, cantaloupe puree samples were cooled on ice and analyzed immediately for survivors as per method described in Section 2.7. Samples were also stored in a refrigerator at 4 ± 1 °C to examine the impact of HHP on pathogens survivability during refrigerated storage for up to 10 days.

2.6. Microbial enumeration

Treated or untreated samples were analyzed to recover the survivors by adding 0.1% sterile PW to each bag containing sample in 1:8 ratio (wt: vol.). The mixture was pummeled in a Stomacher 400 laboratory blender (Seward, Worthington, UK) for 2 min at 230 rpm to obtain a homogenate. Decimal serial dilutions of the homogenate were then prepared in 0.1% PW (pH 6.9). Surviving bacterial populations in puree samples were evaluated by plating 0.1 mL (in duplicate) on nonselective TSA (BD, Difco). Plates were held at room temperature (22°C) for 2 h inside a biosafety cabinet (Nuare™, Plymouth, MN, USA) before overlay of selective agar. The waiting period was to allow for possible recovery of injured bacteria (Juneja et al., 1997). After 2 h, TSA plates were overlaid with the selective medium Xylose-lysine-tergitol 4 (XLT-4,

BBL, BD Difco) for *S. enterica* and PALCAM (Oxoid, England) for *L. monocytogenes*. The plates were then incubated at 37 °C for 24 h. Presumptive colonies of *Salmonella* or *Listeria* formed on the plates were counted. Instances, when there were no detectable viable cells, samples were enriched. For the enrichment methods, approximately, 50 g of all treated samples were placed in a Stomacher bag along with 200 ml Rappaport broth (Oxoid) for *Salmonella* or UVM broth (BBL/Difco) for *L. monocytogenes* followed by incubation for 48 h at 37 °C. Aliquots (0.1 ml) of each undiluted homogenate from the enriched samples were plated on selective media for each bacterium stated above. The experiments were conducted three times with duplicate determination of colony forming units (CFU).

2.7. Soluble Solids (BRIX) analysis

Soluble solids were measured in the control and treated puree sample with or without added AA using a refractometer (AR200 Digital Refractometer, Reichert, Inc, Depew, NY, USA) at room temperature. Sample holder was cleaned and dried using DI water and Kim wipe in-between samples readings. Duplicate reading was taken for each replicate of triplicate experiments. Results were expressed as BRIX-TC.

2.8. pH measurement

A tabletop pH meter (Orion 420A with Orion 9157RN triode refillable pH electrode, Thermo Electron Corporation, Waltham, MA, USA) was used to measure pH of the puree samples. Calibration of the pH meter was done prior to taking readings using the pH 3, 7 and 10 standard buffers.

Cantaloupe puree had a neutral pH of about 6.9 and with the addition of AA (0.1%) the pH was reduced to 6.4. Due to its low acidity (pH> 4.6) cantaloupe is considered as low-acid food. Low acid foods generally favor bacterial growth and hence need to be monitored closely.

2.9. Statistical analyses

Experiments were conducted in duplicate using three cantaloupe puree samples for each treatment, per experimental design, for a total of six data points per treatment. Data were pooled and analyzed using SAS (version 9.2) statistical package (SAS Institute Inc., Cary, NC, USA) for analysis of variance (ANOVA) and the Bonferroni LSD method (Miller, 1981) to estimate significant differences ($p<0.05$) between mean values. Significance was defined at $p<0.05$.

3. Results and discussion

3.1. Effects of pressure magnitude on inactivation of pathogens in cantaloupe puree

The results of high pressure treatment of cantaloupe puree inoculated with *Listeria* and *Salmonella* spp. are shown in Figure 1. The HHP treatment had a lethal effect reducing both microbial prevalence and level. The efficacy of pressure treatment varied as a function of pressure intensity for both bacterial pathogens. A steady decrease in the surviving population of *Salmonella enterica* and *Listeria monocytogenes* was observed at increasing pressure level. Initial inoculation population (~ 6.7 log CFU/g) in the cantaloupe puree sample was reduced significantly ($p<0.05$) due to pressure treatment at 300 Mpa for 5 min at 8 °C and 15 °C for *Salmonella* but the reduction for *Listeria* spp. was not significant. Although, significant reductions ($p<0.05$) for both target pathogens in the puree sample were observed as the pressure magnitude were further increased to 400 Mpa and above (Fig 1). Pressure treatments of 500 MPa

for 5 min at 8 °C inactivated *Salmonella* completely in cantaloupe puree but leaving a low population (1.1 ± 0.2 log CFU/g) of *Listeria* survivors in the puree. However, the pathogens in the puree samples were undetectable (LOD, the limit of detection = 1 log CFU/g) when the pressure treatment (500 MPa) was carried out at 15 °C. Results indicated *Listeria* to be more resistant to pressure treatment compared to *Salmonella* spp. in cantaloupe puree. At 400 MPa, log reductions for *L. monocytogenes* were 2.9 ± 0.5 and 3.1 ± 0.4 logs at 8° and 15 °C, respectively, which were significantly less compared to log reductions (4.5 ± 0.2 and 5.6 ± 0.4 logs) achieved for *Salmonella* spp. under same treatment conditions. Further, a low population of 1.1 logs CFU/g of *Listeria* spp. survived in the cantaloupe puree after pressure treatment at 500 MPa and 8 °C, but no detectable survivors for *Salmonella* were found. Pressure treatment at 500 MPa and 15 °C for 5 min inactivated both pathogens in the puree as the count for *Salmonella* and *Listeria* fell below the detection limit after treatment.

Several reports probed HHP efficacy to control microbial contamination in food and maintain quality. Pasteurization of fruit purees and juices with a HHP treatment has received considerable attention. A recent report on strawberry puree indicated that HHP treatment of 450 MPa pressure at 21°C for 2 min eliminated both *Salmonella enterica* and *Escherichia. coli* O157:H7 in strawberry puree with 6-log CFU/g initial inoculum levels (Huang et al., 2013). The result of this study was similar to our present work with cantaloupe puree although no direct comparison can be made since the food matrix and treatment intensity are somewhat different. High pressure treatment of fruit juices led to inactivation of food borne pathogens to various degrees. HHP treatment of 500 MPa for 5 min at 20 °C provided a 5-log reduction of *E. coli* O157:H7 in apple juice (pH 3.5) and tomato juice (pH 4.1), and only ~2 log reduction in orange juice (pH 3.8) (Jordan et al., 2001). Buzrul et al. (2008) reported inactivation of *L. innocua* and

E. coli O157:H7 by 4 logs in kiwifruit juice but only 1 log reduction in pineapple juice by 300 MPa pressure treatments at 20 °C for 5 min. Pressure treatment of 400 MPa for 10 min provided a 6 log inactivation of *E. coli* O157:H7 in mango juice (Hiremath et al., 2012). Results presented in these reports differ between the studies due to the use of different strains, food matrices, and treatment condition.

3.2. Effects of processing temperature on inactivation of pathogens in cantaloupe puree

The HHP treatment exerts uniform and instant pressure on foods regardless of its shape or size. The extent of temperature increase during HHP depends on the physical properties of the foods and the processing fluid; and for water this ranges from 2-4°C per 100 MPa increase in pressure (Rastogi et al., 2007).

The temperature-pressure profiles of the high hydrostatic pressure processing experiments of cantaloupe puree at 8 °C and 15 °C initial sample temperatures are shown Figure 2A and 2B. In theory, temperature should remain constant during the pressure hold time (processing time), but as in the figure, the temperature actually dropped due to continuous loss of heat to the metal wall surrounding the pressure chamber. Also, during the decompression stage, after the hold period (5 min), the temperature decreased, and dropped below the initial experimental temperature (Fig 2A-B) due to the loss of heat during hold period. The actual sample temperatures at various treatment pressures and initial sample temperatures of 8 ° and 15 °C are given in Figure 2C. According to our results, the temperature increase in cantaloupe puree was dependent on the applied pressure. For 8 °C initial sample temperature, the final temperature obtained were 22.4, 26.2 and 30.2 °C at 300, 400 and 500 MPa operating pressures respectively. Similarly, the sample temperature increased to maximum values of 29.7, 32.7 and 35.8 °C at 300, 400 and 500

MPa pressures respectively when initial temperature was 15 °C (Fig. 2C). This represents an average temperature increase of about 4 °C per 100 MPa pressurizations. Cao et al. (2011) investigated changes in enzymes activity and phenolic contents of strawberry pulps after HHP treatment and reported 3 °C temperature increase per 100 MPa compression. The effect of increased temperature in HHP could be substantial and may play a significant part in log reduction depending on the initial sample temperature, physical property of the food, and the target pressure. The effects of sample temperatures on the inactivation of pathogens at 300, 400 and 500 MPa target pressures are given in Table 1. In general, log reductions increased, but not significantly, for both pathogens as the initial temperature of puree sample was increased for the pressure ranges (300-500 MPa) investigated. At 8 °C initial temperature, and effective sample temperature of 26.2 C (400 MPa), log reduction for *Salmonella* was 4.5 ± 0.2 log CFU/g which was less ($p > 0.05$) compared to log reduction 5.5 ± 0.2 log CFU/g achieved at 15 °C initial temperature and effective sample temperature of 32.7 C (400 MPa) (Table 1). Similarly, at 300 Mpa, the initial temperatures (8 and 15°C) had no significant influence on *Salmonella* log reductions. Also, as evident from Table 1, the effect of experimental temperatures (8 and 15°C) on inactivation of *Listeria monocytogenes* in cantaloupe puree by HHP within the target pressure ranges (300 -500 MPa) was not significant. However, the log reductions for *Salmonella* were significantly higher compared to *Listeria* spp. at experimental temperature (8°C and 15 °C). Attention should be given while designing HHP experiments to minimize the thermal effect of high pressure compression since accumulated heat may adversely alter the quality. In the current study, experiments were designed to perform at near or below 35 °C to minimize the thermal effect of compression.

3.3. Combined effect of HHP and ascorbic acid on the inactivation of pathogens in cantaloupe puree

High pressure experiments were conducted in two separate sets. In one set, cantaloupe puree was treated with AA (0.1%) as a processing aid (USDA, 2009), prior to inoculation and high pressure treatment. Effects of HHP treatment on the survivability of pathogens in cantaloupe puree in the presence and absence of added ascorbic acid is given in Table 2. As indicated in Table 2, AA at 0.1% did not show any significant antimicrobial action against pathogens during high pressure treatment. Survivor counts were, in general, less, but not significantly, for cantaloupe puree containing 0.1% AA compared to puree with no added ascorbic acid (NAA). In presence of AA, counts were approximately 0.4 and 0.2 log CFU/g less for *Salmonella* and *Listeria*, respectively, compared to puree with no AA over the treatment pressures (300-500 MPa) and temperatures (8-15 °C). The measured average pH of cantaloupe puree was 6.9 ± 0.4 and with the addition of 0.1% AA, pH was decreased to 6.4 ± 0.2 . Any mild, insignificant, antimicrobial action of AA during HHP may be due to the pH lowering effects of the acid. Antioxidant and antimicrobial activity of AA on pathogens in food has been reported in the literature. Jordan et al. (2001) conducted HHP experiments (150-350 MPa at 20 °C for 5 min) to inactivate pressure resistant *E. coli* O157 strain C9490 and *L. monocytogenes* in apple juice supplemented with ascorbic acid (5%) and observed no enhanced inactivation in the presence of added ascorbic acid. High pressure treatment (200-600 MPa) was reported to reduce background microbial load (mesophilic aerobic, psychrotrophic and moulds & yeasts counts) and retain the firmness for about a month for nectarine halves when pretreated with either AA (2%) or AA (2%) plus calcium lactate (1%) while non-treated fruits presented microbial spoilage (Miguel-

Pintado et al., 2013). In this study, the presence of ascorbic acid (0.1%) did not show any antimicrobial activity.

3.4. Effect of refrigerated storage on the survival of pathogens in untreated cantaloupe puree and HHP treated puree

In this study, cantaloupe puree containing 0.1% AA (pH 6.4; 11.3 °Brix) and no AA (pH 6.9; 11.2 °Brix) and inoculated with cocktails of *Listeria* and *Salmonella* was pressure treated at 300-500 MPa for 5 min at 8 °C and 15 °C and immediately stored in a refrigerator at 4 °C for 0 (before refrigeration), 6, and 10 days. The effect of refrigeration on survival of bacterial pathogens in HHP treated and untreated cantaloupe puree over a short storage is shown in Table 3. Our results showed that both *Salmonella* and *Listeria* were unable to grow in non-pressure treated (control) puree during short storage at refrigeration temperature, but they were able to survive with full viability. For both pathogens, there was no significant increase or decline in population over 10 days. This is in agreement with previous reports. The results of survival and no growth of *Listeria monocytogenes* in untreated cantaloupe puree supports similar reports of survival but no growth of this pathogen inoculated in fresh-cut cantaloupes stored at 4°C for 15 days (Ukuku and Fett, 2002). The authors concluded that the inability of the pathogen to grow in fresh-cut pieces stored at 4°C due to the antagonistic interaction of the native microflora of the whole cantaloupe rind surface transferred to fresh-cut pieces during fresh-cut preparation. In another study (Ukuku et al., 2015), the authors reported minimal growth in fresh-cut samples stored for more than 9 days at abuse temperature, 10°C and concluded that the growth conditions or inhibition was again due to interaction of native bacterial populations in the sample.

Therefore, the lack of growth of *Listeria monocytogenes* on puree samples stored at 4°C, in the current study, is probably, among other things, due to the antagonistic effect of native bacterial population to the growth.

Flessa et al. (2005) studied survival of *Listeria monocytogenes* on intact and cut surface of strawberries at 4 °C and 24 °C for 7 days and reported that populations on cut surfaces remained constant at both temperatures irrespective of initial inocula levels throughout the storage period and concluded that *L. monocytogenes* is capable of survival but not growth on the surface of fresh intact or cut strawberries throughout the expected shelf life of the fresh fruit. Golden et al. (1993) studied growth of *Salmonella* multiserotypes in fresh cut cantaloupe, honeydew and water melons and observed no growth of *Salmonella* population and little or no decrease in viable populations during 24 hrs incubation at 5°C. Reports also indicated that *Listeria monocytogenes*, *Salmonella* and *E. coli* O157:H7 can survive during cold storage and could be recovered from apple, orange, pineapple and white grape juice concentrates and banana puree through 12 weeks of storage at -23 °C (Oyarzabal et al., 2003). Results of the present work indicate that *Salmonella* and *Listeria* spp. survive in refrigerated untreated cantaloupe products and should not be underestimated. Effective pasteurization of cantaloupe puree is very important to avoid potential consumer safety issues.

For pressure treated cantaloupe puree, a steady significant decline in survivor population of both pathogens was observed as compared to the control during subsequent refrigerated storage (Table 3). The log reductions achieved by the combined process of HHP and refrigeration were often quite substantial compared to high pressure treatment individually. As an example, HHP treatment at 300 MPa and 8 °C for 5 min, provided 2.5 and 1.5 log CFU/g reductions for *Salmonella* and *Listeria* spp., respectively, and 3.6 and 2.6 logs reductions for *Salmonella* and

Listeria spp., respectively, after 10 days refrigeration of pressure treated puree (Table 3). This represents an additional approximately 1 log reduction for both pathogens due to the synergistic effect of high pressure treatment and subsequent refrigeration. For puree samples inoculated with about 6.7 log CFU/g of *Salmonella* and *Listeria*, 500 MPa pressure treatments at 15 °C for 5 min was required to achieve complete inactivation by HHP alone. However, if combined with 10 days of subsequent refrigeration, only 400 MPa pressure treatments would suffice a complete kill. This is probably due to the reason that HHP causes the injury to *Salmonella* and *Listeria* cells and hence remain unable to grow during 10 days storage at refrigeration temperature. Several researchers (Garcia-Graells et al., 1998; Jordan et al., 2001; Yang et al., 2012) reported accelerated death in pressure treated microbial cells during storage above and below refrigeration temperature. Subsequent refrigeration of pressure treated orange juice (pH 3.8) for a day increased the level of inactivation of *E. coli* O157 by 2 logs (Jordan et al., 2001). Similarly, Buzrul et al. (2008) reported 2.5 and 3.5 logs reductions for *E. coli* and *L. innocua*, respectively in pineapple juice but the inactivations were further increased more than 1 log during storage at 4 °C for 24 h for both bacteria. Garcia-Graells et al. (1998) examined the high-pressure inactivation and sublethal injury of pressure-resistant *E. coli* mutant strains in apple, orange and mango juices and observed very limited direct inactivation of mutants with pressure treatments up to 500 MPa but an accelerated inactivation of pressure treated cells during subsequent storage at 8-25 °C for up to 30 days. Results of these previous studies are in general agreement with our findings of death of pressure-treated bacterial cells in cantaloupe puree during subsequent refrigeration, though all of these previous studies were related to high acid food. The inactivation of microorganisms by HHP has been attributed to cell membrane damage, cell wall rupture and chromosome DNA degradation (Yang et al., 2012). The disruptions of cell membrane may

causes leakage of cytoplasmic materials (Guerrero-Beltran et al., 2005) and facilitate entry of compounds that are present in the immediate environment, which may act as natural antimicrobials causing considerable additional inactivation during the refrigerated storage after pressure inactivation.

4. Conclusions

The study indicated effectiveness of high hydrostatic pressure processing to inactivate *Salmonella enterica* and *Listeria monocytogenes* in cantaloupe puree. Pressurization at 300–500 MPa for 5 min at 8 and 15°C brought about different levels of inactivation of pathogens depending on the pressure level. *Listeria* presented higher resistance to HHP compared to *Salmonella* spp. Treatment at 400 MPa and 15 °C for 5 min effectively reduced the number of pathogens to below 1 log in cantaloupe puree during short (10 days) post-pressure refrigeration. The population of natural yeasts and molds and total aerobic microorganisms in the puree also reduced to below detection level with retention of fresh like quality (data not presented). Results demonstrate the ability of high pressure processing. However the production cost (capital and operational) of high pressure batch processing is relatively high compared to thermal treatment which is a real drawback of this process. Since the production cost increases with the increase of pressure, a reduction in operating pressure by incorporating HHP treatment combined with other processing techniques, to achieve the same level of inactivation, remains the main option to make this process economically viable. Study indicated a complete inactivation of *Listeria* and *Salmonella* spp. in the puree is achievable at a lower pressure of 400 MPa at 15 °C if the treatment combined with a 10-day of refrigeration, instead of processing alone at a higher pressure of 500 MPa.

Acknowledgment

The authors thank Jia Zhen for help with high pressure experiments and Dr. John Phillips for assistance with statistical data analysis. This study was funded by the USDA through ARS National Program 108, ARS CRIS project 1935-41000-092.

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Figure Legends

Figure 1. Survivor population of *Listeria* and *Salmonella* spp. in the control and in high pressure treated cantaloupe puree over the pressure ranges of 300 - 500 Mpa at two different temperatures (8° and 15 °C) for 5 min. Recovered mean population (initial) of pathogens was 6.7 ± 0.4 log CFU/g. The limits of detection were 1log CFU/g for plating method. Error bars represent the standard deviation of the mean population. Data with different upper case letters for each treatment are significantly different ($p < 0.05$). ND: No detectable survivor and were negative after enrichment.

Figure 2. Temperature (—) and Pressure (- - -) profiles of high pressure processing of cantaloupe puree at two different initial temperatures, (A) 8 °C and (B) 15 °C; and (C) the thermal effect of HHP treatment on actual temperature of cantaloupe puree sample over 300-500 MPa pressures as a function of initial temperatures.

Figure 1.

A.

B.

C.

Figure 2.

Table 1. Temperature effects of pressure inactivation of *Salmonella* spp. and *Listeria monocytogenes* in cantaloupe puree at different initial temperatures (8 and 15 °C) and pressures (300-500 MPa) for 5 min

Initial temperature (°C)	Pressure (MPa)	Final temperature (°C)	<i>S. enterica</i> Log Reduction (log CFU/g)	<i>L. monocytogenes</i> Log Reduction (log CFU/g)
8	0.1	8*	0	0
	300	22.4	2.4±0.25Bc	1.6±0.17Cd
	400	26.2	4.5±0.24Ab	2.9±0.53Bc
	500	30.2	> 5.7 [§]	5.57Aa
15	0.1	15	0	0
	300	29.7	2.9±0.34Bc	1.8±0.32Cd
	400	32.7	5.6±0.42Aa	3.1±0.41Bc
	500	35.8	> 5.7	>5.7

Data represent mean population reduction (log CFU/g) of replicates ± standard deviations.

*Initial pressure is atmospheric (0.1 MPa)

Recovered mean population (initial) of pathogens was 6.7 ± 0.45 log CFU/g.

The limits of detection (LOD) were 1 log CFU/g for plating method.

§ Log reduction at population below LOD is 6.7- (<1) or >5.7

Data followed by the same upper case letter **in columns** are not significantly different (p > 0.05).

Data followed by the same lower case letter **in rows** are not significantly different (p > 0.05).

Table 2. Pressure inactivation of *Listeria monocytogenes* and *Salmonella* spp. in cantaloupe puree. Cantaloupe puree with 0.1% ascorbic acid (AA) and no ascorbic acid (NAA) was treated at 300–500 MPa for 5 min at 8 and 15 °C

Pressure (MPa)	Temperature (°C)	<i>Salmonella enterica</i> (log CFU/g)		<i>Listeria monocytogenes</i> (log CFU/g)	
		NAA	AA	NAA	AA
0.1*	8	6.8±0.2a	6.7±0.1a	6.7±0.3a	6.7±0.1a
300		4.3±0.3b	4.0±0.2b	5.2±0.3ab	5.1±0.6ab
400		2.2±0.2c	1.8±0.4c	3.8±0.2b	3.6±0.3b
500		ND	ND	1.1±0.2c	ND
0.1*	15	6.8±0.3a	6.7±0.2a	6.6±0.5a	6.6±0.4a
300		3.9±0.7b	3.5±0.3b	4.8±0.4ab	4.7±0.2ab
400		1.2±0.4c	1.1±0.2c	3.6±0.2b	3.4±0.5b
500		ND	ND	ND	ND

Data represent mean survival population (log CFU/g) of replicates ± one standard deviations.

*Initial pressure is atmospheric (0.1 MPa).

AA- Cantaloupe puree with 0.1% Ascorbic acid; NAA-Cantaloupe puree with no added AA

The limits of detection were 1 log CFU/g for plating method. ND: No detectable survivor after enrichment.

Data followed by the same lower case letter in columns are not significantly different ($p > 0.05$).

Table 3. Survival of *Salmonella enterica* and *Listeria monocytogenes* in control and pressure treated cantaloupe puree during 10-day refrigerated storage. Cantaloupe puree inoculated with *Salmonella* and *Listeria* spp. pressure treated at 300-500 MPa for 5 min at 8 and 15°C and then stored at 4°C in a refrigerator

Treatment Pressure (MPa)	Treatment Temperature (°C)	Storage time (day)	<i>Salmonella enterica</i> (log CFU/g)		<i>Listeria monocytogenes</i> (log CFU/g)	
			NAA	AA	NAA	AA
Control	-	0	6.8±0.2a	6.7±0.2a	6.7±0.1a	6.7±0.3a
		6	6.7±0.3a	6.6±0.2a	7.0±0.1a	6.9±0.4a
		10	6.5±0.2a	6.4±0.3a	6.8±0.3a	6.7±0.3a
300	8	0	4.3±0.3b	4.0±0.2bc	5.2±0.3b	5.1±0.6b
		6	4.5±0.3b	3.1±0.2c	4.5±0.3b	4.3±0.3b
		10	3.2±0.2c	2.5±0.3cd	4.1±0.2b	3.9±0.2b
400	8	0	2.2±0.2cd	1.8±0.4d	3.8±0.2c	3.6±0.3c
		6	1.6±0.3d	1.5±0.2d	3.7±0.3bc	3.4±0.6c
		10	1.5±0.1d	1.3±0.4d	2.3±0.cd	1.8±0.3d
500	8	0	ND	ND	1.1±0.2d	ND
		6	ND	ND	ND	ND
		10	ND	ND	ND	ND
300	15	0	3.9±0.8c	3.5±0.3c	4.8±0.5b	4.7±0.2b
		6	3.1±0.3c	3.0±0.2c	4.1±0.3b	3.9±0.6b
		10	2.5±0.2c	2.0±0.4c	3.8±0.2bc	3.1±0.3b
400	15	0	1.2±0.4d	1.1±0.2d	3.6±0.2c	3.4±0.5c
		6	1.4±0.3d	ND	1.9±0.3d	1.7±0.6d
		10	ND	ND	ND	ND
500	15	0	ND	ND	ND	ND
		6	ND	ND	ND	ND
		10	ND	ND	ND	ND

Data represent mean survival population (log CFU/g) of three replicates ± one standard deviations.

*Initial pressure is atmospheric (0.1 MPa).

AA- Cantaloupe puree with 0.1% Ascorbic acid; NAA-Cantaloupe puree with no added AA. The limits of detection were 1 log CFU/g for plating method. ND: No detectable survivor after enrichment.

Data followed by the same **lower case letter in columns** are not significantly different ($p > 0.05$).